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OPERATIONAL NOTE

BASELINE SUSCEPTABILITY AND FIRST OBSERVATION OF KNOCKDOWN RESISTANCE IN *AEDES TAENIORHYNCHUS* POPULATION OF ST. JOHNS COUNTY, FLORIDA

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ABSTRACT. The black saltmarsh mosquito, *Aedes taeniorhynchus*, is a prominent nuisance mosquito within St. Johns County, Florida. Due to their characteristically large outbreaks, and the elevated amount of insecticide application correlated with the outbreaks, local populations of *Ae. taeniorhynchus* are at an increased risk of developing insecticide resistance. This study was established to form a baseline susceptibility of *Ae. taeniorhynchus* against two technical grade materials, permethrin, and chlorpyrifos. Centers for Disease Control and Prevention bottle bioassays were conducted with technical-grade materials during two outbreaks in the fall of 2023. Results indicated a baseline susceptibility against the materials tested, but most notably, the phenotypic expression of knockdown resistance (*kdr*) was observed. Results highlight the need for continued monitoring and investigation into the resistance status and resistance level of this common Florida species.

KEY WORDS *Aedes taeniorhynchus*, chlorpyrifos, insecticide resistance, knockdown resistance, permethrin

The black salt marsh mosquito, *Aedes taeniorhynchus* (Wied.), is a common mosquito along the coastline of the state of Florida (Darsie and Ward 2005). Gravid females lay their eggs in the soil of salt marsh or mangrove habitats, where the soil is periodically inundated because of weather and climatic events (Nielsen and Nielsen 1953, Ritchie and Addison 1992). *Aedes taeniorhynchus* characteristically emerge in large numbers following flooding events, migrating up to 60 mi when aided by wind (Harden and Chubb 1960, Ritchie and Montague 1993, Bataille et al. 2010). Adults are known to be aggressive biters and have shown to be hosts to mosquito-borne viruses such as St. Louis encephalitis, West Nile virus, and Venezuelan equine encephalomyelitis, despite being incompetent vectors (Hodapp et al. 1966, Chamberlain et al. 1969, Hribar et al. 2003).

St. Johns County, located along the coastal region of northeastern Florida, contains numerous mass breeding sites for *Ae. taeniorhynchus*, securing the species as a primary nuisance mosquito within the Anastasia Mosquito Control District's (AMCD) area of operation (Qualls et al. 2022). As such, there is an increased selective pressure placed on the local *Ae. taeniorhynchus* population from the elevated amount of insecticide applications associated with outbreaks. Consequently, insecticide resistance development within *Ae. taeniorhynchus* populations is a primary concern within AMCD's integrative mosquito management program. However, with limited published studies, and no published diagnostic time from the Centers for Disease Control and Prevention (CDC), tracking the resistance status of *Ae. taeniorhynchus* presents limitations.

Within St. Johns County, a main breeding area of *Ae. Taeniorhynchus,* is 1,600 acres (800 ha) of periodically inundated salt marsh and scrub habitat, containing pockets of saline saturated rain and flood water, located within the boundaries of Anastasia State Park. Rugged terrain, chronic tidal and rain flooding, and an arthropod management plan established by the Florida Department of Environmental Protection limit treatments to ground larvicide application within selected areas only. Here we discuss the results of four CDC bottle bioassays conducted on local *Ae. taeniorhynchus* populations collected following outbreaks occurring within Anastasia State Park during the 2023 fall mosquito season. Bottle bioassays were initiated to establish a baseline susceptibility against two commonly used materials in AMCD's treatment program, permethrin (pyrethroid) and chlorpyrifos (organophosphate).

During two outbreaks in August and October 2023, three BioQuip® gravid traps (BioQuip Products Inc., Compton, CA) were placed on top of milk crates, baited with CO_2 (dry ice) and Biogents® BG-Lure's (Biogents Products, Martinsburg, WV) (Fig. 1), and deployed at three separate locations (29.8799, –81.2854; 29.8792, –81.2839; 29.8779, –81.2808) within a 0.4 km radius within Anastasia State Park for 24 h. Adults were targeted as results were required immediately for operational efficacy. Although this trap is traditionally used for the collection of gravid females, AMCD found the replacement of infused water, which typically is placed below the trap, with host seeking lures (BG-Lure and CO_2), to yield the most amount of unharmed host seeking adult females, when compared to other trapping techniques deployed by the district. Collections were returned to AMCD facilities and were pooled together in BioQuip BugDorms for 24 h, with a 10% sucrose solution.

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Fig. 1. Modified gravid trap deployed at three different locations.

Field-collected mosquitoes were stored in a climate-controlled insectary (temperature: 26.6° ± 1° C, RH:70 ± 10%, photoperiod:14Light, 10Dark). Glass Wheaton bottles (250 ml), acquired through the CDC, were prepared the same day as trap collections were returned to AMCD facilities, with technical-grade permethrin at 43 µg/ml and chlorpyrifos at 20 µg/ml diluted with acetone, according to CDC bottle bioassay guidelines (CDC 2023). Four technical replicates were made for each material tested, along with a negative control coated with 1 ml acetone. Bottles were stored overnight, for approximately 12 h, and mosquitoes were tested the next day, aspirating 20–40 mosquitoes of unknown age, that appeared to be physiologically healthy (free flying, responding to stimulation, normal morphology), per bottle. Mortality, as defined here as the inability to properly stand, maintain flight, and/or respond to bottle movement, was recorded every 5 min up until 15 min, and then was recorded in 15-min increments for 1 h and 45 min, resulting in a two-hour test period. After testing, mosquitoes were placed in 12 oz paper cups, serving as holding containers, and were provided a 10% sucrose solution for 24-h *kdr* monitoring. Mosquitoes were considered to be expressing *kdr* if they returned to normal behavior (free flying, responding to stimulation, sugar feeding) after 24 h (CDC 2023).

