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FIELD EFFICACY TRIALS OF AERIAL ULTRA-LOW-VOLUME APPLICATION OF INSECTICIDES AGAINST CAGED *AEDES AEGYPTI* IN MEXICO

FELIPE DZUL-MANZANILLA,[1] FABIAN CORREA-MORALES,[1] ANUAR MEDINA-BARREIRO,[2] WILBERT BIBIANO-MARÍN,[2] JOSE VADILLO-SANCHEZ,[2] MARTIN RIESTRA-MORALES,[2] LUIS FELIPE DEL CASTILLO-CENTENO,[2] EVARISTO MORALES-RIOS,[2] ABDIEL MARTIN-PARK,[2] GABRIELA GONZALEZ-OLVERA,[2] ARMANDO E. ELIZONDO-QUIROGA,[3] AUDREY LENHART,[4] GONZALO VAZQUEZ-PROKOPEC,[5] AZAEL CHE-MENDOZA[2] AND PABLO MANRIQUE-SAIDE[1,6]

ABSTRACT. We evaluated the efficacy of aerial ultra-low-volume (ULV) insecticide spraying in field bioassays with caged *Aedes aegypti* in May 2017 in Puerto Vallarta, Jalisco, Mexico. The insecticides tested included an organophosphate (Mosquitocida UNO ULV) and a neonicotinoid–pyrethroid combination (Cielo). Two *Ae. aegypti* populations were evaluated: a field pyrethroid-resistant local strain (Puerto Vallarta) and an insecticide-susceptible laboratory strain (New Orleans). Knockdown after 1 h by both products was ≥97.0%, and mortality after 24 h was ≥98% for the susceptible laboratory strain. Knockdown of the local Puerto Vallarta field strain by both products after 1 h was ≥96.5%; and mosquito mortality after 24 h was also very high (≥98%). Meteorological conditions during this evaluation were favorable for aerial mosquito control and represented conditions that typically occur during adulticide space spray applications. Temperature oscillated between 24°C and 26°C with winds between 6 and 10 km/h. The majority of droplets met the droplet distribution criteria required for the insecticides. The evaluation demonstrated an acceptable performance of both products for *Ae. aegypti* control when applied undiluted at a rate of 199.4 ml/ha and 73.07 ml/ha for Mosquitocida UNO ULV and Cielo, respectively. The volume median diameter (VMD) droplet size was characterized at 31.3 μm and 37.3 μm, respectively.

KEY WORDS *Aedes aegypti*, aerial application, neonicotinoid, Mexico, ultra-low volume

INTRODUCTION

Chemical control of *Aedes aegypti* (L.), the primary vector of dengue, chikungunya, yellow fever, and Zika, remains a fundamental element of the integrated strategies for prevention and control of *Aedes*-borne diseases in Mexico and worldwide. Specifically, chemical control of adult mosquitoes using ultra-low-volume (ULV) application is the most important strategy for adult *Aedes* control in Mexico, because it can be applied promptly, covering large, urban high-risk areas against the target stage of the vector (DOF 2015). As such, the Mexican health authorities encourage studies on the entomological efficacy of the insecticides employed by vector control programs (DOF 2015). The evidence generated by these studies is crucial for informed selection of products with the greatest potential to perform well under field conditions and, ultimately, to affect vector densities and disease transmission. In addition, evidence supporting strategies for insecticide resistance mitigation and management (particularly in the case of pyrethroids) is needed to promote the rational use of insecticides.

Aerial ultra-low-volume (AULV) application of insecticides for the control of adult *Ae. aegypti* in urban areas involves the application of an adulticide applied with a low-flying aircraft as a cold aerosol with a droplet size ranging between 25 and 40 μm (Bonds 2012). The AULV spraying is a method recommended for rapid control of adult mosquito populations during outbreaks or epidemics over large urban areas, especially where access with ground equipment is difficult and when extensive areas need to be treated very rapidly (WHO 2003, Carney et al. 2008, Bonds 2012, Ruktanonchai et al. 2014, CDC 2017).

One of the key challenges with insecticide-based interventions is that many populations of *Ae. aegypti* have developed resistance to insecticides (Smith et al. 2016), which may compromise the effectiveness of control programs (Hemingway and Ranson 2000, Rivero et al. 2010, Vazquez-Prokopec et al. 2017). In Mexico, the widespread use of permethrin over a 10-year period (late 1990s to 2009) led to the rapid

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[1] Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE) Secretaría de Salud México, Ciudad de México, México.

[2] Collaborative Unit for Entomological Bioassays, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, Yucatan, México.

