OPERATION NOTE

ADULTICIDAL AND LARVICIDAL IMPACTS OF THE MIXTURE OF *BACILLUS THURINGIENSIS ISRAELENSIS* AND BORIC ACID TOXIC SUGAR BAIT (TSB) AGAINST *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Toxic sugar baits (TSBs) can be used to deliver insecticide material via ingestion instead of via contact through spraying of insecticides by targeting the resting and sugar-feeding behaviors of adult mosquitoes. This semi-field study aimed to evaluate the adulticidal and larvicidal dual action of a foliage spray of a TSB with a mixture of *Bacillus thuringiensis israelensis* (Bti), and boric acid against *Aedes aegypti* Puerto Rico (PR) strain (resistant) and Orlando (OR) strain (susceptible), and laboratory colony of *Culex quinquefasciatus* (Gainesville 1995 strain). The larval and adult evaluation of TSB consisted of 11% VectoBac (Valent Biosciences, Libertyville, IL; Bti), 5% boric acid, and 10% sucrose solution. The TSB intervention for adults consisted of the same as the larval intervention but had an addition of a food grade, 5g Blue No. 1 dye (Sigma Aldrich; St. Louis MO) to observe adult feeding. The controls received a 10% sucrose solution. For the larval mortality evaluation, the TSB was applied to the bromeliad with the runoff dripping into pans containing mosquito larvae. At 24 hours post-application, 100% larval mortality was observed. At all-time mortality recordings, 50 larvae were introduced into the larval pans and the bromeliads were sprayed with water to mimic rainfall allowing the remaining TSB to be washed off into the larval pans. After the 4th day of larval introductions, larval mortality was 83.5% ± 14.3 for *Ae. aegypti*_PR, 92.5% ± 6.1 for *Ae. aegypti* OR, and 97 % ±1.7 for *Cx. quinquefasciatus*. Total mean mortality at 72 hours post exposure for the adult TSB evaluation was 52.7% ± 24.2 for *Ae. aegypti*_PR, 34.3% ± 26.5 for *Ae. aegypti*_OR, and 73.7% ± 13.9 for *Cx. quinquefasciatus*. Our study suggests this TSB including Bti is effective against larvae when applied as an adulticide barrier application and could be a dual-action approach to mosquito control.

Key Words: *Aedes aegypti*, *Culex quinquefasciatus*, toxic sugar bait, dengue vector, *Bacillus thuringiensis israelensis*, boric acid

Aedes aegypti L. and *Culex quinquefasciatus* Say are important vectors of diseases impacting global public health (Daep et al. 2014, Alto et al. 2016). Both the geographic distribution and insecticide-resistant populations of these species are rapidly spreading and due to their public health burden as vectors of dengue and West Nile virus (respectively among others), novel control methods are more imperative than ever (Kraemer et al. 2015, Liu et al. 2020). Insecticides are a common control method for reducing vector-borne disease transmission but the development of resistance to these insecticides is an ever-present challenge for control programs (Namias et al. 2021). Additionally, there are limited effective mosquito control insecticides with a need to identify different insecticide formulations or delivery methods for mosquito management. A new alternative for mosquito control is the use of toxic sugar baits (TSB) (Xue et al. 2006, Pearson et al. 2020, Davis et al. 2021).

The application of TSBs to vegetation exploits the resting and sugar-feeding behaviors of adult mosquitoes instead of the flying/host seeking mosquitoes targeted by truck/ aerial adulticide applications. TSBs deliver the toxic material via ingestion instead of via contact through spraying of larvicides and adulticides. TSBs can also be formulated to be attractive (ATSB) to the adult mosquito, likely increasing ingestion of the toxic material resulting in increased adult mosquito mortality (Qualls et al. 2014). Most TSBs and ATSBs evaluations have been performed as barrier sprays (Qualls et al. 2014).

