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OPERATIONAL NOTE

AN OPEN-FIELD EFFICACY TRIAL USING AQUADUET™ VIA AN ULTRA-LOW VOLUME COLD AEROSOL SPRAYER AGAINST CAGED *AEDES ALBOPICTUS*[1]

ARY FARAJOLLAHI[2,3] AND GREGORY M. WILLIAMS[2,4]

ABSTRACT. We conducted an open-field ultra-low volume efficacy bioassay using a water-based formulation AquaDuet™ (prallethrin [1%], sumithrin [5%], and piperonly butoxide [5%]) applied from a truck-mounted cold aerosol sprayer. The adulticide was applied at 90.6 ml/ha (1.23 oz/acre) and 3 replicated treatments were performed using caged *Aedes albopictus* collected from local wild populations. Rotating impingers and mosquito cages were placed in 3 rows stationed at 30.5 m (100 ft), 61.0 (200 ft), and 91.4 (300 ft) downwind of the spray vehicle. Initial knockdown was 95.6%, with overall mortality >99% across all distances, despite low wind conditions. Volume median diameter ($Dv_{0.5}$) and droplet density were 17.4 µm and 110.5 mm², respectively. Our open-field studies against caged *Ae. albopictus* demonstrate that a water-based adulticide formulation is just as efficacious as traditional oil-based formulations.

KEY WORDS Adulticide, ultra-low volume, prallethrin, volume median diameter, water-based pesticide, mosquito control

In the USA, federal and state guidelines for protecting the public during outbreaks of mosquito-borne diseases recommend using adulticides from aircraft or truck-mounted equipment as the most effective method to rapidly reduce the risk of disease transmission to humans (CDC 2003). These adulticide interventions are primarily applied as ultra-low volume (ULV) cold aerosol space sprays, and are an important component of integrated mosquito management (IMM) programs (Mount 1998, Bonds 2012). Adulticides are predominantly applied neat or diluted prior to dispersion with a hydrocarbon solvent such as fuel or mineral oil. However, with the ever-increasing cost of fuels and oil, and more importantly, concern for the environment and society along with requirements for reducing the impact from oil-based solvents, the use of water as a diluent is becoming an attractive and sought-after alternative.

Until recently, water has not been used effectively as a solvent because the vapor pressure of water causes droplets to evaporate and reduce in size (Groome et al. 1989). This effect creates droplets that are too small to impact efficiently on mosquitoes or may simply be lost to the atmosphere and not reach their target. Since droplet size is a principal factor governing the efficacy of adulticide applications, extremely small droplets are inefficient for space sprays (Bonds 2012). But new water-based adulticides are being developed that limit evaporation and increase the stability of the active ingredient.

Droplet optimization technology (DOT) is a new method being used in water-based adulticides that takes evaporation of the water droplet into account, while protecting the active ingredient. This technology permits the evaporation of the ULV aerosols and allows for a more consistent and concentrated delivery of the droplet for impingement on adult mosquitoes (Clarke Mosquito Control 2013). When aerosolized droplets leave a spray nozzle, the aqueous carrier begins to evaporate until it reaches an optimal size where evaporation ceases and the droplet is stabilized. The decrease in droplet volume leads to an increase in density and concentration of the active ingredient as the dimensions of the droplet reduce. Typically, droplets with a volume mean diameter (VMD) of 5–25 µm are considered optimal for adult mosquito control (Mount 1998, Bonds 2012). However, water-based adulticide aerosols will typically lose 30% of their droplet volume (Groome et al. 1989, Bache and Johnstone 1992). Therefore, the AquaDuet™ label recommends calibrating equipment to deliver droplets with a VMD of 8–30 µm to compensate for evaporation. The VMD is also displayed as $Dv_{0.5}$, which is a statistic for the droplet diameter (µm) at which 50% of the spray volume is contained in droplets smaller than this

[1] The mention of trade names or commercial products in this publication are solely for the purpose of providing specific information and does not imply recommendation or endorsement by the writers or other involved parties.

[2] Center for Vector Biology, Rutgers University, New Brunswick, NJ 08901.

[3] Mercer County Mosquito Control, West Trenton, NJ 08628.

[4] Hudson Regional Health Commission, Sccaucus, NJ 07094.

value. Similarly, a $Dv_{0.1}$ and a $Dv_{0.9}$ are values at which 10% and 90% of the spray volume is contained in droplets of that size or less.

