SEMI–FIELD EVALUATION OF ULTRA-LOW VOLUME (ULV) GROUND SPRAY OF AQUALUER® 20-20 AGAINST CAGED *AEDES ALBOPICTUS* AND NON-TARGET HONEY BEE, *APIS MELLIFERA*

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ABSTRACT

Application of permethrin products by ultra-low volume (ULV) spraying against the container-inhabiting mosquito *Aedes albopictus* (Skuse) has been used for many years, but the impact of the insecticides on domesticated honey bees, *Apis mellifera* (Linnaeus) is still lacking. The present study was carried out to evaluate the impact of the permethrin product, Aqualuer® 20-20 (active ingredient: 20.6% permethrin+20.6% Piperonyl butoxide) ULV sprays on caged *Ae. albopictus* and *A. mellifera* in open semi-field conditions with cages spaced at 3 m, 22.8 m, and 45.7 m downwind of the spray-truck path. The results indicated that ULV spray of Aqualuer 20-20 is highly effective against *Ae. albopictus* achieving 94% mortality at 22.8 m and 82% mortality up to 45.7 m downwind distance. The highest mortality of *A. mellifera* was only 72% at 3 m downwind distance, but the spray killed 42% of the exposed bees up to 45.7 m down the spray path. This semi-field study conducted during the day time indicates the high effectiveness of the ULV spray of permethrin against *Ae. albopictus* and its comparatively low impact on the direct exposed non-target honey bee, *A. mellifera*. Further studies designed to be conducted in the natural environment during its real-time operations following label instructions of the insecticide will help establish spraying guidelines to minimize any unfavorable impact on domesticated *A. mellifera* while having expected mortality effects on *Ae. albopictus*.

Key Words: *Aedes albopictus*, *A. mellifera*, ULV, integrated vector management, mortality

INTRODUCTION

Aedes albopictus (Skuse) is a nuisance and a potential disease vector of several emerging and re-emerging arboviral diseases (Paupy et al. 2009, Weaver & Reisen 2010). It has been shown to be capable of transmitting at least 26 arboviruses including dengue (Shroyer 1986, Reiskind et al. 2008, Thavara et al. 2009, Rezza 2012), chikungunya (Bonilauri et al. 2008, Vega-Rúa et al. 2014), Zika (McKenzieI et al. 2019) and yellow fever (Fadila et al. 2016, Amraoui et al. 2018) viruses in tropical and subtropical regions worldwide. With the emergence of Chikungunya virus (CHIKV) in 2006-2007 in many countries, *Ae. albopitus* has been implicated as a main vector of the disease (Gould et al. 2010). Since its discovery in Harris County, Texas, in 1985, *Ae. albopictus* has spread widely in the continental United States (Moore & Mitchell 1997). After the initial invasion of the northern parts in 1986 it has now well established across Florida (O'Meara et al. 1993, Hornby et al. 1994, O'Meara et al. 1995, Juliano et al. 2004, Reiskind & Lounibos 2021). Although low competence vectors for Zika virus, *Ae. albopictus* from Florida was found to be at least two times more susceptible to the infection than *Ae. albopictus* collected in Brazil, where an outbreak of Zika occurred in 2015 (Chouin-Carneiro et al. 2016). Control of the populations of *Ae. albopictus* has thus become a crucial component in any mosquito control program in Florida as well as across the world.

Chemical control using mosquito adulticides applied as ultra-low volume (ULV) sprays is often a key and effective component of integrated vector management (IVM) programs to reduce arbovirus vector and nuisance biting mosquitoes (CDC 2013, Faraji et al. 2016). The ultra-low-volume (ULV) space sprays spread small aerosol particles of insecticides targeting adult mosquitoes as they are flying (CDC 2003). Previous studies report on detrimental effects of direct exposure from both ground and aerial ULV insecticide sprays on non-target organisms like honey bees (Caron 1979, Pankiw and Jay 1992, Hester et al. 2001, Zhong et al. 2003). Some studies report ULV spraying of insecticides had little or no impact on other flying insects with medium to large body mass (Boyce et al. 2007, Kwan et al. 2009, Schleier III and Peterson 2010). In addition, Rinkevich et al. (2017) and Pokhrel et al. (2018) have demonstrated low impact of ground pyrethroid ULV sprays on honey bees while providing effective mosquito control.

