

Effects of sublethal pyrethroid exposure on the host-seeking behavior of female mosquitoes

Lee W. Cohnstaedt and Sandra A. Allan[✉]

USDA-ARS-CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608, U.S.A., Sandy.Allan@ars.usda.gov

Received 7 April 2011; Accepted 7 September 2011

ABSTRACT: A common method of adult mosquito control consists of residual application on surfaces and aerial spraying often using pyrethroids. However, not all insects that contact insecticides are killed. Sublethal exposure to neurotoxic compounds can negatively affect sensory organs and reduce efficiency of host location. Flight tracks of host-seeking female *Culex quinquefasciatus*, *Anopheles albimanus*, and *Aedes aegypti* in a wind tunnel were video-recorded to compare activation of host-seeking and patterns of flight orientation to host odors. During host-seeking flights, all three mosquito species differed significantly in flight duration, velocity, turn angle, and angular velocity. Mosquitoes were then exposed to sublethal levels (LD_{25}) of pyrethroid insecticides to evaluate the effects of the neurotoxicants 24 hours post-exposure. Significant reductions in time of activation to flight and flight direction were observed in mosquitoes exposed to deltamethrin and permethrin. Additionally, pesticide-treated *Cx. quinquefasciatus* mosquitoes flew significantly slower, spent more time in flight, and turned more frequently than untreated controls. *Journal of Vector Ecology* 36 (2): 395–403. 2011.

Keyword Index: *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles albimanus*, pesticide exposure, host orientation.

INTRODUCTION

In a public health setting, pyrethroid-based insecticides are most often used for adult mosquito control programs. Adult mosquito populations are often reduced by aerial sprays and residual treatments and, although effective at preventing disease transmission by repelling or killing mosquitoes, not all mosquitoes that contact the pesticides will die from contact with the aerial droplets or landing on treated surfaces. Some mosquitoes may be pesticide-resistant and others may receive a sublethal dosage of pesticide. The sublethal effects of pesticides on adult mosquitoes is not well reported, although considerable research has been conducted on larval mosquitoes (reviewed in Elliott et al. 1978, Robert and Olson 1989), non-target organisms (Newsom 1967) and beneficial insects (Delpuech et al. 2001, Desneux et al. 2003, Thompson 2003, Prasifka et al. 2007).

The predominantly nocturnal biting *Culex quinquefasciatus* (Say) is an important vector of West Nile and St. Louis encephalitis viruses in the United States (Foster and Walker 2002, Turell et al. 2001) and lymphatic filariasis globally. Another nocturnally biting mosquito, *Anopheles albimanus* (Wiedemann), is the principal vector of malaria in Central America (Breeland 1972). The daytime biting *Aedes aegypti* (L) is the principal disease vector of dengue and yellow fever (Foster and Walker 2002). Although mosquito behavioral patterns, such as being nocturnally active, strong fliers, and preferred host-seeking heights, are broadly reported (Foster and Walker 2002), there has not been a detailed comparison of the differences in host-seeking behaviors. Behavioral differences may affect the optimal delivery of pesticides applied for control of mosquito populations.

Pyrethroid insecticides function by interfering with

the insect nervous system. They prevent sodium channels from closing which leads to trembling or paralysis usually followed by death (Vijverberg and van den Berken 1990). Pyrethroids are more stable synthetic analogs of pyrethrin and are preferred in public health settings because of their high arthropod specificity, a low mammalian toxicity, and a rapid degradation (reviewed by Vijverberg and van den Berken 1990). Modifications to basic pyrethroid structure has increased the effectiveness of the pyrethroids while changing their properties and uses (Carter 2006). Permethrin, a type I pyrethroid without a cyano moiety in the alpha position, generally affects the peripheral nerves, whereas deltamethrin, a type II pyrethroid with the cyano group, affects the entire nervous system (reviewed in Soderlund and Bloomquist 1989). Permethrin is used for intradomestic residual spraying and as a treatment for clothing and bednets to prevent mosquito bites (Schreck and Kline 1989). Deltamethrin applications primarily consist of intradomestic residual spraying, aerial spraying (Clayton and Sander 2002), and as a long-lasting insecticide on the bed-net Permanet®3 (Westergaard-Frandsen). During host-seeking, mosquitoes may encounter sublethal doses of neurotoxic compounds from contact with treated substrates or aerosolized insecticides (ultralow volume sprays or insecticide fogging). While most pesticide applications are evaluated for their direct killing potential, pyrethroid applications may also be detrimental to neurological pathways necessary for host-seeking behavior. Pyrethroid exposure may result in excitation or repellent responses similar to those for several repellents (Greico et al. 2007, Cooperband et al. 2010, Miller et al. 2009), but the effect of this on subsequent host-feeding remains unclear. If sublethal pyrethroid exposure does cause reduced olfactory detection, altered behavior, learning inhibition, and

changes in feeding patterns (Haynes 1988), then a reduction in longevity, host-seeking efficiency, biting behavior, and a change in time flying will reduce the mosquito's probability of transmitting disease based on mathematical modeling, such as for malaria using the classic Ross-MacDonald equation for vectorial capacity (Klempner et al. 2007), West Nile virus (Wonham et al. 2006), and dengue virus (Newton and Reiter 1992). The current study examines the effects of a sublethal exposure to the pyrethroids, permethrin and deltamethrin, on host-seeking activation and orientation of *Cx. quinquefasciatus*, *An. albimanus*, and *Ae. aegypti*.

