Behavioral Responses of Two Dengue Virus Vectors, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), to DUET and its Components

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**ABSTRACT** Ultralow volume droplets of DUET, prallethrin, and sumithrin at a sublethal dose were applied to unfed (nonbloodfed) and bloodfed female *Aedes aegypti* L. and *Aedes albopictus* (Skuse) in a wind tunnel. Control spray droplets only contained inert ingredients. Individual mosquitoes were videotaped before, during, and after spraying and various behaviors analyzed. During the spray periods of all three pesticide treatments, mosquitoes spent a greater percentage of time moving, and the distance moved was greater than for mosquitoes in the control treatments. In the postspray period, the percent of time moving increased for mosquitoes exposed to all pesticide treatments compared with the controls. After treatment, all females spent more time walking compared with controls, with unfed *Ae. aegypti* females walking more after exposure to DUET and sumithrin than after exposure to prallethrin and the control. Pesticide exposure increased flying in both species. Sumithrin exposure increased activity and velocity of unfed mosquitoes more than bloodfed mosquitoes. DUET and sumithrin treatments enhanced activity of *Ae. aegypti* females more than *Ae. albopictus* females.

**KEY WORDS** mosquito, *Aedes aegypti*, *Aedes albopictus*, DUET, prallethrin

*Aedes (Stegomyia) aegypti* L. is the principal epidemic vector for dengue viruses in the world, with substantial range expansion in the Americas during the last 40 yr (Gubler 1998). Among other factors, this reinfestation has contributed to the dramatic increase in dengue and dengue hemorrhagic fever (DHF) in the western hemisphere (San Martín et al. 2010). During the last three decades, in total, 8,491,416 dengue cases, including 183,541 DHF cases, have been reported to the Pan American Health Organization. The annual number of cases caused by one or more of the four dengue serotypes in the Americas continues to rise annually. In addition, *Ae. aegypti* can serve as the vector for yellow fever (YF) virus in many of the *Ae. aegypti*-infested urban centers of the Americas. In 1997–1998, after an absence of 44 yr, the last urban YF outbreak was reported from Santa Cruz, Bolivia (Van der Stuyft et al. 1999). Although the YF virus infection was confirmed in only six residents, five of these cases had a fatal outcome, demonstrating the lethality of this viral disease.

Although not considered as efficient a vector for dengue viruses as *Ae. aegypti*, *Aedes (Stegomyia) albopictus* (Skuse) is also a competent vector of dengue viruses (Lambrechts et al. 2010). Its importance as a vector of dengue and YF viruses was reported after its introduction into Brazil (Miller and Ballinger 1988) and was exemplified by its ability to transmit >20 arboviruses in the laboratory (Paupy et al. 2009). The presence of this second vector of YF virus in Brazil 10 yr after its discovery in the country has caused health officials to be increasingly concerned about future urban YF epidemics (Mondet et al. 1996). The role and importance of *Ae. albopictus* as a vector of these viruses, especially in laboratory studies of chikungunya virus (Turell et al. 1992), is further enhanced by its dramatic expansion in geographic distribution in the United States and internationally (Benedict et al. 2007).

One of the tools commonly used to control *Ae. aegypti* females is the ultralow volume (ULV) application of insecticides (Reiter and Nathan 2001). Several studies of this vector species have reported that it is frequently found indoors (Tidwell et al. 1990, Clark et al. 1994, Kittayapong et al. 1997, Scott et al. 2000, Garcia–Rejon et al. 2008) and beneath houses (Ponlawat and Harrington 2005) in urban areas, which can be a factor in reducing the amount of ULV droplets that penetrate the common resting sites (e.g., bedrooms, living rooms, and bathrooms) (Perich et al. 2000). *Ae. albopictus* has been found in urban, suburban, and rural areas (Lim et al. 1961) and has been aspired from vegetation around homes, bamboo thickets, and areas directly adjacent to villages in Thailand (Ponlawat and Harrington 2005) where it is also...
frequently found indoors. Braks et al. (2003) reported that in Brazil and Florida, *Ae. aegypti* was most prevalent in highly urbanized areas whereas *Ae. albopictus* was found in rural, suburban, and vegetated urban areas.

