EFFECT OF THE COMBINED NOZZLE ORIENTATION AND TRUCK SPEED ON EFFICACY OF ULTRA-LOW-VOLUME SPRAY AGAINST CAGED *AEDES ALBOPICTUS* IN URBAN GAINESVILLE, FLORIDA†

YONGXING JIANG[1], MUHAMMAD FAROOQ[2], JAMES CILEK[3], AND ALEC G. RICHARDSON[3]

[1]Department of Public Works, City of Gainesville Mosquito Control, Gainesville, FL 32609

[2]Anastasia Mosquito Control District, St. Augustine, FL 32092

[3]Navy Entomology Center of Excellence, Jacksonville, FL 32212

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ABSTRACT

A field study was conducted to evaluate the combined effect of nozzle orientation and vehicle travel speed on droplet dispersion and mosquito mortality of an adulticide applied from a truck mounted ULV sprayer in the City of Gainesville, Florida. Three multi-block areas with dense, medium, and sparse vegetation were selected for the study. Aqua-Reslin® was applied in each area in the following treatment combinations: a) horizontal nozzle at 24 km/h travel speed, b) 45° upward orientation (standard) at 16 km/h, and c) 22.5° upward orientation at 24 km/h. Caged, three to five day old *Aedes albopictus* females were used in all evaluations. Spray deposition was determined at various locations inside each application area using Florida Latham Bonds droplet impingers. There was a significant difference in 24-h mortality among the 3 nozzle angle and speed treatment combinations, but not in the interaction between those combinations and application distance. The 22.5° nozzle combination resulted in the greatest mosquito mortality (88.3%) while the 45° combination resulted in the least mortality (63.1%). A significant difference in 24-h mortality among the 3 vegetation densities and application distances occurred with no significant interaction among these two parameters. The greatest *Ae. albopictus* mortality was recorded in the sparse (91.4%) and the lowest in the medium vegetation area (72.2%) at the maximum rate of 0.0015 lb./acre. Adulticide deposition was not significantly different among vegetation levels, but was significantly different among the distances and interactions of those parameters.

Key Words: Adulticide efficacy, Aqua-Reslin, London Fogger 18-20, permethrin, vegetation density

INTRODUCTION

Ground application of ultra-low-volume (ULV) adulticides has been the standard method to combat pestiferous and disease transmitting mosquitoes worldwide for more than 45 years (Bonds 2012). As a key component of Integrated Mosquito Management (IMM), ground application of ULV has been studied extensively, however, control efficacy has varied greatly. Mount (1998) has discussed a number of factors that could affect the control efficacy of ground applied ULV adulticides including droplet size, meteorology (e.g. wind speed and direction, temperature, relative humidity, atmospheric stability and turbulence), vegetation, and structural obstacles (such as homes, solid walls or board fences). Bonds (2012) further discussed the effectiveness of adulticide application timing and whether or not the aerosol plume actually contacted mosqui-

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toes directly. However, no reviews or studies have discussed or tested the potential effect of nozzle discharge direction on the effectiveness of ULV ground applications in controlling adult mosquitoes. The current common practice of ULV application in Florida, and elsewhere, is to orient the spray nozzle at an upward arc of 45° (Teske et al. 2015). To date, no published data are available to support the fact that this angle is the optimal one in terms of control efficacy.

Jiang and Farooq (2016) compared the efficacy of truck mounted ULV sprayer nozzle discharge direction at 45° upward and 0° horizontal in urban Gainesville, Florida against caged *Aedes aegypti* (L.). Results indicated that a horizontally oriented nozzle outperformed a 45° upward nozzle in three out of four field trials. Recently, Farooq et al. (2017) reported from an open field study that the greatest mortality of caged adult *Ae. aegypti* occurred when the nozzle angle was positioned horizontally, followed by a 30° downward angle, while a 45° angle showed the least efficacy. The above two studies point out that nozzle discharge direction could have an actual impact on the efficacy of a ULV spray.