MATERIALS AND METHODS

Insect rearing

Culex quinquefasciatus, *An. albimanus*, and *Ae. aegypti* were raised at the USDA Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, FL, following protocols of Gerberg et al. (1994). The mosquitoes used in trials were non-blood-fed females, seven to ten days of age, maintained on a 10% sucrose solution (w/w). The colonies were provided with blood meals in the form of heated blood sausages which consisted of a lamb skin membrane containing defibrinated bovine blood. Adult mosquitoes were maintained at 28°C and a 14:10 (L:D) photoperiod. Larvae were provided *ad libitum* with an abundance of food while in the colony and during the 24 h post-treatment. Environmental conditions were maintained between 25 to 27°C and 52-82% relative humidity.

Pesticide treatment

Prior to behavioral studies, the optimal sublethal doses were determined for each pesticide. Adult female mosquitoes were topically treated with technical grade permethrin (98% pure, ChemService, West Chester, PA) or deltamethrin (99% pure, ChemService, West Chester, PA) serially diluted in acetone (99% pure, Acros Organics, Morris Plains, NJ). Pesticide was delivered by pipeting 1 µl of acetone containing the insecticide onto the thorax of individual mosquitoes immobilized by chilling (4°C). Controls consisted of acetone treatment without pesticide. After treatment, mosquitoes were held in screened cardboard pint containers with continuous access to 10% sucrose and mortality was scored at 24 h. The surviving mosquitoes were used for behavioral experiments. The

LD₂₅ for each species and pyrethroid was based on a dose response curve calculated by probit analysis using Polo Plus software (LeOra Software, Berkeley, CA). Preliminary testing indicated that LD₂₅ was the highest dose of pesticide that knocked down all mosquitoes but caused minimal external damage (e.g., leg autotomy) to the 75% of the mosquitoes which survived 24 h later.

Females of *Cx. quinquefasciatus* and *Ae. aegypti* received a dose of 1.6 ng/µl and 1.4 ng/µl, respectively, of permethrin and deltamethrin to obtain an average mortality of 25% (LD₂₅) at 24 h. Females of *Anopheles albimanus* were the most susceptible and required a dose of 0.15 ng/µl of permethrin and deltamethrin to obtain the LD₂₅ at 24 h. Cold-immobilized mosquitoes in the control groups had no mortality or less than one per 30 mosquitoes. The replicates were limited to <28 individuals because of the laborious nature of hand treating three species of mosquitoes with three treatments to insure uniform exposure during treatment.

Flight activation and orientation

For host-seeking behavioral trials, treated mosquitoes were released individually 24 h after treatment into a wind tunnel flight chamber between 09:00-13:00, which corresponded to the lighted phase for the day-biting mosquitoes and the dark phase for the photoperiod-adjusted mosquito colony. Mosquito releases were conducted over a one-month period to insure uniform environmental conditions. The Plexiglas wind tunnel measured 121.9 cm long x 29.2 cm wide x 30.5 cm high with laminar air flow at 3.6 m/sec as described by Cooperband et al. (2010) (Figure 1). Carbon dioxide as a flight stimulant (Gillies 1980), was released into the middle of the air stream at 50 ml/min. Additionally, a mixture of host-associated chemicals, known to be attractants for mosquitoes and sand flies, was used to provide a consistent attractive source (Mann et al. 2009). This chemo-attractant mixture consisted of 2.5% 1-octen-3-ol (98% pure, Sigma-Aldrich, St. Louis, MO), 2.5% 1-hexen-3-ol (98% pure, Acros Organics, Morris Plains, NJ) and 95% acetone (99% pure, Acros Organics, Morris Plains, NJ). Odor delivery was achieved by pipeting the attractant mixture (25 ml) onto filter paper (Fisher Scientific, Pittsburgh, PA) placed in a 9 cm diameter Petri dish affixed to the bottom of the wind tunnel 90 cm from the mosquito entry port. The volatilized chemicals were

