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OPERATIONAL NOTE

NIGHTTIME APPLICATIONS OF TWO FORMULATIONS OF PYRETHROIDS ARE EFFECTIVE AGAINST DIURNAL *AEDES ALBOPICTUS*

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ABSTRACT. The successful control of *Aedes albopictus* requires a multifaceted approach using a variety of integrated pest management techniques. Because this species is diurnal, nighttime ultra-low volume adulticide applications seem likely to miss resting mosquitoes and, therefore, are often met with skepticism. The goal of this study was to compare the efficacy of nighttime applications of pyrethroids with and without prallethrin to control caged and field populations of *Ae. albopictus*. During August and September of 2015, 2 adulticide applications were performed, treating 4 urban sites in the city of Trenton. We compared Anvil®, which contains sumithrin and piperonyl butoxide (PBO), to Duet™, which contains sumithrin, prallethrin, and PBO. Because prallethrin excites resting mosquitoes to flight, we hypothesized that Duet would kill more mosquitoes, especially those resting in cryptic harborages. Comparing pretreatment and posttreatment adult mosquito numbers, Biogents Sentinel trap collections revealed twice as many mosquitoes were killed by Duet than by Anvil. For caged *Ae. albopictus*, both products performed comparably, with Duet achieving a slightly higher mortality in front yards and Anvil achieving a slightly higher mortality in backyards. It is clear that nighttime adulticide applications are effective against *Ae. albopictus*, and the need to continue efficacy data collection is important because adulticiding is a key component of disease control response.

KEY WORDS Adulticiding, mosquito control, Asian tiger mosquito, sumithrin, New Jersey

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is a common invasive mosquito species in the southern and eastern USA with a global distribution in temperate and subtropical regions (Kraemer et al. 2015). *Aedes albopictus* is a proven vector of dengue and chikungunya viruses. Recent evidence indicates that *Ae. albopictus* can also transmit Zika virus (Wong et al. 2013). This species readily feeds on humans and other mammals and utilizes habitat within close proximity to humans (Niebylski et al. 1994, Faraji et al. 2014). Female *Ae. albopictus* preferentially oviposit in clean water that has pooled in artificial containers and other cryptic habitats, such as corrugated extension gutters, fence posts, and plant saucers (Unlu et al. 2014).

The oviposition and adult resting behavior of *Ae. albopictus* in urban and suburban sites complicates control efforts that concentrate on ultra-low volume (ULV) applications of adulticides. While source reduction is effective, door-to-door operations are labor intensive, time consuming, and expensive. Accessibility problems and reoccurring trash are also hindrances toward successful control. These challenges lead to the evaluation of alternative strategies. Mist applications of *Bacillus thuringiensis* var. *israelensis* (de Barjac) (VectoBac WDG; Valent BioSciences Corp, Libertyville, IL) in residential areas provided >90% mortality of *Ae. albopictus* larvae, even when applied to row homes, particularly those with alley access and overgrown backyards (Williams et al. 2014). By first monitoring and identifying clusters of high mosquito population density, or "hot spots," a treatment strategy that combined larval source reduction, larvicidal treatment, and pyrethroid treatment of vegetation effectively reduced *Ae. albopictus* burden for up to 4–6 weeks (Unlu et al. 2015).

Another alternative is the use of an agitant as part of an ULV adulticide application to "flush out" mosquitoes resting in foliage or other urban harborages. Ultra-low volume applications are typically conducted at dusk and dawn, when a layer of warm air settles over cooler air, known as a temperature inversion, thereby preventing aerosol droplets from dissipating (Suman et al. 2012). Because *Ae. albopictus* tend to feed during the day and rest during the night, ULV applications conducted at these times are likely to miss resting mosquitoes. Exposure to the pyrethroid insecticide prallethrin increased flight activity and speed in female *Culex quinquefasciatus* Say (Cooperband et al. 2010). We hypothesize that the benign agitation caused by prallethrin would excite resting mosquitoes to flight, thereby increasing their probability of encountering more adulticide droplets.