[3] Secretaria de Salud Jalisco, Guadalajara, Jalisco, Mexico.

[4] Entomology Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA 30333.

[5] Department of Environmental Sciences, Emory University, Atlanta, GA 30322.

[6] To whom correspondence should be addressed. Unidad Colaborativa para Bioensayos Entomológicos (UCBE), Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán. Km. 15.5 Carr. Mérida-Xmatkuil s.n., C.P. 97315. Mérida, Yucatán, Mexico.

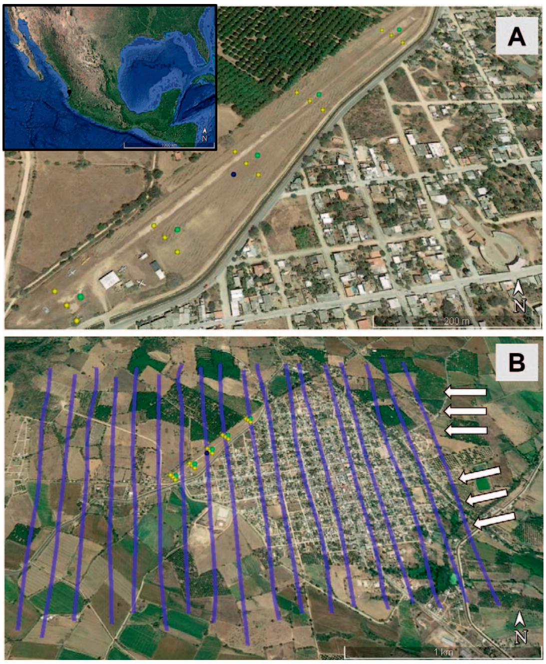

Fig. 1. Study area (A) location in Mexico, (B) location of the cages (yellow), impingers (green), and the station (blue) at Valle de Banderas private aerodrome, Nayarit, Mexico, and (C) path of 1 application. The arrows on the top (right side) show the wind direction (to the west) in May 30, and arrows from the bottom the west–southwest wind direction what prevailed in June 1 of 2017.

emergence and widespread propagation of pyrethroid resistance (Ponce-García et al. 2009), resulting in the decision by the Mexican Ministry of Health to search for other chemical groups to be used as adulticides (DOF 2015).

Prior to the implementation of a large-scale cluster-randomized trial to evaluate the efficacy of AULV spraying to control adult *Ae. aegypti*, we evaluated the efficacy of 2 potential AULV spraying products with the Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE) of the Mexican Ministry of Health under controlled conditions in an aerodrome.

MATERIALS AND METHODS

Study site

The AULV spray trials of 2 different insecticides against caged adult mosquitoes were performed at a private airport Pista de Aterrizaje Ejidal located in Valle de Banderas, Nayarit, Mexico (20.80892, –105.25535), as shown in Fig. 1, using an area of approximately 15 ha within the airport grounds. The trials were performed on May 30 and June 1, 2017 with Mosquitocida UNO ULV (Public Health Supply and Equipment of Mexico, Monterrey, Nuevo Leon, Mexico) and Cielo (Clarke Mosquito Control Products, St. Charles, IL), respectively, 1 insecticide per day.

Insecticides

Mosquitocida UNO ULV (an organophosphate, chlorpyrifos active ingredient [AI] 13.624%) and Cielo (a neonicotinoid, imidacloprid 3%; and the pyrethroid, prallethrin, 75%) were tested in the study. Mosquitocida UNO ULV has been previously used for mosquito control in public health operations in Mexico. Both Mosquitocida UNO ULV and Cielo have the approval and recommendation of the Mexican Ministry of Health to be employed as ULV sprays for public health control of mosquitoes (CENAPRECE 2017).

Adult *Aedes aegypti* cage bioassays

Biological material: *Aedes aegypti* from the insecticide-susceptible New Orleans strain was established at the insectary of the Ministry of Health of Jalisco, derived from eggs coming from a colony established since 2014 and maintained under standard controlled conditions (27 ± 2°C, $\geq$80 ±5% relative humidity [RH], 12:12 light:dark photoperiod). Mosquitoes were reared to adults in standard (0.5 m × 0.5 m) rearing cages on site, and provided a 10% sugar water solution until use in cage bioassays. The mosquitoes of a local field strain (Puerto Vallarta) were obtained from a colony developed from eggs collected from ovitraps in the locality during 2017. Insectary procedures, including mosquito breeding and production, are based on national and international standards recommended by CENAPRECE (SSA 2017).

