COMPARATIVE EFFICACY OF FIVE PERMETHRIN/ PBO 30-30 GROUND ULV INSECTICIDES AGAINST FIELD COLLECTED ADULT *AEDES AEGYPTI*, *AEDES TAENIORHYNCHUS*, AND *CULEX QUINQUEFASCIATUS* IN MANATEE COUNTY, FLORIDA

KATIE F. WILLIAMS[1], EVA A. BUCKNER[2], AMBYR L. MARICANO[1], MARK D. LATHAM[1], AND CHRISTOPHER R. LESSER[1]

[1]Manatee County Mosquito Control District, 2317 2nd Ave W, Palmetto, FL 34221

[2]University of Florida/IFAS, Florida Medical Entomology Laboratory, 200 9th Street SE, Vero Beach, FL 32962

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ABSTRACT

We investigated five formulations containing synergized permethrin/PBO active ingredients, Biomist® 30-30, Evoluer® 30-30, Kontrol™ 30-30, Permanone® 30-30, and Perm-X™ UL 30-30, to determine whether there was variation in efficacy against caged local field collected adult *Aedes aegypti*, *Aedes taeniorhynchus*, and *Culex quinquefasciatus* mosquitoes. Mortality data from field trials with these formulations applied via truck mounted ultra-low volume sprays at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) label rates indicated generally low efficacy against *Ae. aegypti* and *Cx. quinquefasciatus* but generally high efficacy against *Ae. taeniorhynchus*. We discuss potential underlying mechanisms for this variation including effects of meteorology and resistance, and how field-derived efficacy data may be used operationally by mosquito and vector control districts to mitigate cost, environmental impact, and pesticide resistance.

Key Words: Adulticide, pyrethroid, permethrin, resistance

Chemical control with ground ultra-low volume (ULV) adulticides is an effective component of integrated vector management (IVM) to reduce arbovirus vector and nuisance biting mosquitoes (Faraji et al. 2016). Permethrin, a pyrethroid, is an active ingredient in adulticides commonly used in mosquito control due to its relative stability, high toxicity to a wide range of insects at low dosages, and rapid knock-down effects (Smith and Stratton 1986). Field trials with caged sentinel mosquitoes provide data on the potential efficacy of adulticide formulations against natural mosquito populations and, with increased insecticide resistance and environmental concerns, evaluations of different formulations are a necessary component of improved operational planning and decision-making (Farajollahi and Williams 2013). For example, in previous field trials we observed relatively low efficacy of a formulation containing synergized permethrin and PBO against *Aedes aegypti* compared to formulations containing deltamethrin or plant-derived pyrethrins (Buckner et al. 2016). In that study, however, only one permethrin formulation was investigated. Thus, in the present study we investigated whether there is variation in efficacy among five ground ULV adulticide formulations each containing a 30-30 ratio of synergized permethrin and PBO active ingredients against field collected natural populations of adult *Aedes aegypti* (L.) as well as *Aedes taeniorhynchus* (Wiedemann), and *Culex quinquefasciatus* Say mosquitoes.

We conducted truck-mounted ULV spray trials over an open field targeting sentinel mosquito bioassay cages in an unfinished neighborhood, Sanctuary Cove, located in Palmetto, FL (27.516963 N, -82.544865 W) between June 14 and September 27, 2016.

We investigated five formulations, Biomist® 30+30 (Clarke®, St. Charles, IL), Evoluer® 30-30 (AllPro®, Northville, MI), Kontrol™ 30-30 (MasterLine®, Austin, TX), Permanone® 30-30 (Bayer, Research Triangle Park, NC), and Perm-X™ UL 30-30 (Central Life Sciences®, Schaumburg, IL), at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) label rates. For each formulation at each of the two application rates we performed at least two trial replicates. We performed a third replicate for Evoluer and Perm-X at the maximum label rate; however, weather impeded our ability to conduct third replicates for the remaining three formulations.