The assay conducted in August with *Ae. taeniorhynchus* against permethrin resulted in 100% mortality being achieved at 60 min, whereas the assay conducted in October achieved 100% mortality at 45 min. Both assays against permethrin resulted in mosquitoes displaying phenotypic expression of knockdown resistance (*kdr*). In August, 19 out of 152 (12.5%) individuals tested recovered within holding containers, whereas the assay conducted in October resulted in 10 out of 107 individuals (9.3%) recovered after 24 h (Fig. 2). Individuals tested against chlorpyrifos in August achieved 100% mortality at 60 min, whereas mosquitoes tested in October achieved 100% mortality at 75 min (Fig. 2). No control mortalities occurred on either date.

The understanding of the resistance status of *Ae. taeniorhynchus* is vital to the operational efficacy of AMCD. This species is a severe nuisance mosquito within St. Johns County, Florida, with mass emergences occurring in preserve lands located next to residential areas (Qualls et al. 2022). Lucas et al. (2023) tested strains of *Ae. taeniorhynchus* locally acquired at different locations within Collier County, Florida, against technical-grade pyrethrum (15 µg/ml) through CDC bottle bioassays. Diagnostic time reported in their results indicated 100% mortality at 45 min for all three of their strains, closely aligning to our times of 45 min and 60 min against technical-grade permethrin. Navarrete-Carballo et al. (2022) tested two populations of *Ae. taeniorhynchus* collected from separate areas within

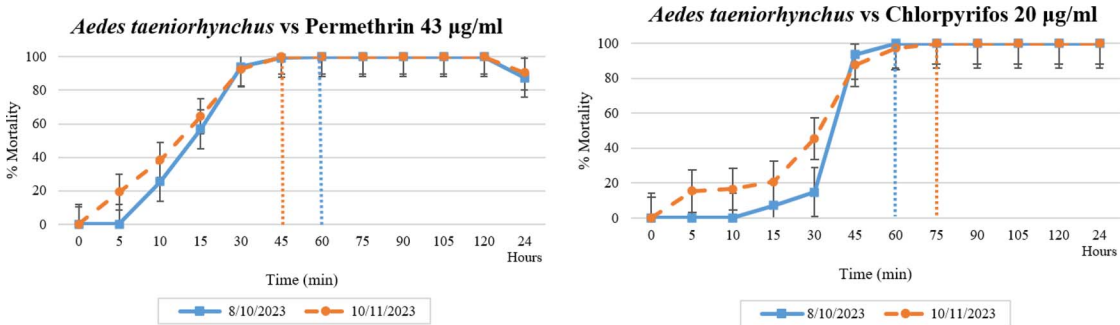

Fig. 2. CDC bottle bioassay results from assays conducted in August and October against permethrin and chlorpyrifos, during outbreaks of *Ae. taeniorhynchus* in St. Johns County, FL. Dotted vertical lines illustrate the time point at which 100% mortality was achieved.

Yucatan, Mexico against technical-grade permethrin (15 µg/ml) and chlorpyrifos (85 µg/ml), among other materials, through CDC bottle bioassays. Diagnostic time reported in their results indicated 100% mortality at 30 min for all materials, illustrating greater susceptibility of their populations of *Ae. taeniorhynchus* to permethrin, especially given the discrepancies between the diagnostic doses used between studies.

Our results for *Ae. taeniorhynchus* against permethrin (100% mortality achieved at 45 min and 60 min) far exceed the diagnostic time of 10 min provided by the CDC bottle bioassay guidelines for *Aedes aegypti* (L.) (REX Colony) and *Aedes albopictus* (Skuse) (LC Colony) (CDC 2023). For chlorpyrifos, our results (60 min and 75 min) are closer to the diagnostic time provided for the *Aedes* genus (45 min) within CDC Guidelines but still surpass the recommended time (CDC 2023). The assay conducted in August against permethrin resulted in over 97% mortality at 45 min, whereas in October, 100% mortality was achieved at 45 min. In October, over 97% mortality occurred at 60 min for chlorpyrifos, whereas in August 100% mortality was achieved at 60 min. As per CDC guidelines (2023), a mortality of over 97% indicates susceptibility. Discrepancies between the two tests may be because of treatment pressure from operational applications of insecticides, variations among the age and health of the mosquitoes, or other environmental and physiological variables. However, our results provide a baseline susceptibility status of *Ae. taeniorhynchus* against technical-grade permethrin (45 min) and chlorpyrifos (60 min), which can be used for future investigation and resistance tracking. This study is also the first documentation of phenotypic expression of *kdr* within *Ae. taeniorhynchus* populations against permethrin in Florida. Substantial analysis of our results cannot be conducted because of the limited amount of reported data on *Ae. taeniorhynchus* susceptibility toward technical-grade permethrin. Further testing must be conducted with a susceptible population of *Ae. taeniorhynchus* to determine resistance status of St. Johns County populations, and molecular testing should be conducted to determine the mechanism behind the recovery observed against permethrin.

Further investigation and monitoring into the resistance status and resistance levels of *Ae. taeniorhynchus* populations across the state need to occur to provide a more inclusive understanding of this common Florida nuisance mosquito, along with factors that may affect operational control of them. Kondapaneni et al. (2021) illustrated the continued concern over high populations of *Ae. taeniorhynchus* that Florida mosquito control districts maintain battle with in their areas of operation, while also highlighting the gap in available research associated with this common species. This trend appears to still be relevant, contributing to a deficiency in the operational knowledge and efficacy of mosquito control districts against *Ae. taeniorhynchus*.

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