Mount (1998) believed that when an effective insecticide dose and appropriate atomization are maintained for a designated swath, dispersal speed (vehicle speed) is not a factor affecting efficacy. However, the most recent study reported by Farooq et al. (2018) showed that 32 km/h travel speed provided the best ULV spray dispersal as indicated by complete mortality of caged female *Ae. aegypti* up to 91 m from the spray line compared with 8 and 16 km/h. Farooq et al. (2018) further discussed that movement of a vehicle creates an air vortex behind it that strengthens with increasing travel speed. This vortex helps mix the spray with air, resulting in higher probability of droplets contacting flying insects. Moreover, the speed of the induced air increases with an increase in travel speed (Farooq et al. 2018). The induced air deflects the spray plume towards the ground and suppresses upward spray movement resulting in better efficacy. Therefore, the objective of this study was to evaluate the effectiveness of a combination of the best nozzle orientation and truck speed for ULV adulticide application against caged *Ae. albopictus* (Skuse) in an urban residential area of Gainesville, Florida.

MATERIALS AND METHODS

Study sites

Previous studies indicated that landscape and housing density have an impact on the effectiveness of ULV spray (Mount et al. 1968; Pant et al. 1971). In order to test the impacts, three communities, namely Ridgeview [29° 41′ 02.7″ N, 82° 20′ 54.7" W], Iron Wood [29° 41′ 48.3" N, 82° 18′ 16.8" W], and Lamplighter [29° 40′ 33.7" N, 82° 15′ 36.1" W] located in Gainesville, Florida were selected for the testing sites. Each community was classified as dense, medium and sparse based on visual estimation of vegetation cover, age of the houses and width of the street. The Ridgeview community was built in the 1960's that consisted of mixed dense vegetation of landscaped palms, ornamental plants, Southern live oaks, and pine trees. Houses were characteristically terraced with shared backyards along a two lane-street without sidewalks. Most front yards were covered with heavy ornamental plants such as holly, Indian hawthorn, and evergreen azaleas. The Iron Wood community was built in the late 1980's with a medium dense vegetation of low shrubs, Southern live oak, and palm trees. These terraced houses are mostly single-family detached homes with shared backyards, two lane-streets and sidewalks. Most of front yards are covered by turf grasses and flower beds. Lamplighter is a single or double-wide mobile home community built in the 1970s with very few shrubs and little to no vegetation present in the front or shared backyards.

Mosquitoes

Mosquitoes used in this study were obtained from an insecticide-susceptible USDA strain of *Ae. albopictus,* reared at Gainesville Mosquito Control headquarters at 26.6 °C, 85 ±5% RH, and a photoperiod of 14:10 (L:

D). Larvae were fed with a 3:2 mixture of bovine liver powder (MP Biomedicals, LLC, OH) and brewer's yeast (MP Biomedicals, LLC, OH). Adult mosquitoes were supplied with 10% sugar water, and 3-5 days old non-blood-fed females were used in all evaluations.

Test product

Aqua-Reslin® (20.0% permethrin AI, 20% PBO, Bayer Environmental Science) a synergized permethrin water-based permethrin was used for this study. The formulation was diluted with water at the ratio of 1:2 and the flow rate is 142 ml/min which is the maximum rate of 0.0015 lb./acre.

Field study

Field studies were conducted following the methods of Farooq et al. (2017) with minor modifications. Briefly, Aqua-Reslin®, mixed with a fluorescent dye (1, 3, 6, 8-pyrene tetra sulfonic acid tetra sodium salt, Spectra Colors Corporation, Kearny, NJ) at 8,000 ppm, was used. The spray mixture was applied with a truck-mounted ULV London Fogger 18-20 (London Foggers, Minneapolis, MN) at a flow rate of 142 ml/min. This equipment produced spray droplets with a volume median diameter ($Dv_{0.5}$) of 15.9 microns and $D_{V0.9}$ of 30.4 microns. Treatments included a) standard nozzle orientation 45° upward at a travel speed of 16 km/h (45° combination), b) 22.5° upward at 24 km/h (22.5° combination), and c) 0° (horizontal) at 24 km/h (0° combination) . Effectiveness of each application combination was assessed by determining *Ae. albopictus* mortality 24 h post-application, spray deposition quantification, and droplet size spectra.

