

## POTENTIAL OF OUTDOOR ULTRA-LOW-VOLUME AEROSOL AND THERMAL FOG TO SUPPRESS THE DENGUE VECTOR, *Aedes aegypti*, INSIDE DWELLINGS

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**ABSTRACT.** A field study investigated penetration of outdoor ground ultra-low-volume (ULV) aerosol and thermal fog adulticide applications into a dwelling to control the dengue vector *Aedes aegypti* (L). Four applications of Kontrol 4-4 (4.6% permethrin active ingredient [AI], 4.6% piperonyl butoxide) at the maximum label rate were made at 25–30 m in front of a house at Camp Blanding Joint Training Center, Starke, FL, during summer 2016. The ULV sprayer and thermal fogger nozzles were oriented horizontally, and vehicle travel speeds were 16 and 24 km/h, respectively. All doors and windows of the house were left open. Spray efficacy was assessed using caged female mosquitoes positioned 30 cm above ground, outside and inside of the house. Interior cages were placed in open areas and cryptic sites (i.e., in a closet or cardboard box). A spinner holding 2 rods sized 3 mm × 75 mm was deployed next to each cage (except cryptic sites) to sample droplets and to quantify AI deposition. Thirty minutes after application, cages were removed, slides collected, and mosquitoes transferred to clean cages in the laboratory where mortality was assessed at 24 h posttreatment. The ULV application to the south side of the house produced 100% mortality in outdoor and indoor cages and 24% mortality at cryptic sites. Similarly applied thermal fog resulted in 85% mortality outdoors, 34% indoors, and only 4% in cages at cryptic sites. Application of either method from the west resulted in 19–61% mortality outdoors and 0.5–6.5% indoors. Droplet volume median diameter ( $D_{v0.5}$ ) on rods from the ULV application was significantly larger compared with the thermal fogger outdoors, but similar indoors. Outdoors and indoors, the AI deposition from ULV was significantly higher than from thermal fog. Our results show the potential for controlling dengue vectors inside houses with outdoor ground ULV applications in areas where doors and windows are left open for ventilation.

**KEY WORDS** Insecticide application, Kontrol 4-4, penetration, permethrin, ultra-low volume

### INTRODUCTION

Dengue is a globally important disease and is reportedly causing more deaths than any other arboviral infection (Jacobs 2000). Hales et al. (2002) predicted that approximately 50–60% of the world's population would be living in areas at risk of dengue transmission by 2085. Unfortunately, this level was surpassed in 2007 (Heintze et al. 2007) due to increasing epidemiological trends and geographic expansion over the last 40 years (WHO 2007), as well as increased urbanization.

Suppression of dengue, in the absence of a vaccine, continues to rely on vector control methods (Jacobs 2000, Heintze et al. 2007, Harburguer et al. 2012, Murray et al. 2013). These methods may include biological and chemical vector control and environmental management strategies, as well as personal protection. Indeed, effective vector control methods are recognized as essential and continue to be promoted globally by the World Health Organization (WHO) through the strategic approaches of integrated vector management. During disease emer-

gencies, ultra-low-volume (ULV) space sprays are the primary method to quickly suppress adult vector populations (Perich et al. 2000, Farooq et al. 2017). In addition, the WHO has included integration of chemical and nonchemical control methods as one component of their global strategy for dengue prevention and control (WHO 2012). This policy remains their focus in managing container-inhabiting *Aedes* vectors in areas where high risk of human-vector contact occurs (Murray et al. 2013).

Dengue vectors such as *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) are found inside and around human habitats (Perich et al. 2000, Chadee 2013, Manzanilla et al. 2017). Suppression of local vector populations can be especially challenging when dwellings are open to the outdoor environment thereby increasing the risk of mosquito-human contact. Such situations are common in many tropical countries as well as on US military forward operating bases.

Traditionally, indoor mosquitoes, primarily *Anopheles* species, have been controlled with indoor residual sprays (IRS). However, this method is not generally recommended for dengue vector control, since specimens of adult *Ae. aegypti* prefer cryptic harborage areas, such as clothing in a closet, instead of resting on walls (Esu et al. 2010).