We conducted field cage bioassays for spray trials with field collected mosquitoes using methods described in WHO (2009). We distributed 9 sentinel cage poles for each trial across a 3 × 3 grid with 30.5 m (100 ft) separation between each pole in each row, with 3 rows of poles at 30.5 m (100 ft), 60.9 m (200 ft) and 91.4m (300 ft) downwind and perpendicular to the spray path. Additionally, we positioned two control sentinel cage poles and a weather station upwind of the spray area. We mounted treatment and control mosquito bioassay cages (8.5-cm diam., 14-cm height; see Buckner et al. 2016) above the level of the vegetation at 1.5 m on poles, with 3 separate bioassay cages containing 20-30 female field collected *Ae. aegypti, Ae. taeniorhynchus*, or *Cx. quinquefasciatus* mosquitoes positioned on each pole for each trial. We paired each treatment and control bioassay cage pole with a rotating impinger based on the Florida Latham-Bonds design holding two 3 mm Teflon-coated acrylic slides to collect spray droplets.

The upwind weather station array consisted of a Kestrel® 4500 NV Model Pocket Weather® Tracker (KestrelMeters.com, Boothwyn, PA) recording temperature, wind direction, wind speed, and relative humidity at 5 sec intervals at ground level, and two DirecTemp® (QTI Sensing Solutions, Boise, ID) temperature probes on a PVC mast at 1.2 m and 10 m above ground and two NM100 Weather Stations (New Mountain Innovations, Inc., Old Lyme, CT) at 1.7 m and 10 m above ground to record wind speed and direction. We used data streams from the weather station array to identify the presence of thermal inversions and whether temperature at both heights would be suitable for keeping the ULV spray at ground-level.

Starting in late May 2016, and throughout the summer we collected mosquitoes as eggs or larvae throughout Manatee County, FL and maintained them at Manatee County Mosquito Control District (MCMCD) under standard laboratory conditions at 28 ± 2 °C and 75 ± 5% RH, and photoperiod of 14:10 (L:D) h. We collected *Ae. aegypti* eggs from little black jars left outdoors in Cortez, FL, (27.459835 N, 82.664212 N) containing a piece of germination paper lining the rim of each jar, approximately 300 mL of water, and 5 g of a 3:2 mixture of liver powder and brewer's yeast. *Aedes taeniorhynchus* larvae were collected by MCMCD inspectors throughout the county,v and we collected *Cx. quinquefasciatus* rafts from a Manatee County Wastewater Plant (27.488016 N, 82.373168 W). We transferred 2 to 10 day-old adult female mosquitoes reared from these eggs or larvae into bioassay cages using a mechanical aspirator (Hausherr's Machine Works, Toms River, NJ) approximately 5-6 h prior to testing and provided mosquitoes access to a cotton ball soaked with 10% sucrose solution. We stored cages in a designated large plastic tote with a lid in the insectary until transportation of the tote to the test site.

Once weather conditions were conducive to spraying at the test site, we activated the rotating impingers and hung mosquito bioassay cages on the poles 5 to 10 min prior to each spray trial. We used a truck mounted 18-20 London Fogger (Adapco® Inc, Sanford, FL) ULV aerosol generator for all spray trials driven at 4.4-8.94 m/s (10-20 mph) depending on the designated application rate, with a flow rate of 192 mL/min (6.5 fl oz/min) monitored using a GeoTracker™ (Adapco Inc.). We collected all slides and cages 10 min post-application and immediately transported them back to the MCMCD laboratory. We exposed all bioassay mosquito cages to CO_2 for 28 sec to knock down and transfer mosquitoes to paperboard holding containers covered with mesh netting, supplied with cotton balls soaked with 10% sucrose solution, and kept at room temperature. We assessed mosquitoes for knockdown at 1 h and mortality at approximately 12 h post-application. After all trials were conducted, the mortality rates caused by the five permethrin/PBO 30-30 ULV products at mid and maximum application rates for each mosquito species were pooled to provide a mean. We calibrated all equipment/formulation combinations prior to the trials to produce a $DV_{0.5}$ or volume mean diameter (VMD), of 15-20 microns as measured using the waved 1-in slide method (Table 1). Additionally, we determined droplet density and $DV_{0.5}$ at each sampling station using DropVision® (Leading Edge Associates, Waynesville, NC) as soon as possible the same morning following each spray trial. Ground ULV trials were only conducted when wind velocity was 0.45 -14.4 m/s (1-10 mph) and a temperature inversion existed. Within each of the seven test periods, ambient temperature ranged from 24.5 to 29.3 °C and local ground-level winds ranged from 0.94 to 3 m/s (2.1 to 6.7 mph).

