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EVALUATION OF AN AERIAL APPLICATION OF DUET HD® AGAINST *AEDES DORSALIS* AND *CULEX TARSALIS* IN RURAL HABITATS OF THE GREAT SALT LAKE, UTAH

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ABSTRACT. The Salt Lake City Mosquito Abatement District (SLCMAD) has been conducting aerial applications using an organophosphate insecticide against adult mosquitoes for several decades. In order to evaluate a potential rotation product, aerial applications of Duet HD™, a pyrethroid, were conducted under operational conditions against wild populations of *Aedes dorsalis* and *Culex tarsalis* and against colony strains of *Cx. pipiens* and *Cx. quinquefasciatus*. The erratic wind patterns of the greater Salt Lake area did not prevent sufficient droplet deposition flux at 9 monitoring locations spread across a 5,120-acre (2,072 ha) spray block within rural habitats. Three separate aerial application trials showed great efficacy against *Ae. dorsalis*. In contrast, *Cx. tarsalis* exhibited inconsistent treatment-associated mortalities, suggesting the presence of less susceptible or resistant field populations as a result of spillover from agricultural or residential pyrethroid usage. Bottle bioassays to diagnose pyrethroid resistance using field-collected *Cx. tarsalis* indicated that some populations of this species, especially those closest to urban edges, failed to show adequate mortality in resistance assays. Despite challenging weather conditions, Duet HD worked reasonably well against susceptible mosquito species, and it may provide a crucial role as an alternative for organophosphate applications within specific and sensitive areas. However, its area-wide adoption into control applications by the SLCMAD could be problematic due to reduced impacts on the most important arboviral vector species, *Cx. tarsalis*, in this area. This study demonstrates the importance of testing mosquito control products under different operational environments and against potentially resistant mosquito populations by municipal mosquito control districts.

KEY WORDS Adulticide, insecticide resistance, mosquito control, organophosphate, pyrethroids

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INTRODUCTION

Federal and state guidelines for protecting the public during outbreaks of mosquito-borne diseases recommend ultra-low-volume (ULV) adulticides from aircraft and truck-mounted equipment as the most effective method of reducing transmission risk to humans (CDC 2003). These adulticide applications play a crucial role in the overall architecture and success of mosquito abatement operations throughout the continental USA. Although ground applications enable responses to localized "hot" spots, the aerosol characteristics at ground level may exhibit reduced efficacy because of insufficient coverage or penetration into habitats (Reddy et al. 2006, Lothrop et al. 2007, Farajollahi et al. 2012, Faraji et al. 2016). These response deficiencies can prove critical during limited time periods to control outbreaks of mosquito vectors and their associated pathogens (Lothrop et al. 2007, Tedesco et al. 2010). Aerial applications of adulticides are more effective at providing greater land area coverage and improved mosquito contact through descending aerosolized droplets (Tedesco et al. 2010, Bonds 2012). This improved coverage is particularly important for the greater Salt Lake, Utah, area, where thousands of acres of wetlands produce immense numbers of *Aedes dorsalis* (Meigen) and *Culex tarsalis* Coquillett in mostly undeveloped areas with limited road coverage. Therefore, aerial adulticide applications remain the most effective and viable option for the Salt Lake City Mosquito Abatement District (SLCMAD) when area-wide coverage of large swathes of mosquito habitat, which are otherwise inaccessible, is of utmost importance. Selection of an appropriate active ingredient and formulation is also pivotal to ensure efficacy where parameters such as climatology, environmental conditions, topography, and insecticide resistance should be considered.