Barrier applications are an effective strategy for mosquito control due to their residual nature, but the insecticide residual is impacted by rain events (Allan et al. 2009), reducing the effectiveness over time. In an effort to combat the loss of adulticidal residual of barrier applications through exposure to rain events, one thought has been to benefit from rain events by combining the

TSB/ATSB with a larvicide (Fulcher et al. 2014, Scott, et al. 2016). *Bacillus thuringiensis israelensis* (Bti) is a common larvicide and is most effective when used as part of an integrated mosquito management program (IPM) (McAllister et al. 2020). A recent study demonstrated that 8% Bti combined in a TSB is successful in controlling adult, female *Ae. aegypti, Aedes albopictus* (Skuse), and *Cx. quinquefasciatus* resulting in an average mortality of 97%, 98%, and 100%, respectively at 48 h (Davis et al. 2021). To follow up on the work by Davis et al. (2021), a semi-field study was conducted to investigate the adulticidal and larvicidal dual action of a foliage spray of TSB combining Bti, and boric acid against *Ae. aegypti* and *Cx. quinquefasciatus.*

Mosquitoes. Two strains of *Ae. aegypti*; the resistant Puerto Rican strain (*Ae. aegypti*_PR) and the susceptible Orlando 1952 strain (*Ae. aegypti*_OR), and *Cx. quinquefasciatus* Gainesville strain were used in this experiment. The eggs of all strains were acquired from the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. All mosquitoes were maintained as colonies at Anastasia Mosquito Control District (AMCD), St. Augustine, Florida's insectary, at 26.6 ± 1°C, 70.0 ± 10% relative humidity, and a 14:10 light: dark (LD) photoperiod.

Larvicidal experiment. Fifty, 2^{nd}-3^{rd} instar larvae of each strain were pipetted into plant saucers containing 500mL of reverse osmosis (RO) water that was placed underneath a bromeliad plant (Bromeliaceae; 3.7 liter pot from Home Depot Store, St. Augustine, FL). The bromeliads and plant saucers were housed inside bug dorms (BioQuip, Rancho Dominguez, CA, 30x30x30 cm.) within the AMCD greenhouse (26.6 ± 1°C, 70.0 ± 10% relative humidity). Three control and three TSB treated bromeliads were placed in separate bug dorms for this evaluation. The TSB intervention consisted of 11% VectoBac (Valent Biosciences, Libertyville, IL; Bti), 5% Boric Acid (Sigma Aldrich, St. Louis, MO), and 10% sucrose solution. Boric acid has been commonly used as a toxicant in the laboratory in TSBs and ATSBs efficacy evaluations against adult mosquitoes (Xue et al. 2006, Pearson et al. 2020). The control plants received a 10% sucrose solution applied to the vegetation. Both the control and intervention solutions were administered by a 1-liter ZEP professional sprayer bottle (Zep Inc. Atlanta, GA) saturating the bromeliad plants until the solution was dripping from the plants (~ 25 ml) into the plant saucer that contained the larvae. Mortality was documented at 24-, 48-, 72-, and 96 h. Dead larvae were removed from the control and intervention saucers at each time period, and 50 more larvae (second to the third instar) were added each day to the saucer up to four days post-treatment. At each time period after 24 h, both control and intervention bromeliads were sprayed seven times with RO water to provide solution runoff into the saucers. The spraying of RO water was to mimic a rain event for observing the longevity of the TSB treated foliage, and to measure the residual TSB effects on larval mortality. The experiment was repeated three times.

Adulticidal experiment. One hundred, five-to seven-day-old male and female adults of each strain were aspirated into bug dorms held in the AMCD greenhouse (26.6 ± 1°C, 70.0 ± 10% relative humidity). Three control and three TSB treated cups containing cotton balls were placed in bug dorms as described above for this evaluation. The intervention cotton balls received a TSB consisting of 11% VectoBac (Bti), 5% Boric Acid, 5g of Blue No. 1 dye (Food grade dye; Sigma Aldrich; St. Louis MO), and 10% sucrose. The 5g of food grade dye was used so that only the dyed, dead mosquitoes were counted for the confirmation of the ingestion on the TSB. The food grade dye was used just in the intervention treatment. The control cotton balls received a 10% sucrose solution. Both the control and intervention solutions were administered by saturating cotton balls in the solution and placing them in a 60 ml plastic cup in each cage. The bromeliad was still included in the cages because it is a common plant and breeding site in residential areas and homes in Florida. Mortality was documented at 24-, 48- and 72 h. Dead mosquitoes were aspirated from the bug dorms at each time period. The experiment was repeated three times.

