SEMI-FIELD EVALUATION OF ULTRA-LOW VOLUME (ULV) GROUND SPRAY OF AQUALUER® 20-20 AGAINST IRRADIATED *AEDES AEGYPTI*

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ABSTRACT

Sterile insect technique (SIT) using irradiated mosquitoes is an effective control method capable of being assimilated into integrated vector management (IVM) programs. Chemical control of mosquitoes using ultra-low volume (ULV) spray applications of pyrethroid and organophosphate insecticides is already an essential component of IVM programs. Prior to their release in nature, irradiation of mosquitoes for SIT use can significantly impact the mosquito's biology, specifically its host-seeking and feeding behavior. Little is known about how radiation exposure might impact a mosquito's susceptibility to pyrethroid insecticides. The present study was carried out to evaluate the influence of Aqualuer® 20-20 ULV applications on irradiated *Aedes aegypti*. Caged mosquito trials indicated that both male and female irradiated *Ae. aegypti* were as susceptible as their non-irradiated counterparts of the same population to Aqualuer 20-20 ULV application, with the highest mean percent mortalities achieved at the first 24h post-treatment period at both 30.5 m and 61 m downwind of the spray application path.

Key Words: Sterile insect technique, irradiated mosquitoes, *Aedes aegypti*, ULV, integrated vector management

INTRODUCTION

Aedes aegypti (L.) is the principal vector of several emerging and re-emerging arboviral disease agents including dengue, chikungunya, Zika and yellow fever viruses in tropical and subtropical regions worldwide (Bonica et al. 2019, Gubler 2002, Higgs and Vanlandingham 2015, Kraemer et al. 2015, Reiskind et al. 2008, Thavara et al. 2009, Weaver and Reisen 2010). In the absence of effective vaccines or drugs to prevent or treat these diseases, the most effective strategy has been to disrupt the virus transmission cycle by reducing the frequency of human-vector contact (Wilder-Smith et al. 2017). Contemporary vector control methods, such as thermal or ULV space spray and larvicide applications to reduce adult and larval vector populations and physical methods used to reduce breeding sites or to deter vector contact with humans, have limited ability to effectively control vector populations. These methods are best applied as part of an integrated vector management (IVM) program (Esu et al. 2010, Lima et al. 2015, Marini et al. 2019), in which the chemical and non-chemical vector control methods are appropriately integrated to achieve the optimal effectiveness (WHO 2020). In fact, there is evidence that currently used insecticide applications have led to the development of insecticide resistance in *Ae. aegypti* (Deming et al. 2016, Ishak et al. 2015). Therefore, the need for novel complementary vector control tools that are effective, sustainable, and environmentally benign is becoming a high priority (Fernández-Salas et al. 2015).

Sterile insect technique (SIT) is an environmentally safe control method, being species specific and without leaving any chemical residues (Alphey et al. 2010). One component of SIT involves chemo-sterilization of male insects. It requires colonization and mass rearing of the target insect, the sterilization of large numbers of the reared male insects by ionizing irradiation using gamma- or X-rays and their subsequent periodic release into the target area, where they compete with wild males for mating with wild females. Those wild females lay only sterile eggs which in turn leads to suppression of the population. Irradiation-based SIT has been used successfully since the 1930's to control many agricultural and other pests such as Mediterranean fruit fly (*Ceratitis capitata,* Weidemann), screw worm (*Cochliomyia hominivorax,* Coquerel), pink bollworm (*Pectinophora gossypiella,* Saunders), and tsetse fly (*Glossina austeni,* Wiedemann) (Cayol et al. 2002, Dowell et al. 1998, Henneberry 1994, Vargas-Teran et al. 2005, Vreysen et al. 2000). Studies have demonstrated that this technique has been successfully used against several mosquito species including *Ae. albopictus* (Skuse), *Anopheles albimanus* (Weidemann) and *Culex quinquefasciatus* (Say) (Bellini et al. 2013, Benedict and Robinson, 2003, Lofgren et al. 1974, Patterson et al. 1970). The optimal use of SIT in vector control should be within an IVM program, with the potential to reduce the vector population below an arbovirus transmission threshold (Alphey et al. 2010).

