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EVALUATION OF BORIC ACID SUGAR BAITS SPRAYED ON PLANTS AGAINST THE SALT MARSH MOSQUITO, *AEDES TAENIORHYNCHUS* (DIPTERA: CULICIDAE)

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The black salt marsh mosquito, *Aedes taeniorhynchus* Wiedemann (Diptera: Culicidae), is an abundant nuisance mosquito distributed throughout coastal regions of the U.S. and Caribbean, and is also an important vector of canine heartworm and Venezuelan equine encephalitis (Apperson 1991). With the increasing human encroachment on coastal habitats, environmentally sensitive control methods are critically needed to balance human needs for mosquito control with those of delicate ecosystems.

Attractive Toxic Sugar Baits (ATSBs) against mosquitoes have been successful (Müller & Schlein 2008). The 'toxic' active ingredient in ATSBs is mostly boric acid, which is an environmentally friendly compound harmless to humans, but toxic to adult mosquitoes (Xue & Barnard 2003; Müller et al. 2010a, b; Beier et al. 2012). Other recent evaluations of ATSBs in tropical environments have shown much promise (Xue et al. 2011, 2013; Qualls et al. 2012; Naranjo et al. 2013).

Aedes taeniorhynchus has been shown to seek and feed on nectar (Van Handel & Day 1990). Concurrently, there is evidence that toxic sugar baits (TSBs) may be used to exploit resting behaviors of *Aedes* mosquitoes (Schlein & Müller 2012). However, there is no information about using these techniques to control *Ae. taeniorhynchus*. TSBs do not contain the additional "attractant", thus focusing on resting and sugar feeding mosquitoes. Resting vegetation commonly found in and around coastal hammocks, such as yaupon holly (*Ilex vomitoria* Sol. ex Aiton; Aquifoliales: Aquifoliaceae) and black mangrove (*Avicennia germinans* (L.) L.; Lamiales: Acantaceae), were selected based on their presence at the field site and other local hammocks. The focus on boric acid toxic sugar baits built upon previous work by Xue et al. (2006, 2008) to advance field-based studies of environmentally friendly adult salt marsh mosquito control technology. The major objective of the present study was to evaluate the efficacy of boric acid sugar baits sprayed on black mangrove and yaupon holly cuttings in the laboratory and common coastal plants in the field against the adult black salt marsh mosquitoes.

The Toxic Sugar Bait (TSB) solution was formulated using 1% boric acid (Sigma-Aldrich, USA) as the active ingredient diluted into a 5% sucrose (Domino Brand, ASR Group, Palm Beach, Florida) solution. The solution was prepared by adding the boric acid to hot (60 °C) tap water until fully dissolved. Eleven liters were mixed for each field application and 100 mL batches were prepared for the laboratory experiments.

Seven to 10-day old adult *Ae. taeniorhynchus* mosquitoes were obtained from the USDA, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida and used for the laboratory experiments. Conditions in the insectary were maintained at 26-28 °C, 70-80% RH and 12:12 h L:D for the duration of the experiment. Both control and treatment cages of mosquitoes were provided with 59 mL Diamond Daily Mini Cups™ containing approximately four water saturated (25 mL) cotton balls during the observation period, while negative controls received 10% sugar.

Experiment 1

One hundred female mosquitoes were aspirated from the colony into each of the six cages (45 × 45 × 37cm) for the black mangrove laboratory evaluation. Three of the cages comprised the experimental group and the other three cages were for controls. Permission from Anastasia State Park, St. Augustine, Florida was granted to collect cuttings of plants (DEP/AMCD 06-01-13). Anastasia State Park was chosen because insecticidal spraying is prohibited at this location and the plant samples were less likely to be contaminated. We immediately placed the

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black mangrove cuttings into small glass cups (500 mL). The cups were supplied with brackish water from the leaf collection site. Aluminum foil was used as a cover and barrier over the cup opening, so the mosquitoes could not reach the water. Each cup contained approximately twelve non-flowering 10-12 cm in length cuttings with 8-10 leaves per cutting. The TSB solution (10 mL/plant) was applied using a handheld 946 mL Zep Professional Sprayers™ (Ace Hardware, St. Augustine, Florida) to the 3 treatment cuttings to the point of run off. The 3 control cuttings were not sprayed. Mortality counts were taken daily until greater than 90% (cumulative) mosquito mortality was observed. The evaluation had 3 repetitions.

Experiment 2

The yaupon holly evaluation was conducted as in experiment 1 with the following modifications: the yaupon holly cuttings were approximately 6 cm long and 4-5 cuttings were placed into small 100 mL glass vials filled with tap water. One vial was placed into each 9.5 L bucket cage in the laboratory. Thirty female mosquitoes were introduced into each of these cages and mortality was recorded at 24, 48 and 72 h. The evaluation had 3 repetitions. Each repetition was composed of 3 treatments and 2 controls.

