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SCIENTIFIC NOTE

EVALUATION OF A BIFENTHRIN-BASED RESIDUAL BARRIER TREATMENT FOR CONTROLLING *AEDES TAENIORHYNCHUS* POPULATIONS ON AN ISOLATED BARRIER ISLAND IN COLLIER COUNTY, FLORIDA

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ABSTRACT. *Aedes taeniorhynchus*, the black salt marsh mosquito, is a significant nuisance in Florida, frequently emerging from mangrove swamps in Collier County. Keewaydin Island, part of the Rookery Bay National Estuarine Research Reserve, contains both private parcels and publicly managed conservation land. As aerial adulticide treatments are not feasible, this study evaluated bifenthrin barrier treatments on private properties. Although leaf bioassays showed high mosquito mortality, no significant reduction in adult populations was observed.

KEY WORDS *Aedes taeniorhynchus*, barrier island, barrier treatment, bifenthrin, leaf bioassay

Aedes taeniorhynchus Wiedermann, or the black salt marsh mosquito, is an aggressive diurnal biter common among the coastal regions in the eastern United States. Considered a nuisance species in Florida (FL), it is also capable of vectoring Venezuelan equine encephalitis (VEE) and eastern equine encephalitis (EEE) viruses, and the dog heartworm, *Dirofilaria immitis* (Drew). Female *Ae. taeniorhynchus* lay eggs in moist soil of mangrove swamps and often leave their emergence sites during large populations explosions (Lucas et al. 2019), migrating up to 60 mi (96 km) when assisted by wind (Harden and Chubb 1960).

In Collier County, FL, most spring adulticide applications by the Collier Mosquito Control District (CMCD) target *Ae. taeniorhynchus* populations originating from conservation lands, including barrier islands and mangrove islets (Lucas et al. 2019). Keewaydin Island, a primary barrier island in Rookery Bay National Estuarine Research Reserve, hosts approximately 30 waterfront private parcels (Fig. 1A). This coastal ecosystem is exceptionally delicate, requiring special consideration during mosquito control operations. The combination of extensive conservation lands, the species' migratory behavior, and its capacity to overwinter as eggs contributes to large emergences and the dispersion of mosquitoes into nearby residential areas, limiting effective control options (Kennedy 1961, Lucas et al. 2019).

Residual barrier sprays may offer localized control and reduced impact on protected ecosystems. Studies have shown bifenthrin-based sprays effectively reduce mosquito populations (Trout et al. 2007, Cilek 2008, Qualls et al. 2012, Fulcher et al. 2015, Richards et al. 2017). In the Florida Keys, Wisdom® TC Flowable (7.9% bifenthrin) (AMVAC Chemical Corp., Los Angeles, CA, USA), showed significant residual activity against *Ae. taeniorhynchus* for up to three weeks after treatment (Boehmler 2021). Here we present our study evaluating bifenthrin residual activity and the ability to reduce *Ae. taeniorhynchus* populations on Keewaydin Island.

Three sites on Keewaydin Island were selected: one treatment site and two control sites (Fig. 1A). The treatment site (26.028922, –81.768878) was a 10.38-acre (5.19 ha) residential parcel with two established homes (Fig. 1B). Control sites were recreational parcels located to the north [Windstar (26.029936, –81.770060) (2.74 acres (1.37 ha)] and south [South (26.019531, –81.765366) (1.96 acres (0,98 ha))] of the treatment site. All sites featured subtropical vegetation dominated by seagrape (*Coccoloba uvifera* (L.)), mangrove (*Rhizophora*), and palmetto (*Sabal*).

From May 21 to September 4, 2024, adult mosquito populations at the three sites were monitored weekly using Centers for Disease Control and Prevention (CDC) miniature light traps (BioQuip, Rancho Dominguez, CA). One CDC trap per site tracked mosquito abundance trends, with the treatment site trap placed centrally to the barrier treatment. Traps were baited with 0.95-watt incandescent bulbs and CO_2 released at 500 ml/min. After 24 h, trap collections were identified by morphology (Darsie and Ward 2005) at the CMCD laboratory. Weekly Landing Rate Counts (LRCs) were conducted at three locations per site by recording the number of adult female mosquitoes landing on an individual over 2 min, as described (Lucas et al. 2019). Daily rainfall data from the Naples Airport (FL) was sourced via National Oceanic and Atmospheric Administration (NOAA). Analyses were done in GraphPad Prism v11.0.0 (GraphPad Software, Boston, Massachusetts, USA), with mean LRC ± Standard Error of Mean (SEM) calculated and two-way ANOVA used to compare biting pressure across sites.