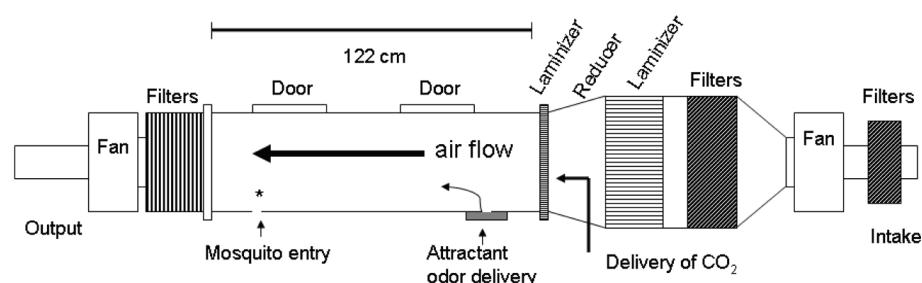


Figure 1. Diagrammatic representation of wind tunnel.

continuously passively diffused through a 3 cm hole in the wind tunnel by the lower pressure caused by the moving air within the tunnel. Pretesting with smoke confirmed the uptake and distribution of the attractant within the wind tunnel.

The temperature and humidity within the chamber was monitored during each trial. Mosquito host orientation was evaluated from video recorded flight tracks of mosquitoes. Mosquitoes were aspirated into a collection vial and the vial held to the entrance port until it flew into the wind tunnel. If the mosquito did not exit the vial in 5 min it was considered a non-responder. Each trial started when the mosquito entered the wind tunnel from the entrance port (3 cm diameter) in the bottom of the wind tunnel and ended when the mosquito ceased moving on the screens at the ends of the tunnel. The attractant release point was 18 cm from the upwind screen. Some mosquitoes did not respond by flying towards the upwind (positive flight) or downwind (negative flight) screens, rather they displayed incomplete flight in one direction and after 2 min. the trial was terminated. A Chi-square test was used to detect significant changes in positive flight activation response between treated and untreated individuals. The behavior of the control mosquitoes was used as the expected response and the observed response was the behavior of the insecticide-treated mosquitoes. Significant differences in behavioral responses were detected using a goodness-of-fit test and reported data is at a 95% confidence interval.

Video recording was conducted using a video camera (Panasonic WV-BP334 equipped with a Rainbow lens 1/3" manual IR-sensitive, 3-8 mm, F14) (Rainbow CCTV, Costa Mesa, CA) using a real-time mpeg encoder (Canopus Digital Video Recorder EMR100) and MediaCruise Software (Version 2.24.001) and a laptop computer. Nocturnally active mosquitoes (*Cx. quinquefasciatus* and *An. albimanus*) were filmed under infrared lighting (920 nm) with a light-absorbing black ABS board as a background. Diurnally active mosquitoes (*Ae. aegypti*) were filmed on a white background with external incandescent illumination (4 - 25 W bulbs). Flight tracks were recorded from the mosquito release point to their final destination (positive flights) and the videos digitized to obtain x- and y-coordinates using Ethovision® (v. 6.1 Noldus, Wageningen, Netherlands) or Motus® (v. 8.5.2 Vicon, Oxford, UK) software. Motus software was used to manually digitize tracks Ethovision software was unable to detect. All digitized flight tracks were analyzed using the same arena parameters in Ethovision to insure uniform movement and calibration for both manual and automated digitizing. From each flight track the following calculations were made: time in flight (s), velocity (cm/s), and total degrees turned (degrees). Time in flight was the duration of time needed to move from the mosquito release area to the end screen of the wind tunnel. The velocity was the total distance divided by time in flight and resulted in the average flight speed of the mosquito. Total degrees turned was defined as the cumulative degrees turned during

Table 1. Numbers of mosquitoes treated as controls or sublethally (LD_{25}) with pesticides responding to chemical attractants combined with carbon dioxide in a wind tunnel.

	Numbers of mosquitoes			
	Positive flight*	Negative flight	Incomplete response	Total
<i>Culex quinquefasciatus</i>				
Control	23	1	1	25
Permethrin [1.6 ng/ μ l]	9	12	4	25
Deltamethrin [1.4 ng/ μ l]	12	9	4	25
<i>Anopheles albimanus</i>				
Control	16	10	2	28
Permethrin [0.15 ng/ μ l]	14	14	0	28
Deltamethrin [0.15 ng/ μ l]	12	16	0	28
<i>Aedes aegypti</i>				
Control	20	0	0	20
Permethrin [1.6 ng/ μ l]	13	1	6	20
Deltamethrin [1.4 ng/ μ l]	16	0	4	20

*Positive flight is defined as movement upwind from the entrance of the chamber to the end of the chamber near the attractants. Negative flight is movement downwind and away from the attractants. Incomplete flight indicates the mosquito did not move down the tunnel completely in either direction.

flight. Mean angular velocity was calculated from the total degrees turned divided by the time in flight. Metrics from flight tracks were compared using ANOVA (Proc GLM) using SAS software and separation of means were tested using Student-Newman-Keuls at $P < 0.05$. Data were visually observed for deviations from a normal distribution using Proc Logistic and none were found.