Ultra-Low Volume (ULV) ground-spray application of adulticides is often a key and effective component of IVM programs to reduce arbovirus vector and nuisance biting mosquitoes (Faraji 2016). Pyrethroids, such as permethrin, are commonly used in ULV adulticide programs (EPA 2019) due to their relative stability and low toxicity to a wide range of insects at low application rates used for mosquito control applications (Elliott 1976). ULV spray of Aqualuer® 20-20 (20.6% permethrin and 20.6% piperonyl butoxide; AllPro Inc., St. Joseph, MO) is one of the main components of the IVM program of the Anastasia Mosquito Control District (AMCD), located in St. Augustine, Florida.

In 2017-2018, AMCD conducted regular ULV applications of Aqualuer 20-20 in response to service requests stating that residents were concerned about an abundance of the nuisance salt marsh mosquito, *Ae. taeniorhynchus* (Wiedemann). The service requests coincided with areas where irradiated male *Ae. aegypti* were being released for SIT trials. However, with SIT trials in progress, very little research had been done to investigate any potential discrepancy in the effects of ULV sprays on released irradiated male mosquitoes compared to wild males of the same species and the implication on how this could impact future SIT releases. The present study was carried out to determine the impact of Aqualuer 20-20 ULV ground application on irradiated *Ae. aegypti.* It would help to determine if ULV spraying could be used to selectively reduce wild males within a SIT program to increase the chances of remaining wild females mating with irradiated males thus warranting the incorporation of SIT into the IVM program.

MATERIALS AND METHODS

Semi-field trials (WHO 2009) were conducted with laboratory-reared, irradiated and non-irradiated, male and female *Ae. aegypti* of the same population (St. Augustine strain) in a 90 m x 90 m grid test site at AMCD. Mosquitoes were reared in insectaries at the United States Department of Agriculture's Center for Medical, Agricultural & Veterinary Entomology (USDA-CMAVE), in Gainesville, Florida. The incubators (Percival Scientific, Perry, IA) were maintained at 28° ± 1°C, 70% relative humidity (RH) and 14:10 L:D photoperiod. Immatures were fed on a diet of pulverized tetramin *ad libitum* and adults were fed *ad libitum* with 10% sucrose solution soaked in cotton balls. Male and female *Ae. aegypti* pupae were irradiated with 50 Gray (Gy) by γ-radiation using a Gammator M (Radiation Machinery Corp., Parsippany, NJ) containing a cesium-137 source that generated 8.8 Gy/min. The radiation doses applied to pupae were 0 and 50 Gy, with the 0 Gy acting as a control. Radiation doses were checked with alanine films applied to petri dishes with pupae for every dose.

Nine sentinel cage poles were distributed in the treatment plot in a 3 x 3 grid with 30.5 m separations between each row. The sentinel poles were placed at 30.5 m, 61.0 m and 90.4 m downwind of the spray-truck path (Fig. 1). Additionally, three control sentinel cage poles were positioned upwind of the spray zone. A weather station (WatchDog 2550, Spectrum Technologies Inc., Aurora, IL) was placed in the treatment plot to monitor wind speed and wind direction to select time of application and the direction of the spray-truck path. Temperature and RH were recorded immediately before and after each application. Twenty mosquitoes from all 4 groups (irradiated males and females, and non-irradiated males and females) were aspirated into 4 separate cylindrical screened paper cages (10 x 4 cm) to make a set. Each set of 4 cages were mounted on the sentinel cage poles approximately 1.2 m above ground level in the treatment plot. A rotating impinger (Leading Edge Associates Inc., Fletcher, NC) with two Teflon-coated glass slides was fixed to each sentinel cage pole for the verification of insecticide reach. A truck-mounted single-nozzle ULV cold aerosol sprayer (Guardian 95ES, Adapco, LLC, Sanford, FL) was driven at 16 km/h perpendicular to the wind direction with an application rate of 2.9 to 3.5 L/hectare and droplet size (mass median diameter) of 25.7 microns. Dilution of the insecticide was 1 part Aqualuer 20-20 to 9 parts water. The spray-tuck started 30.5 m prior to the first cage pole of the row and was stopped 30.5 m after the last cage pole to ensure the spray coverage was sufficient. Paper cages and Teflon slides were collected and brought back to the laboratory 15 min post application. The three sentinel control poles with cages were placed upwind of the spray zone for 15 min just prior to starting the treatment, collected, and returned to the laboratory. All the cages were provided with a cotton pad soaked in 10% sucrose solution and the number of knocked down mosquitoes in each cage was recorded after 1 h. Mortality counts were taken at 24 h and 48 h post application. Three successful replications were conducted in June/July 2019 between 0730 to 0930 with at least one week separating the evaluations.