Figure 1 illustrates the field layout, ULV spraying direction, and relative position of bioassay cages. Two rows of bioassay cages were placed at least 15 m apart, and up to 90 m perpendicular to the spray line. Each row contained six cages with 25 female *Ae. albopictus* per cage, were positioned at 0, 15, 30, 45, 60, and 90 m from the line of application. Alongside, and 1 m away from each treatment cage, a Florida Latham Bonds spinner (model 319; John W. Hock Company, Gainesville, FL) using two 3 mm × 75 mm acrylic rods was deployed to collect sprays for assessing droplet size characterization and deposition. One of the 2 rods was used for

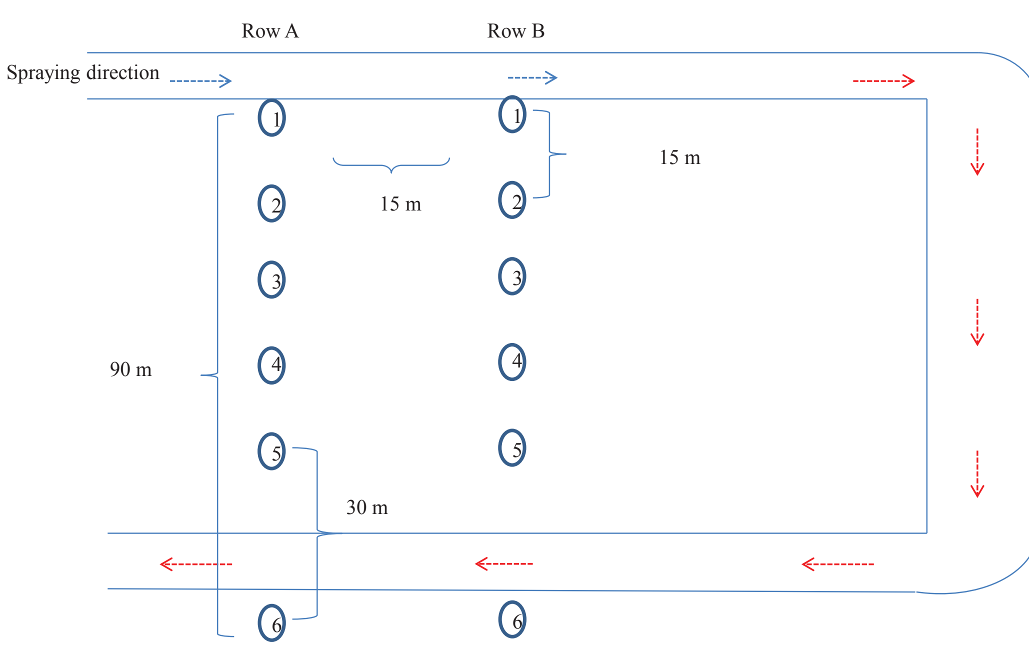

Figure 1. Field layout illustrating relative position of mosquito sentinel cages and spraying directions.

droplet size and the other for quantification of adulticide deposition. Cages and spinners were suspended 1.5 m above ground. On the first day, both rows of samplers in three vegetation levels were randomly assigned to be sprayed with one of the three nozzle orientation and travel speed combinations to make 1 replication of all treatments. The 3 replications were made on 3 different days at least 2 weeks apart and treatments sequentially rotated. The same spray time sequence for sites on each day was maintained. Temperature, RH, wind speed, and wind direction were recorded 1.5 m above the ground.

Control cages were deployed well out and upwind of the spray zone and placed in the same environment for 15 min, then collected before application. Spray cages and rods were placed in the field immediately before spraying. Cages were removed from all stations 15 min post-spray, supplied with 10% sugar solution, transferred to the laboratory, and maintained under normal room conditions until the 24 h mortality count was taken. Along with cages, spinner rods were also removed from the field. One rod from each location was preserved for measurement of droplets and the other stored in a pre-labeled, re-sealable plastic bag. All rods were then stored in a cool and dark environment and transported to the US Navy Entomology Center of Excellence laboratory where they were stored in a refrigerator for later droplet size measurements and determination of deposition.