The application of a chemical that starts or speeds up movement in these diverse adult resting sites, while acting as a “locomotor stimulant” (Dethier et al. 1960), may increase an excitatory behavioral response. This term is reserved for activation of abnormally high kinetic locomotion, like that produced by application of sublethal amounts of certain insecticides in laboratory studies (Miller et al. 2009). In laboratory studies, Cooperband et al. (2010) exposed female *Culex quinquefasciatus* Say to sublethal levels of prallethrin, sumithrin, and piperonyl butoxide applied as ULV droplets and found a positive correlation between mosquitoes that spent more time flying during the time of spray and number of insecticide droplets on their bodies, presumably through increased contact while flying. They also found that excitation in the form of increased speed and duration of flight was more immediate in mosquitoes exposed to prallethrin than sumithrin. This behavior may improve the efficacy of peridomestic space spraying to control *Ae. aegypti* and *Ae. albopictus* and impact dengue virus transmission, an issue that was recently reviewed (Esu et al. 2010).

The focus of this study was on three pyrethroid insecticide treatments—DUET (which contains prallethrin and sumithrin as active ingredients; Clarke, Roselle, IL), prallethrin alone, and sumithrin alone. These insecticides have been evaluated in field studies in the United States. Among other reports are those with prallethrin (Responde) against three species of mosquitoes in Arkansas and Louisiana (Groves et al. 1997) and with sumithrin (Anvil) against *Anopheles quadrinaculatus* Say in Arkansas (Meisch et al. 2007). More recently there has been the report of a field evaluation of DUET from Florida against West Nile virus vectors, including *Ae. albopictus* (Qualls and Xue 2010).

The goal of this laboratory study was to determine whether these insecticides, when applied as ULV sprays, increased excitation in female *Ae. aegypti* and *Ae. albopictus* that have or have not recently taken a bloodmeal. Both species cause important public health problems internationally and the use of ULV insecticides that produce excitation and stimulate them to move from their resting sites may have an important role in their control.

**Materials and Methods**

**Insects.** Mosquitoes used for these studies were females from laboratory colonies of *Ae. aegypti* (Orlando 1952) and *Ae. albopictus* (Gainesville 2003) reared (following Gerberg et al. 1994) and maintained at the U.S. Department of Agriculture (USDA) Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, FL. Pupae were placed in cages (38.7 by 45.7 by 36.8 cm) (Bioquip, Inc., Rancho Dominguez, CA) maintained at 27°C and 70% relative humidity (RH), and with a photoperiod of 16:8 (L:D) h. Emerged adults were provided 10% sucrose ad lib. Assays were conducted with 4- to 8-d-old females that were nonbloodfed (referred to as unfed) or recently bloodfed. Before testing (30–150 min), unfed females were aspirated from the emergence cage to a paper cylindrical carton with a screened lid on the morning of the test. Bloodfed mosquitoes were fed ≈16–18 h before the bioassays. They were transferred by mouth aspirator to a screened carton and were bloodfed for 1 h on defibrinated bovine blood in a sausage casing (DeWeid, San Antonio, TX) warmed by water bath to ≈40°C and placed on top of the carton. Females that had clearly not bloodfed were discarded.

**Wind Tunnel.** This study was conducted in an acrylic wind tunnel (30 by 30 by 120 cm) described in detail in a previous study with *Cx. quinquefasciatus* (Cooperband et al. 2010). This wind tunnel had a detachable acrylic cylindrical spray chamber (15.6 cm in diameter by 16.2 cm in height) that was used to deliver pesticide spray into the wind tunnel from underneath with a plunger. An airbrush nozzle was inserted into the spray chamber through a small hole (1.6 cm in diameter) mid-way up the side of the spray chamber. After spraying into the chamber, the spray hole was sealed with a piece of tape. ULV droplets were produced by a single action airbrush (Badger, model 350, Franklin Park, IL). To produce optimal droplet sizes (8–30 μm) as recommended on the DUET label, a heavy tip and fine needle was used at 40 psi as determined previously by Cooperband et al. (2010).

A small hole (1.3 cm in diameter) in the bottom of the wind tunnel allowed spray from the spray chamber to enter the wind tunnel. The hole was closed with an acetate flap that was closed when the plunger was still. As the plunger was pushed upward, spray from the chamber moved into the wind tunnel. Each treatment had its own spray chamber (and associated floor panel of the wind tunnel) to ensure there was no cross-contamination. In addition, plastic wrap and aluminum foil used to cover the inside of the wind tunnel was replaced between treatments.

