Journal of the American Mosquito Control Association, 30(2):138–142, 2014
Copyright © 2014 by The American Mosquito Control Association, Inc.

SCIENTIFIC NOTE

SUSCEPTIBILITY OF FIELD-COLLECTED MOSQUITOES IN CENTRAL NEW JERSEY TO ORGANOPHOSPHATES AND A PYRETHROID

DEBIN SUN,[1,5] NICK INDELICATO,[2,5] JACK PETERSEN,[3] ERIC WILLIGES,[1,3] ISIK UNLU [2,3] AND ARY FARAJOLLAHI[2,3,4]

ABSTRACT. Chemical insecticides are the primary means to control mosquitoes, and mosquito control programs must regularly monitor for resistance of mosquito vectors to commonly used insecticides to ensure the efficacy and sustainability of active ingredients. We performed insecticide resistance bioassays to test the susceptibility of field-collected mosquitoes in central New Jersey to 1 larvicide (temephos) and 2 adulticides (malathion and sumithrin). Larval susceptibility of *Culex pipiens pipiens* to temephos provided median concentration (LC_{50}) and 95% lethal concentration (LC_{95}) values of 1.108 µg/l and 2.02 µg/l, respectively. Bottle bioassays of adult *Aedes albopictus* showed that 100% mortality was achieved at 35-min exposure to sumithrin and at 40-min to malathion. Baseline values were obtained using both temephos and sumithrin. Our bioassays indicate satisfactory susceptibility to temephos and sumithrin in *Ae. albopictus* and *Cx. p. pipiens* field populations in central New Jersey. Despite constant field use, both products are still effective and can be used adequately for control of the test species. However, the susceptibility of target insects to various formulations should be closely monitored periodically to ensure continual efficacy.

KEY WORDS Insecticide resistance, bioassay, temephos, malathion, sumithrin

Monitoring insecticide resistance is an important component of responsible mosquito control programs. While larvicides and adulticides have traditionally been used to control mosquito populations, long-term use of these insecticides may lead to reduced efficacy caused by insecticide resistance in local mosquito populations. Organophosphates and pyrethroids are 2 main classes of insecticides commonly used in mosquito control, and resistance to these chemicals has been reported in multiple mosquito species worldwide (Brogdon and McAllister 1998, Hemingway and Ranson 2000). However, insecticide resistance may be very focal (Canyon and Hii 1999), which suggests that it is important to monitor insecticide resistance locally.

To reduce the chances of insecticide resistance development in local populations, many mosquito control programs utilize an integrated mosquito management (IMM) plan to monitor and suppress target populations as needed. This approach is a comprehensive mosquito control or prevention strategy that utilizes all available methods to reduce populations to tolerable levels while minimizing the environmental impact of control activities. When used properly, IMM may greatly delay or minimize insecticide resistance in mosquito populations.

[1] Essex County Division of Environmental Affairs, Cedar Grove, NJ 07009.
[2] Mercer County Mosquito Control, West Trenton, NJ 08628.
[3] Center for Vector Biology, Rutgers University, New Brunswick, NJ 08901.
[4] To whom correspondence should be addressed.
[5] Both authors contributed equally to this study.

Bioassays allow for the recognition of insecticide resistance in mosquito populations. The diagnostic dose can be used to discriminate susceptible mosquitoes from resistant, with those that survive the test considered as resistant to the insecticide being tested. A resistance ratio is another way to detect insecticide resistance using bioassays (Paeporn et al. 2004). It is calculated by dividing the toxicity value of the resistant strain by that of the susceptible strain (Selvi et al. 2010). The resistance will be considered as low if the resistance ratio is between 1 and 5, medium if it is between 5 and 10, and high if it is more than 10 (Mazzarri and Georghiou 1995). The Centers for Disease Control and Prevention (CDC) bottle bioassay is a simple and economical method of quickly gauging insecticide resistance in field-collected mosquito populations (McAllister et al. 2012). This method uses bottles coated with an insecticide to detect potential resistance by measuring the time it takes for the insecticide to kill mosquitoes. The World Health Organization (WHO) also provides a standard procedure for laboratory and field testing of mosquito larvicides, and this method has also been adopted worldwide to test larvicide efficacy (Paeporn et al. 2004, Chen et al. 2005, Selvi et al. 2010, Bisset et al. 2013, WHO 2013).

