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OPERATIONAL NOTE

FROM SURVEILLANCE TO CONTROL: EVALUATION OF A LARVICIDE INTERVENTION AGAINST *AEDES AEGYPTI* IN BROWNSVILLE, TEXAS

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ABSTRACT. South Texas is recognized as a potential area for the emergence and re-emergence of mosquito-borne diseases due to recent circulation of Zika, chikungunya, and dengue viruses. During 2017, high *Aedes aegypti* abundance found in the city of Brownsville, TX, in combination with the previous year's local transmission of Zika virus, triggered the activation of the Texas Department of State Health Services Emergency Mosquito Control Contingency Contract. A contract with the Clarke Environmental and Mosquito Control was a response to control *Ae. aegypti*, using a ground-based wide-area larvicide spray (WALS™) containing *Bacillus thuringiensis israelensis*. The WALS application was evaluated through a field-based bioassay and by comparing surveillance data pre- and post-WALS application. The WALS application bioassay demonstrated that the larvicide was effective up to 60 m into the target properties. Additionally, the number of *Ae. aegypti* captured in traps decreased in the WALS intervention areas compared with the untreated control areas, with an estimated 29% control.

KEY WORDS Field evaluation, larvicide, mosquito abundance, surveillance, wide-area larvicide

The Lower Rio Grande Valley (LRGV) of South Texas is a vulnerable area for the emergence and re-emergence of mosquito-borne viruses (Hotez 2018). In 2017, following the local Zika virus (ZIKV) transmission in the LRGV, combined with abnormally high abundance of *Aedes aegypti* (L.) in surveillance traps operated by the City of Brownsville Public Health Department (CBPHD), the Texas Department of State Health Services (TDSHS) Emergency Mosquito Control Contingency Contract was activated. The contract was with Clarke Environmental and Mosquito Control, which had recently assisted in the control of the ZIKV outbreak in Miami, FL, using the wide-area larvicide spray (WALS™) containing *Bacillus thuringiensis israelensis* de Barjac (*Bti*) (Clarke 2019a, 2019b). Given that the WALS application using the truck-mounted Buffalo Turbine sprayer had been successful in controlling *Ae. aegypti* populations in Miami, FL (Stoddard 2018), *Bti* applied as a water-dispersible granular (WDG) was selected for use in Brownsville, TX.

Two methods were used to evaluate the WALS application: 1) a field study using a larval bioassay and 2) a comparison between pre– and post–*Ae. aegypti* surveillance data at the WALS application and untreated control sites in Brownsville. Findings from both methods are presented here.

The City of Brownsville, TX is in Cameron County along the Texas–Mexico border, directly north of the city of H. Matamoros, Tamaulipas, Mexico (Fig. 1A). Brownsville covers an area of 132.33 mi^2 and has a population of 183,392 people, of which 93.9% is of Hispanic or Latino origin (USCB 2018).

The CBPHD personnel conducted weekly mosquito surveillance from January to December 2017. Weekly collections from June 3 (epidemiological wk [EW] 22) to December 1 (EW 48) of 2017 were used for the current study. Mosquito surveillance was done by deploying 50 BG-Sentinel 2® (BGS2) traps (Biogents AG, Regensburg, Germany), baited with