A single mosquito was placed in a test chamber in the wind tunnel. Air flow was measured with a hotwire anemometer (TSI Incorporated, Shoreview, MN) inside and to the side of the test chamber. Beside the test cage, the wind speed was 100 cm/s, while the mesh reduced the wind speed to 50 cm/s inside the test chamber, a speed at which droplet movement is enhanced (Hoffmann et al. 2008). Experiments were conducted at 22°C and 60% RH.

**Test Chambers.** Test chambers to contain mosquitoes within the wind tunnel during their exposure to treatments were fabricated from 0.95-liter paper cylinders (Cooperband et al. 2010). Briefly, both ends and one side of the carton were removed and replaced with nylon mesh to allow airflow and pesticide delivery. A clear acetate sheet was glued to the open side...
so that the behavior of the mosquito could be video-taped. The bottom of the test chamber had a hole (1 cm in diameter) corresponding to a similar hole in the bottom of the wind tunnel for introduction and removal of individual mosquitoes.

**Bioassay Procedures.** Each treatment was conducted with a single (4- to 8-d-old) female mosquito (*Ae. aegypti* or *Ae. albopictus*) that was either unfed or bloodfed. The test chamber containing the single female mosquito was placed 81.3 cm downwind of the spray chamber in the wind tunnel and located in the air stream from the spray chamber so that droplets from the spray chamber moved through the corridor. The optimal location of the test chamber within the wind tunnel was determined previously using sublimated dry ice vapor. Conditions for experimentation were described previously by Cooperband et al. (2010). After the test chamber was placed in the wind tunnel, the mosquito was allowed to come to a resting position within the test chamber before the test and the prespray period began.

Responses of the mosquitoes within the test chamber to the treatments were video-taped for subsequent analysis using a CCD video camera (Panasonic, model WV-BP334, Secaucus, NJ) with an automatic iris lens (CCTV, one-third inch, 3–8 mm, F 1.4, Rainbow, Irvine, CA), an MPEG recorder (Canopus Co., model EMR100, Kobe, Japan), and laptop computer. The video recording for each female lasted 7.5 min and consisted of three periods: prespray, during spray, and postspray. The prespray period recorded mosquito movement for the 2-min period before the initiation of spraying. The start and end of the spray period was perceptible on the video recording. The spray period consisted of depressing the airbrush for 0.5 s to load the spray chamber with droplets, and then the 2.5 min to plunge the droplets into the wind tunnel. The following 3 min consisted of the postspray period for observation of mosquito movement in the absence of droplets.

**Treatments.** Sprays consisted of four treatments, including the full DUET formulation and three subformulations, which consisted of various combinations of ingredients, as supplied by Clarke Mosquito Control (Roselle, IL) (Table 1). Treatments were made at a sublethal exposure level of the formulations at a 0.5 s spray. Doses were measured in a settling chamber, and doses were selected that did not cause immediate knockdown but resulted in <50% mortality at 24 h (Cooperband et al. 2010). The air in the spray chamber was cleared for 5–10 min between replicates of similar treatments. In addition, the foil and plastic linings of the wind tunnel were removed and replaced, and the airbrush was thoroughly cleaned with acetone. Lack of residual effects from the previous treatment was verified by minimal amount of mosquito movement observed in the prespray portion of each video. Ten replicates of each treatment were performed, with a total of 160 females being treated and recorded (10 females × 2 species × 2 bloodfed states × 4 treatments).

**Assessment of Knockdown.** After treatment, each mosquito was removed from the test chamber and placed in a new screened paper carton for observation where knockdown (KD) was assessed every 30 min over an 8-h period. A mosquito was considered knocked down if it was unable to stand and was not differentiated from death.

**Analysis of Video Recordings.** Videotapes were edited into three periods (prespray, during spray, and postspray), contrast enhanced, and reduced in size to five frames per s (Power Director [version 6], Cyberlink, Taipei, Taiwan). Video recordings were analyzed for movement analysis (Motus [version 8.2], Peak Performance Technologies, Inc., Centennial, CO, and Ethovision [version 7.0], Noldus Information Technology, Wageningen, The Netherlands) and specific behaviors (The Observer XT [version 8.0], Noldus Information Technology).