Indoor and outdoor ULV sprays have been previously evaluated for control of adult *Ae. aegypti* inside dwellings. Harwood et al. (2014) reported that

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indoor applications of the adulticide ULD BP-300 (synergized pyrethrins) with hand-held thermal fogger and backpack ULV sprayer provided 93–100% and 88–100% mortality, respectively, of caged *Ae. aegypti*. Another study on indoor application of ULD BP-300 and 9M Plus2 (deltamethrin) indicated that mortality of caged mosquitoes in the middle of a room ranged from 59% to 100% and 94% to 100% from thermal fog and ULV, respectively (Ponlawat et al. 2017). Moreover, the mortality in cryptic areas (inside closed paper bags) ranged from 3% to 46% and 6% to 72% from thermal fog and ULV, respectively (Ponlawat et al. 2017). Such applications, even with their promising efficacy, are limited in their utility to isolated homes and smaller communities due to time and personnel requirements. An effective technique to achieve indoor protection over larger areas does not exist.

Perich et al. (1992) reported that malathion aerosol droplets from ULV ground and aerial applications penetrated into dwellings and further interior areas (i.e., bedroom closets, under beds). Unfortunately, this study did not measure mosquito mortality in relation to droplet penetration. However, the authors found that droplet volume median diameter averaging 4  $\mu\text{m}$  in the interior areas of the house that resulted in approximately 2 min of “potential insecticide exposure” may be sufficient for vector contact. Sudsom et al. (2015) suggested indoor ULV as an effective method of space spraying, which has greater potential to control indoor adult dengue vector populations than outdoor ULV spraying. They found that indoor mosquito density was not significantly different between outdoor and indoor ULV sprays 2 days before and 6 days after space spraying, but the density after the indoor sprays was significantly less than the density after the outdoor sprays at 1 and 2 days postspraying. Chadee (1985) reported that ground ULV application of malathion resulted in 55% control of caged *Ae. aegypti* outdoors but only about 23% in similarly caged mosquitoes indoors. Harburguer et al. (2012) compared indoor control of caged *Ae. aegypti* with that of outdoors when applied with either an outdoor thermal or cold fogger of a mixture of permethrin and pyriproxyfen. The authors found that outdoor control using the cold fogger ranged from about 65% to 86%, while indoor control ranged from about 23% to 58%. These studies showed not only the lack of spray penetration, but also a lack of outdoor control, which may have been due to inappropriate applications. The varying results of these applications indicate the need for improvements in application methodology, as well as the need for studying its potential for indoor control. In previous studies we have optimized outdoor ULV aerosol application efficacy by changing nozzle orientation (Farooq et al. 2017) and travel speed (Farooq et al. 2018). This information encouraged us to evaluate the impact of outdoor ULV adulticide applications on indoor resting mosquitoes. Therefore, the objective of our study reported herein was to

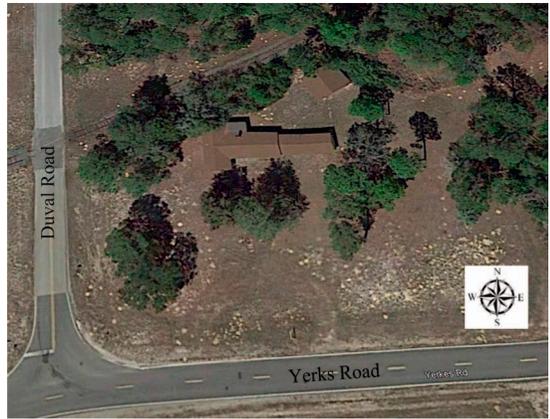


Fig. 1. Aerial view of the study site.

investigate the effectiveness of outdoor adulticide application using optimized settings of conventional truck-mounted ULV aerosol and thermal fog equipment for the control of indoor *Ae. aegypti*.