RESULTS

Flight activation

When released into the wind tunnel, 92% of the untreated control *Cx. quinquefasciatus* mosquitoes flew upwind towards the attractants (Table 1). A single individual (4%) flew downwind away from the attractants and another individual (4%) did not fully respond by flying to either end. After permethrin treatment, females of *Cx. quinquefasciatus* had a significantly different response to the controls (Chi-squared test, $\chi^2 = 138.6$, df = 2, $P < 0.001$) with only 36% of the treated mosquitoes responding positively by moving towards the attractants compared to 92% for the controls. Forty-eight percent of treated females responded negatively by flying away from the attractant and 16% had no response. Treatment with deltamethrin caused significant changes in behavior (Chi-square test, $\chi^2 = 78.3$, df = 2, $P < 0.001$). Positive flight towards attractants was reduced to 48% and negative flight and incomplete responses increased to 48% and 16%, respectively.

The attractants and carbon dioxide did not elicit a consistent positive response from *An. albimanus* mosquitoes. The acetone-treated (control) mosquitoes flew in both directions in the wind tunnel with 57% moving towards the attractants and 36% moving away. Two individuals (7%) had incomplete responses. The responses of the permethrin-treated mosquitoes were not significantly different from the controls (Chi-square test, $\chi^2 = 3.9$, df = 2, $P > 0.05$); however, deltamethrin-treated individuals did differ (Chi-square test, $\chi^2 = 6.6$, df = 2, $P < 0.05$). After permethrin treatment, 50% of the mosquitoes flew towards the attractant and 50% away from the attractant. Treatment with deltamethrin had a similar effect, with 43% moving upwind towards the attractant and 57% flying downwind and away from it.

Female *Ae. aegypti* that received the acetone control treatment (100%) flew towards the attractants. Fewer mosquitoes responded after the permethrin (Chi-square test, $\chi^2 = 27.4$, df = 2, $P < 0.001$) and deltamethrin treatments (Chi-square test, $\chi^2 = 9.8$, df = 2, $P < 0.01$). Fewer permethrin-treated *Ae. aegypti* (65%) flew to the attractants and a single individual (5%) responded negatively while the remaining 30% demonstrated incomplete responses. Fewer deltamethrin-treated mosquitoes flew towards the attractant (80%) and more had incomplete responses (20%).

Host orientation

Not all flight paths of positively responding mosquitoes were usable or digitized because of the difficulty and time consuming nature associated with digitizing flight

tracks on small fast flying insects. Therefore, a subset of the positive flight tracks were digitalized resulting in ten control, eight permethrin, and eight deltamethrin tracks for *Cx. quinquefasciatus* host orientation analysis. *Anopheles albimanus* had eight control, 11 permethrin, and nine deltamethrin digitized tracks. The smaller diurnal *Ae. aegypti* was more difficult to film and resulted in eight control, eight permethrin, and 11 deltamethrin digitized tracks.

The flight path characteristics of the control mosquitoes were compared to determine differences between mosquito species host orientation behavior. Several aspects of the host-seeking behavior of *Cx. quinquefasciatus*, *An. albimanus*, and *Ae. aegypti* were compared. Findings between treated and control flight paths were significantly different for all three species. Untreated *Cx. quinquefasciatus* mosquitoes were in flight for 3.1 ± 0.5 s, significantly shorter than both *An. albimanus* (7.7 ± 2.8 s) and *Ae. aegypti* (5.7 ± 0.9 s) flight times ($F = 4.84$, df = 2, $P < 0.05$) (Figure 2). Similarly, the control *Cx. quinquefasciatus* flight speed (85.6 ± 8.6 cm/s) was significantly faster than the speed of *Ae. aegypti* (44.2 ± 9.8 cm/sec) and *An. albimanus* (35.1 ± 2.9 cm/sec) ($F = 12.26$, df = 2, $P < 0.0001$) (Figure 3). Conversely, the total degrees turned by control *Cx. quinquefasciatus* averaged 726 ± 186 degrees which was significantly fewer than the $1,621 \pm 407$ degrees by *An. albimanus* and $1,804 \pm 262$ degrees by *Ae. aegypti* ($F = 3.99$, df = 2, $P < 0.05$) (Figure 4).