During mid- label rate application trials, Permanone had the smallest mean droplet size (9.03 µm) and a mean droplet density of 26.81 drops/mm², whereas Biomist had the largest mean droplet size (11.52 µm) and a mean droplet density of 43.36 drops/mm² (Table 1). During maximum label rate application trials, Permanone had the smallest mean droplet size (8.94 µm) and a mean droplet density of 22.50 drops/mm², whereas Biomist had the largest mean droplet size (11.72 µm) and a droplet density of 54.84 drops/mm² (Table 1). These findings confirm that truck-mounted ULV applications for all five tested formulations produced droplets of optimal size and concentration meeting label specifications.

It is important to note that droplets were larger on the pre-trial 1-in calibration slides (Table 1). The apparently smaller droplet sizes measured during spray trials is expected and can be explained by two factors: 1) When sampling at a further distance from the nozzle, i.e., 30-90 m during spray trials compared to a few meters during calibra-

Table 1. Mean droplet size ±SE and mean droplet density ±SE for five ground ULV oil-based 30% permethrin 30% PBO adulticide formulations applied at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) application rates.

Treatment	Biomist ml/ha 113	Biomist ml/ha 226	Evoluer ml/ha 113	Evoluer ml/ha 226	Kontrol ml/ha 113	Kontrol ml/ha 226	Permanone ml/ha 113	Permanone ml/ha 226	Perm-X ml/ha 113	Perm-X ml/ha 226
$DV_{0.50}$ (µm; VMD[1])	11.72 ±0.65	11.52 ± 0.38	10.11 ± 0.60	11.10 ± 0.35	9.43 ± 0.25	9.56 ± 0.40	8.94 ± 0.95	9.03 ± 1.00	10.76 ± 0.29	10.71
Droplet Density (mm²)	54.84 ± 20.74	43.36 ± 8.11	29.68 ± 0.84	35.28 ± 3.27	45.55 ± 2.71	20.45 ± 3.22	22.50 ± 1.09	26.81 ± 5.25	40.78 ± 2.20	4.14
Wave Slide $DV_{0.50}$ (µm; VMD)	17.91 ± 2.31	16.83 ± 1.22	18.30 ± 0.69	17.20 ± 0.42	17.22 ± 3.65	15.26 ± 1.68	17.51 ± 0.08	17.51 ± 0.08	18.29	18.43 ± 0.14

[1]VMD, volume mean diameter

tion, larger droplets should settle out prior to reaching the spinning slides, and 2) the narrow (3 mm) slides used during spray trials to better sample the diffuse spray cloud (Bonds et al. 2009) have a biased collection efficiency for small droplets compared to a 1-in slide, causing underestimation of true droplet sizes (Fritz et al. 2011).

Mortality for both *Ae. aegypti* and *Cx. quinquefasciatus* was relatively low for all five formulations (Fig. 1 A and C), not exceeding approximately 67% (mid application rate) and 72% (maximum rate). On the other hand, mortality for *Ae. taeniorhynchus* was relatively high for all five formulations (Fig. 1 B), with up to approximately 96% (mid rate) and 100% (maximum rate). Comparing 1 h knockdown to 12 h mortality, we observed 4% increased mortality in *Ae. aegypti* and 0.66% in *Ae. taeniorhynchus.* In contrast, *Cx. quinquefasciatus* showed a 13% recovery between the knockdown and final mortality counts. Control mortality was <1% during this experiment.

Mosquito control districts including MCMCD leverage ground ULV applications of formulations containing synergized permethrin as a key IVM component to prevent arbovirus transmission and reduce mosquito populations before they become a nuisance (Faraji et al. 2016). It is important that these applications are efficacious while using as little insecticide as possible (Mount 1998), and droplets must drift through areas where mosquitoes are flying or resting to be effective. In this investigation we consistently collected droplets throughout the treatment grid with droplet sizes that met label requirements for both mid- and maximum label rates, so the observed mortalities in the field collected sentinels should be a good indication of the potential relative efficacies of these formulations in an operational setting using similar calibrated pieces of equipment.

