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From surveillance to control: Evaluation of a larvicide intervention against *Ae. 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 thanks to recent circulation of Zika (ZIKV), chikungunya and dengue viruses. During 2017, high *Aedes aegypti* mosquito abundance found in the city of Brownsville, TX, in combination with the previous year's local transmission of ZIKV and continued risk, triggered the activation of the Texas Department of State Health Services (DSHS) Emergency Mosquito Control Contingency Contract. The contract was with Clarke Environmental and Mosquito Control and the response was to control *Ae. aegypti* populations using a wide-area larvicide spray (WALS™) of *Bacillus thuringiensis israelensis* (*Bti*). The WALS application was evaluated through a field-based bioassay and by analyzing surveillance data using a nonparametric comparison of mosquito abundance pre- and post-WALS application. The WALS application bioassay demonstrated that the larvicide affected larval habitats up to 60 m into the target properties. Additionally, the number of *Ae. aegypti* captured in traps decreased in the WALS intervention areas compared to the control areas with an estimated 29% control.

Keywords

Larvicide; mosquito abundance; field evaluation; wide area larvicide spray; surveillance

Introduction

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). This is due to several factors including socio-economic conditions (Monaghan et al., 2016), a subtropical climate (TWDB, 2012), abundant populations of *Aedes aegypti* (Linnaeus) (CDC, 2018; Hahn et al., 2017), and the large movement of people and goods between the United States and Mexico (MPI, 2006; Stoddard et al., 2009). Moreover, there have been several recent reports of dengue (DENV), chikungunya (CHIKV), and Zika virus (ZIKV) transmission along the Texas-Mexico border (Nava-Frias et al., 2016; Salud, 2018; 2019a; b; Zubieta-Zavala et al., 2018).

The LRGV for many years has experienced local transmission of *Aedes*-vectored arboviruses including sporadic local transmission of DENV (Brunkard et al., 2007; CDC, 1996; Hafkin B, 1982; Ramos MM, 2008). Brownsville, located along the Texas-Mexico border, observed locally-acquired cases of dengue fever in 2005 (n=4) and in 2013 (n=21) (TDSHS, 2013; Thomas et al., 2016), chikungunya fever in 2015 (n=1) (TDSHS, 2016), and Zika in 2016 (n=6) and 2017 (n=1) (Martin et al., 2019; TDSHS, 2019a).

In 2017, the inherent threat of continued local ZIKV transmission in the LRGV combined with abnormally high abundance of *Ae. aegypti* in surveillance traps operated by the City of Brownsville Public Health Department (CBPHD), led to the initiation of the Texas Department of State Health Services (DSHS) Emergency Mosquito Control Contingency Contract. 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) (Clarke, 2019). 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), this application was selected for use in Brownsville for the application of *Bacillus thuringiensis israelensis* (*Bti*), a larvicidal spore-forming bacterium that has shown to be highly efficient against mosquito and black fly larvae with no adverse effects on non-target invertebrates and vertebrates (Boyce et al., 2013).

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 control sites. The findings of the WALS application evaluation via the truck-mounted Buffalo Turbine are presented here. Results are presented in the context of LRGV community structure and provide future guidance for *Ae. aegypti* control in highly urban areas of Texas.

Materials and Methods

The City of Brownsville is located in Cameron County along the Texas-Mexico border, directly north of the city of H. Matamoros, Tamaulipas, Mexico (Figure 1). The city of Brownsville covers an area of 132.33 square miles and has a population of 183,392 people of which, 93.9% is of Hispanic or Latino origin (Bureau, 2018). Approximately 31% of Brownsville's population lives in poverty and the median household income for 2017 was $35,636 (Bureau, 2018). The city has an average temperature of 23°C, an average relative humidity of 75 % and the rainfall averages 25.5 mm (Brownsville, 2019).

Mosquito surveillance

Given the six cases of autochthonous ZIKV infection in late 2016 and the threat of established local transmission, the CBPHD conducted weekly mosquito surveillance from January to December, 2017. Weekly collections from June 3rd (epidemiological week (EW) 22) to December 1st (EW 48) of 2017 were used for the current study.