In the past decade, the Anastasia Mosquito Control District (AMCD) of St. Johns County, Florida has conducted regular truck-mounted ULV sprays of the pyrethroidbased Aqualuer® 20-20 (active ingredients-20.6% permethrin and piperonyl butoxide 20%, AllPro Inc., St. Joseph, MO) in response to residential complaints about nuisance problems caused by container-inhabiting mosquitoes. However, the effects of these ULV sprays on domesticated honey bees, *A. mellifera* the crucial pollinators for many economically important fruit crops, has not been evaluated. The domesticated honey bee provides greater economic benefit to people than any other arthropod found in Florida (University of Florida 2018) including the St. Johns county. Florida is also the nation's third-largest honey producer. The honey bees are crucial pollinators of many economically important fruit crops such as strawberries, blueberries, squash, watermelons and avocados. Furthermore, they support several other commercial activities including selling beeswax, pollen, royal jelly and propolis also known as "bee glue" (University of Florida 2018). Therefore, honey bee health is so important to Florida crop production. This preliminary study was carried out to determine the direct spatial impact of ULV ground-spraying of Aqualuer 20-20 under semi-field conditions on caged *Ae. albopictus* and domesticated honey bee, *Apis mellifera* (L.) so that ULV spraying guidelines could be improved accordingly.

MATERIALS AND METHODS

Ae. albopictus pupae were obtained from the United States Department of Agriculture-Center for Medical, Agricultural & Veterinary Entomology (USDA-CMAVE), Gainesville, Florida, and maintained in an insectary at 28±2°C, 40-70% relative humidity, and a photoperiod of 14L:10D until adult emergence. Adults were kept in flight cages in the same insectary and provided with 10% sucrose solution *ad libitum.* Adult females used in the semi-field trials were 4-7 days old. Female *A. mellifera* worker bees >7 d old were collected from frames of beehives from the honey bee apiary of the Entomology and Nematology Department of the University of Florida. Bees were collected and transferred to cages one day prior to the test. They were maintained in a laboratory with windows (natural photo period) at 22±2 °C with and an RH between 40-70%, provided with 50% sucrose solution *ad libitum.*

Semi-field evaluations (WHO 2009) of ULV application against the two species were conducted with in a 90 m x 90 m grid test site at the St. Augustine Gun Club, Florida. Ten female mosquitoes were aspirated into each of the 12 cylindrical screened paper cages (10 x 4 cm). Ten honey bees were introduced into each of 12 separate paper cages similar to the ones used for the mosquitoes. One cage with mosquitoes and one with honey bees were placed on each of 12 pipe stands at ~ 1.2 m above ground level in the field evaluation area. Three pipe stands with control cages were placed upwind of the spray zone, just prior to starting the treatment, and left in place for 15 min. After this period, the control cages were collected and returned to the laboratory to avoid exposure to pesticide applications.

Pipe stands for the treatment experiment were placed in a 3 x 3 grid with the three rows standing 3 m, 22.8 m, and 45.7 m downwind of the spray-truck path. A truck-mounted single-nozzle ULV cold aerosol sprayer (Guardian 95ES, ADAPCO, LLC, Sanford, FL) was driven at 16 kmph in a path perpendicular to the wind direction with a flow rate of 1.18-1.42 L/0.41 hectare and droplet size (mass median diameter) of 25.7 microns. The insecticide was diluted to 1:9 (Aqualuer 20-20:water) as per the label instructions. The treatment started 30.5 m prior to the first pipe stand of the row and was stopped 30.5 m after the last stand to ensure sufficient spray coverage. Cages were collected 15 minutes after the treatment and taken to the laboratory. Once collected, each mosquito and honey bee cage was provided with a cotton pad soaked in 10% sucrose solution (mosquitoes) or 50% sucrose solution (honey bees). The number of knocked-down mosquitoes and honey bees in each cage was recorded at 1 h and 12 h post-treatment. Mortality counts were taken at each 24 h and 48 h post exposure and percent mortalities were used in the analyses. Three successful replications were conducted between 07:30 h to 09:30 h with at least one week separating the evaluations.

