*Journal of the American Mosquito Control Association*, 39(3):212–215, 2023


# OPERATIONAL NOTE

## BASELINE SUSCEPTIBILITY AND EFFECTIVENESS OF ADULTICIDES TO LOCAL *AEDES TAENIORHYNCHUS* FROM COLLIER COUNTY, FLORIDA

KEIRA J. LUCAS, EMORY BABCOCK AND RACHEL BALES

*Collier Mosquito Control District, 600 North Road, Naples, FL 34113*

ABSTRACT. The black salt marsh mosquito, *Aedes taeniorhynchus*, is the primary nuisance mosquito in the coastal regions of Florida. This study aimed to establish the baseline susceptibility of *Ae. taeniorhynchus* to adulticide products used for mosquito control by the Collier Mosquito Control District (CMCD). The Centers for Disease Control and Prevention bottle bioassay was used to test technical-grade and formulated products, and Merus 3.0® was evaluated in semifield cage trials through aerial applications. The results revealed the baseline susceptibility of *Ae. taeniorhynchus* to the tested materials and the effectiveness of Merus 3.0 to effectively control the *Ae. taeniorhynchus*. The study provides important information for the development of an integrated mosquito management strategy for controlling *Ae. taeniorhynchus* mosquito populations in southwest Florida.

**KEY WORDS** *Aedes taeniorhynchus*, organophosphate, pyrethroids, resistance testing

The black salt marsh mosquito, *Aedes taeniorhynchus* Wiedemann, is a migratory pestiferous species found throughout the coastal regions of the southeastern United States. Gravid females lay their eggs in moist soil of the salt marsh and mangrove swamps just above the waterline where hatching is influenced by rainfall or high tide (Ritchie and Addison 1992). Because of their migratory nature, adults often leave their emergence sites during large population explosions (Kennedy 1961, Lucas et al. 2019). Population dynamics of *Ae. taeniorhynchus* is dependent on several factors, including severe weather events (Ritchie 1984, Lucas et al. 2019, Weaver et al. 2020). The Collier Mosquito Control District (CMCD), located in southwest Florida, performs a variety of control measures targeting *Ae. taeniorhynchus* during the spring months (April–June). In Collier County (Florida), nuisance adult females typically derive from local mangrove swamps adjacent to residential neighborhoods (Lucas et al. 2019). Naïve females from nearby preserve lands, such as Everglades National Park, Ten Thousand Islands National Wildlife Refuge, and Rookery Bay National Estuarine Research Reserve, also enter the district by migrating on the wind as far as 60 mi (32–96 km) (Harden and Chubb 1960, Lucas et al. 2019).

In recent years, CMCD began making a shift in its approach to integrated mosquito management, incorporating several new surveillance and control strategies to target both vector and nuisance mosquito species. This included the addition of pyrethroid-based adulticide products for mosquito control and the development of an insecticide resistance monitoring program. In Collier, populations of *Ae. aegypti* L. (Estep et al. 2018, Schluep and Buckner 2021, Lucas and Bales 2022) and *Culex quinquefasciatus* Say (Lucas et al. 2020, Watkins et al. 2021) have shown resistance to pyrethroid-based control materials.

To date, information on pesticide resistance in *Ae. taeniorhynchus* remains limited. Some populations of *Ae. taeniorhynchus* have previously shown resistance to DDT (dichloro-diphenyl-trichloroethane) (Brown 1986) and malathion (Boike et al. 1978, Mekuria et al. 1994). The objective of this study was to establish baseline susceptibility to adulticide products for control of *Ae. taeniorhynchus* mosquitoes. Technical-grade and formulated pyrethroid and organophosphate-based products were evaluated in the laboratory using the Centers for Disease Control and Prevention (CDC) bottle bioassay. Further, Merus 3.0® was tested in semifield cage trials through aerial applications. The results of these evaluations determine baseline susceptibility to the tested products and their ability to effectively control *Ae. taeniorhynchus* mosquitoes.

In July 2019 *Ae. taeniorhynchus* larvae (F0) were collected using a standard 750 ml dipper from production sites adjacent to residential neighborhoods in Naples, Florida: Bayview (0.25 mi radius from 26.10079, −81.78129), Barefoot Williams (0.25 mi radius from 26.04896, −81.70868), and Bayshore (0.25 mi radius from 26.09754, −81.77009). Mosquito larvae were morphologically identified in the laboratory, transferred to the insectary, and held at 28°C, 80% RH, with a 14-h-light:10-h-dark photocycle and allowed to eclose. The CDC bottle bioassay was used to identify baseline susceptibility and establish diagnostic times for *Ae. taeniorhynchus* as previously described (CDC 2017). All experiments described used 3–5-day-old female mosquitoes.