Mosquito surveillance was conducted by CBPHD personnel by deploying 50 BG-Sentinel 2® (BGS2) traps (Biogents AG, Regensburg, Germany), baited with dry ice, within the city limits. Each trap was visited four times per week. Each time captured mosquitoes were collected, and the trap was reset by adding a clean catching net and ~1 kg of dry ice into a modified ½ gallon beverage cooler (Coleman Company Inc., Wichita, KS). Trap collections were sent to the DSHS Arbovirus Laboratory for species counts and arbovirus testing. Data provided on the submission forms included trap location (address and GPS coordinates), habitat description, and date of collection.

From the BGS2-trap surveillance, high mosquito counts (mean >10 *Ae. aegypti*/trap/week) were obtained for six consecutive weeks (EW 27–32). The areas with the highest *Ae. aegypti* counts were selected for the WALS intervention using *Bti*, and will be referred as treatment zones whilst areas not sprayed as control zones (Figure 1). BGS2-trap failures were excluded from the analysis.

Field larval bioassay

In order to evaluate the WALS product delivery to container habitats by the Buffalo Turbine sprayer (Clarke Mosquito Control Products, Roselle, IL, USA), two larvicide field bioassays were conducted. The bioassays were performed on different WALS application days at the same location in one of the neighborhoods within a treatment zone (Figure 2). 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 (Figure 3). For controls, 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 following the larvicide application. Subsequently, plastic cups were covered with plastic film to prevent *Bti* cross contamination by *Bti*-exposed water spillage and transported back to the laboratory at Texas A&M AgriLife Research & Extension Center at Weslaco, TX.

Prior to the field-based bioassay, *Ae. aegypti* Liverpool strain eggs were hatched in double distilled water. Larvae were fed *ad libitum* with a 10% (w/v) liver powder solution (Garcia-Luna et al., 2018). Once the bioassay cups arrived at the laboratory, ten 3rd instar *Ae. aegypti* larvae were placed into each of the treatment cups exposed to the WALS application and into the control cups. Larval mortality was recorded at 24 hours after the WALS application.

Larvicide intervention

The WALS intervention used the truck-mounted Buffalo Turbine sprayer (Clarke Mosquito Control Products, Roselle, IL, USA) for the application of *Bti* in the treatment zones, selected based on the mosquito abundance. Each treatment zone was treated 1 to 3 times over the course of 3 weeks from August 18 to September 4, 2017 (EW 33 and 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) (Figure 1). Based on the larvicide applications, we refer to EW 22 to 36 as the pre-WALS intervention period EW 37 to 48 as the post-WALS intervention period.

Weather and environmental data

To help interpret the mosquito abundance data in the treated versus the control zones, environmental data were incorporated into the analysis. As a proxy for vegetation, we utilized data on normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI), both of which are widely used in ecological studies (Pettorelli et al., 2005). We obtained monthly images for vegetation indices with a 1-km resolution vegetation product (M*D13A3), which is based on Modis satellite images (Didan, 2015) from the NASA server of the Land Processes Distributed Active Archive Center (LP DAAC), United States Geological Survey (USGS)/Earth Resources Observation and Science (EROS) Center, Sioux Falls, SD, using the package *MODIStsp* for the software R (Busetto, 2016). Each image was clipped to the surface of the Brownsville area and stacked into a geotiff using the package *raster* for R (Brunsdon, 2015). For each monthly image, we extracted NDVI and EVI estimates for each trap location during the pre- and post- WALS intervention. Weekly NDVI and EVI estimates were obtained by interpolation based on a nonparametric LOESS regression (Venables & Ripley 2002)

Weather data were obtained from the National Oceanic and Atmospheric Administration (NOAA)/Global Historical Climatology Network (GHCN). We obtained the daily average of the minimum and maximum temperatures from the Brownsville, TX station (USW00012919), located at (25°54’51, −97°25’23) (NOAA/NCEI, 2017). Precipitation was obtained from the NOAA with the CPC Morphing Technique (CMORPH) with 0.25 degrees of resolution. For both temperature and precipitation, we computed the weekly mean for EW 22 to 49 of 2017. The coordinates (lat/lon) and elevation of each trap location were obtained using a Garmin eTrex® 20X GPS (Olathe, KS, USA).