Data analysis. The data was analyzed using SPSS-version 20 (IBM SPSS Statistics). Data sets were arcsine transformed and independent t-tests were performed to determine any significant difference between the control and treatment groups of each species/strain at each time period. One-way ANOVA with Tukey's pairwise multiple comparison tests were also performed to compare the TSB mortality of each species at different time periods and between species/strains.

The larvicidal effect of the TSB resulted in 100% mortality at 24 h for all larval species/strains tested (Figure 1). After four days of larval introductions, the total mean mortality was 83.5% ± 14.3 for *Ae. aegypti*_PR, 92.5% ± 6.1 for *Ae. aegypti*_OR, and 97 % ± 1.7 for *Cx. quinquefasciatus.* The control mortality remained below 5% for all three species/strains at each time period and were significantly lower than corresponding TSB mortality ($P<0.05$ for all). There were no statistically significant differences between the different time periods for the species/strain. The ANOVA performed on data

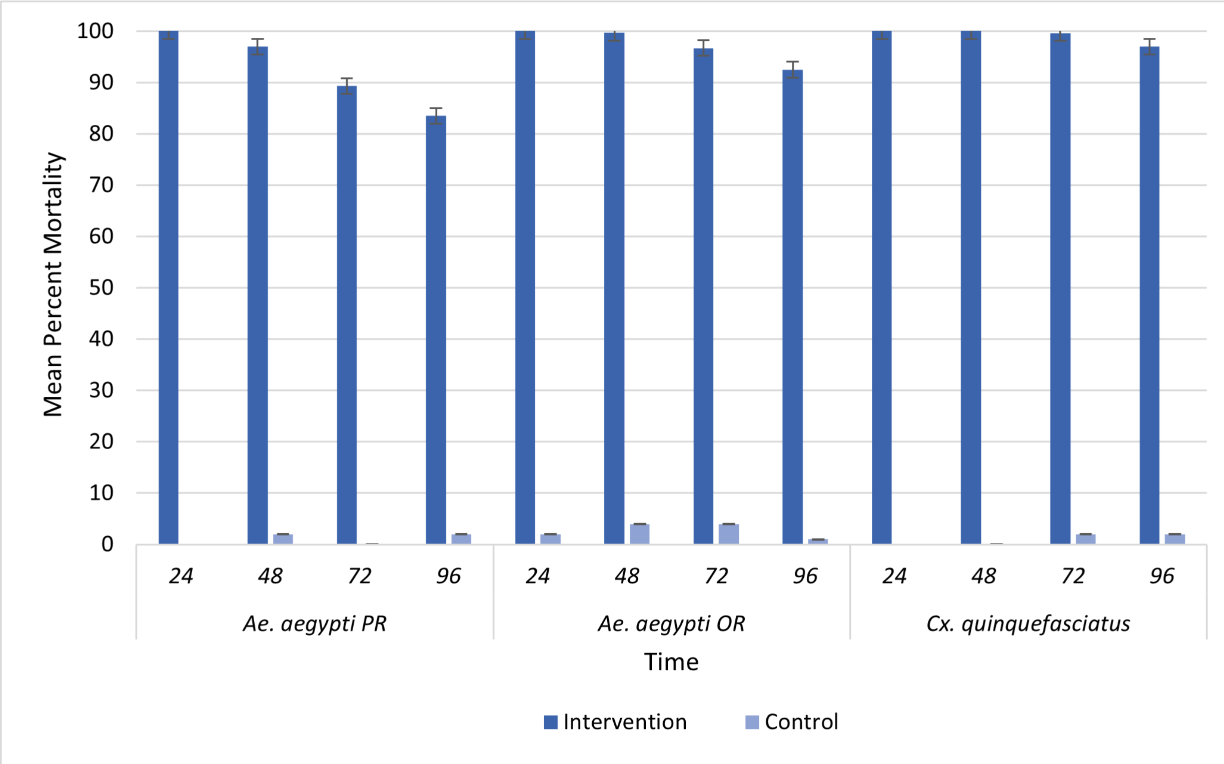

Figure 1. Cumulative mean larval mortality of the three species/strains at 24, 48, 72, and 96-h time periods following wetting events of 25 ml of RO water to simulate run-off of the barrier TSB application. (error bars=standard error)

sets pooled for time periods did not show any significant differences in mortality between species/strains with F_2 = 1.09, and P = .0342.

The TSB resulted in adult mortality at each time period for all three species/strains tested (Figure 2). At the 72-h time check, the total mean mortality for all days was 52.7% ± 24.2 for *Ae. aegypti*_PR, 34.3% ± 26.5 for *Ae. aegypti*_OR, and 73.7% ± 13.9 for *Cx. quinquefasciatus*. The control mortality remained below 5% for all three species/strains. The TSB had significantly higher mortality at all time periods for the three species/strains