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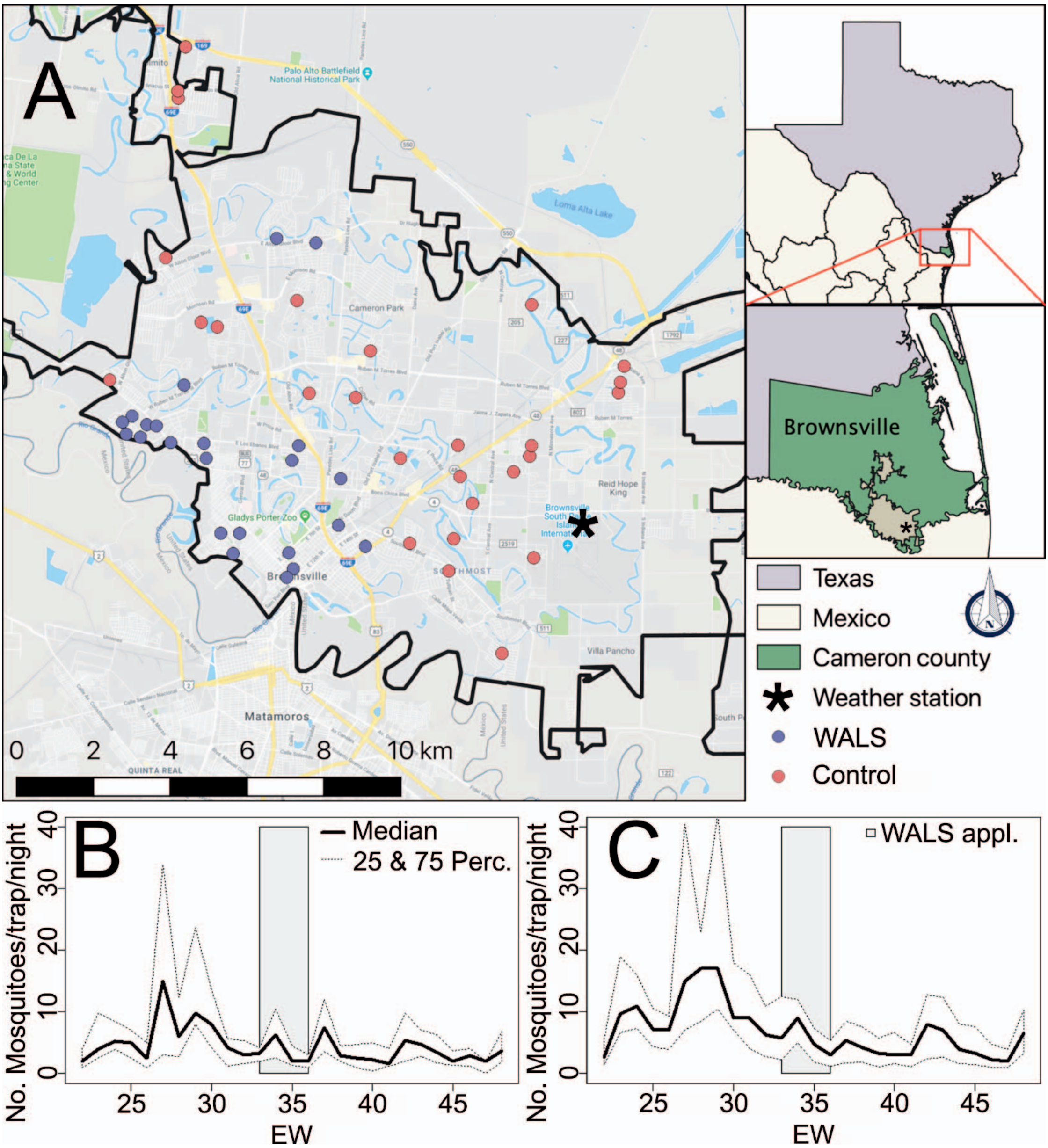

Fig. 1. Study site and *Aedes aegypti* abundance changes following a wide-area larvicide spray (WALS™) of *Bacillus thuringiensis israelensis* (*Bti*). (A) Map showing the location of BG-Sentinel 2® traps within the city of Brownsville, TX. A purple dot represents a trap placed in a *Bti*-treated zone while a red dot denotes a trap placed in an untreated control zone. The black dot denotes the weather station location. (B) *Aedes aegypti* abundance in control area traps. (C) *Aedes aegypti* abundance in WALS intervention area traps. In panels B and C, *Ae. aegypti* abundance is weekly and shows the number of adult mosquitoes/trap-night at Brownsville, TX. Thick black lines are median values, and the areas inside the dotted lines indicate the 25th and 75th percentile of weekly mosquito abundance distributions during the study period. Gray boxes indicate the timing of the WALS application. For further details, refer to the inset legend.

dry ice, within the city limits. Each trap was visited 4 times per week. Trap collections were sent to the TDSHS Arbovirus Laboratory for species counts and arbovirus testing. The BGS2-trap failures were excluded from the analysis.

To evaluate the WALS product delivery to container habitats by the Buffalo Turbine sprayer (Clarke Mosquito Control Products, Roselle, IL), 2 larvicide field bioassays were conducted. VectoBac WDG was diluted to an 18% mixture and applied at

22 kg/acre. The Buffalo Turbine sprayer was calibrated to produce droplets from 120 to 135 μm with a 60-m swath with a vertical height of 38 m. The bioassays were performed on different WALS application days at the same location in one of the neighborhoods within a treatment zone. Access and placement of the bioassay cups in private yards was done after verbal consent was granted by the owner/resident. Thirty-two plastic cups containing 100 ml of water (purified by reverse osmosis) were placed on open terrain at different distances (15 m [$n = 16$] and 30 m, 45 m, and 60 m combined [$n = 16$]) perpendicular to the larvicide truck's route. For control, 10 plastic cups containing 100 ml of purified water were placed in an area with no larvicide applied concurrent with the WALS application. An aquarium pebble was placed into each plastic cup to prevent the wind tipping the cup over. Plastic cups were deployed the day of the larviciding and retrieved the morning after. Subsequently, plastic cups were covered with plastic film to prevent cross contamination by *Bti*-exposed water spillage and were transported back to the laboratory at Texas A&M AgriLife Research and Extension Center at Weslaco, TX.

