Exploring New Thermal Fog and Ultra-Low Volume Technologies to Improve Indoor Control of the Dengue Vector, *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT Control of the mosquito vector, *Aedes aegypti* (L.), inside human habitations must be performed quickly and efficiently to reduce the risk of transmission during dengue outbreaks. As part of a broad study to assess the efficacy of dengue vector control tools for the U.S. Military, two pesticide delivery systems (ultra-low volume [ULV] and thermal fog) were evaluated for their ability to provide immediate control of *Ae. aegypti* mosquitoes with a contact insecticide inside simulated urban structures. An insect growth regulator was also applied to determine how well each sprayer delivered lethal doses of active ingredient to indoor water containers for pupal control. Mortality of caged *Ae. aegypti*, pesticide droplet size, and droplet deposition were recorded after applications. In addition, larval and pupal mortality was measured from treated water samples for 4 wk after the applications. The ULV and the thermal fogger performed equally well in delivering lethal doses of adulticide throughout the structures. The ULV resulted in greater larval mortality and adult emergence inhibition in the water containers for a longer period than the thermal fogger. Therefore, the ULV technology is expected to be a better tool for sustained vector suppression when combined with an effective insect growth regulator. However, during a dengue outbreak, either delivery system should provide an immediate knockdown of vector populations that may lower the risk of infection and allow other suppression strategies to be implemented.

KEY WORDS *Aedes aegypti*, ultra-low volume, thermal fog, indoor space spray, pyriproxyfen

Half of the global population is projected to be at risk of dengue infection, with ≈390 million people estimated to be infected annually (Bhatt et al. 2013). Dengue, an acute systemic viral infection transmitted to humans by *Aedes* mosquitoes, may be fatal for some individuals when the disease develops to dengue hemorrhagic fever or dengue shock syndrome. Despite attempts to develop vaccinations, there are currently none available. The primary method for reducing the risk of dengue infection is through preventing the mosquito vectors from feeding on the human hosts (Eisen et al. 2009). This is achieved primarily through personal protection and mosquito population suppression (Morrison et al. 2008, Eisen et al. 2009). Successful control programs rely on a combination of intradomiciliary adulticide application, larvicidal treatment of breeding sites, and removal of artificial water containers (Pan American Health Organization [PAHO] 1994, World Health Organization [WHO] 2009). However, these methods are labor intensive, often unsustainable in developing countries and during military operations, and may not achieve population control quickly enough to reduce the risk of dengue transmission, as demonstrated by the high number of dengue infections reported annually (e.g., WHO 2009, Bhatt et al. 2013).

Dengue is primarily transmitted by *Aedes aegypti* (L.) in urban endemic regions. *Ae. aegypti*, which is also a vector of chikungunya and yellow fever, is adapted to the urban environment, where it uses artificial containers for breeding (Kittayapong and Strickman 1993), feeds almost solely on humans (Christopher1960), and displays reclusive resting behavior within human dwellings (Perich et al. 2000). Specifically, female *Ae. aegypti* have been shown to rest under furniture, behind hanging objects on walls,

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14. ABSTRACT

Control of the mosquito vector, Aedes aegypti (L.), inside human habitations must be performed quickly and ef?ciently to reduce the risk of transmission during dengue outbreaks. As part of a broad study to assess the ef?cacy of dengue vector control tools for the U.S. Military, two pesticide delivery systems (ultra-low volume [ULV] and thermal fog) were evaluated for their ability to provide immediate control of Ae. aegypti mosquitoes with a contact insecticide inside simulated urban structures. An insect growth regulator was also applied to determine how well each sprayer delivered lethal doses of active ingredient to indoor water containers for pupal control. Mortality of caged Ae. aegypti, pesticide droplet size, and droplet deposition were recorded after applications. In addition larval and pupal mortality was measured from treated water samples for 4 wk after the applications. The ULV and the thermal fogger performed equally well in delivering lethal doses of adulticide throughout the structures. TheULVresulted in greater larval mortality and adult emergence inhibition in the water containers for a longer period than the thermal fogger. Therefore, the ULV technology is expected to be a better tool for sustained vector suppression when combined with an effective insect growth regulator. However, during a dengue outbreak, either delivery system should provide an immediate knockdown of vector populations that may lower the risk of infection and allow other suppression strategies to be implemented.

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among hanging cloths, in bedspreads, on mosquito-net poles, mosquito nets, and inside shoes (Pant and Yasuno 1970, Nelson 1986, Reiter and Gubler 1997, Perich et al. 2000). These resting behaviors make controlling *Ae. aegypti* populations difficult by limiting the effectiveness of outdoor and peridomestic insecticide applications, as the pesticide delivery systems must be able to distribute lethal doses of active ingredient (a.i.) to all secluded areas that may serve as resting sites for *Ae. aegypti* adults (Chadee 1989, Perich et al. 2000, Esu et al. 2010).

Indoor applications of space sprays have been observed to achieve high levels of adult mortality despite the reclusive resting behavior of the species (Perich et al. 2001, Perich et al. 2003). These indoor space sprays also appear to increase the duration of population control compared with peridomestic applications (Koenraadt et al. 2007). However, for a population suppression tactic to be useful in dengue vector control, the pesticides used in space sprays must not only produce high adult mortality, but also provide some level of persistent population control (Gratz 1991). Indoor delivery of contact adulticides by ultra-low volume (ULV) and thermal fog spray systems have been shown to result in high adult mosquito mortality throughout structures (Perich et al. 2001, Perich et al. 2003). However, the mosquito populations appear to quickly rebound following suppression attempts if only adults are targeted (Koenraadt et al. 2007), due to the larval habitats remaining untreated (Reiter 1992). Intuitively, the efficacy of an emergency vector suppression plan should be improved by including a product for persistent immature control (Gould et al. 1970), such as a larvicide or an insect growth regulator (IGR). While individually treating all potential larval habitats through manual insecticide application is often impractical, including larval control agents in a space spray to efficiently deliver a lethal dose to all potential larval habitats in the sprayed area may overcome this logistical hurdle. A pyriproxyfen-based IGR (NyGuard; MGK, Minneapolis, MN) has been approved by the U.S. Environmental Protection Agency for indoor space sprays and the manufacturer's label claims it can be delivered using both the thermal fogger and the ULV. Pyriproxyfen has been shown to inhibit adult emergence from the pupal stage for several months (Sihuincha et al. 2005, Moh Seng et al. 2006) at doses far below the WHO maximum amount allowed in drinking water (WHO 2002).