Droplet size on rods was determined using the DropVision system (Leading Edge Associates Inc., Fletcher, NC) and droplet distribution parameters ($D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$) were determined. The $D_{V0.1}$, $D_{V0.5}$, and $D_{V0.9}$ are the droplet diameters (µm) where 10, 50, and 90% of the spray volume is contained in droplets smaller than these diameters (ASTM Standard E1620, 2004). Adulticide deposition on rods was measured using the methods described by Farooq et al. (2009). Rods were washed inside a plastic bag using 25 ml of deionized water. Fluorescence readings of the solution were determined using a spectrofluorophotometer (Shimadzu, Model RF5000U, Kyoto, Japan) and converted to the amount of dye on the slide using calibrations developed from a set of standardized fluorescence concentrations. The amount of dye in each sample was then divided by the effective sampling area of 63 cm^2 to calculate dye deposition (ng/cm^2). Using the ratio of dye and active ingredient (AI) in the spray tank, deposition was converted to AI deposition (ng/cm^2).

Statistical analysis

Statistical analysis was conducted with Intel® Visual Fortran Composer XE 2013. The Kolmogorov-Smirnov test (Smirnov 1939) showed that all datasets were non-normal and the Bartlett test (Bartlett 1937) showed non-homogeneity of variances. Therefore, mean 24 h *Ae. albopictus* mortality, adulticide deposition, and $Dv_{0.5}$ data were subjected to a 3-way nonparametric Kruskal-Wallis analysis ($\alpha = 0.05$) that was used to assess differences among the nozzle combinations (i.e. 45° upward at 16 km/h, 22.5° upward at 24 km/h, and 0° horizontal at 24 km/h-factor 1), vegetation levels (dense, medium, and sparse-factor-2), and application distances (0, 15, 30, 45, 60, and 90 m-factor 3), as well as the interactions between the nozzle combination × vegetation, the nozzle combination × distance interaction, the vegetation type × distance interaction, and nozzle combination × vegetation type × distance interaction. Subsequent Tukey multiple-comparisons tests were conducted to identify those nozzle combination treatments that were significantly different from each other. Differences from all analyses were considered significant when $P < 0.05$.

RESULTS

Effect of Nozzle Orientation and Speed on Mortality

There was a significant difference in 24-h mortality of caged *Ae. albopictus* among the 3 nozzle/speed combinations ($P = 0.0096$) and distances ($P = 0.0212$) but not the interaction between combination and distance ($P = 0.5863$) (Figure 2). The 22.5° combination resulted in the greatest mortality (88.3%)

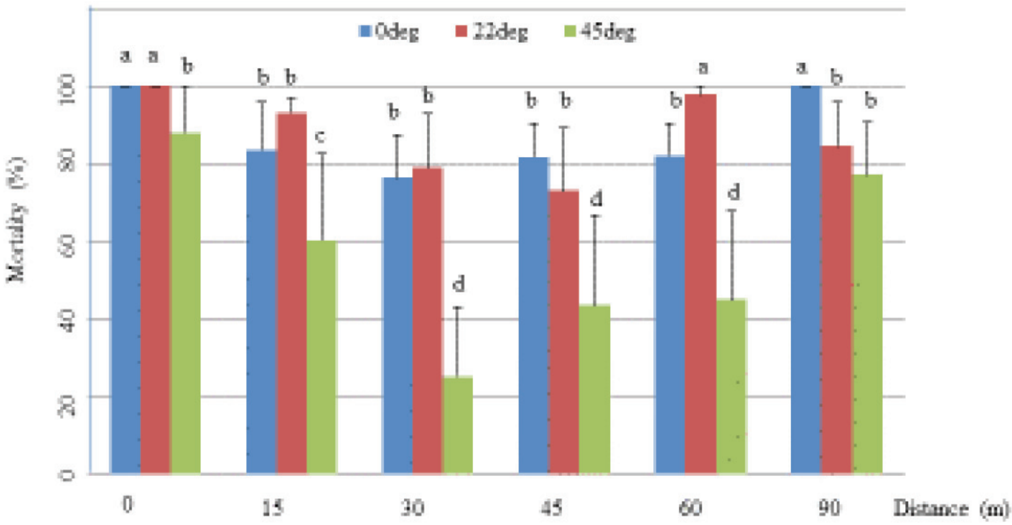

Figure 2. Comparison of 24-h mortality among the combinations of angle and speed and distances.

and 45° combination resulted in the least mortality (63.1%) (Table 1). There was no significant difference between 22.5° combination and 0°combination in terms of 24-h mortality. At every distance, mortality from 45° combination was significantly lower than 22.5° and 0° combinations. At distance 0 m, 22.5° and 0° combinations resulted in 100% mortality; at distances of 15, 30, and 60 m the 22.5° combination slightly outperformed 0° whereas, at 45 m, the 0° combination slightly outperformed the 22.5° combination but these differences were not significant (Figure 2).