compared to the control (Table 1) PR F_2 = 1.68 and P = 0.0264, OR F_2 = 1.66 and P = 0.268, Cx F_2 = 2.1 and P = 0.0204, except *Ae. aegypti*_PR at 72 h, *Ae aegypti*_OR at 24 h and 72 h, compared to controls. There were no statistically significant differences in mortality between different time periods of any species/strain. Analysis of data pooled for all time period revealed a significant difference in mortality between species/strains (P=0.015) and post-hoc tests confirmed the difference was only between *Cx. quinquefasciatus* and *Ae. aegypti*_OR (P=0.11).

This study demonstrated 100% mortality for all three species/strains of larvae at the initial treatment of the TSB. Although larval mortality decreased each post-treatment check, the mortalities of all three species were still high at the four days post-treatment count. The mortality count demonstrates the high residual effects of the TSB foliage spray and its impacts on larval development but further evaluations are needed to demonstrate if this translates over to effective field control. There could be reduced larvicidal impact of the TSB following rain events and would most likely be due to the dilution of the active ingredients in the TSB after each rain event. We demonstrate that continued larval mortality is possible with TSB foliage applications following natural wetting events.

The mortality of the adult mosquitoes was significantly lower in this study compared to previous literature results (Xue et al. 2006, Davis et al. 2021). This study concluded lower cumulative mean mortality of adult mosquitoes of the three species/strains at 72 h when compared to other studies. This study administered the TSB to the male and female adult mosquitoes by saturating cotton balls in the solution and placing them in a 60 ml plastic cup in each cage. Similar studies evaluated this TSB (Xue et al. 2006, Davis et al. 2021,) administered the TSB by spraying the