Aedes aegypti Liverpool strain mosquitoes were used in the bioassays as follows: 10 3rd instars were placed into each of the treatment cups exposed to the WALS application and into the control cups. Larval mortality was recorded at 24 h after the addition of the larvae.

The areas with the highest *Ae. aegypti* counts in the BGS2 surveillance were selected for the WALS intervention using *Bti* (VectoBac WDG) by the TDSHS, referred to hereafter as WALS treatment zones (Fig. 1A). Each treatment zone was treated 1–3 times over the course of 3 wk from August 18 to September 4, 2017 (EW 33 to 36). The WALS application covered an area of approximately 57 km^2. Accordingly, of the 50 BGS2 traps used for surveillance, 27 were located in the control zones (without WALS application) and 23 traps were located in the treatment zones (with WALS application) (Fig. 1A). Based on the larvicide applications, EW 22–36 was referred to as the pre-WALS intervention period and EW 37–48 as the post-WALS intervention period.

Results from the field larval bioassay were compared using a Pearson's chi-square test of homogeneity (Pearson 1900) with the null hypothesis that proportions of dead and live larvae after 24 h were equal in the control and treatment cups, independent of the distance from the truck-mounted Buffalo Turbine sprayer's route. To assess larvicide impacts on mosquito populations, we compared mean mosquito abundance values during the pre- and post-WALS intervention periods. For the comparison we employed Welch's *t*-tests, which correct the degrees of freedom (df) to account for heteroskedasticity, i.e., unequal variance during the pre- and post-WALS intervention periods in this study (Welch 1947).

For *Ae. aegypti* populations, we estimated the proportional abundance change in the WALS-treated and untreated (control) areas. We also estimated the percentage of control after the larvicide application, calculated by the following formula:

$$\text{Percentagecontrol} = 100 - [(T/U) \times 100],$$

where T is the posttreatment mean divided by the pretreatment mean in the WALS intervention area, and U is the posttreatment mean divided by the pretreatment mean in the control area (Fonseca et al. 2013). Meanwhile, the proportional abundance change (PAC) in the treatment area was calculated as $(1 - T) \times 100$ and for the control area $(1 - U) \times 100$. We estimated both the PACs and the percentage of control, because the former quantifies local abundance changes, while the latter accounts for mosquito population seasonality (Fonseca et al. 2013).

During the 1st bioassay trial, 92% larval mortality was observed at 15 m and 78% mortality at 30–60 m. During the 2nd bioassay trial, 95% larval mortality was observed at 15 m and 100% at 30–60 m. In both assays no mortality was observed in the control cups. In both the 1st ($\chi^2 = 76.21$, df $= 2$, $P < 2.2 \times 10^{-16}$) and 2nd trials ($\chi^2 = 85.87$, df $= 2$, $P < 2.2 \times 10^{-16}$), larval mortality differences among the treatments were statistically significant. The results demonstrated that the WALS applied through the Buffalo Turbine reached up to 60 m into the target properties and resulted in >90% mortality regardless of the distance. Placing plastic cups more than 60 m from the application path would have helped identify an upper limit to product delivery to hidden or cryptic containers.

During routine BGS2 surveillance conducted by CBPHD from EW 22 through 48, a total of 98,296 mosquitoes were caught, of which the majority belonged to the *Aedes* spp. (non-*aegypti* and non-*albopictus* species; 31,762). This was followed by *Ae. aegypti* (25,834), *Culex quinquefasciatus* Say (25,573), *Culex* spp. (12,101), *Ae. albopictus* (Skuse) (1,603), *Anopheles* spp. (925), *Psorophora* spp. (595), and other mosquito species (104).