AquaDuet (Clarke®, Roselle, IL) is a newly developed water-based formulation that uses DOT to deliver effective aerosols to adult mosquitoes. Like its precursor, oil-based formulation Duet™ Dual-action Adulticide (Clarke), AquaDuet also combines the pyrethroids prallethrin (1%) and sumithrin (5%) with the synergist piperonly butoxide (5%). Formulations of Duet have been shown to cause a benign agitation (a nonbiting excitation) and may flush mosquitoes from resting places to increase contact with airborne aerosols that are more likely to impinge on flying adults (Cooperband et al. 2010). Prallethrin reportedly induces the excitatory response while sumithrin and piperonyl butoxide produce the lethal effect. These adulticides are effective not only against resting gravid or engorged mosquitoes, but also against diurnal mosquitoes such as *Aedes albopictus* (Skuse) that are increasing their distribution and abundance in temperate North America (Farajollahi and Nelder 2009, Rochlin et al. 2013), and which may be inactive during routine nighttime ULV applications by mosquito abatement programs.

Duet has recently been evaluated in semi–field caged mosquito assays with promising results. Qualls and Xue (2010) reported >70% mortality against field-collected *Culex quinquefasciatus* Say up to 106 m (350 ft) from the point of application in Florida. Field evaluations against caged *Ae. albopictus* in New Jersey have shown >95% mortality up to 92 m (300 ft) downwind from the spray vehicle (Suman et al. 2012). Additionally, nighttime ULV applications of Duet against wild *Ae. albopictus* in a northeastern urban setting have proven effective in reducing field populations by >73% with a single full-rate application, or >85% with a dual application at mid–label rate spaced 1 or 2 days apart (Farajollahi et al. 2012). However, although Duet is being utilized and evaluated by mosquito control programs, no such data exist in the literature on AquaDuet. Since the efficacy of new formulations must be meticulously tested in the field against various mosquito species prior to integration into control programs, the scope of this research was to assess the effectiveness of AquaDuet in an open–field caged study conducted in northeastern USA.

We evaluated the efficacy of AquaDuet against caged *Ae. albopictus* (F_1 generation) placed up to a distance of 91.4 m (300 ft) applied from a truck-mounted Cougar® (Clarke) ULV cold aerosol sprayer using the maximum label rate of 90.6 ml/ha (1.23 oz/acre). The ULV sprayer was calibrated the day prior to the applications with the Army Insecticide Measuring System (Brown et al. 1993) and produced a VMD ($Dv_{0.5}$) of 16.86 μm (2,444 droplets counted), a $Dv_{0.1}$ of 3.89 μm, and a $Dv_{0.9}$ of 35.31 μm. All bioassays were carried out using standard published methods (WHO 2009).

We collected *Ae. albopictus* adults from Mercer County, NJ, and maintained the mosquitoes under standard laboratory conditions at 26 ± 1°C, 70–75% RH, and a photoperiod of 16:8 (L:D) h. The truck-mounted ground ULV applications were conducted over an asphalt surface in a secure and secluded area of Trenton-Mercer Airport, Mercer County, NJ, on September 6, 2012. A 3 × 3 grid design was used for the experiment (Suman et al. 2012). Three rows of adult mosquito cages (14.4-cm diam and 4-cm depth) were placed 30.5 m (100 ft), 61.0 m (200 ft), and 91.4 m (300 ft) downwind and perpendicular to the spray path. Stakes (1.5-m height) holding treatment cages (1 cage at each distance; 3 distance points × 3 replicates = 9 cages total), and Florida Latham-Bonds rotating impingers (Clayson et al. 2010; 6-V DC, 590 rpm, 5.6 m/sec) were activated immediately before and stopped 10 min after the application to collect aerosolized droplets on two 3-mm-wide slides with Teflon® tape at each station (Clayson et al. 2010). All droplet diameter measurements and droplet density calculations were performed under a compound microscope using DropVision® (Leading Edge Associates, Waynesville, NC).

We placed up to 20 adult female *Ae. albopictus* (7–14 days old) in each treatment cage a few hours before the application. Each set of cages were placed into a designated large plastic tote with a lid, and a cotton pad soaked in 10% sucrose solution was placed on top of each cage. Moist towels were placed within each plastic tote to maintain a high RH. Cages were placed on stakes 15 min prior to adulticide applications and remained within the treatment plot for 10 min postapplication as the aerosolized fog moved through the experimental plot. Posttreatment, adults were transferred to cups with the use of a mouth aspirator and kept in 237-ml cardboard ice cream containers covered with mesh netting lids for observations under laboratory conditions. A 10% sucrose solution was provided during the holding period in the form of a soaked cotton pad placed on top of each mesh lid. Mosquitoes that were immobile and remained on the bottom of each cage after a gentle tap were counted for knockdown. Mortality was assessed at 1 h, 24 h, 48 h, and 72 h posttreatment. Treatment applications were replicated 3 times for all 3 distances by setting up 3 rows of cages and the experiment was repeated 3 times. One set of control mosquitoes was placed in the field 30 min prior to 1st adulticide application and handled as mentioned above. Control mosquitoes were not exposed to the insecticide; however, they were