The movement track of each mosquito was manually digitized to produce a movement track from which the two-dimensional vectors, velocity, and percent of time spent in motion could be calculated. From this initial analysis, the percent of movement (based on number of frames in which movement occurred from the previous frame starting with the initial frame) and the average velocity while moving (including flying and walking) was determined. From this, velocity >1 cm/s, previously determined to be an effective assessment of pesticide impact on movement (Cooperband et al. 2010), was calculated. Movement tracks from Motus were analyzed in Ethovision to measure the distance moved.

Using behavioral analysis software (The Observer XT), the time that a mosquito spent in motion (walking and flying), or not in motion (resting) was determined for the three treatment periods and converted to percent time for each treatment period to provide the percentage of time walking, flying, resting, and knocked down.

**Statistical Analyses.** Data were analyzed within each of the two spray periods (during and after spray) using an analysis of variance (ANOVA) model to identify differences between the fixed effects of mosquito species, physiological state (unfed, bloodfed), and treatment (formulation of DUET and its components), and interactions. Nonsignificant interactions were dropped from the model and data reanalyzed (ANOVAPROC GLM) (version 9.2, SAS Institute, Cary, NC). For parameters with no significant effects

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Prallethrin</th>
<th>Sumithrin</th>
<th>PBO</th>
<th>Inert</th>
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<td>5</td>
<td>5</td>
<td>85</td>
</tr>
<tr>
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<td>–</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>Sumithrin</td>
<td>–</td>
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<td>–</td>
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<td>100</td>
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PBO is piperonyl butoxide; inert consists of a proprietary mixture of inert ingredients—indicates absence of an ingredient.

* Full formulation of DUET.
or interaction with state, data from unfed and bloodfed mosquitoes were combined for figures and subsequent analyses. Before analysis, data were analyzed with Levene’s test for homogeneity of variance and data log (x+1) or arcsine transformed as necessary with untransformed means presented in tables and figures. Post hoc means comparison tests were performed using the Student–Neuman–Keuls test or paired t-tests using a Bonferroni adjustment to \( P < 0.05 \). Within each treatment, means for unfed and bloodfed females were compared by paired t-test (\( P < 0.05 \)).

Results

The KD and mortality responses (Fig. 1A) produced in unfed and bloodfed Ae. aegypti following exposure to DUET at the selected sublethal dose were quite similar. The responses of unfed and bloodfed Ae. albopictus were also similar, yet different from those of Ae. aegypti. The KD and mortality responses following exposure to prallethrin were similar in the bloodfed Ae. aegypti and Ae. albopictus. A similar response pattern was observed in the unfed Ae. aegypti and Ae.
albopictus. The KD and mortality response following exposure to sumithrin was similar in the bloodfed Ae. aegypti and Ae. albopictus. A similar response pattern was observed in the unfed Ae. aegypti and Ae. albopictus exposed to sumithrin. The overall control mortality for all sprays was 12%. There was little KD (3 out of 40 individuals) during the spray period, with no differences for knockdown between species, states, or treatments ($P > 0.05$) (Fig. 1B). In the postspray period, however, only treatment significantly affected responses ($F_{3, 154} = 12.49; P < 0.01$). Knockdown of Ae. aegypti females was similar after exposure to DUET and prallethrin and greater than to sumithrin, whereas knockdown after exposure to prallethrin was greater than other treatments for Ae. albopictus (Fig. 1B).

The effect of state (unfed vs. bloodfed) was generally not significant in any of the main effect comparisons; however, several two-way interactions with state were significant. For parameters that had no significant effects involving state, figures are presented with data for unfed and bloodfed females combined. For parameters with significant differences involving state, data for unfed and bloodfed females are presented separately.