## MATERIALS AND METHODS

The study was conducted at Camp Blanding Joint Training Center, Starke, FL (29°55'26.4"N, 81°59'49.7"W) using a single-story house at the intersection of Duval Road and Yerks Road (Fig. 1). The house is 43 m north of Yerks Road and 30 m east of Duval Road and consists of 2 bedrooms, a living room, kitchen, bathroom, and a storage area. This house is used for training drills of law enforcement personnel and simulates a poorly managed house. The dwelling has 8 windows with an entrance door on the south side, 4 windows on the west side, 5 windows on the north side, and another entrance door and window on the east side.

Figure 2 shows that 9 screened 9-cm-diameter 6-cm-deep paper cages containing 25 adult female *Ae. aegypti* were placed inside the house, of which 5 cages (BN2, BS, LW, LE, and KI) were in the open area of 4 different rooms, 1 in a closet (CU), 2 in the cardboard boxes (DR and BN), and 1 in a storage closet (ST). Figure 2 also shows 10 cages were placed outdoors, 3–5 m from the house, of which 3 (FW, FM, and FE) were on the south, 3 (RW, RM, and RE) on the north, 2 (ES and EN) on the east, and 2 (WN and WS) on the west of the house. A Florida Latham Bond (FLB) spinner (John W. Hock, Co., Gainesville, FL) with 2 rods size 3 mm  $\times$  75 mm was deployed 1 m away from each outdoor cage to collect spray for droplet size and deposition analysis. Likewise, a spinner was placed 1 m away from each indoor cage only in the open area (Fig. 2). Each spinner had a Teflon<sup>®</sup> coated rod for droplet size determination and a plain rod for deposition analysis. All cages and spinners were 30 cm above ground because the vast majority of indoor *Ae. aegypti*

