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EFFICACY OF UNMANNED AERIAL APPLICATION OF DELTAMETHRIN AGAINST MOSQUITOES (DIPTERA: CULICIDAE)

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Abstract This is the first reported study on efficacy of aerial ultra-low volume (ULV) application of an adulticide using a small Unmanned Aerial System (sUAS) in the United States. Imperium™ Insecticide (Bayer CropScience LP), containing the active ingredient (AI) deltamethrin, is the only Type II pyrethroid registered in the United States for wide area mosquito control. It is currently registered for application with hand-held, backpack, portable, ground and aerial ULV sprayers. PrecisionVision® unmanned aircraft system (Leading Edge Aerial Technologies, Inc.) is designed specifically for the aerial application of both liquid and granular products as well as ULV adulticide applications. Two trials were conducted to determine the efficacy of ULV spray applications of deltamethrin formulation using the sUAS at 0.00089 lbs AI/acre *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) in Florida (FL) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae) in California (CA). Both trials implemented a similar study design. Treatment stations were placed on a 3x3 grid at various distances downwind from the sUAS path. Three control stations were placed upwind of the sUAS path for each treatment. Each station was equipped with two mosquito cages and a rotating spinner with 1x3 inch Teflon-coated slides for capturing data to determine the droplet spectrum. Mosquito knockdown data was recorded approximately one hour post-treatment and mortality data was assessed approximately 12 hours post-treatment. The trial in FL achieved complete knockdown and kill against *Aedes aegypti*. The trial in CA achieved greater than 94% knockdown and 100% mortality against *Culex quinquefasciatus*.

Key words *Aedes aegypti*, *Culex quinquefasciatus*, pyrethroid, aerial application, Unmanned Aerial System, Ultra low volume

INTRODUCTION

Integrated mosquito control programs rely on effective and efficient measures to control adult mosquito populations. Ground and aerial ULV applications of mosquito adulticides are important components in these programs (Focks et al, 1987; Reiter and Nathan, 2001). Pyrethroid insecticides have been widely used in vector controls due to their high relative potencies which allow greatly reduced application rates compared with organophosphates and carbamates (Elliott et al, 1978; Carter, 1989). Deltamethrin is a type II pyrethroid which has been recommended by the World Health Organization (WHO, 2019) for use as residual, long-lasting insecticidal net and space spray adulticide. It is the only Type II pyrethroid registered in the United States for wide area mosquito control.

In recent years, unmanned aircraft system (UAS) technology has been developed for performing dull, dirty or dangerous missions for the military. But this technology has rapidly expanded into many other areas such as commercial, scientific, recreational, and agricultural applications. UAS includes an unmanned aircraft vehicle (UAV or Drone), a ground-based controller, and a system of communications between the two. The UAVs may operate with various degrees of autonomy: either under remote control by a ground pilot or autonomously by onboard computers. The unmanned aircraft system used in the study is designed specifically for the aerial application of both liquid and granular products as well as ULV adulticide applications. This was accomplished through the integration of the PrecisionVision iOS with Avionics suite (Leading Edge Aerial Technologies, Inc. Waynesville, North Carolina), its UAS and the various payload systems. It has been used for imagery and aerial applications in the forestry, agriculture, noxious weed and mosquito control industries in the United States (unpublished data). However, the use of sUAS for aerial adulticiding has never been documented in the United States.

The objective of this study was to determine the efficacy of deltamethrin formulation against mosquitoes following aerial applications using the sUAS. The study consisted of two trials which were conducted in collaboration

with Manatee County Mosquito Control District (MCMCD) in Manatee County, Florida and East Side Mosquito Abatement District (ESMAD) in Stanislaus County, California of the United States.

MATERIALS AND METHODS

Test Sites The study consisted of two trials: 1) in Manatee County (Palmetto, FL) in August 2018; and 2) in Stanislaus County (Modesto, CA) in June 2018. Each trial consisted of two test sites: treated and untreated. Both the treated and untreated sites were in relatively open fields with vegetation that was well below the mosquito cages and droplet samplers within the plots. Untreated sites were located at least 1.5 miles upwind from treatment sites.

Mosquitoes. Healthy, adult female *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (Benzon Research Inc. Carlisle, Pennsylvania) were selected and used for the study. Prior to each trial the *Aedes aegypti* were shipped directly to test sites in Palmetto, FL while the *Culex quinquefasciatus* were shipped to Modesto, CA. Once adults arrived at the test sites they were maintained with adequate humidity and temperature and provided *ad libitum* with a 10 % sucrose water solution until they were transferred into smaller experimental cages for setting up the trials.