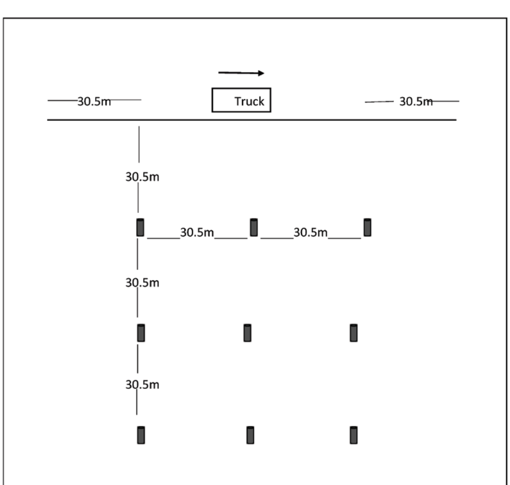

Figure 1. Layout of sentinel cage poles and the direction of spray-truck path.

Data were analyzed using SPSS (IBM® SPSS® statistics, V. 20). A Kruskal-Wallis test and Mann-Whitney U test was used appropriately for comparisons because the Shapiro-Wilk normality test could not confirm the normal distribution of data sets.

RESULTS

Immediate effects of Aqualuer 20-20 ULV application on irradiated and non-irradiated adult mosquitoes were determined by comparing percent knockdown between treatment and control groups at 1 h post application. First, the percent knockdown of the four groups - irradiated and non-irradiated, control and treatment - were analyzed separate to determine any significant differences between the sexes. Since there were no statistically significant differences between the sexes in any of the groups (P>0.05 for all), data for sexes were pooled to compare the effect of the distances from the spray path. Percent knockdown showed a highly significant difference among the downwind distances of both irradiated ($\chi^2_{(2)}$ = 18.98, P<0.001) and non-irradiated mosquitoes ($\chi^2_{(2)}$ = 14.55, P<0.01). Significantly higher knockdown was observed at 30.5 m downwind than at 61 m (Mann-Whitney U = 81.5, P<0.05 for the irradiated mosquitoes and U = 91.0, P<0.05 for the non-irradiated mosquitoes). Knockdown was higher at 61.0 m than 90.4 m only in irradiated mosquitoes ($U = 92.0$, $P<0.05$). As there were significant differences in knockdown between downwind distances, control and treatment groups were compared at different distances to determine the immediate effect of the ULV application. The immediate effects of Aqualuer 20-20 ULV application on both irradiated and non-irradiated mosquitoes were statistically significant only at 30.5 m downwind of the spray path ($U = 55.0$, $P<0.01$ and $U = 52.5$, $P<0.01$, respectively (Fig. 2).

Forty-eight-hour post application mean percent mortalities of the treatment group were below 25% while those in the control group were below 4%. Although there were no statistically significant differences in mortality between the two sexes, corresponding mortality of males was always higher than that of treated females while it was lower than the control females (Table 1). Once the mortality data of the two sexes was pooled, the differences in percent mortality of the treatment group were significant among the downwind distances [$\chi^2_{(2)} = 9.15$, $P<0.05$ and $\chi^2_{(2)} = 7.72$, $P<0.05$ for irradiated and non-irradiated mosquitoes, respectively]. The observed differences were only between the 30.5 m and 90.4 m downwind distances ($U = 75.5$, $P<0.05$ and $U = 67.5$, $P<0.05$ for irradiated and non-irradiated mosquitoes, respectively). Delayed effects of Aqualuer 20-20 ULV application on irradiated and non-irradiated mosquitoes, ascertained by comparing mortality at 48 h post application between the treatment and control groups were statistically significant at 30.5 m ($U = 56.0$, $P<0.001$ and $U = 89.0$, $P<0.001$ respectively) and 61.0 m ($U = 50.5$, $P<0.05$ and $U = 84$, $P<0.01$ respectively) downwind of the spray path. There were no statistically significant differences in mortality between the treatment groups of the irradiated and non-irradiated mosquitoes at any of the distances (Fig. 3). Percent mortalities were significantly higher at the first 24 h period than at the second 24 h period at both 30.5 m ($U = 83.5$, $P<0.05$ for irradiated mosquitoes; $U = 41.0$, $P<0.001$ for non-irradiated mosquitoes) and 61.0 m ($U = 100.0$, $P<0.05$ for irradiated mosquitoes; $U = 81.5$, $P<0.01$ for non-irradiated mosquitoes) downwind of the spray path.