The efficacy of combining adulticides with immature control measures on *Ae. aeygpti* populations has been evaluated previously (Lucia et al. 2009, Harburguer et al. 2011), and comparisons have been made between the effectiveness of thermal fogs and ULV sprays in delivering adulticides within a structure (Perich et al. 2001, 2003). To the authors' knowledge this is the first study to compare ULV and thermal fog technologies in delivering lethal doses of both a contact adulticide and a persistent IGR to simulated *Ae. aegypti* resting sites and breeding sites indoors. In addition, the effect of the delivery method (thermal fog or ULV) on the persistence of the IGR in larval habitats was evaluated. The results generated from these tests will be used to assess the utility and feasibility of the various insecticides and application technologies as vector control tools for mosquito control teams in both civilian and military settings during dengue, chikungunya, and yellow fever epidemics.

Materials and Methods

The equipment and insecticides selected for this study were identified based on their potential for use by military entomologists and their potential to improve the sustainability of dengue vector control operations given the resource constraints faced by both military and global public health systems. The ULV sprayer tested was the Twister XL3, a compact, lightweight (11.8 kg), backpack, aerosol generator manufactured by Curtis Dyna-Fog (Westfield, IN), and claimed to be ergonomically suited for long periods of use. The thermal fogger evaluated was the Patriot, a hand-held, portable thermal fogger manufactured by Curtis Dyna-Fog weighing 12.5 kg. The Patriot has a more compact design than other thermal foggers manufactured by Curtis Dyna-Fog, allowing the machine to be operated in more confined spaces. The contact insecticide used was ULB BP-300 (BASF, Ludwigshafen, Germany) comprised of 3% pyrethrin with a 16% dual synergist. ULB BP-300 was selected for this evaluation as it is commonly implemented in ULV and fog applications for U.S. Department of Defense and it is also labeled for indoor use. The IGR was NyGuard (10% pyriproxyfen; MGK, Minneapolis, and MN).

In total, four spray treatments were tested with both the ULV and thermal fogger, resulting in a total of eight treatments. The four spray treatments were: 1) water ($n = 3$); 2) ULB BP-300 undiluted ($n = 6$); 3) NyGuard diluted with BVA 13 mineral oil (BVA Oils Inc., Wixom, MI [1 part NyGuard to 15 parts mineral oil, $n = 6$]); and 4) mixture of ULB BP-300 and NyGuard (1 part NyGuard to 15 parts ULB BP-300, $n = 6$). A control was also conducted during each spray trial ($n = 9$), which involved placing caged mosquitoes and water containers into buildings for 15 min without being sprayed, thus enabling the observation of environmental effects on adult, larval, or pupal mortality.

All mosquitoes used for the assays were laboratory-reared, Orlando strain, *Ae. aegypti.* The colony was maintained under constant laboratory conditions (26°C, 75% relative humidity [RH], and a photoperiod of 16:8 [L:D] h). Adults were held in large group cages and provided with a 10% sucrose solution. Females were blood fed and subsequent eggs collected on seed germination paper and stored at 26°C and >75% RH in a desiccator. Eggs were hatched by flooding egg papers and the larvae were held in enamel trays with 1 liter of deionized water and 30 mg dried yeast.

Field Trials. All field trials were conducted at the Military Operations in Urban Terrain (MOUT) site North located at Camp Blanding Joint Training Center, Starke, FL (29° 57′6.84″ N, 81° 58′47.64″ W) during February and March 2013. The average temperature recorded during the field trials was 18.4 ± 2.9°C with

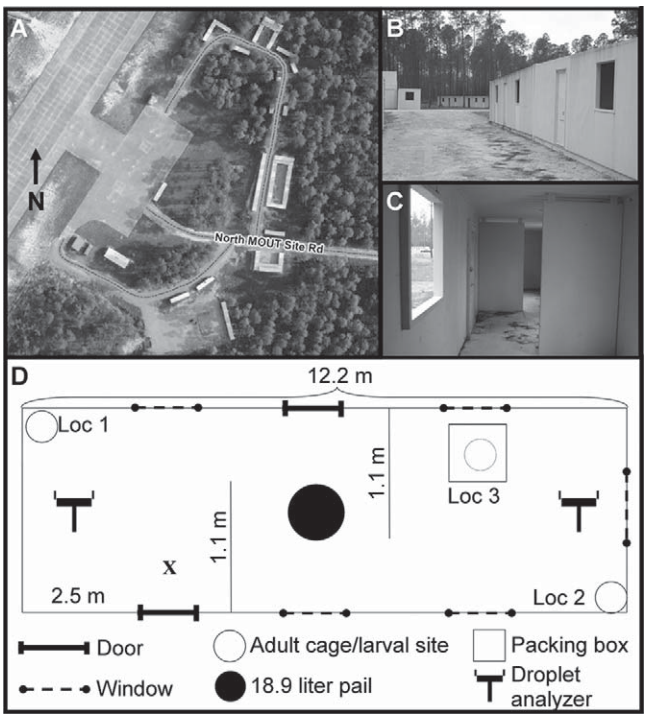

Fig. 1. (A) Arial view of the experimental plot at MOUT site North, Camp Blanding Joint Training Center, Starke, FL., (B) external ground view of the simulated urban structures, (C) interior view of the structures taken from location 2 toward location 1, and (D) the floor plan of each structure along with the locations where adults and 120-ml specimen jars, rotating droplet analyzers and the 18.9-liter pails were placed. The sprayer stood 0.5 m inside the room in front of the door located 2.5 m from the external wall (marked with "X"), while the door opposite in the center of the structure remained shut.