Statistical analysis

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 turbine 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 have a correction in the degrees of freedom (d.f.) to account for heteroskedasticity, *i.e.*, unequal variance during the pre- and post- WALS intervention periods in this study (Welch, 1947). We also compared mean values in the weather and environmental variables measured in Brownsville, TX.

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 a variant of the Henderson's method by the following formula:

$$Percentage\ control = 100 - [(T/U) \times 100]$$

Where T is the post-treatment mean divided by the pre-treatment mean in the WALS intervention area, and U is the post- treatment mean divided by the pre-treatment mean in the control area (Fonseca et al., 2013). Meanwhile the proportional abundance change (PAC) in the treatment area was calculated as (1-T)*100 and for the control area (1-U)*100. We estimated both the PACs and the percentage of control, because the former quantifies local abundance changes, while the later provides a conservative estimate of the control efficiency that accounts for the seasonality of mosquito populations (Fonseca et al., 2013).

Results

Field larvicide bioassay

Two trials for the larvicide bioassay were conducted to evaluate the efficacy of the WALS application to experimental container habitats along the application route (Figure 3). Control cups not exposed to WALS application had 0% larval mortality for both trials (Table 2). During the first trial, plastic cups exposed to the larvicide and placed at 15 m from the truck route demonstrated 92% larval mortality, while plastic cups placed between 30–60 m had a 78% larval mortality, with significant differences among the studied distances (x^2=76.21, df=2, p<2.2 $\times 10^{-16}$). During the second trial, plastic cups placed 15 m from the truck route demonstrated 95% larval mortality, while plastic cups between 30–60 m had a 100% larval mortality. In the second trial *Ae. aegypti* larval mortality differences among the plastic cup placement distances were also statistically significant (x^2=85.87, df=2, p< 2.2 $\times 10^{-16}$).

Mosquito abundance in treatment and control zones

During routine BGS2 surveillance conducted by CBPHD on EW 22–48, a total of 98,296 mosquitoes were caught, of which the majority belonged to the *Aedes* genus (60.1%), followed by *Culex* (38.3 %) with *Anopheles*, *Psorophora*, and other genera of mosquitoes accounting for the remainder of the collections (1.6%) (Gaffigan TV). Non- *aegypti* and non-*albopictus* species were combined and reported as *Aedes* spp. Likewise, other than *Cx. quinquefasciatus* mosquitoes were combined into *Culex* spp. (Table 1).

A significant reduction was documented in the abundance of *Ae. aegypti* adults post- WALS intervention in both the treatment and control zones (Table 3). Prior to the larvicide treatment, the mean number *Ae. aegypti* per BGS2 trap per week was 12.83 ± 15.49 in the treatment zones and 7.19 ± 9.39 in the control zones. The *Ae. aegypti* abundance dropped after the WALS intervention, to 5.65 ± 5.97 trap/week in the treatment zones and to 4.52 ± 4.92 trap/week in the control zones. According to the Henderson’s equation, the WALS intervention resulted in 29% control of the *Ae. aegypti* adult populations.

A significant difference was not observed in the total mosquito abundance pre- and post-WALS intervention in the treatment zones (Table 4). In the control zones an increase in the total mosquito abundance was observed. Regarding *Cx. quinquefasciatus* and *Ae. albopictus*, there was a significant increase post-WALS intervention in both the treatment and control zones (Table 4).

We observed that the mean number of mosquitoes/trap/night was higher in the treatment zones when compared to the control zones for all species, including *Ae. aegypti* (Figure 4). 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 (Figure 4B, Table 4). The overall number of *Ae. aegypti* per trap location was high prior to the WALS intervention and decreased after the WALS intervention. The mean number of *Ae. aegypti*/trap/night decreased after the WALS intervention took place (Figure 4C). The abundance of *Ae. albopictus* was very low (Figure 4D) 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.