Culex quinquefasciatus was the only species to have significant changes in flight path characteristics caused by the sublethal effects of pyrethroid poisoning. *Culex quinquefasciatus* control mosquitoes spent less time in flight (3.1 ± 0.5 s) than permethrin-treated ones (6.9 ± 1.7 s) but not significantly more than deltamethrin-treated mosquitoes (4.9 ± 0.9 s) ($F = 3.60$, df = 2, $P < 0.05$) (Figure 2). Flight velocity of control *Cx. quinquefasciatus* mosquitoes was faster (85 ± 9 cm/s) than either permethrin- (55 ± 11 cm/s) or deltamethrin- (53 ± 11 cm/s) treated mosquitoes ($F = 4.47$, df = 2, $P < 0.05$) (Figure 3). Untreated control *Cx. quinquefasciatus* flew in a less serpentine route with fewer turns (726 ± 187 total degrees) than permethrin-treated mosquitoes (1499 ± 340 total degrees) but not deltamethrin-treated ones (928 ± 279 total degrees) ($F = 3.39$, df = 2, $P < 0.05$) (Figure 4).

Despite an increase in negative flights during flight activation in both species, *Aedes aegypti* mosquito flight tracks were not significantly different between controls and pesticide treatments, demonstrating no long-term effects of the pesticide exposure on time in flight ($F = 0.56$, df = 2, $P > 0.05$), velocity ($F = 0.77$, df = 2, $P > 0.05$), and total turn angle ($F = 0.03$, df = 2, $P > 0.05$). Similarly, *An. albimanus* flight tracks were not significantly different between controls and pesticide treatments for time in flight ($F = 1.39$, df = 2, $P > 0.05$), velocity ($F = 0.54$, df = 2, $P > 0.05$), and total turn angle ($F = 0.71$, df = 2, $P > 0.05$).

DISCUSSION

We found significant differences in the behavior of

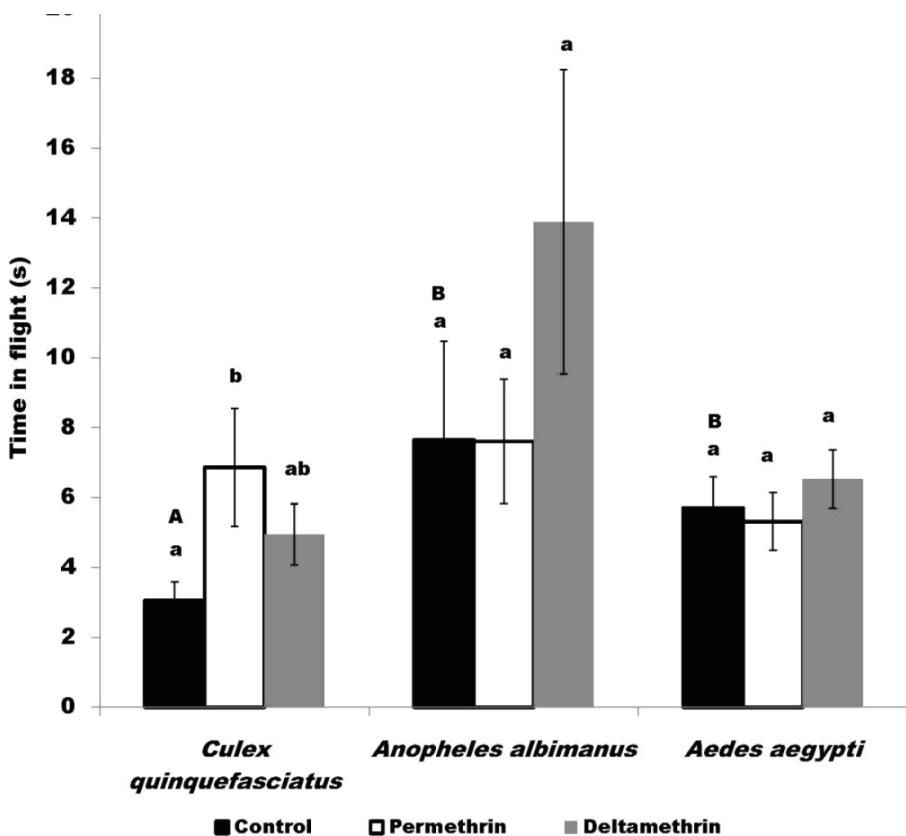


Figure 2. Total time spent in flight (sec) (\pm SE) by mosquitoes that flew upwind towards the attractants after treatment as controls or sublethally (LD_{25}) with pesticides. Different capital letters indicate significant differences between species for control means. Different lower case letters indicate significant differences within species between treatment groups at $P<0.05$.