an average relative humidity of 52.1 ± 15%. The MOUT site consists of several dozen steel intermodal shipping containers (2.4 by 2.4 by 12.2 m), modified to simulate urban structures. Each container has two doors and five windows cut into its walls and two interior walls placed inside, which divide the interior into three equal sized spaces connected with doorways (Fig. 1). Three cages—each holding 25 3–5-day-old laboratory-reared adult female *Ae. aegypti*—were placed in each of the structures selected for the trials. The first cage was placed at location 1, hanging 2.14 m above the ground in the corner directly opposite to the open door, while the second cage was placed at location 2, hanging 2.14 m above the ground in the furthest corner from the door, opposite to location 1 (Fig. 1). The third cage was placed at location 3, inside a cardboard packing box (0.3 m^3), turned on its side, positioned 0.3 m from both the interior wall and the exterior wall in the space farthest from the open door with the box's opening facing toward the interior wall. The box was used to simulate furniture that mosquitoes may rest under for shelter (Perich et al. 2000). An open 120-ml polyethylene specimen jar filled with 90 ml of deionized water was placed below each of the hanging adult cages and next to the adult cage inside the box. By having three cages and three specimen jars in each room, the effectiveness of each sprayer at delivering lethal doses of the pesticides throughout the room and around potential obstructions was evaluated. An additional 18.9-liter plastic pail, filled with 5 liters of water, was placed between the two interior walls and 1.2 m in front of the closed center door. Pails were only placed in one structure for the unsprayed control and the treatments sprayed by only the BP-300, whereas a pail was placed within each of the structures sprayed by the mixture of BP-300 and NyGuard. To measure the droplet size and density delivered by each machine, a rotating aerosol droplet sampler was placed at both ends of the structure 1 m from the walls at a height of 1.3 m in three replicates for every treatment. The rotating aerosol droplet samplers each had two Teflon coated 7.5 by 2.5 cm slides (Summit Chemical Co., Baltimore, MD) attached. While the pesticides were sprayed into the structure, the aerosol droplet sampler rotated at 450 rpm and continued to spin for 15 min after the spray was concluded. In addition to the slides placed on the rotating aerosol droplet sampler, two slides were placed inside the packing box next to the adult cage.

The pesticides were sprayed into each room through the doorway that is off-center (2.5 m from the closest exterior corner), while the middle door was closed (Fig. 1). The spray team stood 0.5 m inside the room, in front of the door, and sprayed toward each end of the structure for the same duration of time. The ULV and the thermal fogger were sprayed for the duration of time estimated for each to fill the volume of the structure (72.5 m^3) with a.i. The Twister XL3

ULV was sprayed at a rate of 168 ml/min, requiring 14.4 s to fill the structure, while the Patriot thermal fogger was sprayed at a rate of 159 ml/min, requiring 15.2 s to fill the structures. After the application of the pesticide or water, the spray team exited the structure and the doors were closed for 15 min. After 15 min, the adult cages, the 120-ml specimen jars, the 18.9-liter plastic pail (when present), and the Teflon coated slides (when present) were removed from the structures. Adult mortality was determined for each cage immediately after removal and again at 24 h. The specimen jars and 18.9-liter plastic pails were sealed and returned to the laboratory to use in the larval and pupal mortality assays.

In addition to these assays, a final set of trials was conducted to determine the deposition (nl/cm^2) of product that could be delivered through the structure by each sprayer type. In these trials, Uvitex OB fluorescent dye (Ciba Corporation, Newport, DE) was mixed at a concentration of 3,000 ppm in BVA-13 mineral oil. Two 1 m in length and 2.5 cm in width biodegradable cotton ribbons (Lab Safety Supply Inc., Janesville, WI) were stretched vertically from the floor and from the ceiling, 0.5 m from each of the corners where the adult cages and the specimen jars were placed. In addition, a biodegradable cotton ribbon was placed inside the packing box, which was positioned as before. Three replicates were conducted for each sprayer.

Laboratory Bioassays. Ten third- to fourth-instar larval *Ae. aegypti* were introduced into each of the 120 ml specimen containers 24 h after the field trials were conducted and maintained in the laboratory for the remainder of the experiment and provided with a larval diet consisting of a three to two ratio of bovine liver extract to brewer's yeast in a 2% solution. Larval and pupal mortality were recorded daily, as the proportion dead, along with adult emergence from the pupae, until all larvae or pupae died or all adults emerged. Because some larvae would die before pupation, pupal mortality was calculated based on the proportion of mosquitoes that successfully pupated. Adult emergence inhibition (E.I.) was calculated from the larval and pupal mortality rates. E.I. was therefore the total percentage of larvae that did not develop into adults. Also, three 90-ml subsamples, were collected from each of the 18.9-liter pails 24 h postspray and placed into separate 120-ml specimen containers. Ten larval mosquitoes were placed in each of these containers and observed for larval and pupal mortality until all larvae or pupae died, or all adults eclosed. Three more samples were collected weekly from each of the 18.9-liter pails over the subsequent 4 wk of observation. E.I. was calculated from the recorded larval and pupal mortality.