Culex pipiens pipiens L. and *Aedes albopictus* (Skuse) are arguably the most important vector species in northeastern USA (Farajollahi and Crans 2012). *Culex p. pipiens* is the primary enzootic vector of West Nile virus in this region, and can be controlled effectively in immature stages using larvicides (Farajollahi et al. 2011). *Aedes albopictus* is a container-inhabiting species

that can be challenging to control via larvicides in the immature stages; therefore, source reduction plays a larger role in larval management (Bartlett-Healy et al. 2011, Farajollahi and Price 2013). It is often necessary to utilize adulticides intensively to reduce populations of both species for the protection of public health. However, insecticide resistance to a large variety of active ingredients has been reported widely for both species, including organophosphates and pyrethroids (APRD 2014). Because *Ae. albopictus* and *Cx. p. pipiens* are a primary concern for mosquito control districts in our region, and because organophosphates and pyrethroids are heavily used against these species, the purpose of our study was to investigate the status of insecticide resistance in these species in order to guide our operational activities and select the best choice of chemical for field use.

Specifically, we used the CDC bottle bioassay method to test the susceptibility of adult *Ae. albopictus* to malathion and sumithrin and used the WHO method to determine the susceptibility of immature *Cx. pipiens* to temephos. Our ultimate goal was to establish susceptibility baselines of field-collected mosquitoes in central New Jersey against these chemicals as part of our IMM plan.

Egg rafts of *Cx. p. pipiens* were collected from Trenton, NJ, during the late summer and early fall of 2012 in a local study site (40.235156, −74.744831) using gravid trap pans filled with a fermented mixture of grass and tap water. The grass infusion was made by adding 1 kg of freshly cut grass to 120 liters of water. Egg rafts were collected in the morning and transported to the laboratory. Each individual egg raft was allowed to hatch in separate larval rearing trays containing 2 liters of dechlorinated tap water. The larvae were reared in separate 20-cm × 30-cm stainless steel pans and fed finely ground rat chow every 2 days (Nelder et al. 2010). Larvae were maintained at 25°C and 70% RH with a photoperiod of 16:8 h L:D. The larvae were allowed to develop to the 3rd instar before species identification confirmation and use in subsequent bioassays. Voucher specimens were also preserved and cataloged for record keeping.

Aedes albopictus eggs were collected from another study site (40.226585, −74.738521) in Trenton, NJ, during the late summer and early fall of 2012. Eggs were collected by using dark-green plastic cemetery vase (400-ml capacity; Eaton Brothers Corp., Hamburg, NY) oviposition cups (ovitraps). The ovitraps were filled with 300 ml of an oak leaf–infused water (5 g of *Quercus alba* oak leaves to 8 liters of tap water) to increase the number of eggs oviposited (Fonseca et al. 2013). Seed germination papers (Steinly et al. 1991) were inserted into the ovitraps to cover the interior surfaces and collected weekly by field crews. Collected germination papers were placed in labeled plastic bags to retain moisture and reduce egg desiccation, and taken to the laboratory where they were submerged in dechlorinated tap water to allow the eggs to hatch. *Aedes albopictus* larvae were reared using the same methods as with *Cx. p. pipiens* larvae above.

Pupae were transferred to a separate 236-ml Styrofoam cup and then placed inside an aluminum adult mosquito holding cage measuring 30 × 30 × 30 cm in size. Upon eclosion, the adult mosquitoes were provided sugar-saturated wicks (10% sucrose solution) ad libitum. Both larvae and adult mosquitoes were maintained at 25°C and 70% RH with a photoperiod of 16:8 h L:D.

Anvil 2+2® (Clarke Mosquito Control, Roselle, IL), with 2% active ingredient (AI) of sumithrin and 2% synergist piperonyl butoxide and Atrapa® (Griffin, Valdosta, GA), with 96.5% AI of malathion were used for adult bioassays. Abate 4E® (Clarke Mosquito Control) with 44.6% AI of temephos was used for the larval bioassays.

The test concentration of malathion used was 474 μg/ml and 22 μg/ml for sumithrin (Petersen et al. 2004; FMCA 2005a, 2005b). The stock solution for temephos was diluted with 99.5% ethyl alcohol (ETOH) to an operational dosage of 1 mg/l. *Culex p. pipiens* larvae were exposed to final test concentrations of 0.6, 0.8, 1, 1.2, 1.4, and 1.6 μg/l to estimate LC_{50} and LC_{95} values.