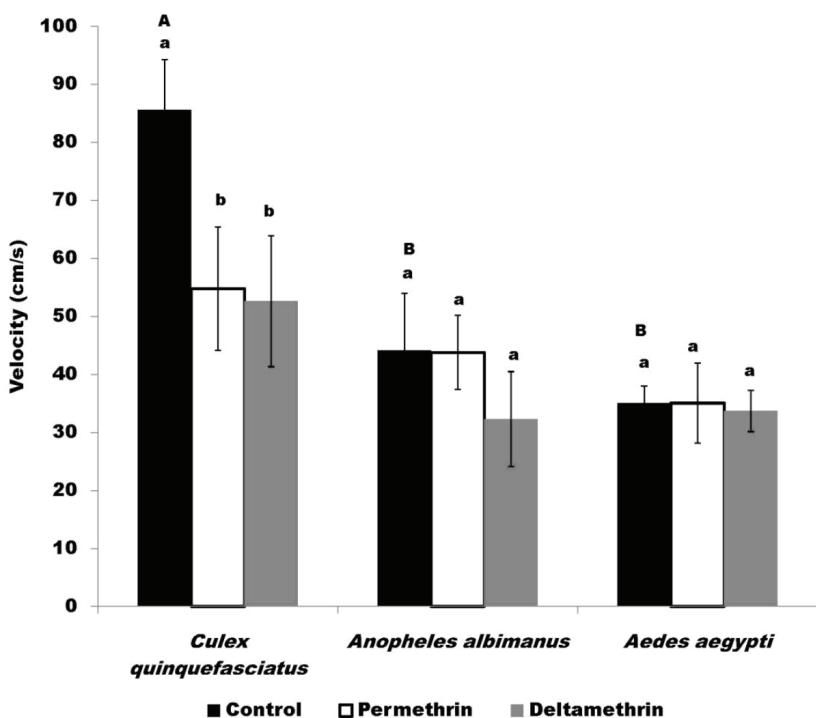


Figure 3. Average flight velocity (cm/sec) (\pm SE) by mosquitoes that flew upwind towards the attractants after treatment as controls or sublethally (LD_{25}) with pesticides. Different capital letters indicate significant differences between species for control means. Different lower case letters indicate significant differences within species between treatment groups at $P<0.05$ level.

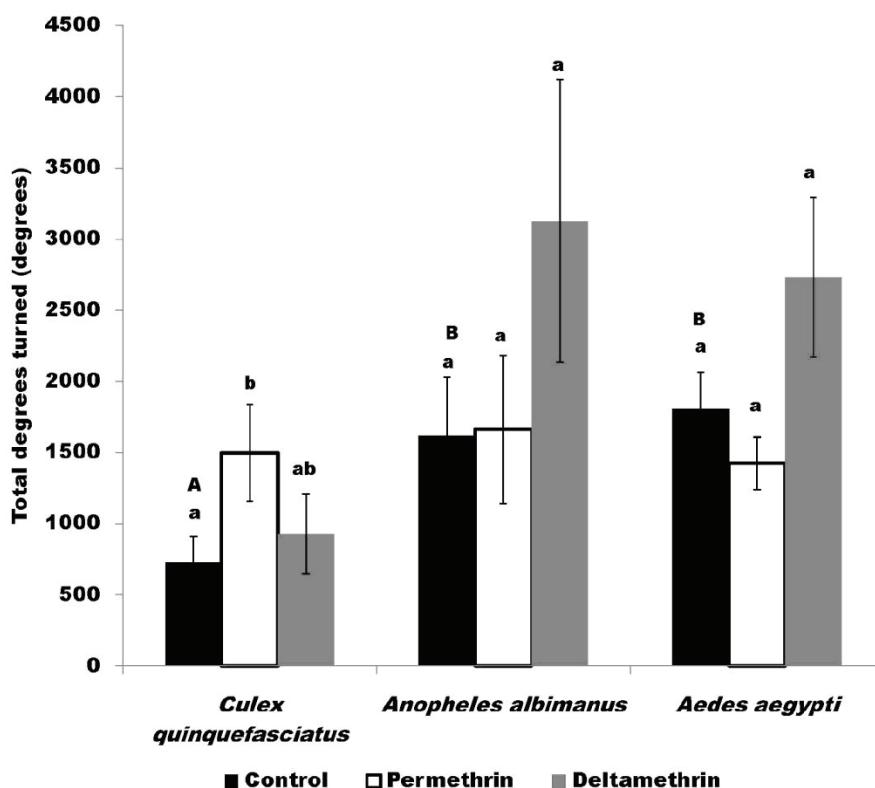


Figure 4. Total number of degrees (\pm SE) turned by mosquitoes that flew upwind towards the attractants treated as controls or sublethally (LD_{25}) with pesticides. Different capital letters indicate significant differences between species for control means. Different lower case letters indicate significant differences within species between treatment groups at $P < 0.05$ level.