Environmental temperature, RH and wind speed at both control and treatment sites for all replicates ranged between 25.5-29.4°C, 69-88% and 3.2-8.0 km/h, respectively. Teflon-coated slide readings recorded that the droplet density ranged from <10 droplets/mm^2 (on slides placed at 30.5 m) to <5 droplets/mm^2 (on slides placed at 91.4 m).

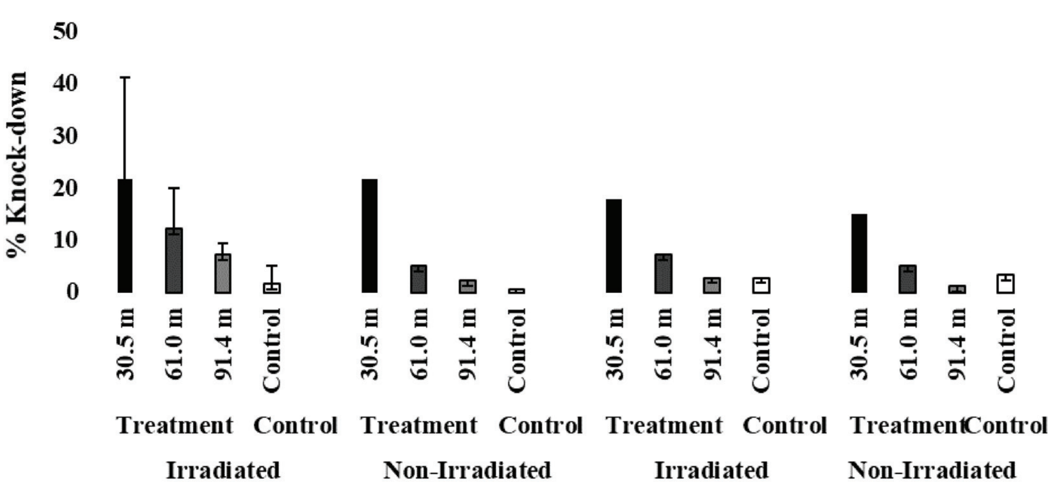

Figure 2. One-hour post-treatment knockdown between treatment and control groups of irradiated and non-irradiated *Aedes aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray at different downwind distances.

Table 1. Forty-eight-hour post-treatment mortalities of male and female irradiated and non-irradiated *Ae. aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray at different distances (mean ± standard error).

	Irradiated *Ae. aegypti*		Non-irradiated *Ae. aegypti*	
	male	female	male	female
30.5 m	21.70 ± 5.59	17.78 ± 5.40	26.67 ± 7.5	17.22 ± 7.08
61.0 m	12.22 ± 5.84	7.22 ± 1.88	11.11 ± 3.41	10.00 ± 4.17
90.4 m	7.22 ± 2.06	2.78 ± 0.88	6.11 ± 2.00	3.89 ± 2.17
Control	1.60 ± 0.83	2.78 ± 0.88	0.56 ± 0.56	3.33 ± 1.17

DISCUSSION

This study demonstrated that both irradiated and non-irradiated *Ae. aegypti* mosquitoes were equally susceptible to Aqualuer 20-20 ULV applications at least up to 61.0 m downwind. The insecticide application did not show any difference in mortality between sex, and the highest mortality was achieved within 24 h post-treatment. This indicates that Aqualuer 20-20 ULV applications would immediately knockdown both male and female *Ae. aegypti* mosquitoes in the environment without regard to sterilization status. Since it is imperative that released SIT male mosquitoes should have a maximum lifespan (Culbert et al. 2020) to disperse well in the environment, find wild females cohorts, and mate successfully, the simultaneous use of ULV applications to control other species in the same area as the release site would likely negatively impact the efficacy of the SIT release. Because of this, SIT might be better at targeting the last remaining vectors rather than targeting when populations are elevated. As the effectiveness of the SIT program is related to the ratio of released males to wild fertile females, and released sterile males will actively seek out wild females, SIT can target these remaining individuals and reduce the population further, probably from low to zero (Alphey et al. 2010).