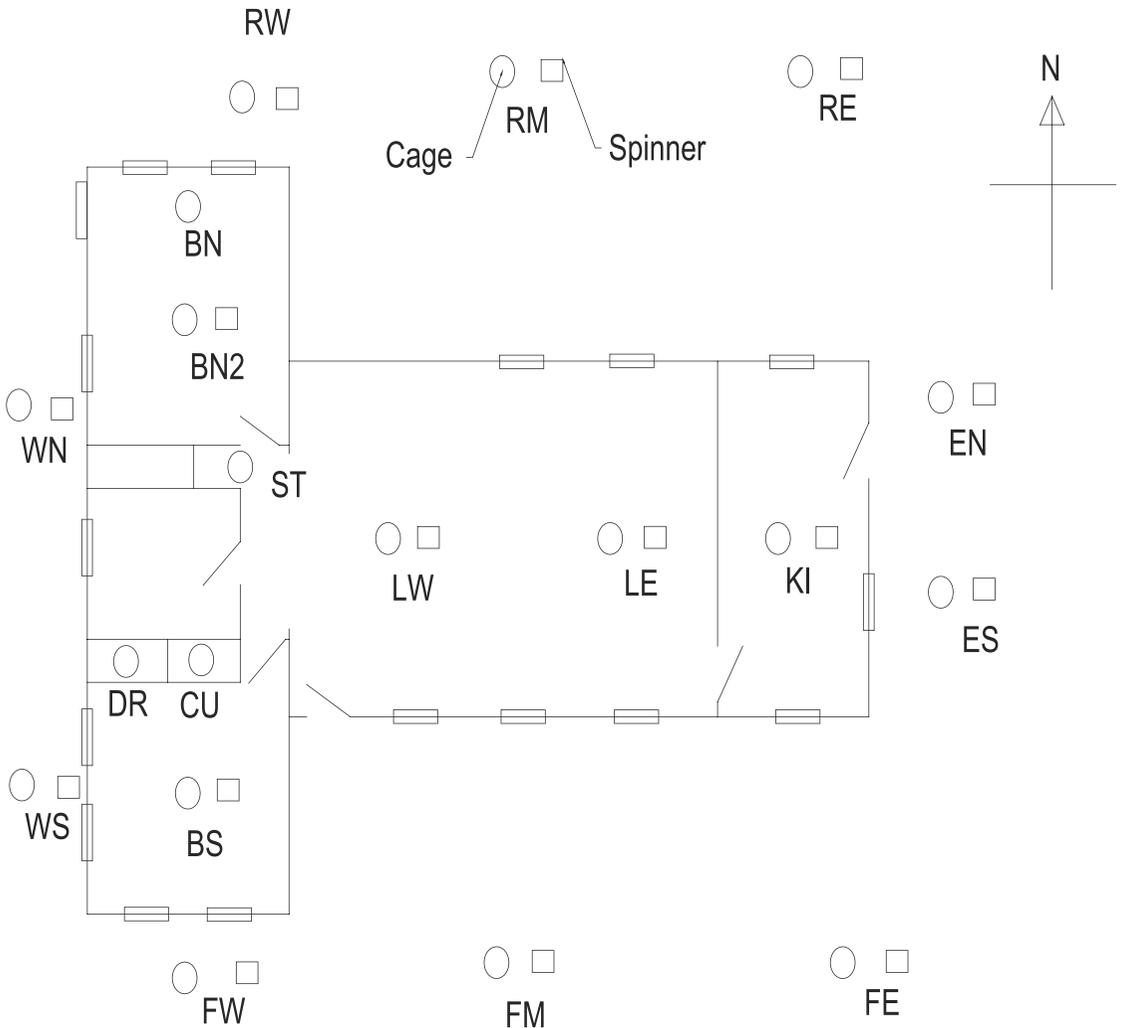


Fig. 2. Building and location of mosquito bioassay cages and droplet sample spinners.

typically rest within 1.5 m from the floor (Manzanilla et al. 2017).

Kontrol 4-4 adulticide (4.6% permethrin active ingredient [AI], 4.6% piperonyl butoxide; Univar Environmental Sciences, Austin, TX) was applied at the maximum label rate (195 ml/ha) as an outdoor ULV space spray and thermal fog. For ULV sprays, oil-soluble Yellow 131SC<sup>®</sup> fluorescent dye (Rohm and Haas Co., Philadelphia, PA) was mixed with the pesticide at 25,000 ppm. These applications were conducted using a truck-mounted Cougar<sup>®</sup> ULV sprayer (Clarke Mosquito Control, Roselle, IL) traveling at 16 km/h, nozzle discharging horizontally and out the back of the truck at 473 ml/min. We elected to adjust the nozzle orientation based on favorable ULV application results where complete mortality of caged *Ae. aegypti* occurred up to 90 m from the spray nozzle (Farooq et al. 2017). For thermal fog sprays, 1 part of pesticide was diluted

with 1.8 parts of BVA-13 mineral oil (BVA Inc., New Hudson, MI), and fluorescent dye was mixed at 12,000 ppm. These applications were made using a truck-mounted Swingfog<sup>®</sup> SN101 thermal fogger (Swingtec GmbH, Isny, Germany) traveling at 24 km/h, nozzle discharging insecticide horizontally and out the back of the truck at a flow rate of 2.0 liter/min. All windows and doors of the house were opened before spraying and remained open until all cages and slides were collected.

During all applications, the sprayer/fogger traveled either 25 m south of the house parallel to Yerks Road or 30 m west of the house on Duval Road, depending on wind direction (Table 1). These distances were selected to avoid trees on the south side and a ditch on the west side. Adulticide application with both pieces of equipment was conducted on each day on April 20, May 4, May 11, and May 18, 2016. On each day, ULV and thermal fog applications were rotated.

Table 1. Calendar dates, times, vehicle travel direction, and ambient weather conditions during ultra-low-volume (ULV) and thermal fog (TF) insecticide applications at Camp Blanding Joint Training Facility, Starke, FL in 2016.