Cx. quinquefasciatus, *An. albimanus*, and *Ae. aegypti* when orienting towards host-associated odors. We also report that mosquitoes treated with sublethal levels of pyrethroids are less responsive to attractants than were controls at 24 h post-exposure. When *Cx. quinquefasciatus* respond, the insecticide-treated mosquitoes' flight is slower and more circuitous than that of controls. Several studies discuss the effects of pyrethroid-based insecticides on non-target insect species (Thompson 2003, Newsom 1967), but few have looked at the sublethal effects of these neurotoxic compounds on target mosquito behaviors such as flight activation, orientation, and blood feeding. Sublethal doses of pyrethroids interfere with sodium ion channels, causing temporary paralysis but not death (Vijverberg and Bercken 1990), however they may also be causing longer term nerve damage to sensory organs or to nerves responsible for flight activation, coordination, and orientation.

Sublethal doses of pyrethroids significantly impacted flight activation (Table 1). As untreated mosquitoes, the combination of CO_2 and the attractants, 1-hexan-3-ol, 1-octen-3-ol, and acetone, induced a high percentage of *Cx. quinquefasciatus* (92%) and *Ae. aegypti* (100%) mosquitoes to flight. However, after application of a sublethal dose of permethrin or deltamethrin exposure, this response was reduced at 24 h after exposure. Permethrin exposure reduced positive *Cx. quinquefasciatus* flights by 56% and *Ae. aegypti* by 45%, indicating compromise of the mosquitoes' ability to detect flight stimulants by exposure to this type

I pyrethroid pesticide that affects the peripheral nerves. Exposure to the type II pyrethroid deltamethrin resulted in similar significant reductions in flight activation and orientation as permethrin in *Cx. quinquefasciatus* and *Ae. aegypti*. After exposure to either of the pyrethroids, *Cx. quinquefasciatus* mosquitoes had an increased tendency of negative flights (away from the attractants) and incomplete flights (flights not ending either upwind or downwind). Female *Ae. aegypti* exposed to either pesticide had an increased number of incomplete flights. Both findings suggest that mosquitoes exposed to sublethal doses of pesticides have more difficulty orienting towards host odors. Flight activation and orientation require detecting subtle differences in attractant concentration to locate the odor source. The lack of flight activation and reduced orientation efficiency may be from pyrethroid damage to sensory nerves and/or natural behavioral variation. After initial flight activation, downwind flight away from the attractants has been observed in untreated *Ae. aegypti* in the presence of a homogenous carbon dioxide plume similar to the one generated in this wind tunnel (Geier et al. 1999). In this study, if the neurotoxicants damaged the palps, the carbon dioxide concentrations may appear homogenous because the small variations in the plume were undetectable.

Anopheles albimanus were generally less responsive to the attractant blend with a low percentage of mosquitoes (57%) demonstrating positive flight in the presence of the flight stimulant and attractants. However, sublethal

applications of pyrethroids altered behavior of treated mosquitoes with a greater tendency to fly downwind away from the attractants after both pesticide treatments. Downwind flight has been observed in a study of *Anopheles* mosquitoes in the Gambia. The study compared mosquito flight direction in unbaited suction traps with wind direction and found only 64% of mosquitoes flew upwind, although the amount varied between 87% and 43% during the duration of the study (Gillies et al. 1978). The reduction in responsiveness to attractants observed in the current species has also been reported in other insects. Application of lambda-cyhalothrin (LD_{20}) altered orientation and oviposition behaviors of the parasitoid wasp, *Aphidius ervi* (Haliday), to the aphid, *Myzus persicae* (Sulzer) (Desneux et al. 2003). The egg parasitoid, *Telenomus busseolae*, did not respond to long distance host pheromones when exposed to cyfluthrin at an LD_{25} level, although this difference in response was not detected after treatment with deltamethrin (Bayram et al. 2010). The observed reduction in flight activation and incomplete flights may be a consequence of damage to sensory neurons causing reduced detection of attractants or CO_2 as the pesticide is transferred from the tarsi to the palps and antennae during grooming behavior, which increases in post-pesticide exposure (Desneux et al. 2007). The incomplete flights may also be important when considering mosquito control efforts because combining bednets with indoor residual spraying may increase bite protection if mosquitoes receive a sublethal dose from a bednet and then land more frequently on treated walls before exiting the domicile.

None of the species had significantly longer flight paths than the others, possibly a consequence of the restrictive dimensions of the wind tunnel. As the length was five times larger than the height, a very serpentine path would be necessary to detect significant differences in distance traveled. Increased flight distance would enhance treatment of individuals that fly through the plumes of insecticide applied as ultralow volume sprays and insecticide fogs.