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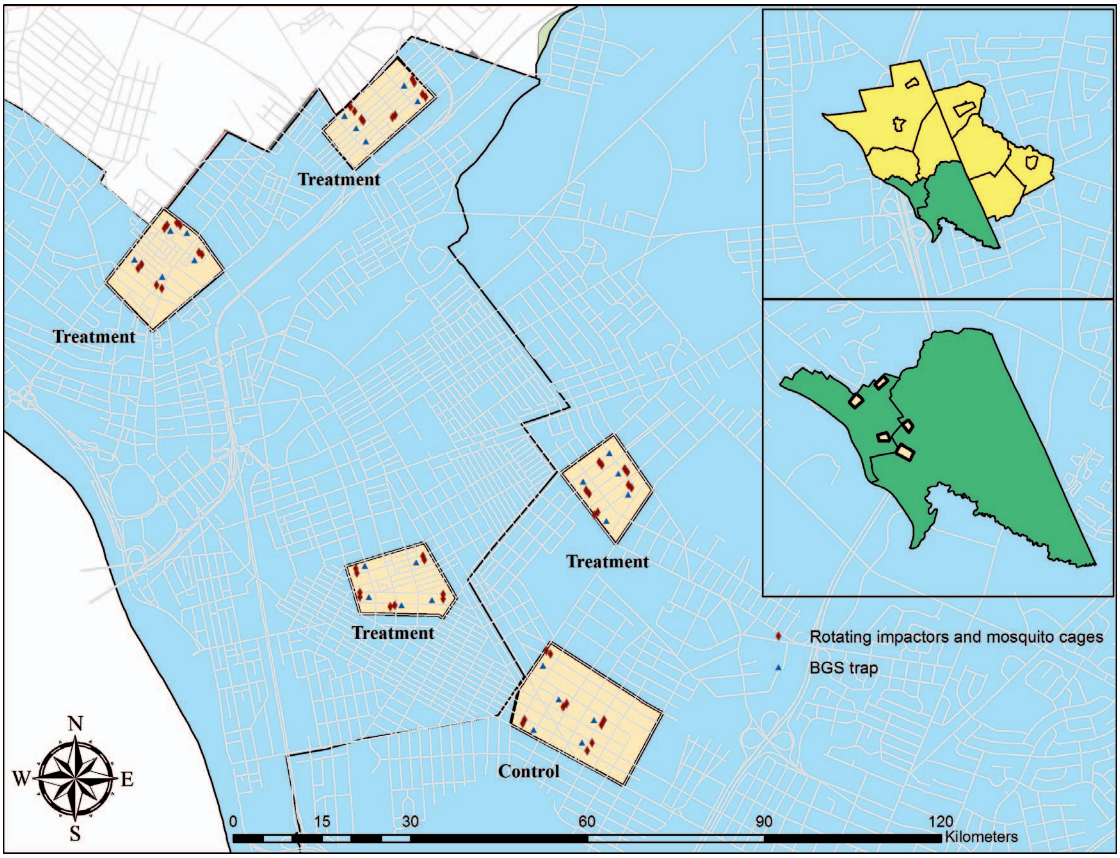

Fig. 1. Map of ULV adulticide treatment site in Mercer County, NJ, USA, August and September, 2015. Inset of Mercer County in the top right displays locations of study sites, and inset below displays locations of the treatment (4) and control (1) sites. A detailed map shows locations of rotating impactors, caged mosquitoes and BGS traps. Typical blocks within these highly urbanized study sites are about 90 m wide and 150 m long, with some blocks divided by a drivable alleyways behind the parcels. All roadways were driven during an adulticide application excluding alleyways.

Study sites were located in Mercer County, which is located along the Delaware River in west-central New Jersey, between Philadelphia and New York. The sites consisted of 4 treatment and 1 control site (Fig. 1). The locations of the treatment sites and control were chosen based on Biogents Sentinel (BGS) (Biogents, Regensburg, Germany) surveillance where high numbers of adult *Ae. albopictus* were observed (Unlu et al. 2015). The BGS trap is proven to provide an effective estimate of host-seeking *Ae. albopictus* populations (Unlu et al. 2011). The study sites are as follows: Brunswick (40°23′N, 74°76′W), Mulberry (40°15′N, 74°44′W), Cummings (40°21′N, 74°74′W), and Ellwood (40°13′N, 74°44′W). South Clinton was used as the control site (40°20′N, 74°72′W). Study sites were at least 0.7 km apart (from edge to closest edge). These were urbanized areas, which consisted of private residences mixed with commercial and industrial properties where all of the surface roads were configured in a standard grid system.