Percent movement, determined as the percent of video frames indicating movement, provided insight into the effects of the pesticide treatments. There was little mosquito movement in the prespray period, with no significant differences among the control and pesticide treatments ($P > 0.05$); and prespray data were excluded from further analysis (Fig. 2). During the spray period, however, model effects for treatment ($F_{3, 148} = 23.93; P < 0.001$) and species ($F_{1, 148} = 25.44; P < 0.001$) were significant. However, significant interactions between treatment $\times$ species ($F_{1, 148} = 3.53; P < 0.05$) and treatment $\times$ state ($F_{3, 148} = 4.32; P < 0.05$) were significant.
females compared with bloodfed females. Exposure to DUET resulted in more movement in unfed compared with bloodfed mosquitoes. Exposure to all pesticide sprays and more than the control resulted in movement by unfed and bloodfed mosquitoes. Exposure to DUET resulted in more movement by unfed and bloodfed Ae. albopictus compared with controls (Fig. 2). For Ae. albopictus, all pesticide treatments enhanced movement, whereas only prallethrin treatments increased movement of bloodfed mosquitoes. Exposure to DUET resulted in more movement by unfed and bloodfed Ae. aegypti compared with controls (Fig. 2). For Ae. aegypti, movement of both unfed and bloodfed mosquitoes are presented separately (Fig. 2). All pesticide treatments resulted in higher percent movement of both unfed and bloodfed Ae. aegypti compared with controls (Fig. 2). For Ae. aegypti, all pesticide treatments increased movement, whereas only prallethrin treatments increased movement of bloodfed mosquitoes. Exposures to DUET resulted in more movement by unfed and bloodfed Ae. aegypti compared with Ae. albopictus (P < 0.05). Exposure to sumithrin resulted in more movement in unfed Ae. albopictus females compared with bloodfed females (t\textsubscript{18} = 3.72; P < 0.01). During the postspray period, model effects for treatment (F\textsubscript{3,151} = 42.62; P < 0.001) and species (F\textsubscript{1,151} = 26.52; P < 0.001) were significant, with significant species x treatment interactions (F\textsubscript{3,151} = 7.11; P < 0.001). There were no differences between unfed and bloodfed mosquitoes, and these data were pooled. For Ae. aegypti, sumithrin spray in the postspray period resulted in more movement than DUET and prallethrin sprays, which, in turn, resulted in more activity than the controls (Fig. 2). In contrast, female Ae. albopictus moved to the same extent after exposure to all pesticide sprays and more than the controls (Fig. 2). Overall, female Ae. aegypti moved more than Ae. albopictus after exposure to both DUET (t\textsubscript{18} = 4.78; P < 0.001) and sumithrin (t\textsubscript{18} = 4.84; P < 0.001) sprays.

Velocity of movement (>1 cm/s) during the spray period had significant main effects for treatment (F\textsubscript{3,151} = 19.18; P < 0.001), with significant interactions of species and treatment (F\textsubscript{3,151} = 4.85; P < 0.01) (Fig. 3). All pesticide treatments increased moving velocity compared with the control during the spray period for both mosquito species (Fig. 3). Velocity of Ae. aegypti was greater than Ae. albopictus after exposure to DUET (t\textsubscript{18} = 3.42; P < 0.05). After the spray period, main effects for treatment (F\textsubscript{3,148} = 45.67; P < 0.001) and species (F\textsubscript{1,148} = 13.43; P < 0.001) were significant; however, there were also significant interactions between species and treatment (F\textsubscript{3,148} = 6.28; P < 0.001) and state and treatment (F\textsubscript{3,148} = 3.17; P < 0.05). After spraying with pesticides, Ae. aegypti females moved more quickly than control mosquitoes (Fig. 3). All insecticide treatments increased moving velocity of unfed Ae. albopictus, whereas only bloodfed females exposed to prallethrin increased velocity. Bloodfed Ae. aegypti females increased velocity more than bloodfed Ae. albopictus females after exposure to DUET spray (t\textsubscript{18} = 3.94; P < 0.001). Velocity increased more in unfed female Ae. albopictus compared with bloodfed females with exposure to sumithrin (t\textsubscript{18} = 3.29; P < 0.05).