Pyrethroids and organophosphates are the only 2 pesticide classes certified for ULV applications for adult mosquito control in the USA (Davis et al. 2007). Pyrethroids exhibit very low mammalian toxicity but can be highly toxic to some aquatic organisms (Davis et al. 2007). Organophosphates are more toxic to terrestrial vertebrates but can be quickly broken down in the environment and are less toxic to aquatic organisms (USEPA 2006, Davis et al. 2007). Dibrom® (87.4% naled, AMVAC Chemical Corporation, Commerce, CA) is an organophosphate insecticide used aerially by the SLCMAD since the 1970s. The unique chemistry of Dibrom and high specific gravity of the formula enable excellent penetration through thermal inversion layers, erratic environmental conditions at ground level, and some vegetative barriers (Davis et al. 2007). Additionally, the active ingredient is susceptible to photolysis and hydrolysis, resulting in a short environmental half-life (USEPA

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2006) and limited or transient effects on nontarget organisms (Rochlin et al. 2022). Consistent rotation of active ingredients, when possible, is also essential to mitigate resistance in wild mosquito populations (Karunaratne et al. 2018). Furthermore, overreliance on singular insecticide classes and the subsequent resistance observed in treated mosquito populations is a ubiquitous occurrence (Karunaratne et al. 2018), which has created a constant need for new formulations that satisfy both environmental and economic stewardship.

The pyrethroid formulated product Duet™ (Clarke Mosquito Control, St. Charles, IL), containing sumithrin (5%), prallethrin (1%), and the synergist piperonyl butoxide (PBO, 5%), was specifically formulated to improve penetration of space sprays (Cooperband et al. 2010). Oil- and water-based ULV applications using Duet have shown efficacy in ground trials (Farajollahi et al. 2012, Suman et al. 2012, Farajollahi and Williams 2013), but the low specific gravity of the product has given some mosquito control programs pause about using the product from the air. The advent of the aerial-specific formulation, Duet® HD (High Density to indicate the greater specific gravity), containing the same percentage of active ingredients above, has generated interest in reinvestigating the Duet product line for aerial applications. This aerial formulation includes a carrier complex to increase weight, minimize vaporization for better spray cloud performance, and enhance cuticular bonding and penetration on mosquitoes (Clarke 2020). The availability of a new aerial formulation that may prove efficacious under the unique geography and environmental conditions of the greater Salt Lake area prompted the evaluation of this product as a potential alternative to Dibrom.

Typically efficacy evaluations are conducted on a small scale typical of most field trials (Vessey et al. 2007, Dzul-Manzanilla et al. 2019). In an effort to be more representative of typical operational environments within a local district, the SLCMAD opted for a large-scale field testing. The main objective of these field trials was to determine if Duet HD can be effective under unpredictable climatology, environmental conditions, and unique geography of the district and against local mosquito populations. Additionally, offsetting flight paths to account for drift is generally infeasible in this region because of proximity with the Salt Lake City International Airport and the mountain ranges that surround the Salt Lake Valley. Even when possible, offsetting leads to unpredictable drift due to the high wind shear and turbulence as wind moves down the front ranges and through the canyons. Therefore, the secondary objective of this study was evaluating aerial applications without offsetting the flight lines to compensate for wind and commercial flight paths.

MATERIALS AND METHODS

Wild-type mosquitoes were collected via SLCMAD carbon dioxide–baited traps in the style of a miniature Centers for Disease Control and Prevention (CDC) trap without a light. Insect nets were supplied with a 10% sucrose solution and a cotton wick, mounted to the net with a magnet glued to the water container, and secured with a second magnet outside the net lining. After a 24-h collection, mosquitoes were returned to the laboratory, anesthetized with CO_2, and sorted on a chill table to separate *Ae. dorsalis* and *Cx. tarsalis*. Wild-type mosquitoes were tested within 72 h of collection. Susceptible controls included 2 laboratory strains: 2016 Salt Lake City *Culex pipiens* L. and Clarke *Culex quinquefasciatus* Say specimens reared at SLCMAD facilities in collection trays. Adults were provided a 10% sucrose solution during regular maintenance. Laboratory-raised adults used in testing were between 5 and 10 days old and not previously blood fed. Both wild-type and laboratory control adults were transferred to circular, mesh-sided testing cages (Clayson et al. 2010) kept at a consistent 27 ± 1°C and 70 ± 5% relative humidity (RH) before use in the field.