Bioassays: Groups of 25 F_2 female adult *Ae. aegypti* (2–3 day old) of either susceptible or field strains were mouth aspirated into standardized, cylindrical cardboard cages (14.4 cm diameter and 4 cm depth; with nylon mesh and 1.2 × 1.2 mesh openings) (Bonds et al. 2010). The cages were placed on stakes 1.5 m above ground level with 2 cages per stake (1 cage containing the susceptible strain and the other cage containing the field strain) (Fig. 2). Five groups of 3 stations were placed 150 m apart (Fig. 1) so that a total of 15 pairs of cages were exposed to each product. Five pairs of control cages (unexposed mosquitoes) were placed in the spray plot and left 60 min, and then they were removed before the treatments started. After spraying, the treated mosquitoes were kept for 60 min at the exposure site. Then treatment cages were collected and taken to insectary facilities, where treated mosquitoes were transferred to clean holding cages using electric aspirator with hose for knockdown and mortality monitoring. Mosquitoes were fed with a 10% sugar water solution and scored at 1 h for knockdown and 24 h for mortality. Mosquitoes were considered knocked down or dead if they

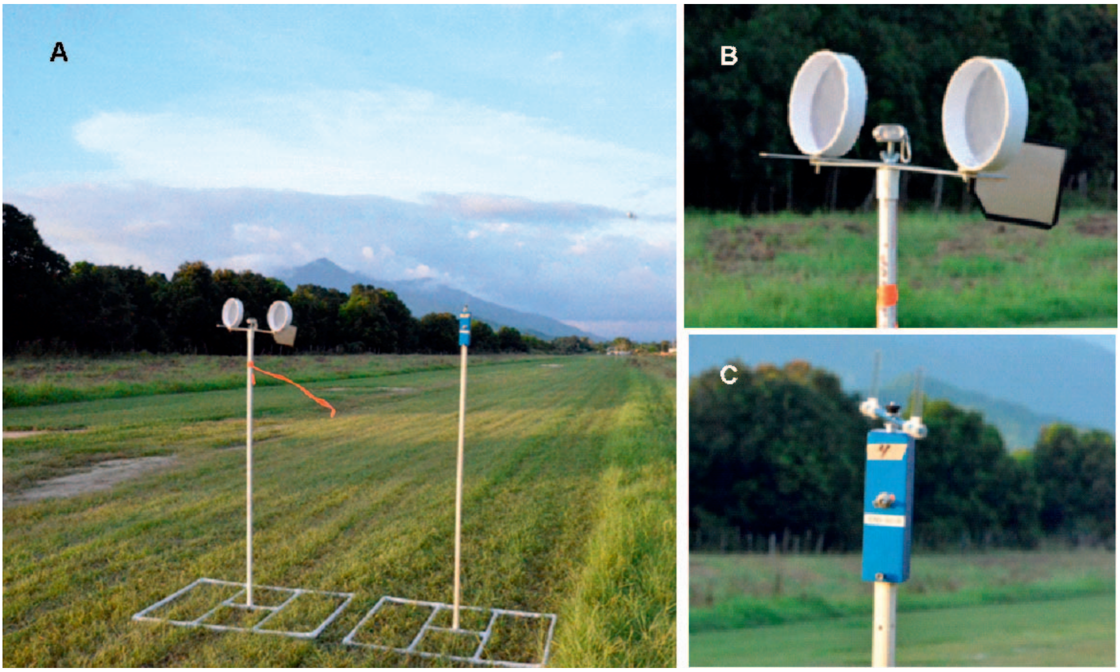

Fig. 2. General layout (A) test system showing a cage pair mounted on a stake with a wind vane and an impinger mounted on a stake, (B) close-up of cage pair, and (C) close-up of impinger.

remained moribund after receiving a slight puff of air from the observer.

AULV spray application

The insecticides were applied using a Cessna 206-H aircraft equipped with Flightmaster drift optimization software, AIMMS-30 wind measurement probe, and 2 Micronair AU 4000 rotary atomizers mounted under each wing (Fig. 3). The equipment was calibrated to provide a droplet size of 25–45 volume median diameter (VMD) and a blade speed of either 7,800 rotations per minute (rpm) or 8,800 rpm, and a flow rate of either 9,390 ml/min or 3,441 ml/min for Mosquitocida UNO ULV or CIELO, respectively. The Cessna 206-H flew at an altitude of 60 m with a swath width of 146 m and a speed of 193 km/h (120 mph). Applications were conducted near dusk/evening during favorable environmental conditions, including temperature and wind and when typical adult mosquito control activities would take place. The spray was applied directly over the cages and continued upwind from the field site for 18 passes according to CENAPRECE standard procedures for AULV spray application (2,500 m) to ensure product reached the evaluation site (Fig. 1).