Additionally, the environmental variables showed a significant difference in the NDVI and EVI before and after the intervention. Rising temperature was associated with a significant increase in mosquito abundance, but this was not observed for rainfall (Table 4).Temperature was approximately 30 °C, during the time of the WALS intervention, with an initial decrease occurring in EW 23 that gradually increased over time (Figure 5A). Three peaks of precipitation (EWs 26–27, 36, and 40–41) totaling 305.9 mm out of 438.7 mm were observed during the study (Figure 5B). The mean NDVI presented a subtle increase after EW 35 that was consistent with the precipitation peak; hence many of the trap locations increased their NDVI after EW 35 (Figure 5C). However, the EVI was constant around 0.3 throughout the study period (Figure 5D).

Discussion

The threat from ZIKV, CHIKV, DENV and other arboviruses, combined with an increase in the vector population during the summer of 2017, triggered the Texas DSHS Emergency

Mosquito Control Contingency Contract to assist the CBPHD in a *Bti*-based WALS intervention. During this intervention, the potential effects of the WALS were evaluated using field-based larval bioassays. The results demonstrated that the WALS applied through the Buffalo Turbine reached up to 60 m into the target properties. 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. *Aedes aegypti* abundance decreased in the treatment zone by 51% going from 12.83 trap/week to 5.65 trap/week after the WALS intervention. However, *Ae. aegypti* population counts also decreased by 29% in the untreated (control) areas.

Others have evaluated the use of larvicide interventions for the control of *Ae. aegypti*, specifically for their efficacy during active ZIKV transmission. In 2016, following mosquito-borne transmission of ZIKV in Miami, FL, *Ae. aegypti* control efforts were initiated. Initially, ground-based insecticide control efforts were used to limit the ZIKV outbreak. However, due to consistently high female *Ae. aegypti* counts within 5–7 days of initiating control efforts, aerial application of Naled and *Bti* were performed. This combination of applications resulted in a mean density of one *Ae. aegypti*/trap/day after the second aerial application. Mosquito numbers increased to high levels (>20 *Ae. aegypti*/trap/day) in places where only the adulticide was used. In contrast, *Ae. aegypti* populations were much lower (5–10 trap/day) for up to one-month post-treatment following the combination of aerial adulticide and larvicide (Likos et al., 2016).

Stoddard (2018) evaluated the control efforts during the 2016 ZIKV transmission in Miami by analyzing mosquito trap data in the treatment areas. Similar to our study, *Bti* was applied with a Buffalo Turbine. Following the WALS application in Miami Beach, *Ae. aegypti* population counts fell to less than 90% of their prior level 17 days after the first *Bti* application and remained close to that level for 13 more days (Stoddard, 2018).

Pruszynski et al. 2017 evaluated the aerial application of *Bti* to control *Ae. aegypti* in the Florida Keys, FL. In that evaluation, five weekly treatments of *Bti* were followed by 4 bi-weekly treatments that resulted in a >50% reduction in female *Ae. aegypti* populations. Additionally, bioassays conducted to assess larval mortality of *Ae. aegypti* demonstrated that the *Bti* droplets reached the bioassay containers under dense canopy leading to >55% mortality on all application days (Pruszynski et al., 2017).

Multi-component approaches to control vector populations, including *Ae. aegypti*, have resulted in a variety of outcomes. For instance, an intervention to control *Ae. albopictus* populations in two suburban sites in New Jersey included a combination of education, source reduction, and insecticides (larvicides and adulticides), which resulted in 75% and 25% control in the two treatment sites compared to the untreated control areas (Fonseca et al., 2013). While to control *Ae. aegypti* population in Caguas, Puerto Rico during the Zika epidemic in 2016 an integrated vector management approach that included community awareness and education, source reduction, larviciding and mass trapping with autocidal gravid ovitraps (AGO) resulted in a decrease of the *Ae. aegypti* mosquitoes from 8 female *Ae. aegypti*/AGO trap/week to a <2 *Ae. aegypti*/AGO trap/week (Barrera et al., 2019b). In addition, Barrera and colleagues have proposed that the evaluation of a control intervention should focus on the reduction of mosquito populations to levels low enough to prevent disease transmission rather than unsustainable elimination (Barrera et al., 2018). Several studies performed in Puerto Rico have suggested that <3 *Ae. aegypti*/AGO trap/week will limit disease transmission (Barrera et al., 2017; Barrera et al., 2019a; Barrera et al., 2014a; Barrera et al., 2014b; Barrera et al., 2018; Lorenzi et al., 2016). However, those levels will be applicable at the community or city-wide scale level applied in Puerto Rico and will differ from those needed on greater scales and at other geographical locations.