Three aerial applications were conducted on July 27, August 2, and August 9, 2022. The spray block consisted of approximately 5,120 acres (2,072 ha) northwest of Salt Lake City (Fig. 1). Nine stations were distributed to create a 3 × 3 grid that covered most of the treated area, leaving roughly a 2,000 ft (610 m) buffer between stations and the edge of the spray block. Three additional stations were placed 3 mi (4.8 km) east of the spray block as untreated controls. Each station consisted of 1 cage each of approximately 25 female *Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. tarsalis*, as well as approximately 15 *Ae. dorsalis*, because of smaller numbers of this species collected from the field. The cages were affixed to a tripod-mounted, self-correcting weather vane (Vessey et al. 2007, Dzul-Manzanilla et al. 2019). On a separate tripod, rotary impingers (Clayson et al. 2010) were loaded with a 3-mm Teflon-coated rod slide and a 1-in. (25.5 mm) flat glass slide, spun at ~600 rpm, and replaced for each replicate. Meteorological stations (Kestrel 5500, Kestrel Instruments, Shawnee on Delaware, PA) were placed independently of the sampling stations at the center and edge of the spray block at 5 ft (1.5 m) and 30 ft (9.1 m) above ground level, with an additional station placed near the edge of the block.

Tinopal® (CBS-X, BASF Corporation, Ludwigshafen, Germany), a fluorescent dye powder, was mixed into the drums of Duet HD (Clarke Mosquito Control, St. Charles, IL) at a rate of 0.09% (209 g per 55 gallons/208 liter of Duet HD) as described previously (Faraji et al. 2016), so that droplets of Duet HD could be distinguished from other aerosolized contaminants that may have been collected by rods. Applications were conducted from a Piper PA-23-250 Aztec airplane equipped with Micronair® AU4000 rotary atomizers at the wing tips. Each atomizer was adjusted to spin at approximately 10,000 RPM to create a droplet spectrum with a volumetric median diameter ($Dv_{0.5}$) less than 60 µm. The pump was calibrated to deliver 0.75 oz (22.2 ml) of Duet HD per acre. The aircraft flew the same pattern for each application, flying north and

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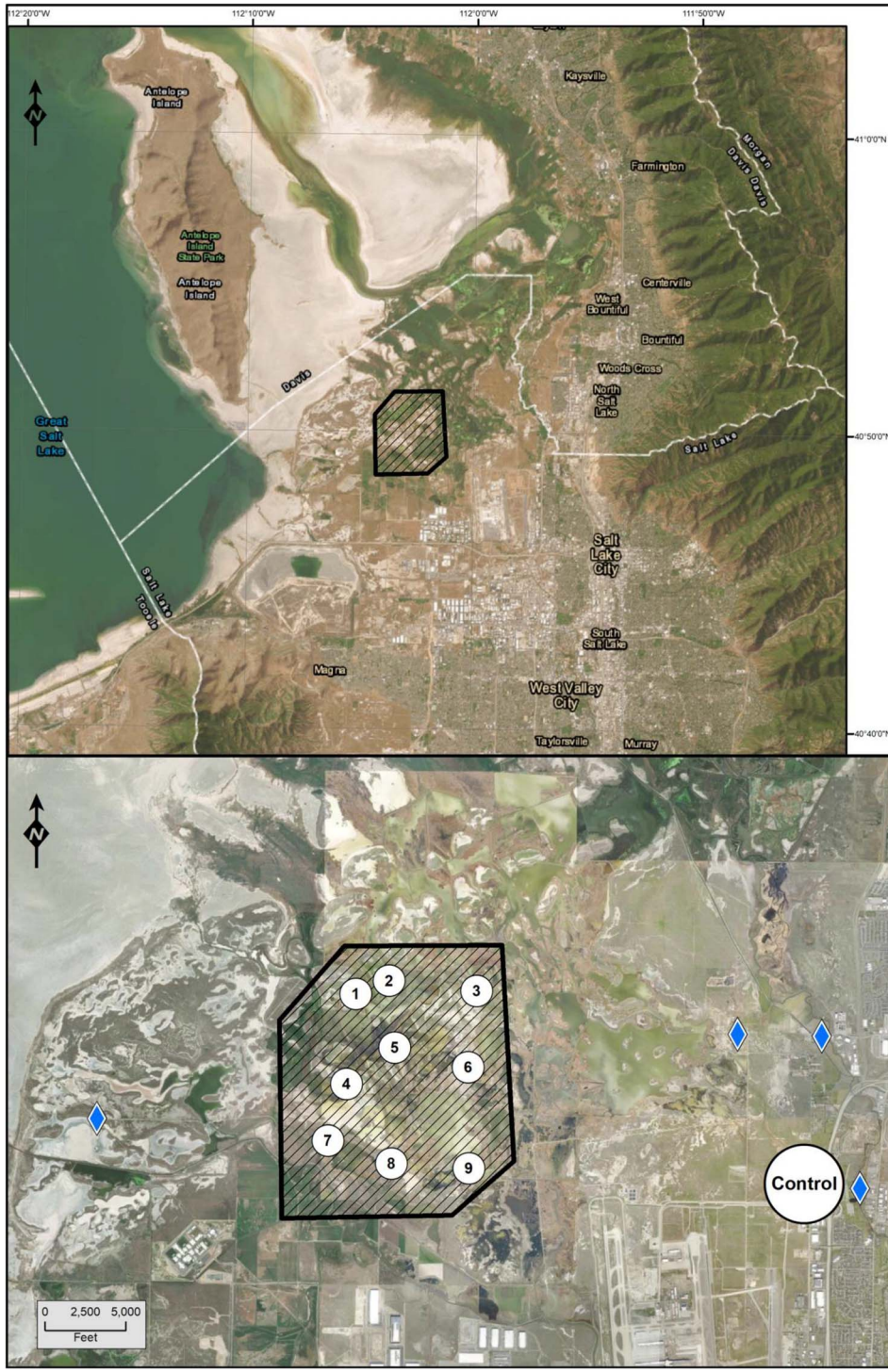