Droplet Analysis. The droplet diameter on the Teflon coated slides was measured with DropVision System (Version 2.2, Leading Edge Associates, Waynesville, NC). The DropVision system software parameters used to score individual droplets required an image size greater than six pixels, roundness one, and spread factor ranged from 0.60 to 0.72. The droplets were measured from a minimum of five locations on the slide surface or until a minimum of 200 droplets were recorded. From these data, the volume median diameter ($Dv_{0.5}$), the $Dv_{0.1}$, the $Dv_{0.9}$, and the average droplet density (drops per square millimeter) were estimated. The $Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$ are the droplet diameters (μm) such that 10, 50, and 90%, respectively, of the spray volume is composed of smaller droplets.

To analyze the droplet deposition, each cotton ribbon sample was placed in a plastic bag and washed with 25 ml of denatured ethanol. The samples were submersed for 5 min in the ethanol, and then shaken. The resulting wash solution was poured into two 10-ml cuvettes and measured with a calibrated spectrofluorophotometer (Shimadzu Scientific Instruments, Durham, NC). The raw spectrofluorophotometer readings were converted to dye concentration (C_{ws}) with calibrations obtained from a standard solution. The surface area (A_s) for each sample was calculated by using the dimensions for the ribbons. Deposition, defined as the spray mass deposited per unit surface area, was calculated with the following formula:

$$Dep = \frac{1{,}000\ C_{ws} V_w}{A_s},$$

where *Dep* is the deposition of dye on a sample's surface (ng/cm^2), C_{ws} is the concentration of dye in the wash solution (ppm or μg/ml), V_w is the wash volume (ml), and A_s is the surface area of the cotton ribbons (cm^2). Using the concentration of dye in the spray tank (ng/nl), dye deposition was converted to deposition of BVA in nl/cm^2, onward referred to as deposition.

Estimated Concentration of A.I. in Water. The concentration of a.i. delivered to each water container by the two sprayers was estimated based on the deposition (nl/cm^2) on the cotton ribbons. The average deposition of the high and low ribbons at locations 1 and 2 was used to estimate the concentration of a.i. in the 18.9-liter pail, as it was placed in the center of the structure. The concentration of a.i. in the 120-ml specimen jars at locations 1 and 2 were estimated based on the average deposition of the high and low ribbons from the respective location. The concentration of a.i. in the 120-ml specimen jars at location 3 was estimated based on the deposition on the ribbon placed on the floor of the packing box. The surface area of the water in the pail and specimen jar was calculated from the container dimensions and volume of water in the containers. It was assumed that deposition of BVA per unit surface area of the cotton ribbon would be similar to that of the pesticides deposited on the surface area of the water. The surface area of the water in containers, volume of water in containers, the deposition, and proportion of a.i. in mixtures were then used to estimate the concentration of a.i. in the water as parts per billion (ppb).

Statistical Methods. All Statistical tests were performed in Intel Visual Fortran Composer XE 2013 with an $\alpha = 0.05$. Separate Kruskal–Wallis (K-W) hypoth-

Table 1. Average proportion (±SE) of caged adult female *Ae. aegypti* that died 15 min and 24 h after applications of each indoor space spray treatment

Spray treatment	Adult mortality at 15 min		Adult mortality at 24 h	
	Thermal fog	ULV	Thermal fog	ULV
Water	0.00 ± 0.00a	0.00 ± 0.00a	0.03 ± 0.01a	0.01 ± 0.01a
BP-300	0.85 ± 0.06b	0.56 ± 0.09b	0.93 ± 0.07b	0.88 ± 0.07b
NyGuard & mineral oil	0.03 ± 0.01a	0.02 ± 0.01a	0.03 ± 0.01a	0.02 ± 0.01a
BP-300 & NyGuard	0.85 ± 0.06b	0.71 ± 0.07b	1.00 ± 0.00b	1.00 ± 0.00b

Sprays were conducted in Feburary and March 2013 at the Camp Blanding Joint Training Center, Starke, FL. Different letters next to each value represent significant differences among the treatments within each time period (15 min or 24 h), as calculated with Tukey multiple-comparisons test.

The average mortality of the environmental control was 0.005 ± 0.004 at 15 min, and 0.014 ± 0.007 at 24 h, with a Tukey grouping of "A" for both times.

esis tests (Zar 1999) were applied to the adult mortality measured at 15 min and 24 h after the pesticide applications, and the initial larval mortality and %E.I. from the 120-ml specimen jars. Sampling among the six buildings and two replicates were assumed randomized, so that sample size, n = (3 buildings) × (2 reps) = 6. Two-way K-W assessed for differences in adult, larval, and pupal mortality due to treatment (sprayer × pesticide mixture), location, and the treatment × location interaction term.

Additional three-way K-W tests were applied to the weekly larval mortality and percent adult E.I. data gathered from the subsamples of each 18.9-liter plastic pail to test the effect of the sprayers, the pesticide mixtures, the five weekly observations (wk 0, 1, 2, 3, and 4), the two-way interactions (sprayer × pesticide, sprayer × time, and pesticide × time), and the three-way interaction (sprayer × pesticide × time). The sample size n = (3 buildings) × (3 subsamples) × (2 reps) = 18. A simple linear regression analysis (SLRA) was conducted for the larval mortality and adult E.I. data (y) versus time since application (x, weeks; y = a + b × x; n = 5 time measurements: 0, 1, 2, 3, 4 wk) for each treatment, for a total of n = 2 × 2 = 4 regression equations for both larval mortality and adult E.I. (n = 5 times). Magnitude and sign of the regression coefficient for time after treatment were obtained to assess the degrees of sensitivity and directional impacts of time on larval mortality and adult E.I. A comparison of regressions analysis was performed for each of these SLRAs to test for differences in regression slopes, elevations (i.e., regression intercepts), and coincidental regression among the regressions (Zar 1999).