Effect of Vegetation Density on Mosquito Mortality

There was a significant difference in 24-h mosquito mortality among the 3 vegetation levels ($P = 0.0319$) and distances ($P = 0.0212$) but no significant difference among the interactions of vegetation level and distance ($P = 0.5863$). The greatest mortality (91.4%) was recorded in the sparse and the lowest in medium vegetation density (72.2%) (Table 1). At 0 m, no significant difference in 24-h mortality among the three vegetation levels occurred. At 15 m, *Ae. albopictus* mortality in the sparse vegetation community remained close to 100% but significantly lower mortality occurred in the medium and dense vegetation communities (Figure 3). At 30 m, mortality in sparse vegetation areas decreased to 80% while in medium and dense locations mortality was further reduced to 60% and 40%, respectively.

Effect of Nozzle Orientation and Travel Speed Combination on Adulticide Deposition

Adulticide deposition was not significantly affected by nozzle orientation and

Table 1. Mean mosquito mortality from nozzle angle combinations in different vegetation densities.

	Mortality, % (Mean ± SD) from angle combinations			
Vegetation Density	0°	22.5°	45°	Average
Dense	74.9 ± 23.2	90.4 ± 23.4	59.7 ± 45.0	75.0 ± 30.5
Medium	86.6 ± 15.3	96.5 ± 6.5	33.5 ± 41.9	72.2 ± 21.2
Sparse	100.0 ± 0.0	78.1 ± 29.7	96.1 ± 7.4	91.4 ± 12.4
Average	87.2 ±12.8	88.3 ± 19.9	63.1 ± 31.4	79.5 ± 21.4

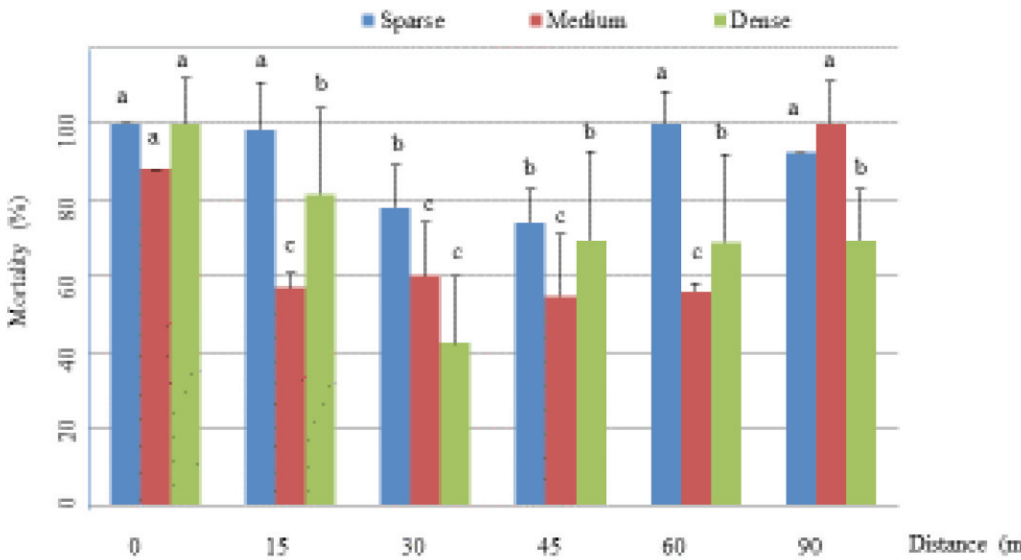

Figure 3. Comparison of 24-h mortality among the vegetation levels and distances.

travel speed combinations ($P = 0.2066$), or interaction of these combinations with distance ($P = 0.2806$). Deposition was significantly affected by application distance ($P < 0.0001$) (Figure 4). Overall, 0° combination resulted in the greatest average deposition, followed by 22.5° and 45° combinations. At 0 m, significantly higher deposition occurred from 0° combination whereas at the remaining distances, there were no significant differences in deposition between nozzle/speed combinations (Figure 4).