Fig. 1. (Top figure) Outer area view, with the Great Salt Lake on the west (left) and the Wasatch Mountain front on the east (right), with the polygon showing the spray block. Coordinates for the grid are annotated in the margins. (Bottom figure) Spray block with annotated sampling sites 1–9. Untreated controls were stationed east of the spray block at the Salt Lake City Mosquito Abatement District property. Diamonds indicate collection sites for mosquitoes used in study.

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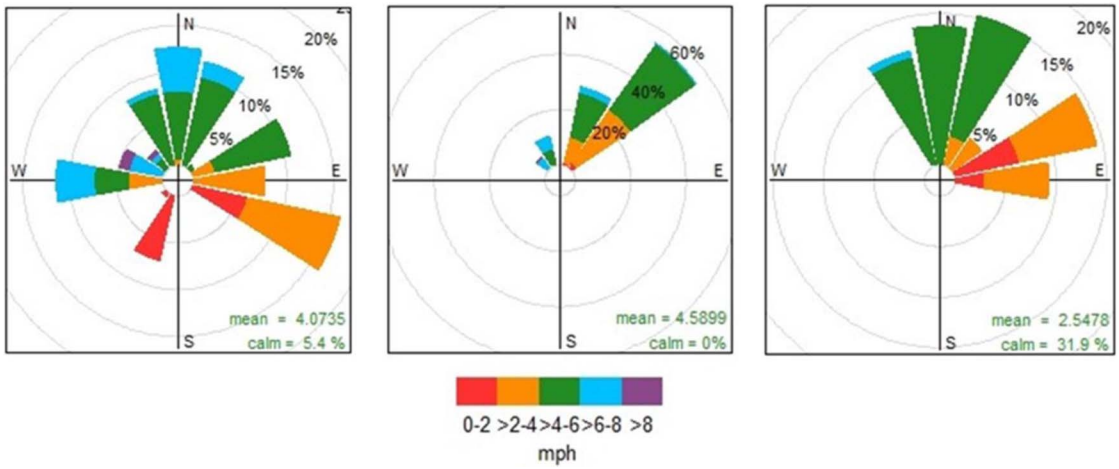


Fig. 2. Wind direction showing frequency of counts during 3 aerial treatment applications (1–3 from left to right), Station 5, 30 ft (9.1 m) above ground. Three panels (from left to right) correspond to July 27, August 2, and August 9, 2022, applications.

south and working from an eastward to westward direction. This allowed for the longest flight lines and most efficient use of flight time. Applications were made at a release height of 100 ft (30.5 m) with a swath width of 1,000 ft (305 m). Each spray mission used approximately 30 gallons (114 liters) of Duet HD, with a new drum being loaded for each replicate.