Test Substance Deltamethrin formulation (EPA registration No. 432-1534) is an emulsion concentrate which contains 20 g active ingredient per L. The product was loaded directly into the spray mixing tank without adding any water or other solvents or stabilizers. The application rate of 0.00089 lbs AI/acre was targeted for both trials.

Aerial Application Equipment. The route of administration for the deltamethrin formulation was an unmanned aerial ULV spray application using a sUAS with PrecisionVision 600P (Leading Edge Aerial Technologies, Inc., Waynesville, North Carolina). The sUAS was equipped with two Micronair micromizer 10 nozzles (Micron Group, Herefordshire, United Kingdom), one mounted on the end of each side of the UAS (Figure 1). A flow rate calibration was performed on the Micronair micromizer 10 spray nozzles used for this study on the same day of or one day prior to the applications. Once a desired flow rate was achieved, an aerial calibration spray was conducted by flying directly over Teflon-coated spinners at approximately 10 ft above ground. A slide that captured the most droplets was selected and analyzed for droplet size using DropVision (Leading Edge Aerial Technologies, Inc. Waynesville, North Carolina). A spread factor of 0.61 was used to calculate the droplet size for deltamethrin formulation on the Teflon slides. The registered deltamethrin formulation label indicates that the aerial application equipment should be calibrated to produce droplets that are less than 60 microns for $DV_{0.5}$ and less than 115 microns for $DV_{0.9}$. A detailed calibration summary is presented in Table 1. The sUAS was fully equipped with an on-board system PrecisionVision Avionics Suite (Leading Edge Aerial Technologies, Inc. Waynesville, North Carolina) that mapped out the complete flight path and recorded numerous application-specific parameters.

Figure 1. Unmanned aerial ULV application equipment using a sUAS.

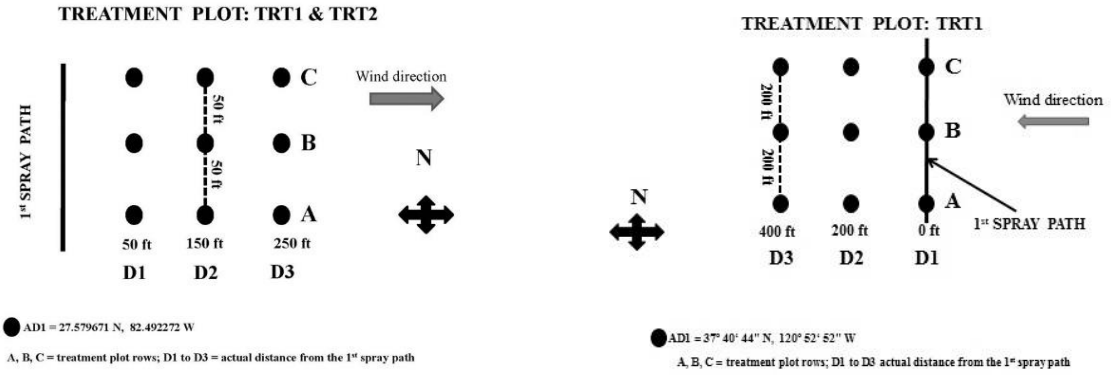

Figure 2, 3. Treated plots.

Study Design. The deltamethrin formulation was first dispersed at approximately 50 ft aloft using three flight passes in mid-air for treatment applications. Dispersed test item droplets then descended and drifted onto the ground level where the mosquito cages were set up in the treatment plots. Spray drift software AGDISP (USDA Forest Service) was used to calculate spray offset from test plot, based on real-time weather data. The actual application rate of approximately 0.00089 lbs AI/acre was achieved by using its respective aircraft speed, flow rate, and swath width during each trial. The application parameters for each trial were presented in Table 3.

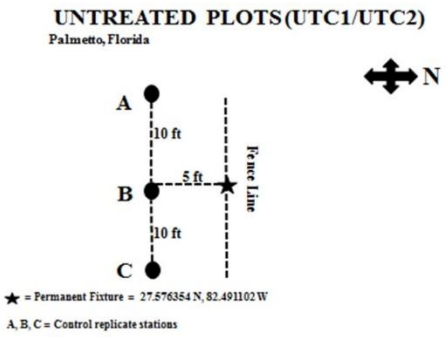

Figure 4. Untreated plots.

Figure 5. Bioassay cages.