Aedes aegypti abundance decreased significantly in the WALS treatment zone by 51%, going from 12.83 trap/wk to 5.65 trap/wk after the WALS intervention (Table 1). However, *Ae. aegypti* population decreased by 37% in the control untreated areas. The percentage control equation demonstrated a 29% control of the *Ae. aegypti* adult populations following the WALS intervention. Similar to our study, Stoddard (2018) evaluated the control efforts during the 2016 ZIKV transmission in Miami, FL, by analyzing mosquito trap data from the treatment areas. Following the WALS application in Miami Beach, *Ae. aegypti* population counts fell to less than 90% of their prior level 17 days after the 1st *Bti* application and remained close to that level for 13 additional days (Stoddard 2018).

Table 1. Mean mosquito abundance in Brownsville, TX, before and after the wide-area larvicide spray (WALS™) intervention treatment.[1]

Parameter	Pre-WALS intervention	Post-WALS intervention	t	df	P-value
Aedes aegypti—WALS intervention	12.83 ± 15.49	5.65 ± 5.97	7.51	403.32	4.0×10^{-13}*
Ae. aegypti—Control	7.19 ± 9.39	4.52 ± 4.92	4.44	487.05	1.1×10^{-05}*
Total mosquito abundance—WALS intervention	26.39 ± 28.59	25.68 ± 34.58	0.266	523.35	0.79
Total mosquito abundance—Control	21.08 ± 27.25	37.16 ± 46.26	−5.142	455.44	4.04×10^{-7}*
Culex quinquefasciatus—WALS intervention	6.92 ± 8.21	9.27 ± 11.83	−2.741	471.04	0.006*
Cx. quinquefasciatus—Control	3.19 ± 3.72	8.60 ± 10.41	−8.358	353.12	1.481×10^{-15}*
Ae. albopictus—WALS intervention	0.11 ± 0.33	0.20 ± 0.46	−2.661	482.35	0.008*
Ae. albopictus—Control	0.47 ± 1.22	0.93 ± 2.07	−3.33	455.86	0.001*

[1] All parameters are presented as mean ± SD. t indicates the Welch's t-statistic comparing the pre- and post-WALS intervention mean values; df, degrees of freedom; and P-value the significance of the test. The pre-WALS intervention period was from epidemiological wk (EW) 22 to 36 and the post-WALS intervention period was from EW 37 to 48.
* $P < 0.05$.

The mean number of all mosquitoes/trap-night was higher in the WALS treatment zones when compared with the control zones for all species, including *Ae. aegypti* (Fig. 1B, 1C). For *Cx. quinquefasciatus,* we observed that the WALS intervention (treatment zones) had a higher mean number of mosquitoes/trap-night than in the control zones and that the number of mosquitoes per trap was even higher after the WALS intervention (Table 1). The abundance of *Ae. albopictus* was very low (Table 1) and therefore difficult to compare in the treatment and control zones. However, an increase was observed in the number of *Ae. albopictus* individuals by trap location after the WALS intervention.

The observed increases in mosquito abundance might be related to the immediate WALS impact on larval mortality that initially decreased abundance and the optimal conditions created by the washing, a combination of events that can lead to increased larval fitness (Chaves et al. 2011) and large abundance fluctuations (Hayes 1975, Hayes and Hsi 1975, Hayes and Downs 1980). Moreover, rainfall peaked during the WALS application, which might have affected *Bti* applications in open larval habitats colonized by mosquito container species of interest (Chaves and Kitron 2011, Nguyen et al. 2014).

This study encountered some limitations with the field bioassay evaluation. The study was designed quickly, given the need for emergency mosquito control measures to stop a potential ZIKV outbreak in Brownsville, TX. Placing the plastic cups at >60-m distances and under canopy would have provided more information about the potential for *Bti* to reach larval habitats under those conditions. During the WALS evaluation period, the catastrophic Hurricane Harvey made landfall near the LRGV, resulting in heavy winds and rainfall for a period of days, disrupting the WALS intervention. Not only was there an increase in rainfall in the Brownsville area, increasing potential *Ae. aegypti* larval sites, but the Emergency Mosquito Control Contingency Contract was also activated to assist the Hurricane Harvey–impacted jurisdictions. This required Clarke Environmental and Mosquito Control to adjust their teams from the response in Brownsville to assist with aerial mosquito spraying of the Coastal Bend areas of Texas impacted by Hurricane Harvey in 2017. Overall, we present an operational study where a decrease in *Ae. aegypti* abundance was observed after a WALS intervention in Brownsville, TX, a hot spot area for arbovirus introduction and establishment along the Texas–Mexico border.

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