Three replicates of approximately 20–25 adult female *Ae. taeniorhynchus* for each of the 3 locations were exposed to the CDC diagnostic dose of the

Table 1. CDC bottle bioassay against *Aedes taeniorhynchus* using technical-grade and formulated products.

| AI | Diagnostic Dose | Product | Strain | Corrected % mortality at time (min) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 10 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
| Naled | 2.25 ug | Technical Grade | Bayview | | 93.30 ± 3.51 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Barefoot Williams | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Bayshore | | 94.4 ± 3.85 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | **Average** | | **95.9 ± 3.59** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| | | Dibrom Concentrate | Bayview | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Barefoot Williams | | 96.02 ± 3.52 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Bayshore | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | **Average** | | **98.67 ± 2.30** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| Sumithrin | 20 ug | Technical Grade | Bayview | 86.75 ± 8.95 | 95.58 ± 3.97 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Barefoot Williams | 82.06 ± 11.49 | 97.78 ± 3.85 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Bayshore | 94.28 ± 1.75 | 98.18 ± 1.79 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | **Average** | **87.70 ± 6.16** | **97.18 ± 1.4** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| | | Anvil 10-10* | Bayview | 88.98 ± 1.78 | 98.55 ± 2.51 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Barefoot Williams | 87.10 ± 3.66 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Bayshore | 76.32 ± 1.39 | 98.41 ± 2.75 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | **Average** | **84.13 ± 6.83** | **98.99 ± 0.88** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| Pyrethrum | 15 ug | Technical Grade | Bayview | | 92.56 ± 1.50 | 95.93 ± 4.71 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Barefoot Williams | | 91.94 ± 10.55 | 95.83 ± 7.22 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Bayshore | | 92.75 ± 3.39 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | **Average** | | **92.42 ± 0.42** | **97.25 ± 2.38** | **100** | **100** | **100** | **100** | **100** | **100** |
| | | Merus 3.0 | Bayview | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Barefoot Williams | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | Bayshore | | 96.30 ± 6.42 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | **Average** | | **98.77 ± 2.14** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |

* Anvil 10-10 contains the synergist, piperonyl butoxide (PBO).

technical-grade materials of either or naled (2.25 µg/ml), d-phenothrin (Sumithrin®) (20 µg/ml), or pyrethrum (15 µg/ml) (CDC 2017); acetone was used as a control treatment. Technical-grade products were diluted with acetone to deliver the CDC diagnostic dose of active ingredient in 1 ml of solution. In additional experiments using formulated products, Dibrom® Concentrate (87.4% naled) (AMVAC Chemical Corp., New Port Beach, CA), Anvil 10-10® (10% Sumithrin, 10% piperonyl butoxide [PBO]) (Clarke Inc., St. Charles, IL), and Merus 3.0 (5% pyrethrins) (Clarke Inc.) were diluted in acetone to yield the equivalent CDC diagnostic dose of AI. Wheaton media bottles (250 ml) were coated with one of the test solutions or acetone control solution. Bottles were dried for 2 h in a dark space and used immediately for the assay. Knockdown was recorded every 15 min for 2 h, and an additional reading at 10 min was performed for sumithrin and Anvil 10-10. Knockdown was recorded if mosquitoes could no longer stand, displayed erratic behavior, or couldn't maintain flight. Percent mortality for each location was calculated and corrected using Abbott's formula (Abbott 1925).

Next, because of its specific use in targeting *Ae. taeniorhynchus* in CMCD's adulticide program, semifield cage trials were conducted for Merus 3.0. Merus 3.0 was delivered at 0.83 oz/acre (0.0024 lb AI/acre) using an MD 500 rotary wing aircraft (MD Helicopters LLC, Mesa, AZ) outfitted with a single-boom spray system equipped with a Micronair® AU6539 atomizer (Micron Group, Bromyard, Herefordshire, UK). Prior to the semifield trial, the aircraft's spray system was calibrated to the desired flow of 1.59 liter/min (0.42 gal/min) for a swath of 106.68 m (350 ft) at a speed of 41.13 m/s (80 knots). Droplets were characterized using a 1-inch Teflon slide with a rotating impinger and analyzed using DropVision® FL (Leading Edge, Daytona Beach, FL). The Dv 0.5 and Dv 0.9 were measured as 38.99 μm and 71.84 μm, respectively. The field test plot was located within an AgNav (AgNav, Ontario, Canada) spray block, using 3 × 3 design with rows of 3 sampling stations positioned 7.62 m (25 ft) apart. Each station contained caged replicates with 20–25 adult female mosquitoes for the susceptible *Ae. aegypti* ORL 1952 strain and Bayshore *Ae. taeniorhynchus*. Applications were performed using the preset drift model for Merus 3.0 in the AgNav system, with the test plot downwind of the ultra-low volume (ULV) spray. Nontreated controls were placed in an area outside the treatment block. After 20 min of exposure, cages were brought back into the laboratory. Exposed mosquitoes were transferred to holding cages and monitored for mortality. Knockdown was recorded at every 15 min for 120 min and at 24 and 48 h post exposure. Percent mortality was calculated and corrected using Abbott's formula.