The applied doses of products were 199.4 ml/ha of Mosquitocida UNO ULV (24.5 g AI of chlorpyrifos/ha) and 73.07 ml/ha of Cielo (2.49 g AI of imidaclorprid/ha and 0.62 g AI of prallethrin/ha). These flow rates are indicated by the manufacturer on the label and the doses (ml/ha and g (AI)/ha) are those permitted in Mexico.

Droplet characterization

Rotary slide impingers with Teflon-coated slides were placed on top of the 1.5 m stakes holding the spray cages for each replicate (Fig. 2). Droplet size volume median diameter (VMD) and density (drops/cm^2) were determined for each location within the replicate with the software REMSpc Slide Analysis (http://www.remspc.com/SlideAnalysis/). Teflon-coated slides (25 mm × 75 mm) were read within 24 h posttreatment, starting at 1 end of the slide about 3 mm from the long edge (ocular micrometer in vertical position), moving the slide from one side of the stage to the other (defined as a pass or sweep). Measuring each droplet in ocular (eyepiece) divisions as it passes through the micrometer. Each pass or sweep read an area of 25 mm by 1.01 mm (0.2525 cm^2). At a minimum, 200 droplets were measured to obtain an adequate sample. Every droplet that passed through the micrometer was measured.

Meteorological observations

Meteorological data were recorded using 2 Kestrel DROP model D2 (1.5 m and 10 m) and 1 Kestrel model 5000 Series (1.5 m). These data included temperature, RH, wind direction, and speed (m/s) at 1.5 m above ground. Data were recorded at 1-min intervals after the initial insecticide release (Chris-

Fig. 3. (A) Aircraft employed in the trials with arrows indicating the 4 nozzles, (B) the rotary atomizer mounted under a wing, and (C) the aerial view at the time of application.

tensen et al. 1972). With the temperature data at both heights and wind speed, the atmospheric stability index (also known as stability ratio, SR) was calculated using the following formula and interpreted according to the criteria defined by Yates et al. (1974):

$$SR = \frac{t_2 - t_1}{v^2} 10^5,$$

where t_1 = temperature on 1.5 m (in °C); t_2 = temperature on 10 m (in °C); v = wind speed on 1.5 (in m/s).

Atmospheric stability conditions as a function of SR ranges (Yates et al. 1974) are characterized as unstable (–1.7 to –0.1), neutral (–0.1 to 0.1), stable (0.1 to 1.2), or very stable (1.2 to 4.9).

Statistical analyses

The mortalities were corrected according to the Abbott's formula (1925) when mortality (>5%) was observed in the control group. Mean knockdown and mortality were quantified for each insecticide and mosquito strain following standard procedures (Bonds 2012). All analyses were performed using R statistical software (https://www.r-project.org).

RESULTS

Product efficacy

The percentage knockdown and mortality of the different products on the 2 strains of *Ae. aegypti* are summarized in Table 1. For both mosquito strains and insecticides, mean 24-h mortality values were >98% and significantly higher compared with the control (95% confidence intervals did not overlap with controls, Table 1). Furthermore, all insecticides had mortalities higher than the minimum threshold of efficacy of 80%, recommended by Mexican Official Standards (NOM) as minimum threshold for efficacious AULV applications (DOF 2015). Similarly, irrespective of the mosquito strain, both insecticides had significantly higher knockdown compared with the control.

Spray droplet size distribution and stability ratio

The droplet sizes that are effective for AULV are between 25 and 40 µm (Bonds 2012). The majority

Table 1. Mean mortality (24 h) and Knockdown (1 h) with 95% confidence intervals (in parentheses) of the 2 products applied as AULV spraying against caged *Ae. aegypti* in a semi-field conditions in Mexico.

Aedes aegypti strain	AULV treatment	Mortality, % (95% CI)	Knockdown, % (95% CI)
New Orleans (susceptible)	Mosquitocida UNO ULV	98.0 (96.4–99.3)	97.3 (95.7–99.0)
	Cielo	99.2 (98.3–100)	99.0 (97.5–99.8)
	Control	1.3 (1.0–0.0)	0.0 (0.0–1.0)
Puerto Vallarta (field strain)	Mosquitocida UNO ULV	98.1 (96.7–99.5)	96.5 (94.6–98.4)
	Cielo	99.4 (98.7–100)	99.5 (98.7–100)
	Control	1.0 (1.3–0.0)	0.0 (0.0–0.0)

of droplets sampled demonstrated that the droplet size distribution for both products met these criteria: Mosquitocida UNO ULV, droplet size 28.5 μm [27.3–29.6] and drop density 117.3 drops/cm^2 [102.1–132.4]; Cielo, droplet size 36.1 μm [34.9–37.2] and drop density 139.0 drops/cm^2 [123.8–154.2]. During the AULV spraying, the atmospheric conditions were neutral, with wind speed between 6 and 10 km/h and temperature between 24°C and 26°C.