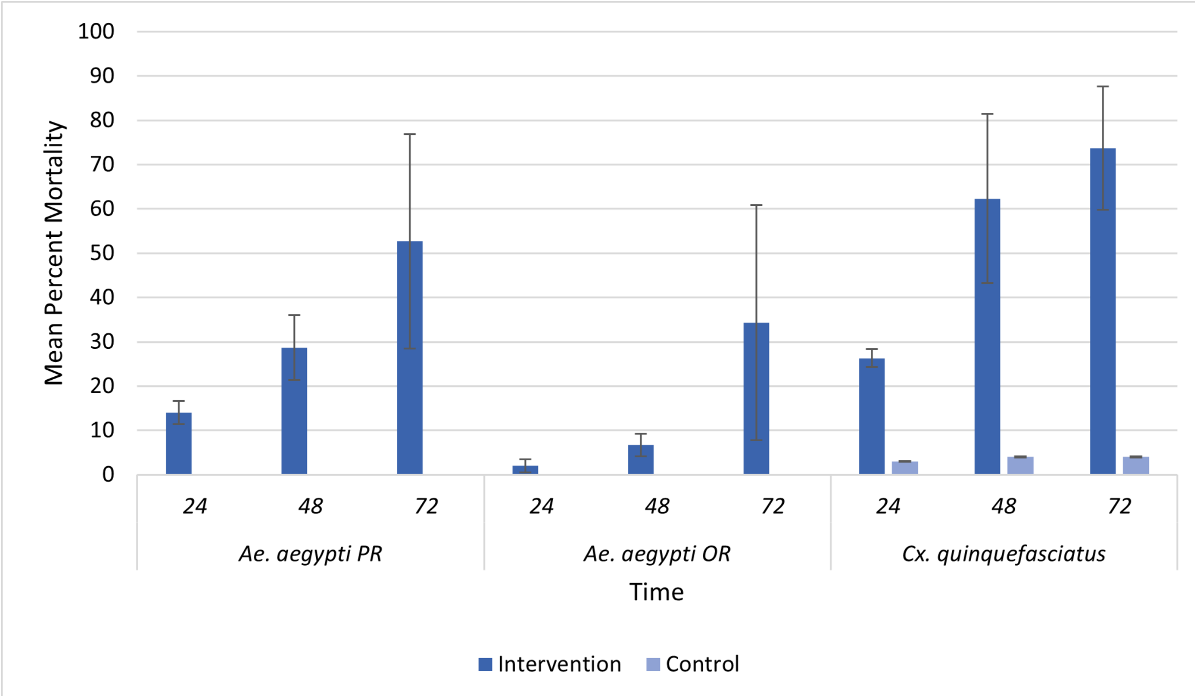

Figure 2. Cumulative mean adult mortality of the three species/strains at 24, 48, and 72-h time periods following exposure to a TSB solution (11% VectoBac (Bti), 5% Boric Acid, 5g of Blue No. 1 dye (Food grade dye; Sigma Aldrich; St. Louis MO). The control received only a 10% sucrose solution. (error bars=standard error)

foliage in the mosquito cages with the solution, possibly being the reason for the differences in mortality observed in the current study. The difference in how the TSB was administered may have altered the ingestion of the toxic solution based off the mosquito's typical resting habits being on foliage and the bromeliad plant may impact the mortality of adult mosquitoes (Xue et al. 2018). The adult TSB evaluation presents a few limitations as there was not a positive control for both the adult and larval trials. In future studies, trials need to be conducted to evaluate the impacts on adult mortality using boric acid and a sugar solution excluding Bti, and Bti and a sugar solution excluding boric acid.

Importantly, the findings demonstrate that adding a larvicide to a TSB can result in larval mortality if larvae are in the surrounding habitat. The results are similar with previous reports by Fulcher et al. (2014) and Scott et al. (2016) about TSB and the insect growth regulator, pyriproxyfen against *Ae. albopictus*. Future studies are planned to demonstrate the concentration of the larvicide to be added to the TSB for effective larval control in settings where the TSB may be flushed into storm drains or ditches.

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Table 1. Statistical significance of adult TSB mortality compared to control mortality at all three time periods for the three species/strains.

	24 hr	48 hr	72 hr
PR	$t_{(4)} = 10.07$	$t_{(4)} = 6.60$	$t_{(4)} = 2.64$
	P = 0.001	P = 0.022	P = 0.118
OR	$t_{(4)} = 1.66$	$t_{(4)} = 4.34$	$t_{(4)} = 1.85$
	P = 0.171	P = 0.012	P = 0.206
Cx	$t_{(4)} = 7.169$	$t_{(4)} = 3.10$	$t_{(4)} = 4.23$
	P = 0.002	P = 0.036	P = 0.013

t=test value, P=probability of significance

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