Out of all five formulations tested, Kontrol performed the best against all three species when applied at the maximum label rate, resulting in mortality rates of 70%, 100%, and 72% for *Ae. aegypti, Ae. taeniorhynchus*, and *Cx. quinquefasciatus*, respectively. If

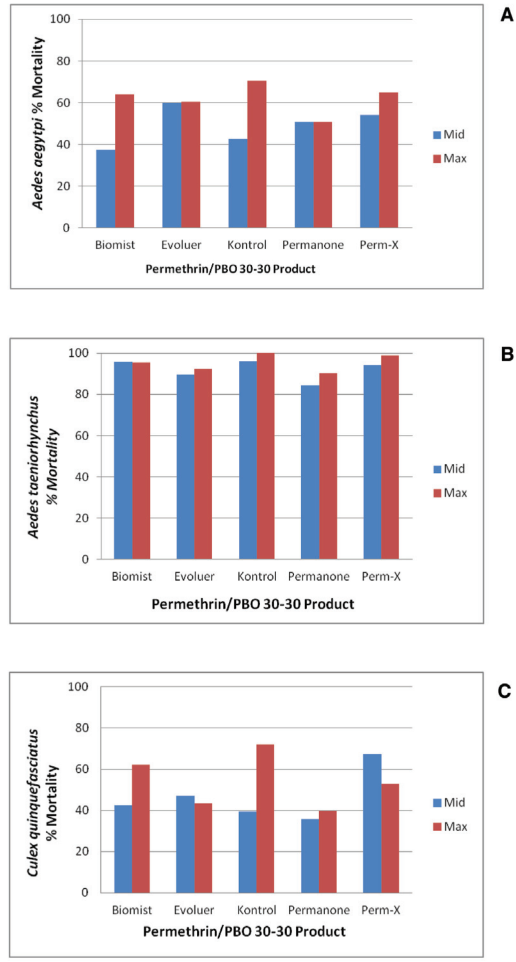

Figure 1. Mean mortality 12 h post-application for caged (A) *Ae. aegypti,* (B) *Ae. taeniorhynchus,* and (C) *Cx. quinquefasciatus* mosquitoes exposed to five ground ULV oil-based 30% permethrin + 30% PBO adulticide formulations applied at mid (113 mL/ha [1.55 oz/A]) and maximum (226 mL/ha [3.10 oz/A]) application rates.

we only consider targeting *Ae. taeniorhynchus* using a permethrin/PBO 30-30 product, our results indicate that four of the formulations, Biomist, Evoluer, Kontrol, and Perm-X, achieved mortality rates ≥ 90% when applied at mid-rate, while Permanone only met that benchmark at the maximum rate – important considerations in cost comparisons across formulations balanced with minimizing introduction of pesticides into the environment.

Biomist, Evoluer, Perm-X, and Permanone resulted in mortality <70% against *Ae. aegypti* and *Cx. quinquefasciatus*, independent of application rate. This could have been due to permethrin resistance developing in local *Ae. aegypti* and *Cx. quinquefasciatus* populations (Kasai et al. 2014) that may be more pronounced with certain formulations of permethrin and PBO active ingredients. Future studies should test natural populations of these species in Manatee County to evaluate permethrin resistance levels across these formulations. Aside from resistance, unfavorable weather conditions could have decreased efficacy in some trials. Wind direction and velocity, as well as temperature play important roles in successful ground ULV applications (Cornine 2015). During application for each trial, wind velocity was 1-10 mph at ground level and we confirmed the presence of a thermal inversion that should have trapped droplets near the ground and the sentinel cages as they drifted through the treatment area (Mount 1998). However, in the field weather can change rather rapidly and if the wind shifts or temperature changes droplets may not reach all of the cages in the treatment grid. This may have contributed to the results we saw when spraying Perm-X against *Cx. quinquefasciatus*, where we observed increased control (67.52%) at the mid rate compared to 52.86% at maximum rate. Also, the majority of these products were only tested at each application rate twice instead of three times due to unfavorable weather conditions, and additional replicates could have revealed different patterns of efficacy.

Overall, the results of this investigation provide evidence that ground ULV applications of formulations containing permethrin/PBO 30-30 active ingredients may be effective against local populations of mosquitoes in Manatee County, with variation depending on target species and application rate. Looking forward, MCMCD should continually monitor the efficacy of these and other adulticide formulations used in their IVM program. Effective IVM programs should incorporate efficacy data such as presented here along with resistance testing and cost analyses to design seasonal rotations of active ingredients to mitigate resistance.

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