Separate three-way K-W tests were applied to the droplet size distributions ($Dv_{0.1}$, $Dv_{0.5}$, and $Dv_{0.9}$), and droplet density measured for three treatments (BP-300, BP-300 + NyGuard, and NyGuard + mineral oil), two sprayers (Twister and Patriot), and three locations for two slides and three replicates. Sampling among the two slides and three replicates were assumed randomized, so the sample size, n = (2 slides) × (3 reps) = 6. The various two-way interactions (treatment × sprayer, treatment × location, and sprayer × location), and the three-way interaction (treatment × sprayer × location) were evaluated. A final three-way K-W test was also applied to the droplet deposition data to test for the effect of sprayer, location (1 and 2 only), height (high and low), and the two-way and three-way interactions, where n = 3. Subsequent Tukey multiple-comparisons tests were conducted to identify the specific pairs of variables that were different for each of the K-W tests that were performed.

Results

Adult Mortality. Within 15 min of application, the BP-300 achieved 56–85% knockdown of the caged *Ae. aegypti*, and killed 88–100% of the female mosquitoes within 24 h (Table 1). The location of the adults within the structures did not influence the 15 min or 24 h adult mortality. BP-300 resulted in similar adult knockdown at 15 min and adult mortality at 24 h when mixed with NyGuard, and when sprayed from the ULV and the thermal fogger (Table 1). The adult mortality that resulted from the application of both undiluted BP-300 and BP-300 in a mixture with NyGuard was significantly greater than the adult mortality in the unsprayed control and following applications of water and NyGuard diluted in mineral oil at 15 min (χ^2 = 117.88; df = 8; $P < 0.0001$) and at 24 h (χ^2 = 124.15; df = 8; $P < 0.0001$; Table 1).

Larval Mortality and E.I. The application of undiluted BP-300 and the mixture of BP-300 and NyGuard resulted in similar rates of larval mortality, ranging from 15 to 100%, and killed significantly more larvae than when NyGuard was applied alone, and compared with all of the controls ($F_{8,161}$ = 13.66; $P < 0.0001$; Fig. 2). The number of larvae killed by the application of BP-300 with and without NyGuard was highest in jars placed at location 1 (closest to applicator) and the lowest at location 3 (under the box; χ^2 = 6.72; df = 2; P = 0.04; Fig. 2). In addition, the ULV applications of BP-300 and BP-300 mixed with NyGuard killed more larvae than when applied using the thermal fogger (χ^2 = 97.09; df = 8; $P < 0.0001$). The thermal fogger achieved the greatest larval mortality when spraying the mixture of BP-300 and NyGuard, while BP-300 undiluted only achieved larval mortality greater than that of the controls at location 1 (Fig. 2). Because the ULV spray of BP-300, regardless of mixture, killed 100% of larvae in several replicates, pupal mortality could not accurately be estimated. Therefore, the per-

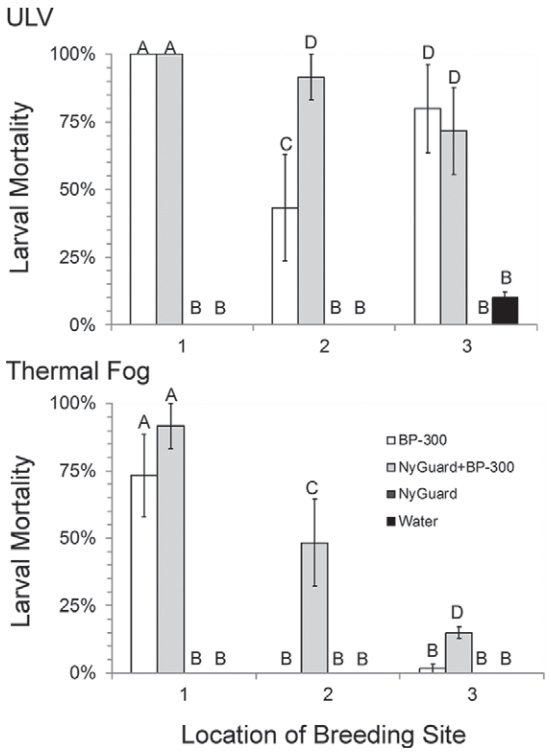

Fig. 2. Average percentage (±SE) of *Ae. aegypti* larvae that died (larval mortality) when placed in 120 ml artificial breeding sites that were located at three locations within a structure during space spays of undiluted BP-300, a mixture of NyGuard and BP-300, NyGuard diluted in mineral oil, and water conducted in February and March 2013 at the Camp Blanding Joint Training Center, Starke, FL. Differences in average mortality for each treatment are represented by different letters, as determined through Tukey post hoc tests.

centage of adult E.I. was used to estimate the efficacy of the treatments that included NyGuard in the pesticide mixtures.

The adult E.I. was similar across all of the locations within the structure. The application of NyGuard, with and without BP-300, resulted in ≈98% adult E.I. in the specimen jars. The presence of NyGuard in the space spray greatly improved the percentage of adult E.I. compared with when BP-300 was sprayed undiluted and compared with the controls ($\chi^2 = 93.57$; df = 4; $P < 0.0001$; Fig. 3).