Experiment 3

Fish Island is an undeveloped peninsula along the Matanzas River (N 29° 51' 42" W 81° 18' 04"), St. Augustine, Florida. This coastal area is surrounded concentrically by tidal salt marsh vegetation at its edge, typical yaupon holly barrier island-type habitat moving inland, and has inner reaches populated by more upland species such as hickory (*Carya* spp.; Fagales: Juglandaceae). Four Mosquito Magnet™ X (MMX) traps were set in different locations in pairs on Fish Island, St. Augustine, FL to verify the presence of *Ae. taeniorhynchus.* Traps were spaced approximately 100 m apart, baited with CO_2 (dry ice), and left overnight. Mosquitoes collected were killed by freezing, identified and counted. The data collected was then used to determine the best sites for the field application of the TSB solution.

TSB was applied from an 11 liter B & G Pest Pro 2050 Back Pack Sprayer in a vertically oscillating pattern from ground coverage to a height of no more than 2 meters on non-flowering vegetation. Effort was made to ensure coverage to a depth of 3-5 m from the trail edge. A section of approximately 280 m in length was sprayed. Following a 200 m buffer zone, another 280 m was considered to be the control length. Landing rate counts by two or three people in the morning were taken at three sites in the sprayed area for 1 min before spraying and post spraying day 1, day 2, day 3, and 1 week (AMCD's IRB protocol#10-13-2005 as approved by the Board of Commissioners for use of human subjects).

Differences between the treatments and controls were assessed using a series of one-way analysis of variance (ANOVA) determinations. All analysis was done using SAS v9.4.

In the mangrove study, overall mortality was greatest at 48 h with a cumulative mortality of 52% (Fig. 1, $F_{3,12} = 6.21$, $P < 0.01$). However, the exposure time did not result in significant differences in the mortality between treatments and controls. We hypothesize that mangrove was not a good nutritional resource for *Ae. taeniorhynchus*. In the yaupon holly experiment, a direct comparison of the treatment and control showed significant mortality (68%) at 48 h post treatment (Fig. 1, $F_{2,2} = 20.72$, $P < 0.05$), but the exposure time did not result in a significant difference in the mortality between treatments and controls. Also, yaupon holly was not a good nutritional resource for this species of salt marsh mosquitoes.

At the field site, there was a very diverse mosquito population (total of 13 species) sampled at Fish Island. The experimental site was chosen because *Ae. taeniorhynchus* was the most prevalent species (> 30%). The field experiment showed that boric acid sugar baits sprayed on plants within a hammock island near salt marshes resulted in the significant reduction of landing rate counts on human subjects (Fig. 2, $F_{1,52} = 4.46$, $P < 0.05$).

In the black mangrove laboratory experiment we found 52% mortality at 48 h. This percent mortality is less than that reported by Xue et al. (2006, 2008), who found > 80% mortality in the application of boric acid to vegetation against a laboratory-reared population of *Ae. taeniorhynchus* in the laboratory and in a semi-field trial. The different species of plants used and experimental conditions may be the reason why different mortalities were induced. Salt excretion by black mangrove leaves may reduce the toxicity of boric acid sugar baits against salt marsh mosquitoes. The impact of plant species and habitants on efficacy of ATSB against adult mosquitoes needs to be further addressed.

Our desire to investigate mosquito-resting behavior could be greatly enhanced by expanding the scope of future studies to include sugar-feeding behavior as in attractive toxic sugar baits (ATSBs). The addition of an attractive component in the laboratory studies may encourage exposure of the mosquitoes to the solution presented on the plants. Future evaluations directly comparing exposure of mosquitoes to TSBs versus ATSBs in the natural environment are needed.