Adulticide applications took place on August 26 and September 16 by using 2 trucks, each treating 2 sites (2 sites per adulticide) with 2 different adulticides. The 2 trucks carried Clarke® Cougar ULV machines equipped with SmartFlow® (Clarke Mosquito Control Products Inc., Roselle, IL) to ensure accurate application rates as the speed of the vehicle changed (target speed was 10 mph). One truck was assigned Duet™ Dual-action Adulticide (Clarke, Roselle, IL), while the other was assigned Anvil® 2+2 ULV. Duet combines the pyrethroids sumithrin (5%, 44.94 g/liter AI) and prallethrin (1%, 8.99 g/liter AI) with the synergist piperonyl butoxide (PBO) (5%, 44.94 g/liter AI), while Anvil 2+2 ULV combines sumithrin (2%, 16.79 g/liter AI) with PBO (2%, 16.79 g/liter AI). The applications were performed at the maximum label rate for each product and a fluorescent tracer dye was added, Uvitex® OB (Ciba Corporation, Newport, DE). Tracer dye was used to limit corruption of the collection slides with other airborne pollutants (e.g., sap, dew, fuel residue, etc.). The fluorescent tracer

Table 1. Summary of the result of Wilcoxon–Mann–Whitney rank sum test. Evaluation of the efficacy of *Aedes albopictus* populations in treatment sites to control sites before and after application of Duet™ and Anvil®, August and September 2015.

Study period 1	Study period 2	*P* value	Difference in mean ± SD
Control before	Control after	0.6129	6.27 ± 28.72
Control before	Duet before	0.5699	−7.32 ± 45.53
Control before	Anvil before	0.0639	3.68 ± 32.28
Control after	Duet after	<0.0001	−12.95 ± 51.09
Control after	Anvil after	<0.0001	−6.08 ± 21.84

dye was mixed with the pesticide at a 0.125% weight-to-volume ratio, or 1.32 g/liter. This dye does not alter the pesticide formulation, droplet spectrum, or movement through the environment (Schleier et al. 2010). On August 26, the Brunswick and Mulberry sites were treated with Duet, while Cummings and Ellwood were treated with Anvil. On September 16, we switched the products applied to the sites so that each treatment site received both products. Brunswick and Mulberry were treated with Anvil, while Cummings and Ellwood were treated with Duet. Each application took approximately 2 h to complete and was conducted between 12:00 and 5:30 a.m. The spray route was designed to cover all surface roads excluding alleyways.

Five residential properties were chosen in each site that could accommodate 2 cages of adult *Ae. albopictus*. The cages were disc shaped, measuring 14.4 cm in diameter and 4 cm wide, with mesh covering each side allowing adequate airflow (Farajollahi and Williams 2013). Protocol for assessing the mortality rate for caged mosquitoes was described by Farajollahi and Williams (2013); briefly, field-collected *Ae. albopictus* eggs were hatched in the laboratory to obtain adult mosquitoes for this trial. Each cage received 15–20 adult female *Ae. albopictus* (5–7 days old) a few hours before the application. Each set of cages was transported to the field in plastic totes. Cages were hung from chain-link fences 15 min prior to adulticide applications and remained in the treatment plot for 30 min postapplication. Adults were transferred to cups with the use of a mouth aspirator and kept in 237-ml cardboard ice cream containers covered with mesh netting lids to record the mortality rates at 1 h, 24 h, 48 h, and 72 h posttreatment. Adults were held on a 10% sucrose solution provided by a soaked cotton pad placed on top of each mesh lid. Control mosquitoes were held separately but cared for in the same manner as the treatment.

Droplet size and density were determined by using Florida Latham Bonds (FLB) rotating impactors (John W. Hock Company, Gainseville, FL). Two FLB rotating impactors were placed at each preselected parcel (one in the front yard and one in the backyard). The FLB rotating impactors were outfitted with 3-mm Teflon-coated acrylic rods (slides) rotating at 5.6 m/sec (Clayson et al. 2010). The FLB rotating impactors were deployed immediately preceding the applications and retrieved 30 min after completion of adulticide applications for each site. Upon retrieval, rod slides were removed from the spinners and placed in an opaque container to avoid light exposure. They were held for droplet analysis, which was performed using a compound microscope equipped with Drop Vision (Leading Edge Associates Inc., Waynesville, NC).

A Shapiro–Wilk test was run for the adult mosquito count data and all *P* values were found to be <0.05, which indicated the data violated the normal distribution. Therefore, a nonparametric alternative to the *t*-test, the Wilcoxon–Mann–Whitney rank sum test was adopted to compare the mosquito numbers before and after the adulticide applications. For statistical analysis, data from 2 adulticide applications were combined in regards to adulticide used (not based on site). To investigate the difference of mortality rates between the caged mosquitoes placed in the front yard and backyard of a residential property, a binomial proportional test was conducted. The Wilcoxon–Mann–Whitney rank sum test was also used to examine whether there was a significant difference between front yard and the backyard with respect to volume median diameter (VMD) and droplet density. The overall VMD and droplet density in the Duet and Anvil sites were separately analyzed. The statistical analysis was conducted in R 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria). Lastly, there was no trap, cage, or rotating droplet impactor malfunctions detected; nor was there any predeployment mortality or adverse meteorological conditions such as rain or high winds.