Laboratory studies indicate that *Ae. albopictus* from the LRGV are highly competent vectors for ZIKV transmission (Azar et al., 2017), while *Ae. aegypti* appear to be slightly less competent (Roundy et al., 2017). However, it is not likely that the observed abundance of these species in the current study would initiate or sustain ZIKV transmission. But the more relevant parameter is vectorial capacity, where several peridomestic and anthropophilic characteristics give *Ae. aegypti* and advantage for transmission of human-amplified arboviruses, as seen in ZIKV transmission in southern Mexico (Azar et al., 2019; Guerbois et al., 2016).

Additionally, the *Culex*-transmitted West Nile virus (WNV) is also present in the LRGV region (TDSHS, 2019b). The WALS intervention resulted in a 52% control of *Cx. quinquefasciatus* in the WALS treatment zones according to the Henderson’s equation calculations in contrast to the 29% control observed for *Ae. aegypti*. The City of Brownsville had specific areas where WNV vectors were abundant; mainly, the historic downtown Brownsville area where pier and beam foundation housing is prominent. This type of housing may be associated with increased larval and resting sites leading to high mosquito populations.

Besides land use, geographical characteristics may influence vector presence. For instance, one unique characteristic of Brownsville is the presence of bodies of water known as esteros or resacas, geographically a delta river system or an ox-box lake (Zavaleta, 2018). The resacas may naturally be prone to retain water creating WNV vector larval habitats. In addition, the resacas may contribute to increased vegetation which will likely increase the sites that favor mosquito breeding (Wong et al., 2014). Therefore since 2013, the CPBHD, has implemented a resaca restoration project which aims to improve the water quality, flow and removal of debri to prevent stagnant water reducing the likelihood of mosquito breeding (BPUB, 2013).

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. However, many of the backyards were fenced, restricting entry to place the bioassay cups in backyards at distances >60 m. 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 mosquito abundance was observed after a WALS intervention.

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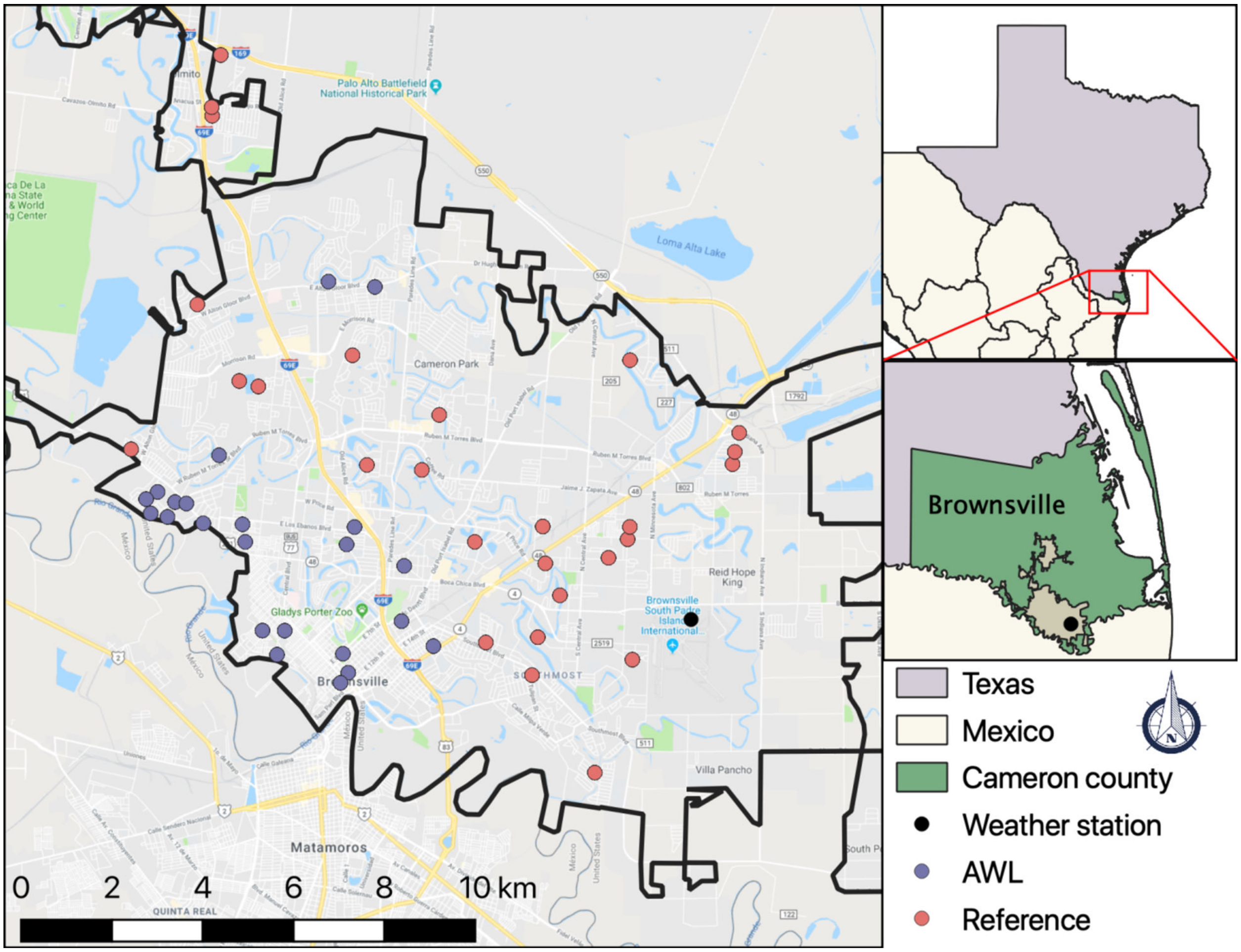