After applications, mosquitoes were retained in the field for 45 min to ensure adequate coverage of the treatment area. They were then transferred to clean holding cages for knockdown and mortality monitoring. Mosquitoes were provided a 10% sucrose solution and monitored at 1 h for knockdown and at 12, 24, and 36 h for mortality. Mosquitoes were considered knocked down or dead if they were incapable of coordinated movement or unable to right themselves after receiving a slight puff of air or a gentle tap of the holding cage.

Supplementary insecticide resistance bioassays were conducted according to CDC bottle bioassay protocols (Brogdon and McAllister 1998, CDC 2022). Samples tested were *Cx. tarsalis* collected from across the SLCMAD (Fig. 1). Populations were exposed to 43 µg/ml of technical grade permethrin for a general pyrethroid baseline. In follow-up poststudy bottle bioassays using mosquitoes collected from high-resistance risk areas, permethrin was combined with 400 µg/ml of PBO. Results were then compared to concurrent bottles treated with 20 µg/ml of Duet HD.

DropVision® Fluorescence (Leading Edge Associations, LLC, New Smyrna Beach, FL) was used to analyze slides for maximum collection and the presence or absence of droplets, while rods were analyzed for droplet size (µm) and droplet density (drops/cm^2) and subsequent correlation with mortality (Faraji et al. 2016). All analyses were performed using R Statistical Software (v4.2.0; R Core Team 2021).

RESULTS

Temperatures and RH ranged from 73 to 82°F and 42–67%, respectively, during the study period. A temperature inversion between the ground and 30 ft (9.1 m) above ground levels had occurred by the time of the initial insecticide release within 10 min of sunset for each application. Wind rose plots (Fig. 2) indicated the following conditions: 1) The first application experienced a wind shift of roughly 150 degrees around 30 min after the start of the application. The wind speed on the ground dropped to below 2 mph (3.2 kmph) for much of the shift, which is not conducive to penetration of cages by the spray droplets (Fritz et al. 2010). 2) The second application exhibited the most consistent wind speed and direction, but the direction of the wind was parallel to the aircraft flight path. 3) The third application experienced the lowest wind speeds overall averaging 2.5 mph (4 kmph) at 30 ft (9.1 m) above ground level, compared to 4.1 (6.6 kmph) and 4.6 mph (7.4 kmph) for the first 2 applications. The wind was calm (<0.5 mph or <1 kmph) above ground level for 31.9% of measurements, compared to 5.4% and 0% of wind measurements being calm for the first 2 applications. As previously noted, low wind speeds are not conducive to penetration of cages by spray droplets.

Whereas both slide and rod methods were utilized to measure droplet density, the slide method can be more difficult to use accurately in windy conditions, as the slide can be moved by the wind and affect the droplet collection (Johnson and Penrose 2006). Droplet density data from rods were thus used in association with caged mosquito mortality. Droplet density means ranged from 415 to 677 drops/cm^2 with standard deviations ranging from 1.47 to 4.57. Droplet densities of greater than 100 drops/cm^2 were recorded from all stations except Station 3, on both the 1st and