We followed the WHO standard procedures for larval bioassays (WHO 2013). Briefly, 10 3rd instars were placed into individual plastic cups containing 250 ml of dechlorinated tap water under the various insecticide concentrations. Temephos, along with 1 ml of 99.5% ETOH, was tested at 6 different concentrations (0.6, 0.8, 1, 1.2, 1.4, and 1.6 μg/l) with 3 replicates per concentration. A control cup was used with 250 ml of dechlorinated tap water containing 1 ml of 99.5% ETOH for each concentration. The test and control cups were held at 25°C with a photoperiod of 16:8 h L:D. Larval mortality was recorded after 24 h and subjected to the Pearson goodness-of-fit chi-square test by PoloPlus 2.0 (LeOra software, El Cerrito, CA) to estimate LC_{50} and LC_{95} values.

Adult mosquito bioassays were conducted based on the CDC guidelines for evaluating insecticide resistance in vectors (CDC 2010). Briefly, 50 female mosquitoes (held 3 days postemergence without blood meal) were transferred from their holding cage into control and treatment bottles using a mechanical aspirator (Hausherr's Machine Shop, Toms River, NJ). The adults were distributed equally (10 females in each bottle) among 5 250-ml glass bottles with Teflon-lined caps. Of the 5 bottles, 4 were evenly coated with 1 ml of the test concentration that contained the commercial product and acetone while the control was coated only with 1 ml of acetone. A single dose was used for each bioassay, with adult mortality recorded every 15 min in the first 30 min and every 5 min after that. The bioassay was repeated 4 times and data were

Table 1. Larval bioassays of temephos[1] against 3rd instars of *Culex pipiens pipiens* in the laboratory.[2]

Insecticide	*n*	Slope ± SE	Statistical test (Pearson goodness-of-fit)	LC_{50} µg/l (95% CI)	LC_{95} µg/l (95% CI)
Temephos	210	6.307 ± 0.863	$\chi^2 = 10.972$ df = 16 $P = 0.05$	1.108 (1.024–1.201)	2.02 (1.745–2.573)

[1] Bioassay data were analyzed by PoloPlus 2.0.
[2] CI, confidence interval; LC, lethal concentration; *n*, mosquito larvae.

pooled for analysis. Bottles were soaked in a 10% bleach solution for 1 h, washed with a mild detergent, triple rinsed, and oven-dried to ensure the removal of all foreign substances after each assay before being used in bioassays (CDC 2010).

We established a susceptibility baseline for *Cx. p. pipiens* exposed to temephos following our bioassays. The LC_{50} and LC_{95} values for temephos were estimated to be 1.11 µg/l and 2.02 µg/l, respectively (Table 1). The heterogeneity obtained from Polo Plus was 0.69, indicating the data fit the model.

According to WHO guidelines (WHO 2013), mosquitoes are considered susceptible to adulticides if the observed mortality within 60 min is between 98–100%, resistant if mortality is less than 90%, and the existence of resistance is suggested if mortality is between 90–97%. The bioassays performed with sumithrin showed that *Ae. albopictus* specimens used in our tests were susceptible to this chemical (Table 2). At 30 min after treatment, 99.38% of test mosquitoes were killed and 100% mortality was achieved at 35 min after combining data from all replicates. No mortality was observed in control groups. The bioassays performed with malathion showed that *Ae. albopictus* were also susceptible to this chemical (Table 2). At 30 min after treatment, 98.75% of test mosquitoes were killed and 100% mortality was achieved at 40 min in all replicates. No mortality occurred in control groups.

The recommended diagnostic concentration for temephos is 12 µg/l within 24 h (WHO 2013). In our bioassays, LC_{95} value of temephos for *Cx. p. pipiens* within 24 h was estimated to be 2.02 µg/l. The locally observed diagnostic concentration for temephos was determined as 4.04 µg/l, which was less than the recommended 12 µg/l, suggesting local populations of *Cx. p. pipiens* are susceptible to temephos.

Table 2. Adult bioassay and time-mortality of *Aedes albopictus* exposed to the adulticides malathion and sumithrin in the laboratory.