Figure 1.-

Map showing the location of BG-Sentinel traps within the City of Brownsville, Texas. 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. Black dot denotes the weather station location.

Figure 2.-
Neighborhood where the field bioassays were conducted.

Figure 3.-

Map showing the set-up of the field bioassays.

A yellow dot indicates where a plastic cup was placed at a 15 m distance from the larvicide deployment route while a blue point denotes a plastic cup placed at a greater than 15 m distance from the larvicide deployment route.

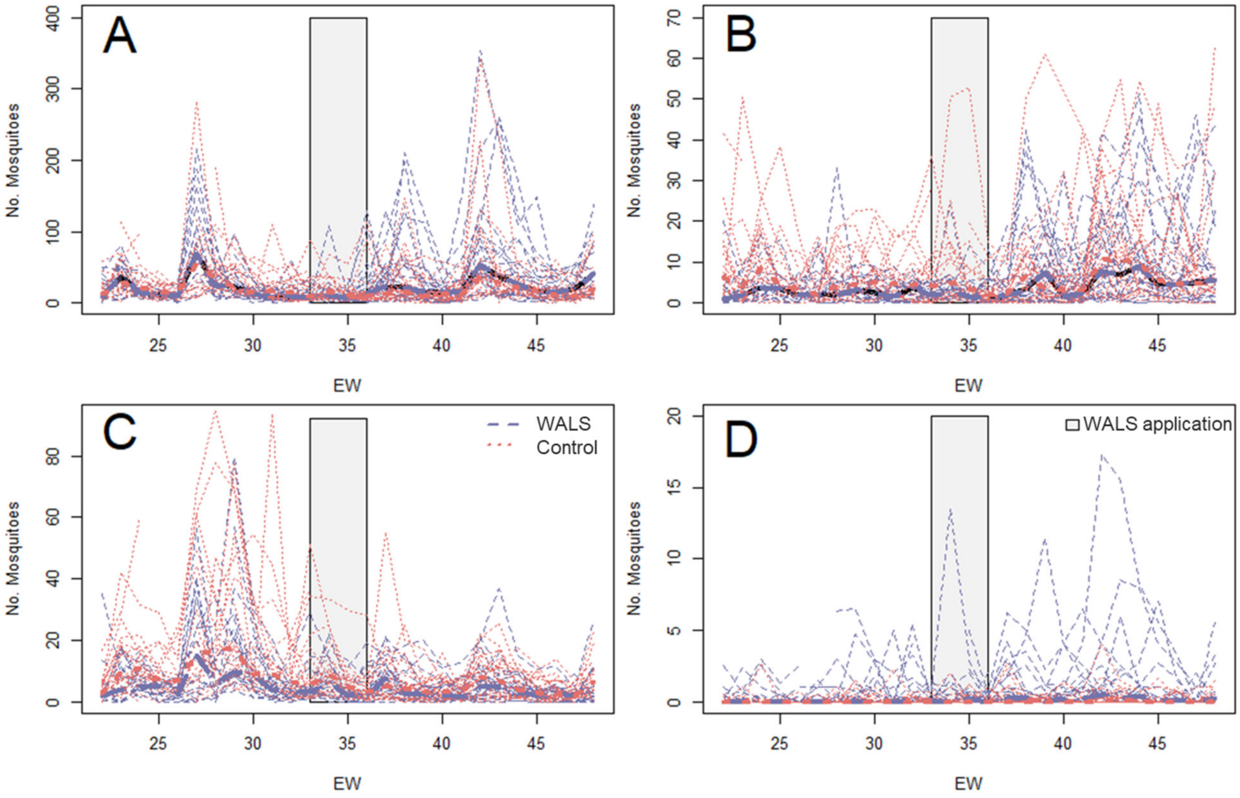

Figure 4.-

Weekly mosquito abundance/trap/night at Brownsville, TX.

(A) All species, (B) *Culex quinquefasciatus*, (C) *Aedes aegypti*, (D) *Aedes albopictus*. In all panels, thick black lines indicate mean values, dashed lines are values estimated for each trap location. The inset legend of panel C indicates the dashing pattern for locations subjected to the WALS intervention and those not treated (control). A gray box indicates the period (EW 33–36) when the WALS took place. In all panels EW stands for “epidemiological week”.