The distance traveled by mosquitoes during the spray period had significant main effects of pesticide treatment (F\textsubscript{3,151} = 32.66; P < 0.001) and mosquito species (F\textsubscript{1,151} = 20.46; P < 0.001), with significant interactions of species x treatment (F\textsubscript{3,151} = 9.34; P < 0.001). During the spray period, exposure to all of the pesticide treatments resulted in more distance covered by Ae. aegypti compared with controls (Fig. 4). For Ae. albopictus females, responses were greatest to
prallethrin sprays (Fig. 4). Female *Ae. aegypti* traveled a greater distance than *Ae. albopictus* females in the presence of DUET ($t_{18} = 4.15; P < 0.01$) and sumithrin ($t_{18} = 3.48; P < 0.05$). In the postspray period, the main effects of treatment ($F_{3,151} = 33.59; P < 0.001$) and species ($F_{1,151} = 15.22; P < 0.001$) were significant with significant interaction between species and treatment ($F_{3,151} = 5.17; P < 0.01$). Female *Ae. aegypti* covered more distance after exposure to sumithrin compared with DUET and prallethrin, both of which resulted in more distance covered than the control (Fig. 4). For *Ae. albopictus*, all pesticide treatments resulted in greater distances traveled than the controls (Fig. 4). Females of *Ae. aegypti* moved greater distances than *Ae. albopictus* females after exposure to sprays containing DUET ($t_{18} = 4.71; P < 0.001$) or sumithrin ($t_{18} = 5.03; P < 0.001$).

The percent of time spent resting during the spray periods had significant main effects of treatment ($F_{3,148} = 20.49; P < 0.001$) and mosquito species ($F_{1,149} = 20.09; P < 0.001$), with significant interaction between species and treatment ($F_{3,148} = 3.45; P < 0.05$) and state and treatment ($F_{3,149} = 3.37; P < 0.05$) (Fig. 5). For *Ae. aegypti*, all pesticide treatments resulted in less resting than controls (Fig. 5). For *Ae. albopictus*, resting was significantly reduced after exposure to prallethrin (unfed and bloodfed females) and sumithrin (unfed females) compared with controls (Fig. 5). Unfed females of *Ae. albopictus* rested more than *Ae. aegypti* females during exposure to DUET ($t_{18} = 3.92; P < 0.05$) and sumithrin ($t_{18} = 3.72; P < 0.05$). Resting was lower for unfed *Ae. aegypti* females compared with bloodfed females during sprays with sumithrin ($t_{18} = 3.36; P < 0.001$). After the spray period, main effects of treatment ($F_{3,151} = 42.29; P < 0.01$) and species ($F_{1,151} = 45.64; P < 0.001$) and species by treatment interactions ($F_{3,151} = 5.63; P < 0.001$) were detected (Fig. 6). Resting by *Ae. aegypti* females, reduced by about one-half in all pesticide treatments, was lower than the untreated controls (Fig. 6). Although resting of *Ae. albopictus* females after exposed to pesticides was lower than the controls, there was only a 20–30% reduction. Resting of *Ae. aegypti* females was more reduced than for *Ae. albopictus* females in the presence of DUET ($t_{18} = 6.17; P < 0.001$) and sumithrin ($t_{18} = 4.48; P < 0.05$) sprays.

The percent of time spent walking during the spray period had significant main effects of treatment ($F_{3,152} = 19.86; P < 0.001$), species ($F_{1,152} = 19.48; P < 0.001$), and interactions between treatment and state ($F_{3,152} = 3.35; P < 0.05$) (Fig. 5). For unfed and bloodfed *Ae. aegypti* and unfed *Ae. albopictus*, more time was spent walking during the pesticide sprays compared with the control sprays (Fig. 5). For bloodfed *Ae. albopictus*, this increase in walking was only seen with DUET and prallethrin treatments. Bloodfed *Ae. aegypti* spent more time walking than did bloodfed *Ae. albopictus* during the DUET sprays ($t_{18} = 5.46; P < 0.05$). Also, unfed *Ae. aegypti* spent more time walking than did unfed *Ae. albopictus* during sumithrin sprays ($t_{18} = 5.19; P < 0.05$). Unfed *Ae. aegypti* females spent more time walking while being sprayed with sumithrin compared with bloodfed mosquitoes ($t_{18} = 4.07; P < 0.05$).
In the postspray period, the percent of time walking had significant main effects of pesticide treatment ($F_{3,154} = 38.61; P < 0.001$) and species ($F_{1,154} = 50.18; P < 0.001$) (Fig. 6). All pesticide treatments resulted in more walking by female *Ae. aegypti* exposed to DUET and sumithrin compared with those treated with prallethrin, which walked more than the controls (Fig. 6). For *Ae. albopictus*, all pesticide treatments resulted in more time walking than the controls. Female *Ae. aegypti* responded to all pesticide treatments, with significantly more walking than *Ae. albopictus* females ($P < 0.05$).