During the 4 wk after the initial pesticide application, larval mortality in water from the large pails was dependant on interactions of time with the sprayer type ($\chi^2 = 3.04$; df = 4; $P = 0.02$) and the pesticide mixture ($\chi^2 = 7.43$; df = 4; $P < 0.0001$; Fig. 4a). Specifically, the ULV only resulted in higher rates of larval mortality than the thermal fogger on week 0 ($\chi^2 = 10.40$; df = 1; $P = 0.002$), while the pesticide mixture of NyGuard and BP-300 only resulted in higher rates of larval mortality at weeks 1 ($\chi^2 = 14.05$; df = 1; $P = 0.0002$) and 2 ($\chi^2 = 15.45$; df = 1; $P < 0.0001$) than undiluted BP-300 (Fig. 4a). According to the comparison of regression analysis of the SLRA, no

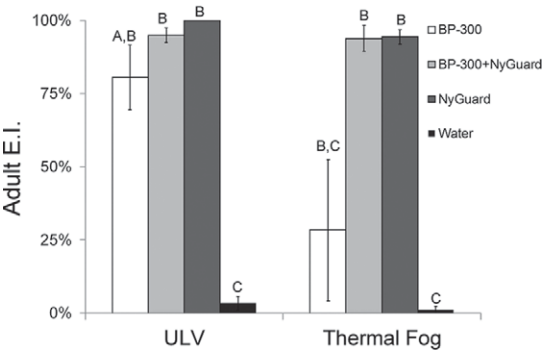

Fig. 3. Average percentage of adult E.I. (±SE) of *Ae. aegypti* placed in 120 ml artificial breeding sites that were located at three locations within a structure during space spays of undiluted BP-300, a mixture of NyGuard and BP-300, NyGuard diluted in mineral oil, and water conducted in February and March 2013 at the Camp Blanding Joint Training Center, Starke, FL. Differences in average mortality for each treatment are represented by different letters, as determined through Tukey post hoc tests.

differences in the regression slopes were observed. Consequently, the rates of change in larval mortality were similar regardless of the sprayer type and pes-

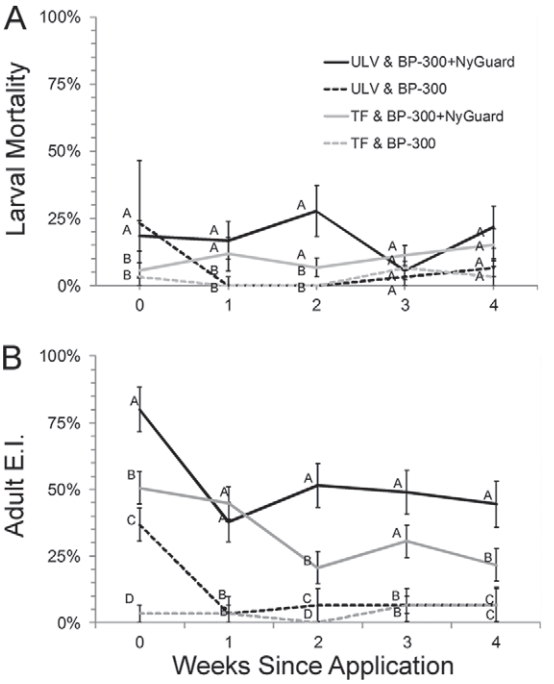

Fig. 4. Average percentage (±SE) *Ae. aegypti* larval mortality (A) and adult E.I. (B) from subsamples taken from pails containing 5 liters of water each week following the initial application (week 0) of undiluted BP-300, or a mixture of BP-300 and NyGuard when delivered by a ULV or thermal fogger (TF) into simulated urban structures at the Camp Blanding Joint Training Center, Starke, FL. Differences in average mortality for each treatment within each time period are represented by different letters, as determined through Tukey post hoc tests.

Table 2. The average size (μm) and ±SE for the median droplet diameter ($Dv_{0.5}$), $Dv_{0.1}$, and $Dv_{0.9}$ of each pesticide and mixture and the droplet density (number of drops per square millimeter) delivered by each sprayer type to three locations within each structure

Pesticide	Thermal fog			ULV		
	Location 1	Location 2	Location 3	Location 1	Location 2	Location 3
BP-300						
$Dv_{0.1}$	4.8 ± 0.1	4.1 ± 0.1	3.6 ± 0.1	8.5 ± 0.5	6.9 ± 0.4	3.7 ± 0.1
$Dv_{0.5}$	8.0 ± 0.4	6.3 ± 0.2	5.5 ± 0.4	16.4 ± 1.1	10.5 ± 0.9	5.9 ± 0.2
$Dv_{0.9}$	13.6 ± 1.2	10.7 ± 0.3	15.2 ± 2.5	29.1 ± 2.1	16.3 ± 1.8	10.7 ± 0.9
Droplet density	144.8 ± 43.1	32.3 ± 8.3	6.6 ± 1.4	393.3 ± 29.1	299.3 ± 45.7	70.6 ± 6.5
NyGuard and mineral oil						
$Dv_{0.1}$	5.1 ± 0.1	4.6 ± 0.1	3.7 ± 0.1	8.3 ± 0.2	7.5 ± 0.2	4.0 ± 0.1
$Dv_{0.5}$	8.5 ± 0.1	7.5 ± 0.1	5.4 ± 0.1	14.4 ± 0.3	11.5 ± 0.4	6.8 ± 0.3
$Dv_{0.9}$	14.1 ± 0.3	12.4 ± 0.3	9.2 ± 0.3	25.2 ± 0.6	17.1 ± 1.4	11.4 ± 3.6
Droplet density	157.3 ± 35.5	88.2 ± 12.8	7.8 ± 0.9	399.3 ± 48.0	222.9 ± 48.7	39.11 ± 7.7
BP-300 and NyGuard						
$Dv_{0.1}$	5.8 ± 0.2	5.4 ± 0.2	3.4 ± 0.1	10.5 ± 0.3	7.2 ± 0.4	3.8 ± 0.1
$Dv_{0.5}$	10.2 ± 0.2	8.9 ± 0.4	5.1 ± 0.1	21.3 ± 0.9	10.9 ± 0.6	6.0 ± 0.1
$Dv_{0.9}$	17.7 ± 0.3	14.5 ± 0.3	7.9 ± 0.3	39.6 ± 2.1	15.8 ± 1.1	9.5 ± 0.1
Droplet density	229.4 ± 34.4	120.6 ± 8.4	20.4 ± 4 0.2	308.3 ± 22.5	252.8 ± 35.4	78.9 ± 8.7

Sprays were conducted in February and March 2013 at the Camp Blanding Joint Training Center, Starke, FL.

ticide application (pooled regression equation: larval mortality = 0.098 + 0.002 × time since application).