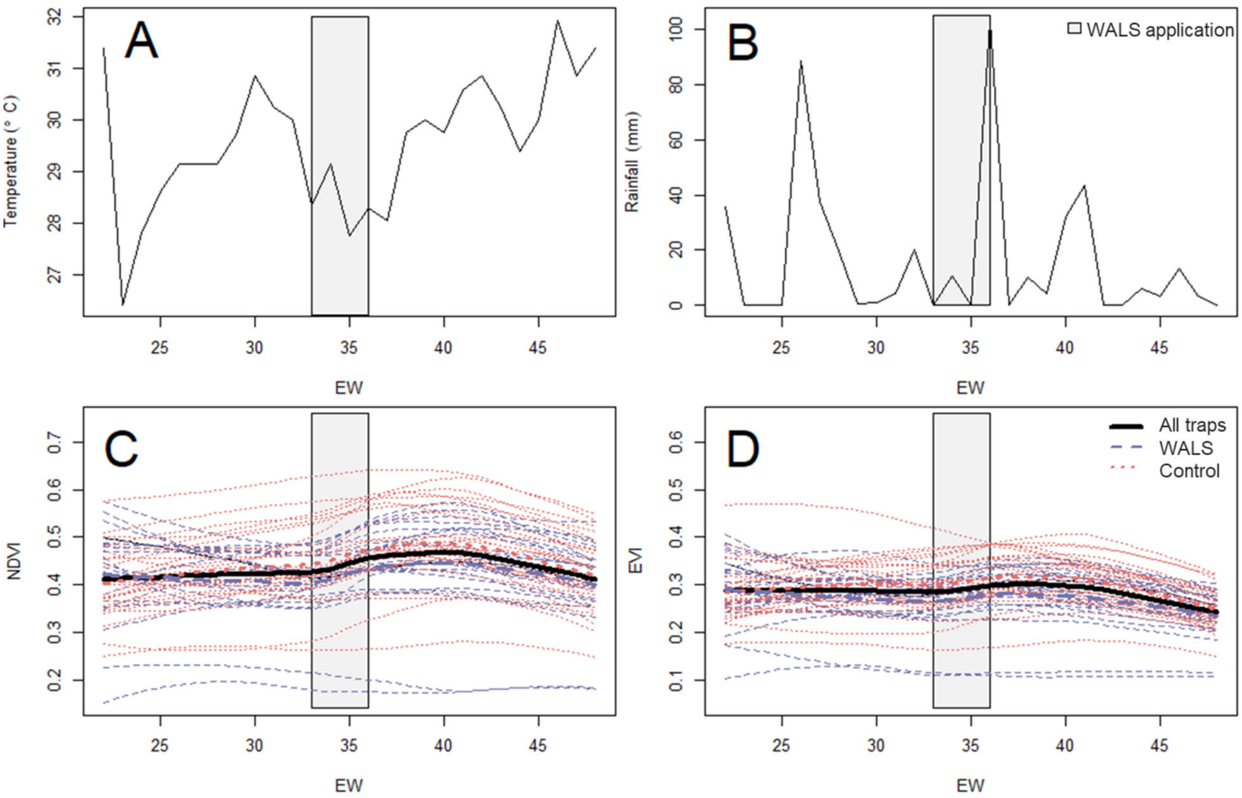

Figure 5.-

Environmental variables at Brownsville, TX.

(A) Temperature, (B) Rainfall, (C) NDVI, (D) EVI. In panels C and D thick black lines indicate mean values, while dashed lines are values estimated for each trap location. The inset legend of panel D indicates the dashing pattern for locations subjected to the WALS intervention and those not treated (control). A gray box indicates the period (EW 33–36) when the WALS took place. In all panels EW stands for "epidemiological week".

Table 1.-

Aedes aegypti larval mortality in cups exposed to WALS intervention at different distances from the Buffalo Turbine sprayer in Brownsville, TX.

	Distance (m)	**Dead**	**Total**	**Mortality (%)**
Trial 1	15	141	154	92
	30–60	121	155	78
	Control	0	100	0
Trial 2	15	139	146	95
	30–60	159	159	100
	Control	0	100	0

Table 2.-

Composition of mosquitoes collected during the length of the study.

ID	**N (%)**
Aedes spp.	31,762 (32.3)
Ae. aegypti	25,834 (26.2)
Ae. albopictus	1,603 (1.6)
Culex spp.	12,101 (12.3)
Cx. quinquefasciatus	25,573 (26)
Anopheles spp.	925 (0.9)
Psorophora spp.	595 (0.6)
Other species	104 (0.1)

Table 3.

Mean abundance of *Ae. aegypti* in Brownsville, TX, pre- and post-WALS intervention.

	Aedes aegypti	
	Control Mean ± SD	**WALS intervention Mean ± SD**
Pre- WALS intervention	7.19 ± 9.39	12.83 ± 15.49