SPSS (IBM SPSS Statistics for Windows, version XX (IBM Corp., Armonk, N.Y., USA) software package was used to analyze the arcsine transformed percent mortality data. Normality of data sets was determined by the Kolmogorov−Smirnov normality test. Means were compared using the independent t-test to determine the effect of the insecticide compared to controls. The differential effects of the insecticide at different upwind distances during different post-exposure periods were determined by using the two-way ANOVA test with post hoc Tukey pairwise comparisons. Significance level was maintained at 0.05.

RESULTS

ULV spray of Aqualuer 20-20 has induced a significant immediate (knockdown) effect followed by high mortality on *Ae. albopictus* and *A. mellifera* at both post exposure periods at all three downwind distances of the spray path, compared to respective controls ($P<0.05$ for all) (Table 1). The two-way ANOVA confirmed no interactions between the distance and post-exposure period on either knockdown or mortality of both species ($P>0.05$). As observed by simple main effects, the effects on different post-exposure periods of knockdown and mortality were not significantly different in both species (P>0.05 for all). However, the downwind distance had a significant effect on both knockdown (F=3.453, P=0.04) and mortality (F=3.83, P=0.03) of *A. mellifera* only. The knockdown of *A. mellifera* was significantly higher at 22.8 m than 45.7 m (P=0.033) while the mortality was significantly higher at 3 m than 45.7 m (P=0.029). The highest knockdown of *A. mellifera* was at 22.8 m (91.1 ± 4.84) (mean ± standard error) while the highest knockdown of *Ae. albopictus* was observed at both 3 m and 22.8 m (84.4 ± 11.07) (Figure 1). With some recoveries of knocked-down *A. mellifera*, the highest mortality occurred at 3 m (72.2 ± 9.83) followed by 22.8 m (68.9 ±7.89) and 45.7 m (41.67 ±15.14) (Figure 2). Without any recovery, the highest mortality of *Ae. albopictus* was at 22.8 m (94.4 ± 4.44) and the lowest mortality was at 45.7 m (82.2 ± 10.9) with no significant differences between any pair of distances (P>0.05 for all).

As there were no significant interactions of distance and the species on both knockdown and mortality, the simple main effects of the two variables were observed. The effects of distance were significant on knockdown (F=3.552, P=0.032) as well as on mortality (F=3.452, P=0.036) of the two species. Although the effects of the two species on knockdowns were not different (P>0.05 for both), *Ae albopictus* had a significantly higher mortality (F=23.009, P<0.0005) than *A. mellifera* both at 22.8 m (t= 4.323, P<0.0005) and at 45.7 m (t=3.454, P=0.002).

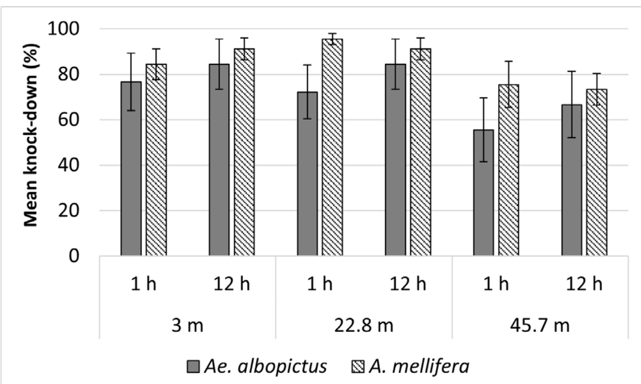

Figure 1. Mean percent knock-down of *Aedes albopictus* and *Apis mellifera* at different post-exposure periods of Aqualuer 20-20 at different downwind distances from the spray path (error bars indicate the standard error of the mean).

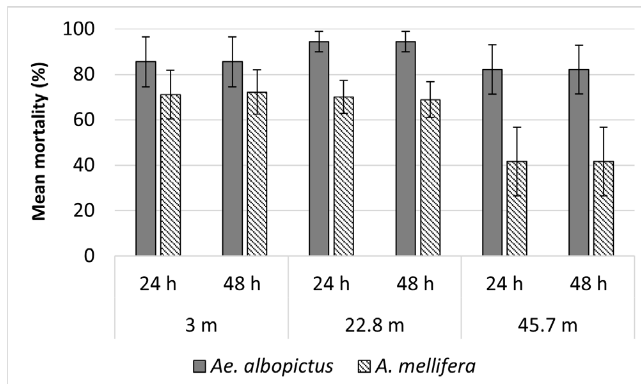

Figure 2. Mean percent mortality of *Aedes albopictus* and *Apis mellifera* at different post-exposure periods of Aqualuer 20-20 at different downwind distances from the spray path (error bars indicate the standard error of the mean).