The adult E.I. in treated water from the large pails was greatest 24 h after the applications were made (week 0; $\chi^2 = 4.28$; df = 4; $P = 0.002$), with the applications of NyGuard mixed with BP-300 leading to greater adult E.I. than the undiluted BP-300 each week ($\chi^2 = 2.62$; df = 4; $P = 0.04$; Fig. 4b). The effect of the sprayer on adult E.I. was dependent on time ($\chi^2 = 6.32$; df = 4; $P < 0.0001$), so that the ULV was found to provide greater percent adult E.I. than the thermal fogger each week, except for weeks 1 and 3, when they achieved similar percent adult E.I. (Fig. 4b). According to the comparison of regression analysis of the SLRA, no differences in the regression slopes were observed. However, there was a significant difference in the relative elevation ($F_{3,15} = 20.1$; $P < 0.0001$), resulting in a noncoincidental regression ($F_{1,12} = 11.07$; $P < 0.0001$). Therefore, at week 0, the initial adult E.I. was greatest for ULV applications of NyGuard mixed with BP-300 and least for thermal fog and ULV applications of undiluted BP-300, as also observed from the K-W tests (Fig. 4b), while the rates of decline in adult emergence were similar over time (pooled regression equation: adult E.I. = 0.344–0.044 × time since application).

Droplet Analysis. The ULV delivered larger droplets throughout the structure than the thermal fogger, across the $Dv_{0.1}$ ($F_{1,107} = 21.07$; $P < 0.0001$), the $Dv_{0.5}$ ($F_{1,107} = 22.15$; $P < 0.0001$), and the $Dv_{0.9}$ ($F_{1,107} = 12.83$; $P = 0.0005$) droplet sizes (Table 2). The pesticide mixture did not affect the droplet size each sprayer delivered. The slide location affected the $Dv_{0.1}$ ($F_{2,107} = 36.14$; $P < 0.0001$), the $Dv_{0.5}$ ($F_{2,107} = 35.06$; $P < 0.0001$), and the $Dv_{0.9}$ ($F_{2,107} = 12.83$; $P = 0.0005$), so the largest droplets deposited at location one and the smallest at location 3.

The ULV generated a greater droplet density than the thermal fogger ($F_{1,107} = 27.37$; $P < 0.0001$), and the greatest droplet density was observed on slides placed closest to the sprayer ($F_{2,107} = 28.88$; $P < 0.0001$; Table 2). The pesticide mixture did not affect the droplet density

The ULV generated greater droplet deposition than the thermal fogger ($\chi^2 = 3.88$; df = 1; $P = 0.05$), but the droplet deposition was not influenced by the location or height within the structure (Table 3). Based on the droplet deposition, the concentration of a.i. delivered to the water containers (ppb) was estimated to be greater in the 90 ml of water in the specimen jars than in the 5 liters of water in the pails. In addition, the ULV was estimated to deliver a higher concentration of a.i. to all containers than the thermal fogger (Table 4).

Discussion

The pyrethrin insecticide, BP-300, was equally effective in killing caged female *Ae. aegypti* when delivered from the ULV and the thermal fogger, with

Table 3. Average deposition (nl/cm^2) and the ±SE of BVA mineral oil by both sprayers to each location at two heights: either near the ceiling (high) or the floor (low), during indoor space sprays conducted in February and March 2013 at the Camp Blanding Joint Training Center, Starke, FL

	Thermal fog			ULV		
	High	Low	Avg.	High	Low	Avg.
Location 1	0.81 ± 0.19	0.21 ± 0.05	0.51 ± 0.16	0.37 ± 0.24	0.84 ± 0.32	0.61 ± 0.21
Location 2	0.05 ± 0.02	0.16 ± 0.02	0.10 ± 0.03	1.80 ± 1.57	0.49 ± 0.11	1.14 ± 0.76
Location 3	–	–	0.31 ± 0.12	–	–	1.70 ± 0.90
Avg.	0.43 ± 0.20	0.18 ± 0.03	0.30 ± 0.08	1.10 ± 0.80	0.67 ± 0.17	1.04 ± 0.35

Table 4. Estimated concentration (ppb) of two a.i.s, pyriproxyfen from NyGuard and pyrethrin from BP-300, expected to be delivered to all of the water containers in the structures during indoor space sprays with ULV and thermal fog delivery systems conducted in February and March 2013 at the Camp Blanding Joint Training Center, Starke, FL

Water container	Pyriproxyfen		Pyrethrin	
	Thermal fog	ULV	Thermal fog	ULV
Pail (5 liter water)	0.3	0.8	1.2	3.5
Jar (90 ml water)				
Location 1	0.8	1.0	3.8	4.5
Location 2	0.2	1.9	0.8	8.4
Location 3	0.5	2.8	2.3	12.5
Avg.	0.5	1.9	2.3	8.5

Amounts are estimated from average deposition (nl/cm^2) of dye sprayed from each delivery system and the proportion of a.i. in each pesticide's formulation.

successful adult knockdown beginning as early as 15 min following the spray, and ≈100% adult mortality achieved within 24 h. In addition, both the thermal fog and the ULV space sprays were capable of delivering lethal doses of pyrethrin throughout the structure and to females that were placed in seclusion under the packing box. These rates of adult mortality can be attributed to the delivery of lethal doses of a.i., as there was nearly no mortality in the controls owing to the environmental conditions at the field site, or owing to transportation back to the laboratory, where the final 24-h mortality rates were recorded. Furthermore, the observed adult mortality is not because of any potential emissions generated from the sprayers or from the mosquitoes being exposed to an aerosol, as nearly no adult mortality was observed when water alone was sprayed. Based on these results, we speculate that both thermal fog and ULV sprays can efficiently knockdown populations of adult *Ae. aegypti* in an indoor environment of up to 72.5 m^3 when applying a contact adulticide through a single opening over a short application time. This finding also demonstrates that individual rooms of larger structures can be successfully treated from a single point when sprayed for ≈15 s.