Low mortality rates (<25%) observed during the Aqualuer 20-20 ULV spray could be due to several reasons: spray trials might

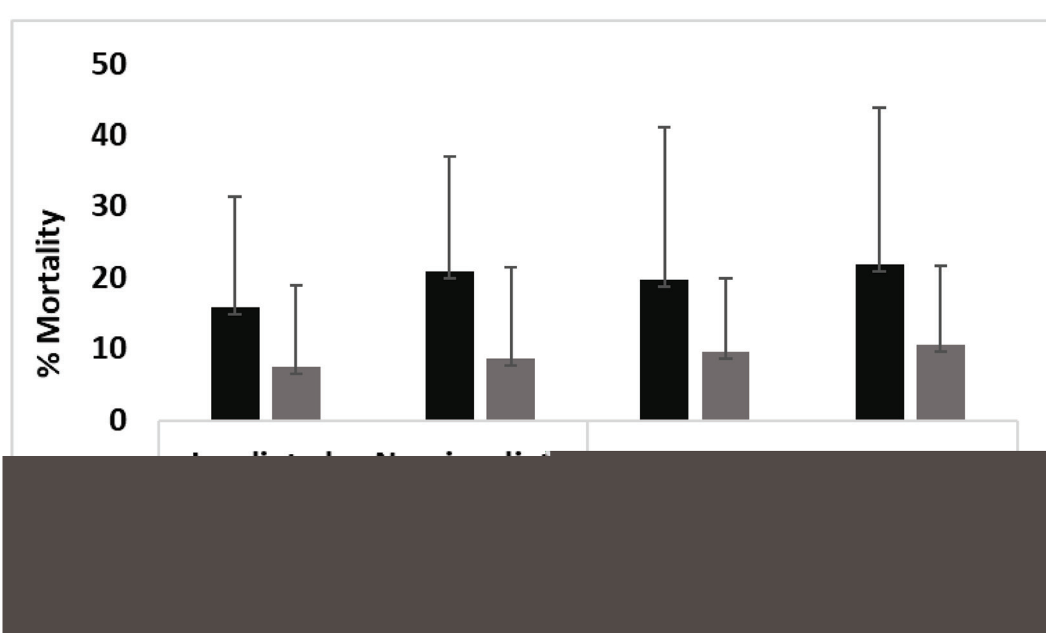

Figure 3. Cumulative mortality between irradiated and non-irradiated *Aedes aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray at different downwind distances.

Table 2. Comparison of cumulative mortality between treatment and control sets of irradiated and non-irradiated *Aedes aegypti* exposed to Aqualuer® 20-20 ultra-low volume spray (Mann-Whitney test).

	Irradiated *Ae. aegypti*						Non-irradiated *Ae. aegypti*					
	30.5 m		61.0 m		90.4 m		30.5 m		61.0 m		90.4 m	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
U	60.0	56.0	95.5	89.0	145.5	115.0	48.0	50.5	87.0	84.0	144.0	117.5
p	0.001	0.000	0.019	0.013	0.519	0.104	0.000	0.000	0.010	0.008	0.502	0.116

U = Mann-Whitney U, p = significance level

have been affected by the sub-optimal wind speeds or the tested strain of *Ae. aegypti* might have developed resistance to pyrethroids. According to WHO (2009), outdoor small-scale insecticide spray applications should not take place when wind speeds falls below 3 km/h. Pesticide drift potential is lowest at wind speeds between 4.8 and 16 km/h (Fishel and Ferrell 2010). Two of the three replicates of this study were conducted at 3.2 km/h and the low number of droplets on Teflon slides may indicate a spray drift, although there was a significant difference in mortality between control and treatment groups. Insecticide resistance status of this strain of *Ae. aegypti* is not known and unfortunately the study did not compare the effect of ULV application between a susceptible strain and the test strain. Such a comparison using CDC bottle bioassay would have provided information to ascertain whether the low mortality rates are due to acquired insecticide resistance. However, these results clearly show significant differences in mortality between control and treatment mosquitoes of both irradiated and non-irradiated groups. Further studies need to be conducted at optimal environmental conditions, especially at higher wind speeds, allowing for optimal downwind insecticidal spread to better characterize the influence of the ULV application and with a pyrethroid susceptible strain of *Ae. aegypti* to compare the effect with the test strain. As this is a semi-field experiment conducted with laboratory-reared mosquitoes, large scale field studies with released irradiated and wild males would be a supplement to the findings.

We believe that this is the first study to evaluate the impact of insecticide ULV spray on irradiated *Ae. aegypti*. Our results provide the first scientific evidence to support the commonly accepted belief that simultaneous use of SIT and ULV control strategies are not compatible for the control of *Ae. aegypti* populations, hence, SIT would be well suited toward the end of a IVM program to target the last remaining individuals of a population. This information will be of value in planning IVM programs that wish to incorporate SIT and adulticiding spray operations.

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