Effect of Vegetation Density on Adulticide Deposition

Deposition on rods was not significantly different among the 3 vegetation densities

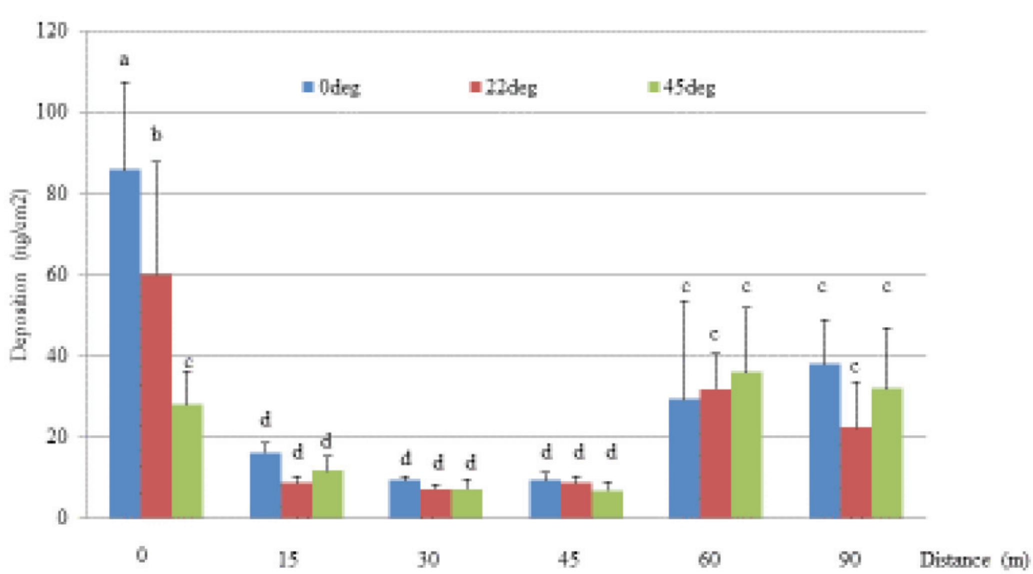

Figure 4. Comparison of deposition among the angel combinations and distances.

among sampling distances ($P < 0.0001$) and interaction of vegetation density and distance ($P = 0.0337$). Generally, deposition was greatest (27.5 ng/cm²) for medium and lowest (23.6 ng/cm²) for sparse vegetation (Figure 5). Also, deposition was greatest at 0 m and lowest at 30 m, and highest for 0° combination at 0 m and lowest at 45° combination at 30 m (Figure 4).

DISCUSSION

Farooq et al. (2017, 2018) demonstrated that nozzle orientation or travel speed alone resulted in a significant impact on adulticide efficacy in an open field. Overall, horizontal nozzle spraying with a truck mounted ULV sprayer achieved the greatest efficacy against adult *Ae. aegypti,* followed by a 30° downward nozzle angle while a 45° angle upward showed the least effectiveness (Farooq et al. 2017). Jiang and Farooq (2016) showed that a ULV nozzle oriented horizontally outperformed those upward at 45° in three out of four field trials, although those differences were not statistically significant. In addition, Farooq et al. (2018) recently reported that a travel speed of 32 km/h achieved the highest efficacy against caged *Ae. aegypti* followed by 16 km/h and then 8 km/h. Our study found that a ULV nozzle oriented at 22.5° at a travel speed of 24 km/h resulted in the greatest mortality against *Ae. albopictus* whereas, the standard nozzle orientation of 45° at speed of 16 km/h (10 mph) resulted in the least mortality. These results confirmed that by changing nozzle orientation and travel speed together one can significantly improve adulticide efficacy. Farooq et al. (2017) explained that when spray nozzles are oriented at 45° upward, most spray material remains above the mosquito fly zone, so no droplets <40 µm would descend to the space 1.4 m above ground (which is habitat for most humans and mosquitoes) before traveling 100 m in a horizontal direction. By setting the nozzle orientation lower than 45° upward, enhanced mixing of spray into the air by the truck wake may have also resulted in an increase of spray efficacy. Likewise, improvement in application effectiveness with increased travel speed is due to the resultant combination of two physical phenomena. First, the induced air movement due to vehicle travel occurs in an opposite direction and increases with an increase in travel speed. The induced air deflects the spray plume towards the ground and suppresses upward