Date	Spray type	Spray time	Wind speed (km/h)	Wind direction	Spray travel path	Temperature (°C)		Relative humidity (%)
						Air	Ground	
Apr 20	ULV	10:58 a.m.	6.0	ESE	South	23.4	39.3	56.0
	TF	9:30 a.m.	2.0	S	South	20.2	24.3	79.6
May 4	ULV	8:12 a.m.	4.7	W	West	22.1	22.6	93.5
	TF	10:00 a.m.	2.0	NW	West	24.4	32.1	86.6
May 11	ULV	10:00 a.m.	4.0	S	South	27.3	44.1	65.2
	TF	8:33 a.m.	4.0	S	South	24.8	33.1	75.0
May 18	ULV	6:53 a.m.	4.3	W	West	20.5	20.7	100.0
	TF	8:13 a.m.	7.6	SW	West	23.4	31.1	89.0

Wind speed, direction, temperature, and relative humidity were recorded 3 m above ground. Based on ambient wind conditions, sprayer travel direction was changed. Sprayer travel path and weather conditions for all applications on different days are provided in Table 1. After each application, 3 exhaust floor fans were deployed inside the house for 30 min to clear the air out of the building before preparation for the next application.

Thirty minutes after application, mosquito cages were removed and transported to the onsite laboratory. Mosquitoes were lightly knocked down with CO<sub>2</sub>, transferred to clean cages, supplied with 10% sugar solution, and moved to Navy Entomology Center of Excellence (NECE) laboratory, where they were kept at room conditions until 24-h mortality was recorded. Control cages were exposed to the same environment but away from the application area and subjected to the transfer process. At the time of cage retrieval, Teflon coated slides were removed and stored in pre-labeled boxes for droplet size measurements while plain slides were stored in pre-labeled resealable plastic bags and stored in a cool, dark place. All slides were then returned to NECE and stored in a refrigerator until analyzed.

Droplets on rods were measured with DropVision system (Leading Edge Associates Inc., Fletcher, NC) and droplet distribution parameters ( $D_{v0.1}$ ,  $D_{v0.5}$ , and  $D_{v0.9}$ ) determined. The  $D_{v0.1}$ ,  $D_{v0.5}$ , and  $D_{v0.9}$  are the droplet diameters ( $\mu\text{m}$ ) where 10%, 50%, and 90% of the spray volume is contained in droplets smaller than these diameters (ASTM Standard E1620 [ASTM 2004]). Insecticide deposition was quantified using procedures described by Farooq et al. (2009). Rods were washed using 20 ml hexane inside the plastic bag, and fluorescence readings of the resultant solution were measured with a spectrofluorophotometer (Model RF5000U; Shimadzu, Kyoto, Japan). The amount of dye on each rod was determined using calibration curves developed from standard concentrations. The amount of dye on each slide was then converted to AI deposition ( $\text{ng}/\text{cm}^2$ ) using a sampling area of 63  $\text{cm}^2$  and the ratio of dye and AI in the spray tank.

### Statistical analyses

Mean treatment mortality data were corrected prior to analyses with that of controls using the formula of Abbott (1925). Intel® Visual FORTRAN Composer XE 2013 software was used to statistically analyze the data with  $\alpha = 0.05$ . Initial Kolmogorov–Smirnov tests (Smirnov 1939) performed on all data sets revealed that the data were nonnormally distributed, while the Bartlett test (Bartlett 1937) showed nonhomogeneity of variances. For analysis, mean mortality data were grouped into 3 zones (outdoor, indoor [cages in the open], and indoor cryptic sites). Mean droplet size and deposition data were grouped into 2 zones (outdoor and indoor), because there were no spinners at cryptic sites. A 3-way Kruskal–Wallis (KW) analysis (Kruskal and Wallis 1952) was conducted to assess differences in 24-h mortality,  $D_{v0.5}$ , and AI deposition for the 2 sprayers, 2 or 3 zones, and 4 application dates. Since the application date significantly affected these data, a 2-way nonparametric KW analysis was conducted to study the effect of sprayer and zone on data for each application date. Subsequent post hoc Scheffe multiple-contrast tests (Scheffe 1959) were conducted to identify effects of those sprayers and zones, which were significantly different from each other.