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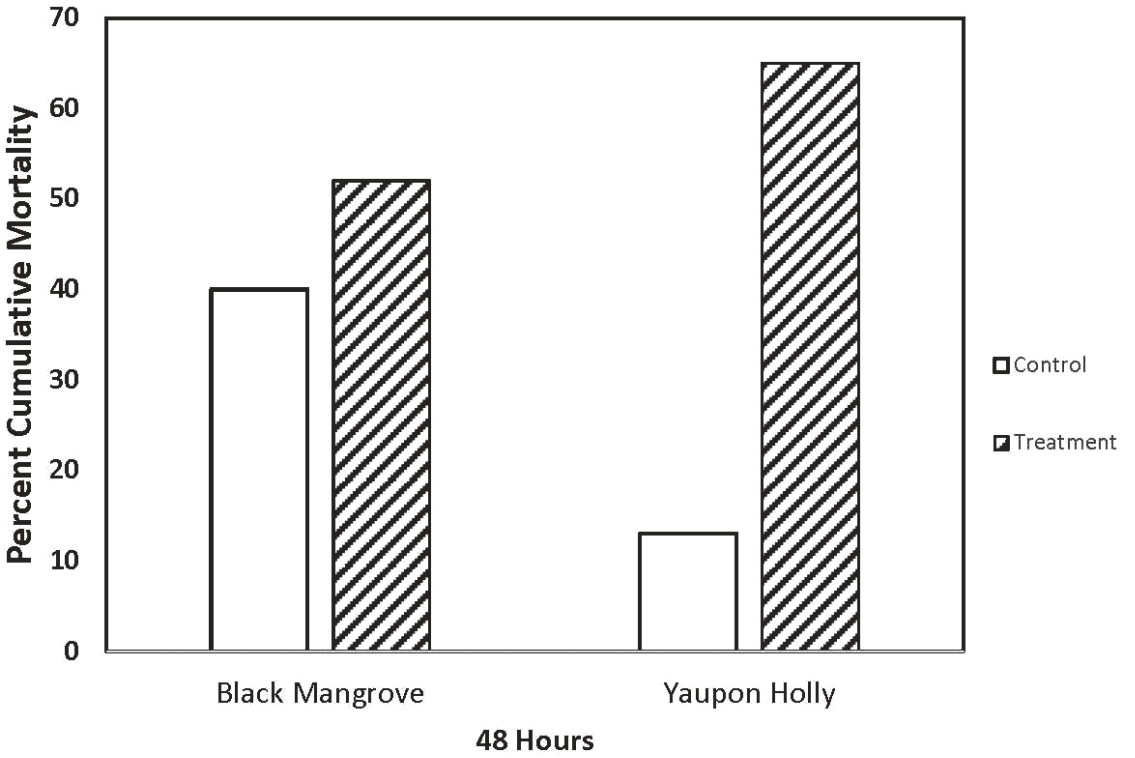


Fig. 1. Percent mortality of *Aedes taeniorhynchus* at 48 h after exposure to 1% boric acid sugar baits sprayed on black mangrove and yaupon holly cuttings in the laboratory.

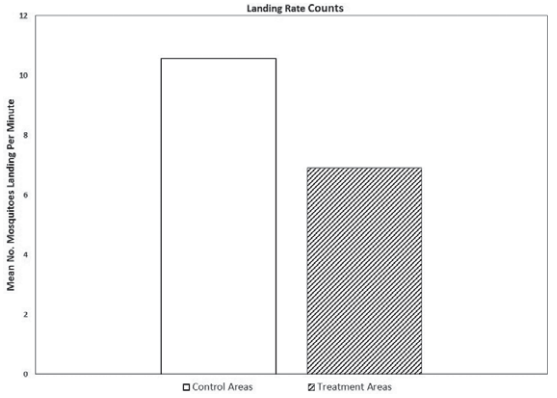


Fig. 2. Mean landing rate counts/minute by adult mosquitoes on human subjects after 1% boric acid sugar baits was sprayed on plants at Fish Island, St. Augustine, Florida.

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Summary

A Toxic Sugar Bait (TSB, active ingredient 1% boric acid) was evaluated against *Aedes taeniorhynchus* (Diptera: Culicidae) in the laboratory and the field at St. Augustine, Florida. The laboratory component was comprised of plants located in known *Ae. taeniorhynchus* resting areas, i.e., black mangrove (*Avecennia germinans* L.) and yaupon holly (*Ilex vomitoria* Ait.). The results indicated that TSB sprayed on black mangrove and yaupon holly cuttings at 48 h resulted in significant mortality of resting *Ae. taeniorhynchus,* compared with the mortality of mosquitoes in the control group under the laboratory conditions. Also, the field studies indicated a significant reduction in mosquito populations after TSB was applied on plants.

Key Words: *Aedes taeniorhynchus*, attractive toxic sugar bait, boric acid

Resumen

Un Cebo Tóxico de Azúcar (CTA, ingrediente activo el ácido bórico al 1%) fue evaluado contra *Aedes taeniorhynchus* (Diptera: Culicidae) en el laboratorio y el campo en St. Augustine, Florida. El componente de laboratorio consistía de plantas

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ubicadas en áreas conocidas donde *Ae. taeniorhynchus* reposa, como en mangle negro (*Avecennia germinans* L.) y acebo yaupon (*Ilex vomitoria* Ait.). Los resultados indicaron que el CTA rociado sobre recortes de mangle negro y el acebo yaupon a las 48 horas resultó en una mortalidad significativa de *Ae. Taeniorhynchus* en descanso, en comparación con la mortalidad de los mosquitos en el grupo control en las condiciones de laboratorio. Además, los estudios de campo indicaron una reducción significativa de las poblaciones de mosquitos después de la aplicacióon de CTA sobre las plantas.

Palabras Clave: *Aedes taeniorhynchus*, atractivo cebo tóxico de azúcar, ácido bórico

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