DISCUSSION

We report results of a field efficacy trial evaluating nonpyrethroid insecticides applied aerially for the control of adult *Ae. aegypti* in Mexico. The results showed that both products, one containing an organophosphate and the other containing a neonicotinoid–pyrethroid combination, proved to be effective at killing both the susceptible and local pyrethroid-resistant strain of *Ae. aegypti* (Correa-Morales et al. 2016, Kuri-Morales et al. 2017).

Efficacy trials with malathion and naled applied aerially on exposed caged *Ae. aegypti* usually show high levels of efficacy, ca. 100% mortality after 24 h (Kilpatrick et al. 1970, Lofgren et al. 1970, Uribe et al. 1984, CDC 1987, Britch et al. 2018). No previously published studies report the efficacy of chlorpyrifos and neonicotinoid–pyrethroid formulations applied aerially against *Ae. aegypti*. Consistent with the literature, the results reported here suggest that their use can achieve a high level of efficacy.

Assessment of insecticide susceptibility among *Ae. aegypti* populations before vector control for dengue, chikungunya, and Zika as implemented is required by current Mexican government policy (NOM-032-SSA2-2014). Recent insecticide susceptibility tests have shown complete susceptibility of *Ae. aegypti* from Puerto Vallarta to chlorpyrifos in the state of Jalisco (CENAPRECE 2016, Correa-Morales et al. 2016, Kuri-Morales et al. 2017). To date, there are no broadly accepted diagnostic doses for prallethrin or imidacloprid to test for susceptibility of *Ae. aegypti,* so it should be considered a priority to determine the diagnostic doses for susceptibility bioassays and to begin monitoring the susceptibility of populations where products containing these insecticides might be considered.

The current strategy in Mexico for integrated *Ae. aegypti* control to reduce the risk of dengue, chikungunya, and Zika transmission includes the use of insecticides applied as ULV as one of its most important tactics (CENAPRECE 2015). According to the global plan for resistance management in mosquito vectors (WHO 2012), vector control programs should include, in addition to resistance monitoring, a plan of interventions to minimize the evolution of resistance. In this sense and as part of the needs established by the Mexican health authorities (DOF 2015) to have a portfolio of products and interventions of proven efficacy, this study showed promising results to incorporate the AULV for the control of *Ae. aegypti* populations into this portfolio. This study provides important evidence to consider regarding the role that nonpyrethroid and neonicotinoid–pyrethroid insecticides may play when considering AULV interventions to control *Ae. aegypti*, an intervention that can be integrated to support strategies for the mitigation and management of resistance to insecticides. Our study provides evidence that these products could be expected to have a high degree of efficacy when applied as AULV sprays to control *Ae. aegypti*. Our initial criteria for determining a successful AULV spraying effect was mortality of adult *Ae. aegypti* $\geq$80% in caged-mosquitoes bioassays (based on the Mexican regulations; DOF 2015). Other insecticide formulations for AULV spraying, different from the ones studied here, may be considered for further testing and recommendation in Mexico, with the provision of their approval by health and environmental authorities.

Space spray efficacy studies using caged-mosquito bioassays are part of the routine preliminary assessments recommended for determining ULV efficacy (Reiter and Nathan 2001), since they allow for an evaluation of insecticide effectiveness at an optimum insecticide application rate, but often these do not reflect the real impact on wild mosquito populations. The impact of AULV spraying on wild *Ae. aegypti* populations is not well characterized. The findings presented here will be used to inform the design of a cluster-randomized controlled trial to evaluate the impact of AULV spraying over a large urban area and quantify its impact on both entomological and epidemiological indicators.

The importance of effective *Ae. aegypti* control in urban areas remains pertinent, especially because effective vaccines for most *Aedes*-borne arboviruses are not likely to be available for many years to come. There is a particular need to develop improved vector control strategies that target adult *Aedes* populations. We demonstrate here that serial applications of insecticides in Mexico may prove to be an effective strategy. However, further research is needed to arrive at a practical public health conclusion regarding the use of AULV space spraying for *Ae. aegypti* control and to provide clear guidelines for appropriate implementation in programmatic settings.

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