Minutes posttreatment	Percent mortality[1]	
	Sumithrin	Malathion
15	90.6	90
30	99.4	98.8
35	100	99.4
40	100	100

[1] Diagnostic concentration of sumithrin at 22 µg/ml and 474 µg/ml for malathion.

The WHO guidelines also suggest doubling the observed LC_{99} value to obtain a diagnostic concentration in local tests. However, we instead defined this as 2 times the LC_{95} value (Petersen et al. 2004) because a reliable estimate of the LC_{99} value requires an impractically larger sample size than the estimate of the LC_{95} (Robertson et al. 2007). The bioassays in this study allowed us to create LC_{50} and LC_{95} values within the suggested dilution series. In the future, insecticide resistance in local mosquito populations will be measured by comparing the LC_{50} value estimated in this study with future values in calculating a resistance ratio.

During our adult *Ae. albopictus* bioassays, 99.38% mortality was observed for sumithrin within 30 min and 100% mortality was achieved within 40 min. This suggests that *Ae. albopictus* is highly susceptible to sumithrin in our study areas, although resistance to pyrethroids has been reported for this species elsewhere (Chen et al. 2005, Bisset et al. 2013). To be conservative in detecting possible resistance in mosquito populations, 100% mortality at 40 min after treatment will be used as a baseline for sumithrin in the future.

For our adult *Ae. albopictus* bioassays against malathion, we used a concentration 9.5 times higher than the recommended diagnostic concentration (CDC 2010). We relied on a time-tested standardized protocol developed and used routinely in Florida (FMCA 2005a, 2005b). In future studies, we will test the assumption of 474 µg/ml as the diagnostic concentration by dose–response tests in bottle bioassays employing local *Ae. albopictus*.

Culex p. pipiens and *Ae. albopictus* are 2 species that are major concerns for mosquito control practitioners because of their vectorial status and their subsequent importance in public health. Since *Ae. albopictus* control is difficult in the larval stage because of the ubiquity and abundance of container habitats in peridomestic environments, we concentrated our resistance investigations on adulticides used against this species. Conversely, temephos has been prominently used against juvenile *Cx. p. pipiens* in the past, and our testing was geared toward this larvicide. Nonetheless, temephos, malathion, and sumithrin are frequently

used to control both of these species in New Jersey. However, the consistent and continual use of any insecticide such as organophophates and pyrethroids against target species will lead to resistance in local mosquito populations. There is a need to monitor the susceptibility of mosquito populations for local mosquito control programs and develop baselines for various insecticides against different mosquito species.

In conclusion, our tests did not show insecticide resistance to temephos and sumithrin in wild mosquito populations of *Ae. albopictus* and *Cx. p. pipiens* in central New Jersey. We will continue to monitor insecticide resistance closely within our mosquito populations and use the data accordingly to make informed decisions about practical and efficacious IMM. Continued monitoring using the bioassays reported here will be used as baselines to monitor insecticide resistance and develop a solid foundation for future work. Although there is no need to change the insecticides currently in use, continuation of insecticide resistance monitoring is necessary to maximize insecticide efficacy, maintain environmental quality, and minimize selection pressure on target populations.

We thank the numerous full-time and seasonal employees of Mercer County Mosquito Control for their dedication and professionalism in all aspects of our surveillance and control operations.

REFERENCES CITED

APRD [Arthropod Pesticide Resistance Database]. 2014. *Search : species* [Internet]. East Lansing, MI: Michigan State Univ [accessed March 1, 2010]. Available from: http://www.pesticideresistance.org/.

Bartlett-Healy K, Hamilton G, Healy S, Crepeau T, Unlu I, Farajollahi A, Fonseca D, Gaugler R, Clark GG, Strickman D. 2011. Source reduction behavior as an independent measurement of the impact of a public health education campaign in an integrated vector management program for the Asian tiger mosquito. *Int J Environ Res Public Health* 8: 1358–1367.

Bisset J, Marín R, Rodríguez M, Severson D, Ricardo Y, French L, Díaz M, Perez O. 2013. Insecticide resistance in two *Aedes aegypti* (Diptera: Culicidae) strains from Costa Rica. *J Med Entomol* 50:352–361.

Brogdon WG, McAllister JC. 1998. Insecticide resistance and vector control. *Emerg Infect Dis* 4:605–613.