Two bifenthrin treatments were performed on May 23 and July 17, 2024, corresponding to treatment 1

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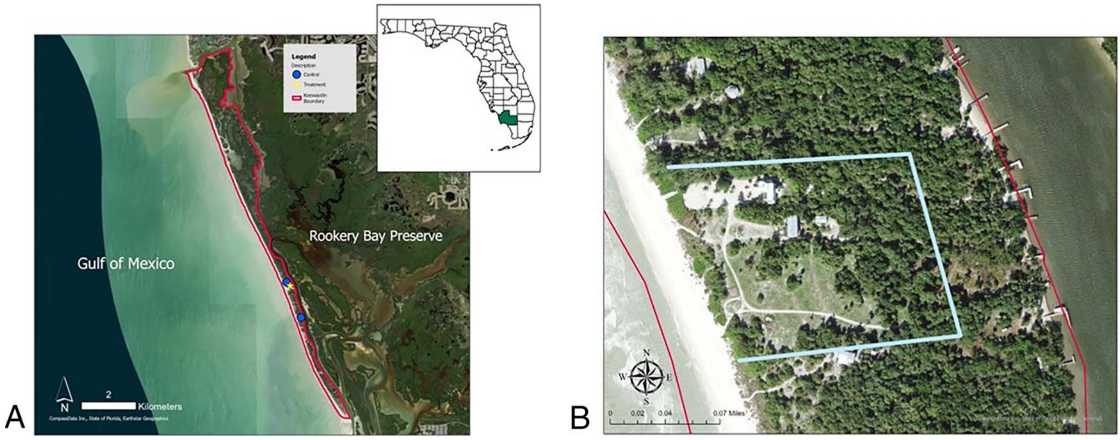

Fig. 1. Map of Keewaydin Island with relative location in Collier County. (A) Red border outlines the island; control sites are marked in blue, and the treatment site with a yellow star. (B) Blue border indicates the treatment path within the test plot.

and treatment 2, respectively. Treatment 1 was initiated when *Ae. taeniorhynchus* activity reached preestablished LRC threshold values of 12 mosquitoes/min, representing 3-fold over baseline (Lucas et al. 2019). Wisdom TC Flowable was diluted at 15.63 ml/liter water (2 oz/gal) and applied at a rate of 1 oz/92.90 sq m (1 oz/1000 sq ft) (Fig. 2). Applications covered a 396.4 m (1,300 ft) barrier totaling 1,207.74 sq m (13,000 sq ft) using a 6.10 m (20 ft) swath and a 15.24 m/min (50 ft/min) walking speed. The product was applied with an AS1200 AC2 electric sprayer (Birchmeier Sprühtechnik AG, Stetten, Switzerland) retrofitted with a TeeJet #4 brass nozzle (878-TX4) (TeeJet Technologies, Glendale Heights, Illinois, USA) delivering 1.89 liters/min (0.5 gal/min). Spraying occurred at least 30 m (98.43 ft) from water and under wind speeds below 16.09 kph (10 mph) to avoid environmental contamination.

Leaf bioassays were conducted to evaluate the barrier spray's effectiveness and residual activity against *Ae. taeniorhynchus*, following Boehmler (2021). Ten leaves were collected from each site before treatment (control), 1 h post-treatment (wk 0) and weekly for 6 wk (wk 1-6). Samples were taken from different trees or shrubs spaced at least 5 m (16 ft) apart, with at least 50% from seagrape because of its abundance and suitable leaf shape. Leaves were cut at the base using a razor blade, with only stems handled to avoid surface contact, and placed in individual plastic containers. Each leaf's adaxial surface was kept free from contact with container walls.

Leaves were cut into disc shapes and affixed adaxial side up in glass 110 mm × 15 mm Petri dishes using double sided carpet tape, then stored at –20°C until use. Each dish contained discs from at least two different leaves, covering the base, with five replicates per site weekly using approximately ten unique leaves. Wild *Ae. taeniorhynchus* used in assays were collected as 4th instars from local mangrove swamps, using a standard larval dipper. Larvae were identified morphologically, reared in the CMCD insectary at 28°C, 80% RH, and a 14:10 light-dark cycle. They were fed a diet of dog chow, lactalbumin, and brewer's yeast; adults received 20% (wt/vol) sucrose ad libitum.