For treated plot, three replicate rows of sampling stations were established 50 to 200 ft apart from each other (Figures 2 and 3). Also, three columns of stations were set up at three increasing distances from the first spray pass (FL trial = 50 ft, 150 ft, and 250 ft; CA trial = 0 ft, 200 ft, and 400 ft). These distances from the spray path were determined based on each application's respective spray offset. For the untreated plot, three control sampling stations were established in a straight line at distances approximately 10 ft apart (Figure 4). Each of the sampling stations in the treated and untreated plots were equipped with rotating Teflon-coated slides (1 x 3 in) and two bioassay cages containing approximately 10 to 40 adult female mosquitoes (Figure 5). Droplet sampler slides and mosquito cages were collected approximately 15 to 20 mins after the end of application. The mosquitoes were transferred out of the experimental cages and into fresh untreated assessment containers using an aspirator and/or CO_2. Transferred mosquitoes were provided with a cotton ball soaked with sugar water solution as a food source and kept at room temperature. All collected mosquitoes were assessed for knockdown and mortality at approximately 1 hr and 12 hrs after application, respectively. Collected Teflon-coated slides were analyzed for droplet spectrum using DropVision.

Test Conditions. During the entire duration of each trial, environmental data was taken at the ground level (approximately 5 ft). A detailed weather summary is presented in Table 2 for both trials.

Assessments. For both trials, collected mosquitoes were assessed for knockdown at 1 hour after application and assessed for mortality at 12 hrs after application. Knockdown mosquitoes were identified as individuals that cannot walk or fly and show only very feeble movements of legs and antennae in response to stimulation; mosquitoes may sometimes recover from being knockdown. Mortality was recorded when an individual was observed to be dead and showed no movement in response to stimuli.

Data Analysis. Mosquitoes that died in the cages sometime between being loaded and start of application were left inside the cages because it was difficult to extract them without harming the caged live mosquitoes. The values for the total mosquitoes used, total knockdown and total dead were adjusted by subtracting the mosquitoes that died prematurely because that mortality was not relevant to the test item application. After this adjustment, the percentage of knockdown/mortality was calculated for each distance from the spray path for each treatment group. Replicate cages and replicate rows were pooled for each distance from the spray path for each treatment group.

Table 1. Droplet assessments during sUAS spray calibrations when deltamethrin was applied at 0.00089 lb AI/acre

Trial location	Calibration date	$DV_{0.5}$ (microns)	$DV_{0.9}$ (microns)
Florida	21 August 2018	45.9	63.3
California	27 June 2018	44.8	58.5

Table 2. Environmental conditions at ground level when deltamethrin formulation was applied by sUAS

Trial location	Trial Date	Wind direction	Average wind speed (mph)	Average temperature (°C)	Average relative humidity (%)
Florida	21 August 2018	West to North-west	2.2	31.2	54.8
California	28 June 2018	North-west	6.5	16.8	66.4

Table 3. Aerial application parameters when deltamethrin formulation applied at 0.00089 lb AI/acre using sUAS in Florida and California

Location	Application start to end time	Number of passes	sUVS speed (mph)	sUVS altitude (ft)	Swath width (ft)	Offset value (ft)	Actual flow rate (fl. oz/min)
Florida	19:21 to 19:24	3	19	50	200	50	5
California	06:59 to 07:03	3	14	50	300	0	6

RESULTS AND DISCUSSIONS

Mosquito Knockdown and Mortality. For both trials, there were no noticeable differences in knockdown and mortality as the deltamethrin formulation drifted through the three distances from the first spray path (Tables 4 and 5). The knockdown at 1 hr after application for *Aedes aegypti* was at 100 % and was noticeably greater than that for untreated control (1.3 %) (Table 4). The knockdown at 1 hr after application for *Culex quinquefasciatus* ranged from 94.5 to 99.4 % and was noticeably greater than that for untreated (1.3 %) (Table 5). A complete mortality was achieved at 12 hr after applications for both trials against *Aedes aegypti* and *Culex quinquefasciatus*. In contrast, untreated control mortality remained at 1.3% for both species at 12 hr after applications.