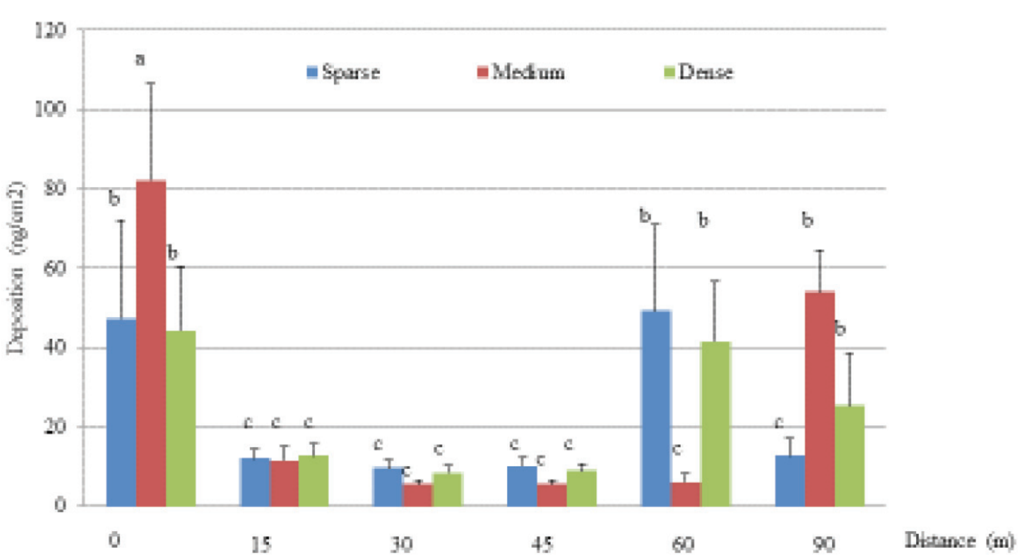

Figure 5. Comparison of deposition among the vegetation levels and distances.

droplet movement resulting in better efficacy. Second, movement of a vehicle creates an air vortex behind it that strengthens with increasing travel speed. This vortex helps to better mix the spray with air, resulting in a higher probability of droplets contacting flying insects (Farooq et al. 2018). At an upward nozzle of 45°, the spray cloud is generally not expected to interact with the vortex behind the vehicle.

Vegetation and housing density is another factor that has a significant impact upon control effectiveness as reported by many authors. Taylor and Schoof (1971) obtained twice the level of kill for 3 species of mosquitoes exposed to 95% malathion aerosols in an open area compared with those exposed in moderately dense wooded areas. Andis et al. (1987) stated that the average mortality of caged *Ae. aegypti* was 95.5% and 49% in open and sequestered locations, respectively. Linley and Jordan (1992) obtained greater percent kills of caged *Culex quinquefasciatus* Say exposed to aerosols of malathion, naled, and resmethrin plus piperonyl butoxide in open compared with vegetated terrain. Results from our study showed that 0° and 22.5° combinations resulted in 75 and 90% mortality of *Ae. albopictus*, respectively in dense vegetation, compared with 60% from the standard 45° combination. Comparing our results with those reported by Taylor and Schoof (1971), Andis et al. (1987), and Linley and Jordan (1992) indicated that adjusting nozzle orientation increased mortality in vegetation.

In summary, significant improvement in performance of a ULV applied adulticide was achieved when the combination of an optimal nozzle orientation was paired with an increase in travel speed. Importantly, Farooq et al (2017) pointed out that this application optimization does not require a structural change, takes only a few minutes to accomplish, and has a significant impact on spray efficacy.

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