Table 1. Effectiveness of ultra-low volume spray using Aqualuer 20-20 against *Aedes albopictus* and *Apis mellifera* compared to respective controls at different downwind distances and post-exposure periods (mean knock-down/mortality ± standard error of the mean).

	Aedes albopictus				*Apis mellifera*			
		Treatment				Treatment		
	Control	3 m	22.8 m	45.7 m	Control	3 m	22.8 m	45.7 m
1 h	1.11±1.11	76.7±12.7	72.2±11.9	55.6±14.1	0	84.4±6.7	95.6±2.4	75.6±10.2
12 h	1.11±1.11	84.4±11.1	84.4±11.1	66.7±14.7	1.11±1.11	85.6±11.2	91.1±4.8	73.3±6.9
24 h	3.33±2.36	85.6±10.9	94.4±4.4	82.2±10.9	2.22±2.22	71.1±10.7	70.0±7.3	41.7±15.1
48 h	3.33±2.36	85.6±10.9	94.4±4.4	82.2±10.9	5.56±3.77	72.2±9.8	68.9±7.9	41.7±15.1

DISCUSSION

This study was conducted to determine the effectiveness of ULV spray of Aqualuer 20-20 against *Ae. albopictus* and its impact on the non-target pollinator, *A. mellifera.* The semi-field experiments using caged insects exposed to the direct spray demonstrated its high effectiveness of 82 - 94% mortality against *Ae. albopictus* within 24 h, up at least to 45.7 m from spray-truck path, which is the maximum distance we tested. This result corroborates the findings of previous semi-field and field studies conducted with Aqualuer 20-20 and other pyrethroids (Farajollahi et al. 2012, Suman et al. 2012, Xue et al. 2013, Bengoa et al. 2014).

In contrast to the insignificantly higher knockdowns of *A. mellifera*, the effects of Aqualuer 20-20 ULV sprays were significantly higher on mortality of *Ae. albopictus.* This indicates a significant recovery of *A. mellifera* from immediate effects of the treatment which could be attributed to its larger body mass (Sanchez-Arroyo et al. 2019), as heavier insects exhibit decreased sensitivity to insecticides applied using ULV technique (Schleier and Peterson 2010a). Decrease in bee mortality with the increasing distance can be explained as a combined effect of the generally expected decrease in insecticide droplet concentration (Schleier and Peterson 2010b, Rinkevich et al. 2017) and the larger body mass of the bees. The highest mortality (72%) of direct exposed *A. mellifera* at 3 m downwind and 69% mortality at 22.8 m downwind are unlikely in operational conditions as many mosquito control programs create buffer zones around beehives. For example, AMCD applicators turn off sprayers at 30.5 m away from notified beehives. It should be noted that bee keepers across the county are encouraged to notify AMCD about locations of their beehives. The results of this semi-field, daytime experiment indicate that Aqualuer 20-20 ULV sprays could be used with high effects against *Ae. albopictus* at least up to 45.7 m while having ~50% less effects on *A. mellifera.* However, the noteworthy impact of 42% mortality of direct exposed *A. mellifera*, up to 45.7 m downwind distance should be further investigated in operational conditions. During operational mosquito control, ULV spraying is only limited to night and early morning applications when mosquito flight activity is high while honey bees are inside their hives and not active, thus not exposed directly to the air-borne insecticides. Therefore, carefully planned operational ULV spraying should have little opportunity to contact and kill honey bees while having a high impact on mosquitoes. Pokhrel et al. (2018) have demonstrated that operational ULV applications of different pyrethroid insecticides made just after sunset (between 7:00 pm to 10:00 pm) following label regulations and using properly calibrated equipment had no significant impact on *A. mellifera* in terms of mortality or brood development. Further studies under operational conditions and in the natural environment will help to look over these preliminary results obtained using caged insects that directly intercept insecticide droplets. Experiments that do not use forced exposure situations that are not normally encountered by bees would provide better insight on potential exposure hazards for bees. These results can help mosquito control programs interested, in taking extra precautions such as determining the buffer zone distance, time of spraying, when planning control operations in order to minimize the impact on honey bees.

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