Droplet Spectrum. In Florida trial against *Aedes aegypti*, the average droplet sizes found in the treated plot ranged from 38.00 to 50.20 microns across the three distances of 50, 150, and 250 ft from the first spray path (Table 4). The droplet sizes found in the untreated plot (average of 8.72 microns) were noticeably smaller than those found in the treatment plot at all distances. The average droplet density ranged from 1.29 to 1.76 droplets/mm^2 across the three distances from the first spray path. The droplet density found in the untreated (1.09 drops/mm^2) was similar to that

found in the treatment plot (at all distances). However, the droplets found in the untreated plot were much smaller than those found in the treatment plot. In California trial against *Culex quinquefasciatus*, the average droplet sizes found in the treated plot ranged from 31.15 to 50.18 microns across the three distances of 0, 200, and 400 ft from the first spray path (Table 5). The average droplet density ranged from 1.12 to 1.34 droplets/mm^2 across the three distances from the first spray path. No droplets were observed on the droplet sampler slides in the untreated plot.

Table 4. Summary of knockdown, mortality and droplet spectrum for sUAS application of deltamethrin formulation against *Aedes aegypti* in Florida

Group and Actual Application Rates (lbs/acre)	Distance from Spray Path (ft)	% Knock-down[A]	% Mortality[A]	Avg. Droplet Density[B] (droplets/mm^2)	Avg. Droplet $DV_{0.5}$ Size[B] (microns)
Treatment = 0.00088	50	100.0	100.0	1.76	40.56
	150	100.0	100.0	1.29	50.20
	250	100.0	100.0	1.48	38.00
Untreated = 0	NA	1.3	1.3	1.09	8.72

[A] Knockdown and mortality percentages displayed here are a result of pooling two replicate cages and three replicate rows (A, B, and C) within each spray distance for each group.
[B] Droplet density and size are presented as averages of replicate rows A, B and C within each spray distance for each group.

Table 5. Summary of knockdown, mortality and droplet spectrum for sUAS application of deltamethrin formulation against *Culex quinquefasciatus* in California

Group and Actual Application Rates (lbs/acre)	Distance from Spray Path (ft)	% Knock-down[A]	% Mortality[A]	Avg. Droplet Density[B] (droplets/mm^2)	Avg. Droplet $DV_{0.5}$ Size[B] (microns)
Treatment = 0.00089	0	99.3	100.0	1.34	32.78
	200	94.5	100.0	1.32	31.15
	400	99.4	100.0	1.12	50.18
Untreated = 0	NA	1.3	1.3	0.00	NA

[A] Knockdown and mortality percentages displayed here are a result of pooling two replicate cages and three replicate rows (A, B, and C) within each spray distance for each group.
[B] Droplet density and size are presented as averages of replicate rows A, B and C within each spray distance for each group.

The differences in the overall droplet spectrum at the untreated control plots in comparison to the treatment plots indicated that the droplets in the control plots were likely substances that were not relevant to the test item applied. The similarity in the droplet spectrum of the three distances from the spray path between two trials indicated that the amount of deltamethrin formulation had been evenly dispersed in a large portion of the target treatment plots.

CONCLUSIONS

Aerial calibration sprays determined that the droplet sizes dispersed from the nozzles of the Small Unmanned Aircraft System (sUAS) were within the range of values of $DV_{0.5}$ < 60 microns and $DV_{0.9}$ < 115 microns specified in the deltamethrin product label. The consistency in the droplet spectrum observed among the three spray path distances for both trials indicated an even dispersion of the deltamethrin formulation on a large portion of the two treatment plots. Greater than 94% knockdown at 1 hr after exposure and complete mortality at 12 hrs after exposure were achieved in both trials. These results indicated that the small unmanned aircraft system application of deltamethrin formulation at low rate of 0.00089 lbs AI/acre was effective at adulticiding against *Aedes aegypti* and *Culex quinquefasciatus*.

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REFERENCE CITED

Carter, S.W. 1989. A review of the use of synthetic pyrethroids in public health and vector pest control. Pestic. Sci. 27:361-374.

Elliott, M., N.F. Janes, C. Potter, 1978. The future of pyrethroids in insect control. Ann. Rev. Entomol. 23:443-469.

Focks, D.A., K.O. Kloter, G.T. Carmichael, 1987. The impact of sequential ultra-low volume ground aerosol applications of malathion on the population dynamics of *Aedes aegypti* (L). Am. J. Trop. Med. Hyg. 36:639-647.

Reiter, P., M.B. Nathan, 2001. Guidelines for assessing the efficacy of insecticide space sprays for control of the dengue vector *Aedes aegypti*. WHO/CDE/CPE/PVC2001.1. World Health Organization, Geneva, Switzerland.

World Health Organization, 2019. List of WHO prequalified vector control products. https://www.who.int/pq-vector-control/prequalified-lists/en.