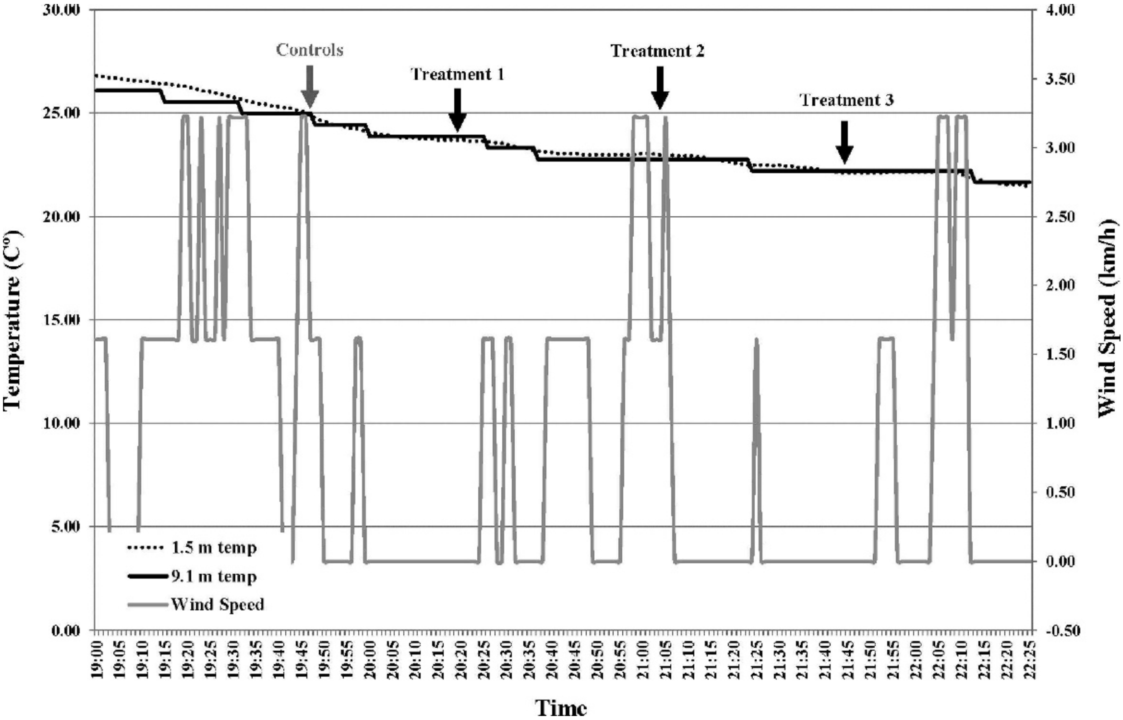

Fig. 1. Meteorological conditions during three open-field ultra-low volume applications of AquaDuet™ against caged *Aedes albopictus*, Mercer County, NJ.

otherwise handled in the same manner as treatment mosquitoes. Knockdown and mortality of mosquitoes were corrected with controls by using Abbott's formula (Abbott 1925) prior to analysis. Data for each cage distance from all 3 replicates of the experiment were pooled to provide a mean. A meteorological station (Davis Instruments, Vernon Hills, IL) was placed in the field 4 h prior to the experiment to measure thermal inversion with temperature at 1.5 m and 9.1 m, RH, and wind speed/direction. Measurements were recorded at 1-min intervals for all variables.

Wind speed was virtually negligible during the course of the experiment (Fig. 1). Average wind speed during the 3 treatment replicates was 0.55 km/h, signifying only the presence of an infrequent gust during the applications. Average RH was recorded at 82.9% and average temperature on the ground (1.5 m) was 22.84°C (73.11°F) and 22.76°C (72.96°F) at the 9.1-m height; only a slight thermal inversion was observed (Fig. 1). Overall $Dv_{0.5}$ values across all 3 replicates and distances were 17.4 μm, while $Dv_{0.1}$ and $Dv_{0.9}$ were recorded at 10.6 μm and 30.7 μm, respectively (Table 1). Mean droplet density (n = 29,694; mean = 1,100 drops per slide) for each slide was 110.5 mm^2. Mosquito mortality was excellent during all treatments, with an overall mortality of 99.9% (Table 1). We did not observe any differences in mortality between the overall counts and the initial knockdown, 24-h, or 48-h recordings; only 1% of adult mosquitoes recovered after initial knockdown. Control mortality was <1% during this experiment. These findings exhibit that a truck-mounted application of AquaDuet at 90.6 ml/ha (1.23 oz/acre) produced droplets of optimal size and concentration, while initiating nearly 100% mortality in caged *Ae. albopictus* up to 91.5 m from the point of application.