Baseline susceptibility determined through the CDC bottle bioassay is depicted in Table 1. The lowest time point with 100% mortality at each of the 3 locations was identified as the diagnostic time. The diagnostic times were determined to be 30 min for technical-grade naled, sumithrin, and formulated Dibrom Concentrate, Anvil 10 + 10, and Merus 3.0. Technical-grade pyrethrum had a diagnostic time of 45 min. These results are similar to previously established diagnostic times for *Ae. albopictus* (Skuse) laboratory colony, but slightly longer than the diagnostic times established for the *Ae. aegypti* REX colony (CDC 2017). Further, for formulated products, such as Merus 3.0, it is not uncommon for their diagnostic times to be shorter than technical-grade insecticides (CDC 2017). These data establish baseline susceptibility and diagnostic times for evaluating changes in susceptibility over time in Collier County's *Ae. taeniorhynchus* populations.

In semifield cage trials, Merus 3.0 was effective almost immediately against the susceptible *Ae. aegypti* ORL 1952 strain and Bayshore *Ae. taeniorhynchus*, with a knockdown of 97.0% and 91.1% on average, respectively, after 1 h post exposure (Fig. 1). By 24 h and continuing through 48 h post exposure, the susceptible *Ae. aegypti* ORL1952 strain and Bayshore *Ae. taeniorhynchus* reached 100% mortality (Fig. 1). These results indicate Merus 3.0 is highly effective for operational control against *Ae. taeniorhynchus* in Collier County.

Information on baseline susceptibility, diagnostic times, insecticide resistance monitoring, and field efficacy in *Ae. taeniorhynchus* is limited. One study has reported baseline susceptibility status on *Ae. taeniorhynchus* in Mexico using the diagnostic doses and time previously established for *Ae. aegypti*

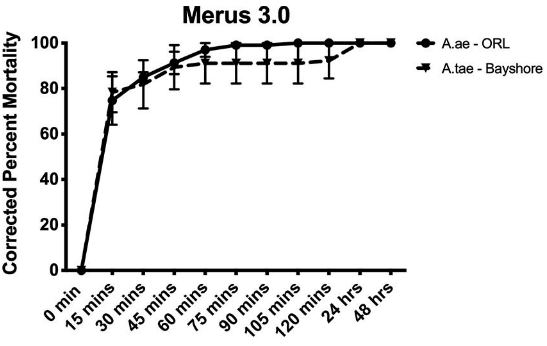


Fig. 1. Semifield cage trials using Merus 3.0 against *Aedes aegypti* ORL 1952 strain (A.ae – ORL) and Bayshore *Aedes taeniorhynchus* (A.tae – Bayshore).

(Navarrete-Carballo et al. 2022). In St. Johns County (Florida), *Ae. taeniorhynchus* has shown susceptibility to a synergized sumithrin-based product in bioassays (Qualls and Xue 2010). Field trials of several synergized permethrin-based products have shown overall high mortality against *Ae. taeniorhynchus* in Manatee County (Florida) (Williams et al. 2021), while naled-based products have shown high effectiveness against *Ae. taeniorhynchus* in the Florida Keys (Zhong et al. 2010). Altogether, these data provide baseline susceptibility of Collier's *Ae. taeniorhynchus* to the 3 main adulticide active ingredients used by CMCD and validate the effectiveness of Merus 3.0 in targeting *Ae. taeniorhynchus*. As CMCD continues to perform more pyrethroid-based treatments to control *Ae. taeniorhynchus*, these data will assist with monitoring changes in susceptibility status over time.

The authors thank the Collier Mosquito Control District (CMCD) Board of Commissioners and Patrick Linn. We also thank all the employees at CMCD who participated in sample collection and technical assistance.

## REFERENCES CITED

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267.

Boike AH Jr, Rathburn CB Jr, Hallmon CF, Cotterman SG. 1978. Insecticide susceptibility tests of *Aedes taeniorhynchus* and *Culex nigripalpus* in Florida, 1974–1976. *Mosq News* 38:210–217.