Post- WALS intervention	4.52 ± 4.92	5.65 ± 5.97
Welch's *t*	4.44	7.51
d.f.	487.05	403.32
P	1.1×10^{-05}[*]	4.0×10^{-13}[*]
Proportional abundance decrease	37%	51%
% of control	29%	

The pre-WALS intervention period was from epidemiological week 22 to 36 and the post-WALS intervention period was from epidemiological 37 to 48. d.f.: degrees of freedom.

[*] $P < 0.05$.

Table 4.

Mosquito abundance and environmental parameters in Brownsville, TX pre- and post-WALS intervention.

Parameter	Pre-WALS intervention	Post-WALS intervention	*T*	d.f.	P-value
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	$4.04\times10^{-7}{}^{*}$
***Cx. 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	$1.481\times10^{-15}{}^{*}$
***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*
NDVI	0.424 ± 0.081	0.450 ± 0.089	−5.346	1218.8	$1.075\times10^{-7}{}^{*}$
EVI	0.288 ± 0.059	*0.279 ± 0.058*	2.681	1293.9	0.007*
Temperature	29.07 ± 0.93	30.24 ± 1.19	−2.651	24.99	0.014*
Rainfall	21.44 ± 32.95	9.76 ± 14.36	1.236	19.75	0.231

All parameters are presented as mean ± SD. *t* indicates the Welch's *t* statistic comparing the pre- and post-WALS intervention mean values, d.f., degrees of freedom and *P*-value the significance of the test. The pre-WALS intervention period was from EW 22 to 36 and the post-WALS intervention period was from EW 37 to 48.

$^{*}P < 0.05$.