Prior to the assay, samples were thawed for 1 h. Clear World Health Organization (WHO) cones were laid atop the samples and secured to Petri dishes using Parafilm (Amcor, Zurich, Switzerland). At least 10 female *Ae. taeniorhynchus* (3–5 day post-emergence) were aspirated into each dish. Mosquitoes received 20% sucrose via soaked cotton balls placed on the cone divots. Mortality, defined as inability to stand or fly, or exhibiting erratic behavior, was recorded at 1 h and 24 h exposure periods. Percent mortality was calculated and corrected using Abbott's formula (Abbott 1925). Untreated control sites had $<5\%$ mortality and were used to calculate corrected percent mortality. One-way ANOVA assessed average mortality across 6 wk post-treatment, with Tukey's HSD used for post hoc comparisons.

Residual activity for treatment 1 was evaluated over seven weeks, with significant residual efficacy lasting up to two weeks post-application. Control leaves displayed 0% mortality at both 1 h and 24 h exposure periods. For samples collected post-treatment on the same day, mortality was 48.00 ± 14.97% at 1 h ($P = 0.0202$) and 100% ($P < 0.0001$) at 24 h (Fig. 2A, 2B). At 1 wk post-treatment, residual activity remained with 50.00 ± 19.24% (P = 0.0136) and 96.00 ± 2.45% ($P < 0.0001$) at 1 h and 24 h exposure period, respectively (Fig. 2A, 2B). By week two, mortality declined to 65.31 ± 15.34% ($P = 0.0086$) at 24 h (Fig. 2B), but remained statistically significant. Week three data were unavailable because of a severe storm event on June 12 (14.96 cm rainfall), resulting in a gap in the dataset. By week four, mortality dropped and was no longer statistically significant.

Treatment 2 exhibited significant residual efficacy for at least four weeks. Control leaves displayed 0%