Canyon D, Hii J. 1999. Insecticide susceptibility status of *Aedes aegypti* (Diptera: Culicidae) from Townsville. *Aust J Entomol* 38:40–43.

CDC [Centers for Disease Control and Prevention]. 2010. *Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay* [Internet]. Atlanta, GA: Centers for Disease Control and Prevention [accessed March 1, 2014]. Available from: http://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf.

Chen C, Nazni W, Lee H, Sofian-Azirun M. 2005. Weekly variation on susceptibility status of *Aedes* mosquitoes against temephos in Selangor, Malaysia. *Trop Biomed* 22:195–206.

Farajollahi A, Crans SC. 2012. A checklist of the mosquitoes of New Jersey with notes on established invasive species. *J Am Mosq Control Assoc* 28:237–239.

Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM. 2011. "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect Genet Evol* 11:1577–1585.

Farajollahi A, Price DC. 2013. A rapid identification guide for larvae of the most common North American container-inhabiting *Aedes* species of medical importance. *J Am Mosq Control Assoc* 29:203–221.

FMCA [Florida Mosquito Control Association]. 2005a. *Formulation of malathion for the bottle bioassay* [Internet]. North Palm Beach, FL: Florida Mosquito Control Association [accessed March 1, 2010]. Available from: http://www.floridamosquito.org/Library/Memoranda/Formulation_of_Malathion_for_the_Bottle_Bioassay.pdf.

FMCA [Florida Mosquito Control Association]. 2005b. *Dilution of Anvil 2plus2 for the bottle bioassay* [Internet]. North Palm Beach, FL: Florida Mosquito Control Association [accessed March 1, 2010]. Available from: http://www.floridamosquito.org/Library/Memoranda/Dilution_of_Anvil_2plus2_for_the_Bottle_Bioassay.pdf.

Fonseca DM, Unlu I, Crepeau T, Farajollahi A, Healy SP, Bartlett-Healy K, Strickman D, Gaugler R, Hamilton G, Kline D, Clark GG. 2013. Area-wide management of *Aedes albopictus*. Part 2: gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. *Pest Manag Sci* 69:1351–1361.

Hemingway J, Ranson H. 2000. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol* 45:371–391.

Mazzarri MB, Georghiou GP. 1995. Characterization of resistance to organophosphate, carbamate, and pyrethroid insecticides in field populations of *Aedes aegypti* from Venezuela. *J Am Mosq Control Assoc* 11:315–322.

McAllister JC, Godsey MS, Scott ML. 2012. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus* from Port-au-Prince, Haiti. *J Vector Ecol* 37:325–332.

Nelder MP, Kesavaraju B, Farajollahi A, Healy S, Unlu I, Crepeau T, Ragavendran A, Fonseca D, Gaugler R. 2010. Suppressing *Aedes albopictus*, an emerging vector of dengue and chikungunya viruses, by a novel combination of a monomolecular film and an insect-growth regulator. *Am J Trop Med Hyg* 82:831–837.

Paeporn P, Ya-umphan P, Supaphathom K, Savanpanyalert P, Wattanachai P, Patimaprakorn R. 2004. Insecticide susceptibility and selection for resistance in a population of *Aedes aegypti* from Ratchaburi Province, Thailand. *Trop Biomed* 21:1–8.

Petersen J, Floore T, Brogdon W. 2004. Diagnostic dose of synergized d-phenothrin for insecticide susceptibility testing by bottle bioassay. *J Am Mosq Control Assoc* 20:183–188.

Robertson JL, Russell RM, Preisler HK, Savin N. 2007. *Bioassays with arthropods*. 2nd edition. Boca Raton, FL: CRC Press.

Selvi S, Edah M, Nazni W, Lee H, Tyagi B, Sofian-Azirun M, Azahari A. 2010. Insecticide susceptibility

and resistance development in malathion selected *Aedes albopictus* (Skuse). *Trop Biomed* 27:534–550.

Steinly B, Novak R, Webb D. 1991. A new method for monitoring mosquito oviposition in artificial and natural containers. *J Am Mosq Control Assoc* 7: 649–650.

WHO [World Health Organization]. 2013. *Malaria: test procedures for insecticide resistance monitoring in malaria vector mosquitoes* [Internet]. Geneva, Switzerland: World Health Organization [accessed March 1, 2010]. Available from: http://www.who.int/malaria/publications/atoz/9789241505154/en/.