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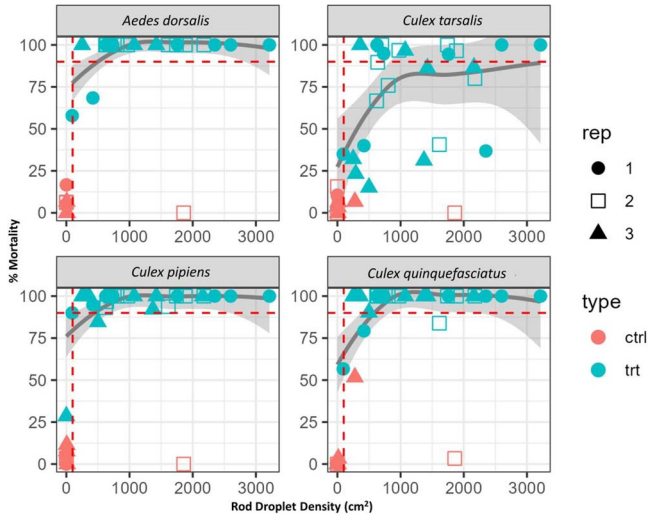


Fig. 3. Mosquito mortality measured at 24-h post application versus droplet density. Four panels correspond to 4 mosquito species used in monitoring. Each point corresponds to an individual cage. Shape indicates application replicate (rep, 1–3 corresponding to July 27, August 2, and August 9, 2022, applications), and color (type) indicates whether the cages were placed in control (ctrl) or treatment (trt) areas. The best fit line for mortality is shown in dark grey with 95% CI in lighter shade. The vertical red dashed line corresponds to 100 droplets/cm^2 on the x-axis, whereas the horizontal red dashed line indicates 90% mortality on the y-axis.

3rd applications. Surprisingly, 2 fluorescing droplets were detected among control stations, further indicative of erratic shifting winds in the area.

For all species, the mean droplet density correlated significantly with a mean mortality, generalized binomial linear model ($Z = 25.3$, $P < 0.001$). For *Ae. dorsalis*, *Cx. pipiens*, and *Cx. quinquefasciatus*, mean mortalities at droplet densities > 100 drops/cm^2 exceeded 93% compared to that of *Cx. tarsalis* at 70%. These differences between the former 3 species and *Cx. tarsalis* were statistically significant after pairwise comparisons with Bonferroni adjustment for multiple comparison, all $P < 0.001$. For *Cx. tarsalis*, results were more variable, with many mortality observations below the 90% threshold recommended by WHO (2019), but some stations did record mortalities above 90% (Fig. 3).

In bottle bioassays (Brogden and McAllister 1998, CDC 2022) conducted during 2022, wild-type *Cx. tarsalis* had reduced susceptibility to permethrin as compared to colony *Cx. quinquefasciatus* (Fig. 4). After 45-min exposure to 43 µg/ml permethrin, *Cx. quinquefasciatus* mortality was 100% compared to 6.3, 14.1, 50.5, and 53.8% mortalities for *Cx. tarsalis* from 4 different field locations (Fig. 4).

DISCUSSION

Some important implications of this study are evident. First, it is imperative to test new products under a variety of local environmental conditions with real-world, large-scale applications. Second, it is crucial to use multiple local mosquito species in conjunction with susceptible colony controls to identify potential issues. It is clear from this study that Duet HD applied at a midlabel rate of 0.75 oz/acre (55 ml/ha) performed well under challenging semidesert and mountainous environments against susceptible species, particularly *Ae. dorsalis*. Similar to most field experiments, there were certain outliers in the data (all outliers were retained for analysis). One station during the first application exhibited a 95% *Cx. tarsalis* knockdown rate with high droplet density (7.1 drops/mm^2); however, the 24-h mortality was only 37%. This level of knockdown recovery was not observed in any other cages (Fig. 3). A possible explanation could be a critically timed reduction in wind speed at this station, along with a directional shift in wind. Impingement anomalies are known to occur on mosquito test cages where the cage itself prevents droplet contact because of poor air circulation within the mesh, even if the droplet impingers, which vortex the surrounding air, would detect those same droplets (Bunner and Perich 1989, Bonds et al. 2010, Fritz et al. 2010).