In addition to various other factors, efficacy of ULV adulticide applications during open field trials are also highly governed by air movement. Insecticide labels, such as the one for AquaDuet, require that ULV adulticide applications be conducted when meteorological conditions are conducive to keeping the spray cloud close to the ground, to avoid applications in calm air conditions, and to apply when ground wind speed is greater than or equal to 1 mi/h or 1.61 km/h (http://www.clarke.com/images/pdf/Labels/2012Labels/aquaduet.pdf). However, the logistics of operational applications often dictate otherwise, and most often, ULV adulticide sessions may experience conditions where wind velocity may vanish completely (Farajollahi et al. 2012). We experienced such an event during our experimental treatments when, although we had waited until ground wind speed exceeded

Table 1. Adulticide droplet characterization and mosquito mortality data from a truck-mounted ultra-low volume application of AquaDuet™ against caged *Aedes albopictus*.

	Treatment 1[1]			Treatment 2[1]			Treatment 3[1]			Mean
	A	B	C	A	B	C	A	B	C	
$DV_{0.10}$ (μm)	10.1	10.9	9.9	11.1	10.7	10.5	10.5	10.9	10.8	10.6
$DV_{0.50}$ (μm; VMD[2])	15.7	17.8	16.6	19.9	18.0	17.0	16.7	17.0	18.2	17.4
$DV_{0.90}$ (μm)	27.6	30.6	30.6	38.1	30.0	28.4	27.8	26.7	34.5	30.7
Total no. drops measured[3]	3,169	3,378	3,144	3,457	3,615	3,106	3,214	3,209	3,402	1,100
Droplet density (mm[2])	156.8	167.1	179.5	197.3	141.2	41.2	125.5	45.8	114.8	110.5
Mosquito mortality[4] (%)	100	100	100	100	95.6	100	100	100	100	99.9

[1] A = 30.5 m (100 ft); B = 61.0 m (200 ft); C = 91.4 m (300 ft).
[2] VMD, volume mean diameter.
[3] Total of 29,694 drops measured (550 per slide, 2 slides per station).
[4] Mortality in control groups <1%.

1.61 km/h to initiate our replicates, wind velocity quickly diminished to less optimal conditions (Fig. 1). We opted to continue the experiments because favorable wind velocities were recorded sporadically, and we were expecting meteorological conditions to improve based on local weather forecasts. However, despite calm air conditions for all 3 replicates, we recorded high mortality in our caged mosquitoes. The lack of wind movement may have contributed to increased mortality, concentrating the aerosolized droplets within the treatment plot and increasing contact with caged mosquitoes. We also recorded higher concentrations of droplet density on our slides (110.5 mm[2]) using AquaDuet than a previous study (22.6 mm[2]) using an oil-based Duet formulation (Suman et al. 2012), but the latter study was conducted when wind speeds averaged between 4.8 to 11.3 km/h (3.0 to 7.0 mi/h). Since wind velocity is a critical factor determining the longitudinal distance and concentration of the spray cloud through the target area (Mount 1998, Bonds 2012), the lower density counts recorded by Suman et al. (2012) could be explained by the faster movement of the aerosolized droplets through the treatment plot by higher wind speeds. Wind (when present) direction also changed irregularly during our treatment applications, further explaining the lower droplet density during Treatment 3 at 61.0 m than at 91.4 m (Table 1). But overall, our $Dv_{0.5}$ (VMD) value of 17.4 μm for AquaDuet was consistent with our precalibration value of 16.86 μm and also with previous studies that recorded $Dv_{0.5}$ values of 17.8 μm (Suman et al. 2012) and 20 μm (Qualls and Xue 2010) for Duet. Our $Dv_{0.5}$ and $Dv_{0.9}$ values were also consistent with the AquaDuet pesticide label, which requires a $Dv_{0.5}$ value of between 8 to 30 μm and a $Dv_{0.9}$ value of <50 μm.

Applying ULV adulticides is often a last resort for professional organizations that use IMM to protect public health and comfort. However, they are a very necessary component that may greatly aid local agencies when adult mosquito populations must be reduced immediately. With increasing insecticide resistance and environmental concerns, evaluations of new formulations are imperative for responsible IMM, which increases environmental stewardship and accountability with no loss in efficacy. Our open-field studies utilizing an ULV adulticide application against caged *Ae. albopictus*, demonstrate that a water-based formulation (AquaDuet) is just as efficacious as traditional oil-based formulations. Further operational evaluations within actual field settings will elucidate the efficacy of water-based adulticides against endemic mosquito populations.

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