Brown AW. 1986. Insecticide resistance in mosquitoes: a pragmatic review. *J Am Mosq Control Assoc* 2:123–140.

CDC [Centers for Disease Control and Prevention]. 2017. *Insecticide resistance* [Internet]. Atlanta, GA: Centers for Disease Control and Prevention [accessed July 3, 2017]. Available from: https://www.cdc.gov/zika/vector/insecticide-resistance.html.

Estep AS, Sanscrainte ND, Waits CM, Bernard SJ, Lloyd AM, Lucas KJ, Buckner EA, Vaidyanathan R, Morreale R, Conti LA, Becnel JJ. 2018. Quantification of permethrin resistance and *kdr* alleles in Florida strains of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse). *PLoS Negl Trop Dis* 12:e0006544.

Harden FW, Chubb HS. 1960. Observation of *Aedes taeniorhynchus* dispersal in extreme South Florida and the Everglades National Park. *Mosq News* 20:249–255.

Kennedy JS. 1961. A turning point in the study of insect migration. *Nature* 189:785–791.

Lucas KJ, Watkins A, Phillips N, Appazato DJ, Linn P. 2019. The impact of Hurricane Irma on population density of the Black Salt-Marsh Mosquito, *Aedes taeniorhynchus*, in Collier County, Florida. *J Am Mosq Control Assoc* 35:71–74.

Lucas KJ, Bales RB. 2022. Insecticide resistance evaluation of *Aedes aegypti* mosquitoes from Collier County, Florida. *Arthropod Manage Tests* 47:1–2.

Lucas KJ, Bales RB, McCoy K, Weldon C. 2020. Oxidase, esterase, and *kdr*-associated pyrethroid resistance in *Culex quinquefasciatus* field collections of Collier County, Florida. *J Am Mosq Control Assoc* 36:22–32.

Mekuria Y, Williams DC, Hyatt MG, Zack RE, Gwinn TA. 1994. Malathion resistance in mosquitoes from Charleston and Georgetown counties of coastal South Carolina. *J Am Mosq Control Assoc* 10:56–63.

Navarrete-Carballo J, Chan-Espinoza D, Palacio-Varges J, Gonzalez-Olvera G, Che-Mendoza A, Martin-Park A, Manrique-Saide P. 2022. Insecticide susceptibility tests of *Aedes taeniorhynchus* in Yucatan, Mexico. *J Am Mosq Control Assoc* 38:224–225.

Qualls WA, Xue R. 2010. Evaluation of a new formulation of adulticide, Duet, against West Nile virus vector mosquitoes. *J Am Mosq Control Assoc* 26:219–222.

Ritchie SA. 1984. Record winter rains and the minimal populations of *Aedes taeniorhynchus* (Wiedemann): cause and effect? *J Fla Anti-Mosq Assoc* 55:14–21.

Ritchie SA, Addison DS. 1992. Oviposition preferences of *Aedes taeniorhynchus* (Diptera: Culicidae) in Florida mangrove forests. *Environ Entomol* 21:737–744.

Schluep SM, Buckner EA. 2021. Metabolic resistance in permethrin-resistant Florida *Aedes aegypti* (Diptera: Culicidae). *Insects* 12:866.

Watkins A, Babcock E, Lucas KJ. 2021. Ornamental bromeliads of local Botanical Gardens serve as larval production sites for pyrethroid-resistant *Culex quinquefasciatus* in Collier County, Florida. *J Fla Mosq Control Assoc* 68:14–23.

Weaver JR, Xue RD, Gaines MK. 2020. Populations outbreaks of mosquitoes after Hurricanes Matthew and Irma and the control efforts in St. Johns County, Northeastern Florida. *J Am Mosq Control Assoc* 36:28–34.

Williams KF, Buckner EA, Maricano AL, Latham MD, Lesser CR. 2021. Comparative efficacy of five permethrin/PBO 30-30 ground ULV insecticides against field collected adult *Aedes aegypti*, *Aedes taeniorhynchus*, and *Culex quinquefasciatus* in Manatee County, Florida. *J Florida Mosq Control Assoc* 66:68–72.

Zhong H, Hribar LJ, Daniels JC, Feken MA, Brock C, Trager MD. 2010. Aerial ultra-low-volume application of naled: impact on nontarget imperiled butterfly larvae (*Cyclargus thomasi bethunebakeri*) and efficacy against adult mosquitoes (*Aedes taeniorhynchus*). *Environ Entomol* 39:1961–1972.