However, Duet HD was less effective against local populations of *Cx. tarsalis* using the same application rate. The split groupings of mortality data from the field trials implicate a potential widespread pyrethroid tolerance in *Cx. tarsalis* populations in the Salt Lake City area (Fig. 3; Lucas et al. 2020). Given the tolerance for pyrethroids in local *Cx. tarsalis* populations, further studies are needed to identify if a higher label rate (up to 0.99 oz/acre or 72 ml/ha label max) would be more effective or if other suitable alternatives to Dibrom and other organophosphates are viable for the SLCMAD. Previously, in 2020 the SLCMAD also detected reduced susceptibility to naled (AI in Dibrom) in bottle bioassays with *Cx. tarsalis* populations sampled from Salt Lake City, Box Elder County, and Utah County (Fig. 5),

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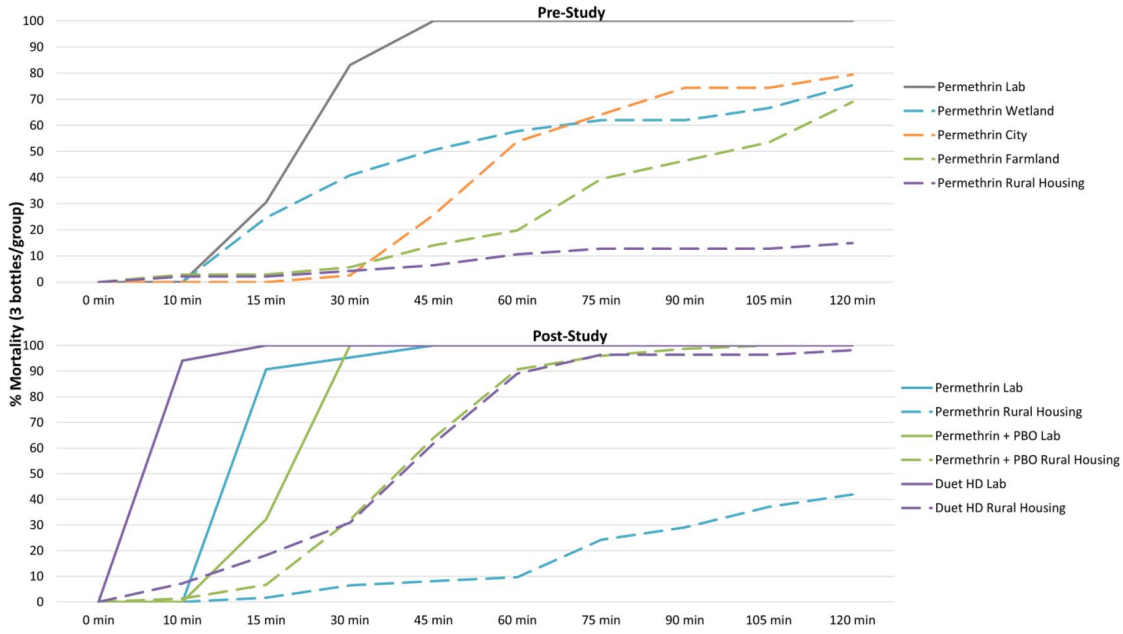


Fig. 4. Bottle bioassays using *Culex quinquefasciatus* laboratory colonies for the susceptible mosquitoes and various collections of wild *Culex tarsalis* from within the Salt Lake City Mosquito Abatement District. Prestudy results are for various sample sites used to collect mosquitoes for inclusion in study. Poststudy results are specific comparisons of Duet™ HD and technical-grade permethrin using the lowest mortality site from the prestudy sampling. All permethrin treatments were 43 µg/ml; PBO treatments were 400 µg/ml; Duet HD treatments were 20 µg/ml.

prompting heightened concern for the continued use of this product in central Utah.