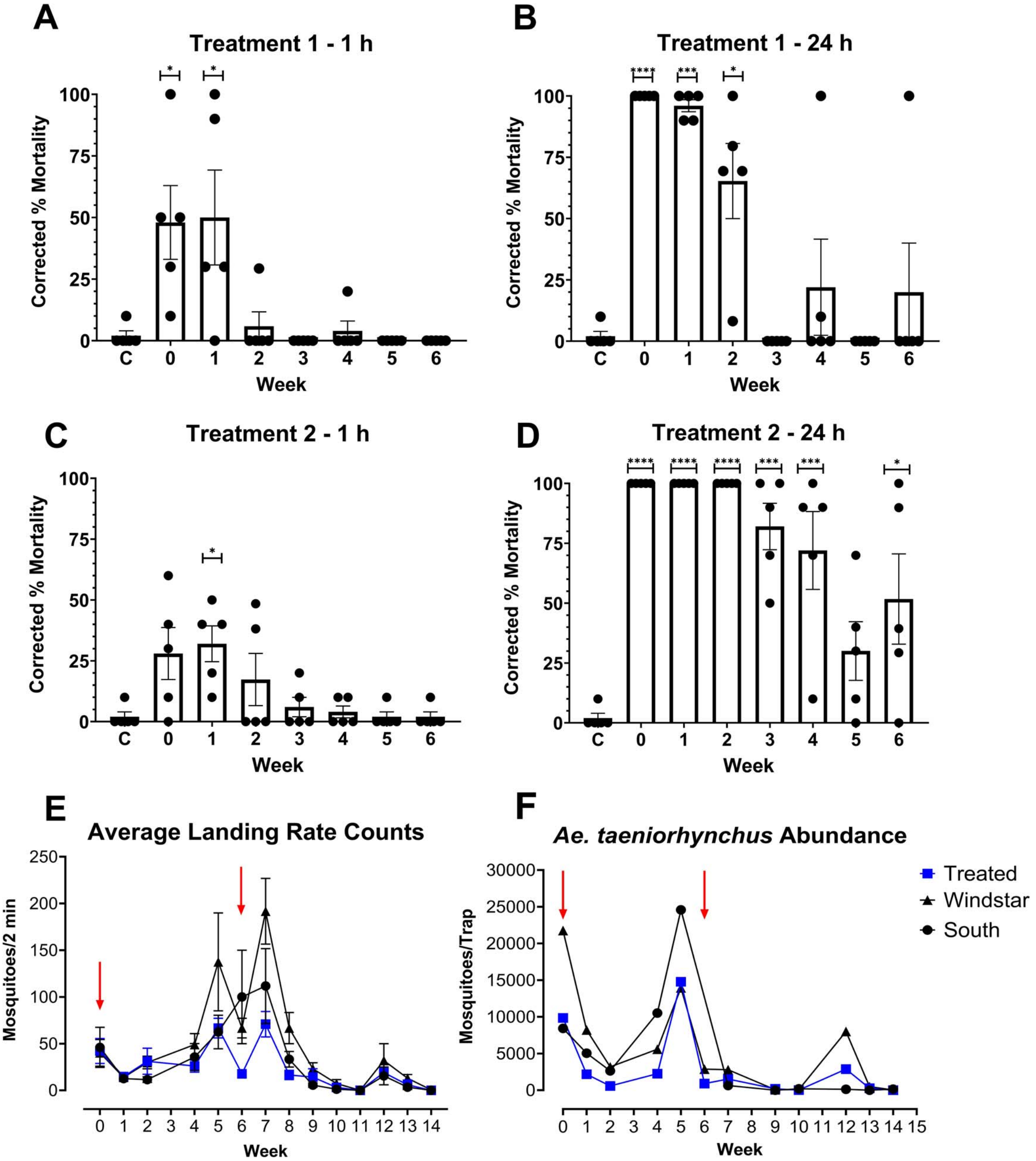

Fig. 2. Corrected percent mortality from leaf bioassays for treatments 1 and 2. (A-B) Corrected percent mortality at 1 h (A) and 24 h (B) exposure periods for treatment 1. Week three data missing because of storms (lightning bolt symbol) (C-D) Corrected percent mortality at 1 h (C) and 24 h (D) exposure period for treatment 2. (A-D) Data represent 5 technical replicates shown as mean ± SEM. Statistical significance: * $P < 0.05$, *** $P < 0.001$, and **** $P < 0.0001$. (E) Mean LRC and (F) CDC trap data across 15 wk for control (Windstar, South) and treatment sites; treatment timing marked with red arrows, treatment site shown in blue.

mortality at both exposure periods. Although 1 h mortality was minimal, the 24 h exposure period yielded significant results. Immediately following treatment, 24 h mortality reached 100% ($P < 0.0001$) and persisted through wk one and two ($P < 0.0001$) (Fig. 2D). Efficacy declined to 82.00 ± 9.70% ($P = 0.0001$) by wk three and 72.00 ± 16.25% ($P = 0.0010$) by week four. By week five, mortality was no longer statistically significant At week six, efficacy rebounded to 51.72 ± 18.87% ($P = 0.0363$), suggesting bifenthrin may remain effective for 4–6 wk in semi-field conditions without heavy rainfall.

Although bifenthrin was effective in semi-field assays, LRC and CDC trap data over the 14-wk period showed no reduction in mosquito populations within the treatment area (Fig. 2E-F). Mean LRC values were not significantly different from the control sites (F = 1.198, $P = 0.2481$), and CDC trap trends were similar across all sites. These findings indicate that the barrier spray did not reduce biting pressure or mosquito abundance.

Although 24 h leaf bioassays showed strong residual activity of bifenthrin for up to 4–6 wk, 1 h mortality remained low, supporting Boehmler's (2021) hypothesis that shorter exposure may better reflect field efficacy. This aligns with our field data, which showed no significant differences in *Ae. taeniorhynchus* abundance between treated and control sites. Treatment 2 yielded particularly strong bioassay results, consistent with previous studies reporting extended bifenthrin efficacy (Trout et al. 2007, Cilek 2008, Allan et al. 2009). However, unlike some prior work showing population reductions after barrier sprays (Trout and Brown 2009, Fulcher et al. 2015, Richards et al. 2017), our study did not observe field-level suppression. Factors such as an untreated beach strip (Fig. 1B), post-hurricane vegetation loss, and limited mosquito contact with treated surfaces are likely reduced effectiveness. Since bifenthrin requires contact to be lethal, mosquitoes flying through without landing on treated vegetation may have escaped exposure.

Environmental conditions also likely played a role (Allan et al. 2009). Heavy rainfall in June likely diminished bifenthrin's efficacy, especially during the third- and fourth-wk post-treatment during treatment 1. Treatment 2 maintained significant mortality following an August storm, indicating some resilience. These findings underscore the challenges of using residual barrier treatments in subtropical, weather-variable environments. Although bifenthrin demonstrated strong residual activity, its limited field impact highlights the need for refinements. Strategies like increased vegetation coverage, reapplication after storms, and better adaptation to local conditions could enhance barrier treatment success, making it a more viable option for remote areas like Keewaydin Island.

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