### RESULTS

The data showed a significant difference in outdoor 24-h mortality ( $X^2 = 9.00$ ,  $df_1 = 1$ ,  $df_2 = 78$ ,  $P = 0.0035$ ), and a nonsignificant difference in indoor 24-h mortality ( $X^2 = 0.9849$ ,  $df_1 = 1$ ,  $df_2 = 36$ ,  $P = 0.3474$ ), as well as in mortality at cryptic indoor sites ( $X^2 = 2.5976$ ,  $df_1 = 1$ ,  $df_2 = 30$ ,  $P = 0.1118$ ) between the 2 sprayers (Table 2). Generally, mean mosquito mortality was higher in all indoor and outdoor cages from the ULV application when compared with the thermal fogger application, but this difference was only significant outdoors. The  $D_{v0.5}$  was significantly affected by sprayers outdoors ( $X^2 = 11.7654$ ,  $df_1 = 1$ ,  $df_2 = 69$ ,  $P < 0.0001$ ) but not indoors ( $X^2 = 0.6915$ ,  $df_1 = 1$ ,  $df_2 = 31$ ,  $P = 0.4319$ ). Droplet  $D_{v0.5}$  from the ULV sprayer was always

Table 2. Comparison of overall mean ( $\pm$ SE) adult *Aedes aegypti* mortality,  $D_{v0.5}$ , and active ingredient (AI) deposition between 2 sprayers at different zones.

Sprayer Type	Zone <sup>1</sup>		
	Outdoor	Indoor	Cryptic sites
Volume median diameter ( $D_{v0.5}$ ) ( $\mu$ m)			
Ultra-low volume (ULV)	14.7 $\pm$ 0.5 a	12.4 $\pm$ 0.5 a	Not measured
Thermal fogger	12.1 $\pm$ 0.7 b	11.3 $\pm$ 0.9 a	Not measured
AI deposition (ng/cm <sup>2</sup> )			
ULV	2.12 $\pm$ 0.44 a	1.87 $\pm$ 0.37 a	Not measured
Thermal fogger	0.74 $\pm$ 0.26 b	1.43 $\pm$ 0.64 b	Not measured
24-h mortality (%)			
ULV	80.6 $\pm$ 5.1 a	48.4 $\pm$ 11.1 a	14.8 $\pm$ 4.5 a
Thermal fogger	52.1 $\pm$ 7.0 b	17.1 $\pm$ 6.4 a	2.3 $\pm$ 1.0 a

<sup>1</sup> Means with different letters in a column for each parameter measured within a zone were significantly different (Scheffe 1959).  $\alpha = 0.05$ .

larger than from the thermal fogger, but again the difference was only significant outdoors. In contrast, the AI deposition was significantly affected by sprayers outdoors ( $X^2 = 5.4758$ ,  $df_1 = 1$ ,  $df_2 = 38$ ,  $P = 0.0208$ ) and indoors ( $X^2 = 15.6094$ ,  $df_1 = 1$ ,  $df_2 = 78$ ,  $P < 0.0001$ ). The AI deposition from the ULV sprayer was significantly higher than from the thermal fogger both indoors and outdoors.

Table 1 shows considerable differences in ambient environmental conditions including prevailing wind direction occurred on different days during the study. Thus, mosquito mortality (the ultimate indicator of effectiveness of an application) was examined on individual spray days. Figure 3 shows mosquito mortality from 2 sprayers in 3 sampling zones on 4 spray days, which indicated that applications performed on May 4 and May 18 resulted in poor mortality and AI deposition in all zones for both sprayers, especially the thermal fogger, compared with the other 2 dates. Figure 3 and Table 2 show that the thermal fogger in general performed poorly.