One of the study's limitations was the use of wild-collected mosquitoes. This makes it difficult to age the mosquitoes, which can be problematic for determining susceptibility and product effectiveness. However, wild-caught blood-seeking females represent the epidemiologically important cohort of vector populations

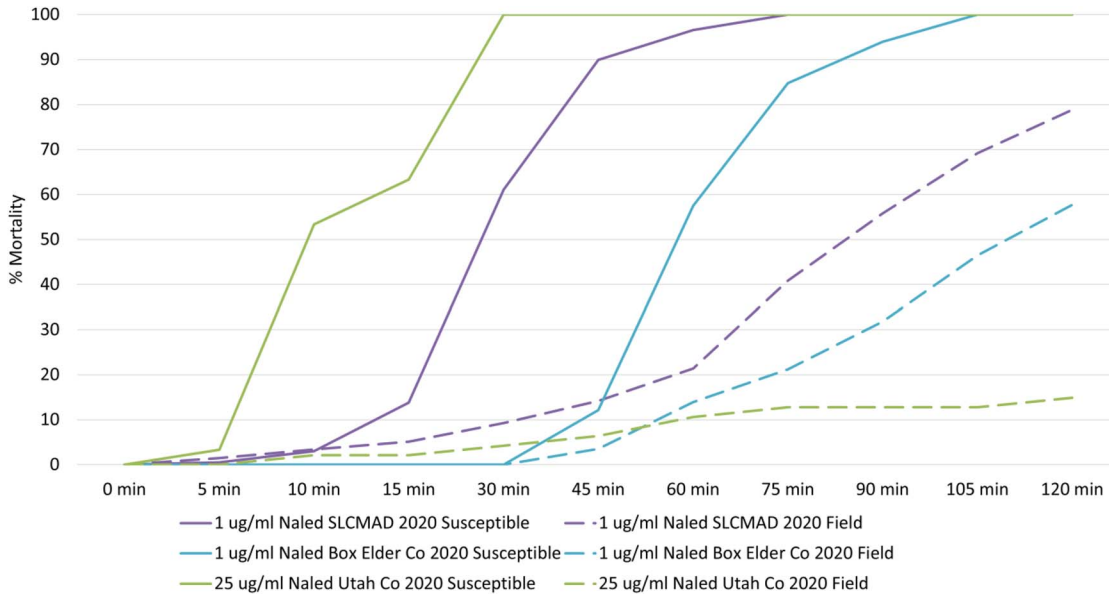


Fig. 5. Bottle bioassays from 2020 using *Culex quinquefasciatus* laboratory colonies for the susceptible mosquitoes and various collections of wild *Culex tarsalis* from within the Salt Lake City Mosquito Abatement District (SLCMAD), Box Elder Mosquito Abatement District, and Utah County Mosquito Abatement.

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and are thus applicable to be tested for pesticide resistance using established procedures (WHO 2016, 2022, Corbel et al. 2023). Another limitation was that we did not test mosquitoes separately for prallethrin resistance. Prallethrin, in addition to its excitatory and agitation benefits, can also exhibit insecticidal properties and is used in mosquito control (Chen et al. 2018).

The resistance seen to both pyrethroids, in this current study, and the organophosphate naled in a prior assessment underscores the pressure to find other means to protect the public from mosquito-borne pathogens. In the SLCMAD area, more commercial developments are occurring adjacent to and within the wetland habitats where mosquitoes are extremely abundant, which will necessitate the need for mosquito management techniques that will be efficacious for years to come. Unfortunately, it appears that the growing urban-rural gradient may also accelerate the loss of susceptibility in mosquitoes of central Utah (Fig. 4, prestudy). Subdividing results based on collection site revealed a probable urban-rural gradient, with reduced susceptibility climbing with proximity to city edges and agricultural lands on the perimeter of the metroplex (Fig. 4). Explicit comparisons between permethrin, permethrin + PBO, and Duet HD implicate that *Cx. tarsalis* from the more tolerant locations may be building up enzymatic resistance (Fig. 4, poststudy).

Although Duet HD did not perform optimally during these investigations, the utility and value of another class of an insecticide that can be rotated as part of an integrated mosquito management approach cannot be understated. Duet HD may also prove instrumental in targeted applications within certain areas where aversion to organophosphates may exist from the general public or environmental groups. We recommend that Duet HD may be a suitable rotational product in aerial operations in the interim, but the urgency exists for the development of new insecticide classes that can be used effectively as mosquito adulticides by agencies tasked with public health protection.

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