During the study 3,069 *Ae. albopictus* adults were collected from 5 study sites. We collected 1,443 mosquitoes prior to the adulticide treatments and 750 mosquitoes postadulticide from treatment sites. When compared to the control site, a significant reduction in adult numbers was observed from pre- to posttreatment ($P < 0.0001$; Table 1). We collected 369 mosquitoes preadulticide treatment and 507 mosquitoes postadulticide treatment from the control site, indicating that control site adult populations increased by 1.4 times from pre- to posttreatment (Table 1). We did not observe a significant difference of adult numbers between treatment and control sites prior to adulticide applications (Table1).

In addition to monitoring adult *Ae. albopictus* with BGS traps in the 5 study sites, we deployed cages of 15–20 *Ae. albopictus* adults. For caged adult *Ae. albopictus*, the overall knockdown was 60%, and when separated by product, knockdown rates for Duet and Anvil were 66% and 56%, respectively. The total mortality at 36 h postapplication was 68% overall (760 of 1,133 caged adult mosquitoes). The overall mortality for Duet was 65%, while for Anvil was 63%. The mean mortality for caged mosquitoes placed in the front yard of residential properties was 0.78 ± 0.12 (mean ± SE), and 0.58 ± 0.15 for the cages placed in the back. Significantly higher mortality was observed in the cages located in the front ($P < 0.001$). The same trend was observed when the caged mosquito mortality was analyzed by adulticide. In front yard cages we achieved 0.80 ± 0.05 mortality for Duet and 0.76 ± 0.06 mortality for Anvil. In backyard cages we achieved 0.56 ± 0.06 mortality for Duet and 0.60 ± 0.05 mortality for Anvil.

There were 35,000 droplets collected from all of the slides during Anvil and Duet applications, with a mean value of 875 drops per station and 437 drops per slide. For Duet, 16,635 droplets were analyzed from all slides, with a mean value of 831 drops per station and 415 drops per slide. For Anvil, 18,405 droplets were analyzed from all slides, with a mean value of 920 drops per station and 460 drops per slide. The VMD and droplet density was measured for all applications. Analysis of Duet resulted in a VMD of 11.37 μm, and a droplet density of 50.69/mm^2. For Anvil, a VMD of 15.33 μm and droplet density of 50.69/mm^2 was observed. Droplets were collected consistently from both stations (front yard and backyard) with no significant differences in VMD and droplet density.

Our goal was to compare the efficacy of nighttime applications of pyrethroids with and without prallethrin to control *Ae. albopictus* in a suburban habitat. We compared Anvil, which contains sumithrin and PBO, to Duet, which contains sumithrin, prallethrin, and PBO. Because prallethrin excites resting mosquitoes to flight (Cooperband et al. 2010), we hypothesized that Duet would kill more mosquitoes, especially those resting in cryptic harborages, than Anvil alone. The VMD and droplet density for both products were comparable in both front and backyards. Because droplet characteristics were similar and both products contain sumithrin (Anvil 2%, Duet 5%) and PBO (Anvil 2%, Duet 5%), we infer that the differences observed in mortality were due to the prallethrin in Duet.

Biogents Sentinel trap field collections of mosquitoes before and after ULV adulticiding revealed roughly twice as many were killed by Duet than by Anvil. For caged *Ae. albopictus,* both products performed comparably, with Duet achieving a slightly higher mortality in front yards, while Anvil achieved slightly higher mortality in backyards. Our results reiterate those of Farajollahi et al. (2012), who found that nighttime applications of Duet effectively suppressed natural populations of *Ae. albopictus*, while experiencing uniform droplet penetration. The major difference between Farajollahi et al. (2012) and our study was the former's approach covered both surface roads and alleyways, whereas our experimental design covered only surface roads. While both studies demonstrated uniform slide results, our addition of caged mosquitoes played a key role in determining that both products were more effective in front yards compared to backyards.

Our results indicate that both Duet and Anvil provide effective knockdown and mortality of *Ae. albopictus* in urban and suburban environments. Conventional wisdom of successful adult *Ae. albopictus* management recognizes nighttime ULV adulticiding as an important component to keep gravid female numbers low, especially in the event of a disease outbreak. Therefore, applying ULV adulticide at night as part of an integrated mosquito control program would be more likely to control diurnal or crepuscular species that seek harborage after sundown.

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