On April 20, the ULV sprayer resulted in significantly higher indoor and outdoor mortality of caged *Ae. aegypti* than the thermal fogger (Fig. 3). The ULV application also resulted in higher mortality at cryptic sites, but the difference was not significant. Also, on this day, the ULV had similar mortality outdoors and indoors but lower mortality at cryptic sites. On May 4, the ULV had significantly higher mortality outdoors and at cryptic sites than the thermal fogger, but the overall mortality was not satisfactory. On May 11, the ULV and thermal fogger resulted in complete mortality outdoors, but the thermal fog failed to penetrate the dwelling (Fig. 3). In contrast, the ULV had complete mortality indoors and considerable mean mortality (29%) at cryptic sites. On May 18, both sprays had similarly low mortality.

**DISCUSSION**

The dengue vector *Ae. aegypti* is commonly found in human dwellings. Manzanilla et al. (2017)

reported that the vast majority (98%) of this species rested in bedrooms, living rooms, bathrooms, and kitchen. Because individuals predominately (82%) rest within 1.5 m of the floor, and not necessarily on walls, IRS is not a suitable option for their control (Esu et al. 2010, Manzanilla et al. 2017). However, indoor ULV sprays have shown promise (Mani et al. 2005, Harwood et al. 2014, Ponlawat et al. 2017) but are neither logistically suitable nor scalable for larger areas. With the adjustment of nozzle orientation and travel speed of truck-mounted ULV sprayers, complete mortality of caged female *Ae. aegypti* was achieved in an open area beyond 90 m from the spray (Farooq et al. 2017, 2018). Encouraged by these results, and by the Perich et al. (1992) study where ULV ground application of malathion penetrated into dwellings and further interior areas, this study was planned to examine the potential of ground ULV sprays to penetrate dwellings to control these vectors.

Our results indicate that the ULV application of Kontrol 4-4 produced higher adult *Ae. aegypti* mortality compared with thermal fog. Furthermore, the spray performed considerably better when applied from the south versus the west side of the house. These results show that the right conditions (wind speed and direction) provided complete mortality of caged *Ae. aegypti* indoors and outdoors with the ULV applications (April 20, May 11). Also, on these dates, ULV spray produced considerable mean mortality (29%) at cryptic locations further inside the house. It should be noted that these sprays were conducted in the morning, mostly in the absence of temperature inversion.

Based on these results, the authors can envision that by using the right combination of sprayer and weather conditions, a single ULV spray can control *Ae. aegypti*, not only outdoors, but also inside buildings. The results also indicate that a properly managed, single application can make it a 2-in-1 application to control day time biting mosquitoes resting indoors and outdoors. However, more studies are needed to refine the technique.