The ability to deliver lethal doses of pyrethrin to artificial breeding sites was influenced by the location of the water source relative to the location of the sprayer, with larval mortality being highest in the corner closest to sprayer and lower in the farthest corner and under the artificial obstruction, regardless of the delivery system. This spatial effect on larval mortality appears to be correlated with the deposition and size of the droplets delivered to each location, as the greatest droplet deposition and largest droplets are delivered to the location closest to the sprayer. The differences in droplet size, droplet density (number of droplets per square millimeter), and droplet deposition (nl/cm^2) of the thermal fogger and ULV may also explain the differences in the two delivery systems' larvicidal potential. The ULV generates larger droplets, which are expected to contain more a.i. (Mount 1970), and produces a greater droplet density, resulting in a higher probability of more a.i. and larger droplets being delivered into each of the water containers. In addition, the larger droplets delivered by the ULV exhibit a higher settling velocity and hence shorter atmospheric residence time owing to gravity (Curtis and Beidler 1996), and are therefore more likely to settle into the larval breeding sites on the ground. This is further supported by the ULV generating greater droplet deposition near the floor than the thermal fogger, resulting in a greater concentration of a.i. being delivered to the containers in structures sprayed by the ULV compared with the thermal fogger. No larval mortality was observed in the artificial breeding sites when only pyriproxyfen was applied, regardless of the delivery system and location within the structure. This result was expected because pyriproxyfen is a juvenile hormone analog that disrupts the normal development of mosquitoes during the pupal stage and kills them before they emerge as adults.

The duration of the pyrethrin's larvicidal affects appears to be improved by mixing with the pyriproxyfen, NyGuard, so that the mixture results in greater larval mortality for a longer period after the application than when the pyrethrin was delivered alone. The mechanism increasing the duration of pyrethrin's larvicidal potential when delivered in a mixture with pyriproxyfen is unclear. It cannot be explained through an additive effect because pyriproxyfen delivered as a mixture with mineral oil resulted in no larval mortality, and the droplet sizes and deposition rates were not affected by the pesticide mixture types. Consequently, there is no increase in the delivery of the a.i.. Similar synergistic interactions between pyriproxyfen and other insecticides have been observed previously (e.g., Darriet and Corbel 2006, Basit et al. 2013). As pyriproxifen is a juvenile hormone mimic, it may retard the growth of larvae or weaken the larvae in some way, making them more susceptible to pyrethrin; however, the physiological mechanism of this synergistic interaction has not yet been determined (Basit et al. 2013).

The ULV and thermal fogger were equally successful at delivering lethal doses of pyriproxyfen to all of the 120 ml artificial breeding sites throughout the structure. Adult E.I. was not improved or diminished when pyrirpoxyfen was sprayed in a mixture with pyrethrin, so the synergistic interaction that improved the larval morality of pyrethrin did not influence E.I. The ULV applications that included pyriproxyfen provided greater adult E.I. in subsamples from the 18.9-liter pails than the thermal fogger most weeks following the application. However, the greatest adult E.I. occurred when the larvae were introduced to the treated water 24 h after the application, but then quickly decreased over the subsequent weeks. Both sprayers were estimated to deliver doses of pyriproxyfen above the expected LC_{50} of 0.012 ppb for *Ae. aegypti* to all of the water containers (Sihuincha et al. 2005). However, the concentration of pyroproxyfen delivered by both sprayers appears not to have reached the concentrations reported to inhibit emer-

gence for several months (Sihuincha et al. 2005, Moh Seng et al. 2006). The improved adult E.I. achieved through delivering pyriproxyfen as an ULV spray in these larger water containers may be attributed to the larger droplet size and increased droplet deposition generated by the ULV compared with the thermal fogger (Mount 1970). Because most *Ae. aegypti* larvae are assumed to be found in larger containers (Kittayapong and Strickman 1993), the ability of the ULV to provide better adult E.I. in the 18.9-liter plastic pails than the thermal fogger suggests the ULV technology is better suited for indoor control of *Ae. aegypti* populations.

Effective dengue vector suppression requires two criteria be met through adulticide space spraying: 1) initial mortality of the adult vector population and 2) a significant level of persistent population control to allow other suppression interventions (e.g., habitat modification and larviciding) to be implemented (Perich et al. 2003). By including the IGR in an adulticide space spray, the overall effectiveness of both pesticide delivery systems for *Ae. aegypti* population suppression is improved. However, based on these results the ULV technology is expected to be a better tool for indoor vector suppression because of its improved larvicidal effects throughout the structures and its improved adult E.I. during the weeks following pesticide application. Even so, both insecticide delivery systems can achieve initial mosquito population suppression with minimal time and labor. During a dengue, chikungunya, or yellow fever epidemic, these methods can provide an immediate population knockdown, and may reduce population numbers for several weeks following the pesticide application, thus allowing time for other population suppression strategies to begin. In conclusion, the positive results presented here warrant further investigation regarding each delivery system's and pesticide's potential for indoor *Ae. aegypti* control. Specifically, field trials against wild populations in dengue endemic regions are required to further support that these methods will lead to successful *Ae. aegypti* population suppression.

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