The percent of time in flight during the spray period had significant main effects of treatment ($F_{3,151} = 10.76; P < 0.001$) and species ($F_{1,151} = 5.72; P < 0.05$), with significant interaction of species and treatment ($F_{3,151} = 6.23; P < 0.001$) (Fig. 5). All pesticide treatments increased the percent of time flying by *Ae. aegypti*, in contrast to prallethrin, which was the only pesticide increasing flight of *Ae. albopictus*. Greater increases in flight occurred with *Ae. aegypti* compared with *Ae. albopictus* for DUET ($t_{15} = 3.54; P < 0.001$) and sumithrin ($t_{15} = 3.11; P < 0.01$) treatments. In the postspray period, the percent of time in flight had...
significant main effects of treatment ($F_{3, 148} = 16.52; P < 0.001$), species ($F_{3, 148} = 8.71; P < 0.01$), and state ($F_{1, 148} = 12.13; P < 0.001$), with significant interactions of species and treatment ($F_{3, 148} = 8.56; P < 0.001$) and state and treatment ($F_{3, 148} = 2.81; P < 0.05$) (Fig. 6). The percent of time flying by unfed Ae. aegypti was twofold greater after sprays containing sumithrin than to other pesticide treatments (Fig. 6) and exposure to all pesticides increased flight compared with controls. For Ae. albopictus, however, flight was enhanced modestly in response to pesticide exposure (Fig. 6). In addition, after exposure to sumithrin, flight was increased more for unfed female Ae. aegypti ($t_{18} = 4.03; P < 0.01$) and Ae. albopictus ($t_{18} = 3.50; P < 0.01$) females than for bloodfed females.

**Discussion**

Populations of mosquitoes consist of individuals in a variety of different ages and physiological states (i.e., teneral, unfed, bloodfed, and gravid), with differences in insecticide susceptibility reported among different ages (Davidson 1958, Rowland and Hemingway 1987, Hodjati and Curtis 1999, Glunt et al. 2011, Rajatileka...
et al. 2011), between sexes (Davidson 1958, Doyle et al. 2009), and among blood-feeding status (Davidson 1958, Rawlings et al. 1981, Polsomoon et al. 2008). Within homes or their immediate surroundings, the common physiological states of $Ae. aegypti$ and $Ae. albopictus$ are unfed and freshly bloodfed individuals (Scott et al. 2000). Previous studies have indicated that insecticide susceptibility is lowest just after blood-feeding (24 h for $Ae. aegypti$, 48 h for Culex, and 72 h for Culiseta: Eliason et al. 1990), with a gradual increase in susceptibility in unfed females (Eliason et al. 1990, Rajatileka et al. 2011). Hadaway and Barlow (1956) reported similar results for $Ae. aegypti$ and Anopheles stephensi Liston to DDT. Ramifications of this have been reported previously (cited in Eliason et al. 1990) with the rapid appearance of egg rafts and larvae shortly after a spray application, indicating that the application did not effectively kill bloodfed and parous adults. Because of the difficulty in effectively controlling these recently bloodfed females, it was important to compare the sublethal exposure of unfed and bloodfed $Ae. aegypti$ and $Ae. albopictus$ females to the individual and combined pyrethroids in this study on flight and thus the potential exposure to ULV droplets during the period of time before the insecticide spray has settled (Cooperband et al. 2010).

Overall, for nearly all of the parameters measured, the unfed or bloodfed state was not significant in analyses, indicating that all pesticide spray treatments were as effective against unfed females as against bloodfed females. The exception was the higher flight activity observed with unfed females compared with bloodfed females. Unlike the other pesticide treatments and indicated by significant state and treatment interactions, sumithrin exposure enhanced activity of unfed females with increased time walking and flying during spray periods, greater velocity of movement, more flight, and less time resting after the spray period. Sprays of prallethrin and DUET were equally effective in activating bloodfed and unfed females of both species.