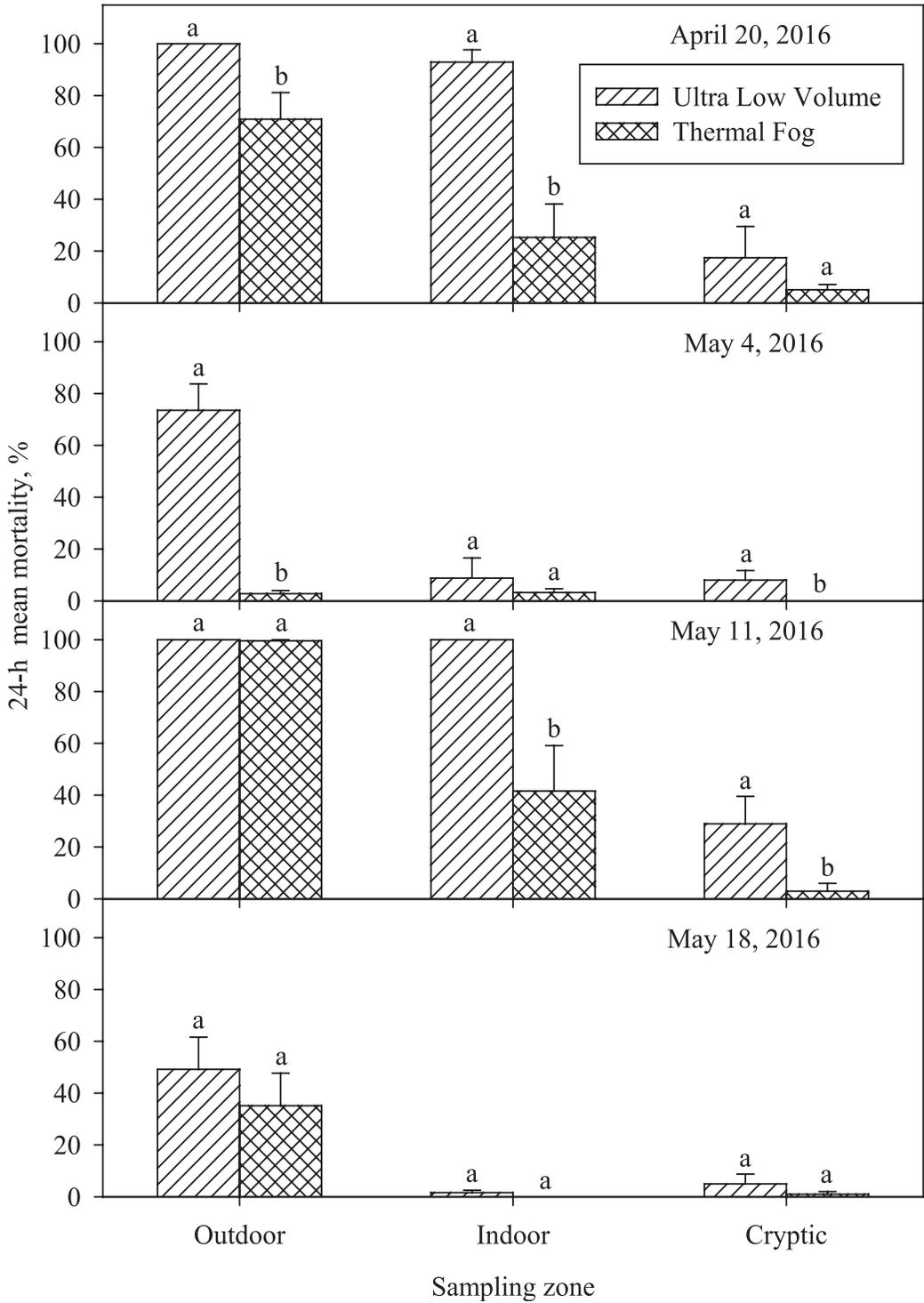


Fig. 3. Mean ( $\pm$ SE) comparison of adult *Aedes aegypti* mortality from ultra-low-volume and thermal foggers in cages placed at different zones on different dates. Means with different letters at each date, within a zone, are significantly different (Scheffé 1959).  $\alpha = 0.05$ .

Elaboration on some of the factors that played a role during this study may be helpful to understand these results and the potential of this type of application. The available sprayer was able to produce a ULV spray, but the droplet spectrum was inclined toward the upper acceptable limit (Hoffmann et al. 2012). The pesticide was selected from the Department of Defense National Stock List, which has a 5-fold higher application rate than other commercially available adulticides, increasing droplet size (Farooq et al. 2016) and requiring larger amounts of spray to penetrate indoors. Also, due to the limit of maximum flow rate of the sprayer, the intended 24 km/h travel speed could not be achieved. Using lower-dose adulticides may improve the efficacy and penetration.

There were few openings for the spray to enter the house from the west side. The penetration was also subdued by thick bushes between the spray release and the house. The location of the house was isolated with a forest border surrounding most of its northern side. Local wind patterns often render it difficult to target such a small area of a single house. Changing wind direction can affect the efficacy of the treatment, providing highly variable results between treatment dates. A spray that misses the target structure is lost. If this type of application occurred in an operational setting and if a spray misses a house, it can potentially move through the neighborhood, penetrate another house, and still impact local mosquito populations. It is expected that this spray technique can perform better in a real, more natural environment, and future work should include trials in villages or operational settings.

Because of a ditch between the house and the road on the west side, distance between the house and truck travel path had to be increased. That might have contributed to poor efficacy from sprays on the west side. This shows the need to investigate the effect of this distance on control of vectors indoors.

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