The species of target mosquitoes can clearly affect the efficacy of treatment as demonstrated in laboratory (Pridgeon et al. 2008, Cooperband and Allan 2009) and field studies (Groves et al. 1997, Trout et al. 2007). Behavioral studies on sublethal exposure to different pyrethroids demonstrated differences in flight duration, velocity, turn angle, and angular velocity in three species of mosquitoes (Cohnstaedt and Allan 2011). In the current study with sublethal exposures to these pesticides, species was a significant factor in various behavioral attributes (e.g., percent of time in movement, time resting, and distance moved), with $Ae. aegypti$ more responsive and active in the presence of DUET and sumithrin sprays than $Ae. albopictus$. However, there was no difference between species in the percent of time knocked down. Clearly, species can affect some aspects of behavioral response to pesticides, particularly at sublethal doses. As indicated by significant interaction effects, the behavioral responses to pesticide treatments differed between species. Female $Ae. aegypti$ in the presence of DUET or sumithrin sprays demonstrated more activity (percent movement, moving velocity, distance covered, and percent of time flying and walking) in some of the measured parameters than $Ae. albopictus$ females. Conversely, $Ae. albopictus$ spent more time resting after those treatments.

Similar to Cooperband et al. (2010), results in this study support the role of the two pyrethroid insecticides (prallethrin and sumithrin) as locomotor stimulants (Dethier et al. 1960). The activating effect of sumithrin in the postspray period documented in Culex (Cooperband et al. 2010) was evident in unfed $Ae. aegypti$ but not in bloodfed or unfed $Ae. albopictus$. In a prior laboratory study evaluating DUET and its components on behavior of Cx. quinquefasciatus, excitation in the form of increased speed and flight duration was immediate in mosquitoes exposed to prallethrin (Cooperband et al. 2010). In contrast to the other treatments in that study containing prallethrin or DUET, exposure to sumithrin did not increase exposure to ULV droplets. As mosquito species vary with their responsiveness to sublethal exposures of pyrethroids, with Culex tending to respond or act more slowly than other species after exposure (Cooperband and Allan 2009, Cohnstaedt and Allan 2011), it was unclear how Aedes mosquitoes would respond to DUET and its components. Similar to previous studies with unfed Culex (Cooperband et al. 2010), unfed $Ae. aegypti$ and $Ae. albopictus$ females exposed to prallethrin and DUET moved more than controls both during and after spray periods. In a bottle-assay evaluation on the contact toxicity of DUET using field-collected mosquitoes, Qualls and Xue (2010) reported differences in species susceptibility; $Ae. albopictus$, Aedes taeniorhynchus (Wiedemann), and Psorophora columbiae (Dyar & Knab) were more susceptible than Cx. quinquefasciatus.

The value of ULV insecticide treatments that increase the movement and flight of exposed mosquitoes is clear particularly with respect to control efforts that target mosquitoes in unexposed and semiexposed places (Perich et al. 2000, Khadri et al. 2009). Peridomestic spraying remains a widely used tool for mediation of dengue transmission (Esu et al. 2010), and enhancement of efficacy of control of bloodfed as well as unfed females could greatly improve population reductions. Enhanced mosquito flight can result in greater exposure to ULV droplets (Cooperband et al. 2010). The behavioral (excitation) responses of $Ae. aegypti$ and $Ae. albopictus$ to excitatory active ingredients such as prallethrin and sumithrin expose them to more ULV droplets and reflects their potential value in controlling these dengue virus vectors. This impact might best be seen when they were used in outdoor habitats with $Ae. albopictus$ present such as in Singapore (Lim et al. 1961), Thailand (Ponlawat and Harrington 2005), and Hawaii (Effler et al. 2005). Furthermore, $Ae. aegypti$ control might be enhanced through the indoor use of these pyrethrins (if approved by appropriate regulatory agencies) where others have been used previously in India (Mani et al. 2005), Taiwan (Teng et al. 2007), Thailand (Pant
et al. 1974, Koenraadt et al. 2007), Honduras (Perich et al. 2001), and Costa Rica (Perich et al. 2003). Regardless, it should be apparent that a single approach to public health vector control will not eliminate these important vectors of human and animal pathogens but should be considered as part of an integrated vector management framework (WHO 2004).

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References Cited


Miller, J. R., P. Y. Siegert, F. A. Amimo, and E. D. Walker. 2009. Designation of chemicals in terms of the locomotor

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