Pesticide exposure altered the *Cx. quinquefasciatus* flight path characteristics more than the other species tested. Permethrin exposure caused *Cx. quinquefasciatus* mosquitoes to spend more time in flight at a slower velocity and to take more turns. Deltamethrin exposure decreased flight velocity. The effect of enhanced turning in response to sublethal pesticide exposure has been reported previously. When orienting to oviposition pheromones, pesticide-exposed gravid *Cx. quinquefasciatus* females demonstrated higher rates of turning and slower flight speed (Pile et al. 2008). In the case of honey bees, parathion poisoning has been suggested to affect their ability to orient relative to gravity but not their overall navigation (Stephen and Schricker 1970). The flight tracks of pesticide-treated *Anopheles* and *Aedes* were not significantly different from controls although the effects of pesticide exposure in changing flight behavior is well documented using video analysis in other species. Prasifka et al. (2007) found after exposure to pyrethroid-based pesticides (lambda-cyhalothrin and tefluthrin) the carabid beetle, *Scarites*

quadriceps (Chaudhury), demonstrated an increase in total distance travelled, maximum velocity, and increased time moving. The effects of permethrin-exposed honey bees attempting to return to their colony were similarly disrupted as they were unable to follow the visual cues and learn new directions (Cox and Wilson 1984).

Exposure to deltamethrin resulted in slower flight velocities in *Cx. quinquefasciatus*, possibly because the treated mosquitoes were less able to detect the synthetic lure, track them efficiently, or their flight coordination was compromised. Deltamethrin affects the nervous system systemically, which explains why a similar finding of altered homing-flight ability was reported with deltamethrin-treated bees that did not return to the colony (Vandame et al. 1995). These bees had incorrect spatial perception or information retrieval problems that may also have happened to the mosquitoes. However, deltamethrin does not appear to affect all insects equally. The wasp, *Trichogramma brassicae* (Bezd), was able to detect and orient towards its corresponding sex pheromone after a $LD_{0.1}$ treatment of deltamethrin and no differences in distance moved, linear speed, or angular speed was found (Delpuech et al. 2001). Therefore, effects appear to vary among insect species as was observed among the three species of mosquitoes depending on the dose and duration of pesticide exposure. In general, no differences were detected in the flight path characteristics between mosquitoes treated with type I and type II pyrethroids.

Most studies with non-target insect studies report some degree of recovery from a sublethal insecticide exposure after 24 h. Desneux et al. (2003) observed that the parasitic wasp, *Aphidius ervi* (Haliday), recovered oviposition and host-seeking behavior 24 h post-lambda-cyhalothrin exposure. Similarly, the effects of permethrin, cypermethrin, and parathion were negligible 24 h post-exposure with no permanent effects on either memory function or foraging efficiency in bees (Rieth and Levin 2008, Schricker and Stephen 1970). However, in this study the mosquitoes did not recover full coordination within 24 h post-exposure. Activation to flight and flight track data were significantly different from controls, indicating a residual effect of the pesticide exposure. Specific coordinated activities may take longer to recover. Honey bees recovered learning within 24 h, but their responses remained slow for more than 3 days post-exposure (Taylor et al. 1987, Mamood and Waller 1990). In this study, the normal host-seeking behavior of mosquitoes remained altered at 24 h post exposure. The loss of sustenance for this duration of impaired sensory perception likely affects the mosquitoes' fitness or longevity and would likely reduce its capacity to transmit disease. In most mathematical models of disease transmission, reduced mosquito longevity lowers vectorial capacity by reducing the likelihood of disease dissemination within the mosquito and the probability of a second infectious bite. Therefore, mosquito population reduction methods directly reduce the probability of infectious bites by killing mosquitoes, but also indirectly affect those that received a sublethal dose that have a reduced probability of survival

and disease transmission because of a loss of host-seeking flight and orientation abilities. The authors do not advocate for sublethal application of pesticides as this will lead to the evolution of insecticide resistance. However, these results should be considered when calculating the evolutionary rates because of the influence on insect longevity and behavior. Similarly, pesticide applicators should not apply sublethal doses of insecticide to influence mosquito behavior instead of source reduction. However, the effect of sublethal doses may be used to better understand why some treatments are more effective than others on certain species. In conclusion, models should take into account the sublethal effects of pesticide exposure on the targeted mosquitoes when evaluating the efficacy of insecticides.

Acknowledgments

We thank Erin Vrzal and Matt Aubuchon for assisting with experiments and Haze Brown for providing mosquitoes. This study was funded by the Deployed War-Fighter Protection Program.

REFERENCES CITED

- Bayram, A., S. Gianandrea, A. Onofri, and E. Conti. 2010. Sub-lethal effects of two pyrethroids on the biological parameters and behavioral responses to host cues in the egg parasitoid *Telenomus busseolae*. *Biol. Contr.* 53: 153-160.
- Breeland, S.G. 1972. Studies on the ecology of *Anopheles albimanus*. *Am. J. Trop. Med. Hyg.* 21: 751-754.
- Carter, S.W. 2006. A review of the use of synthetic pyrethroids in public health and vector pest control. *Pest. Sci.* 27: 361-374.
- Clayton, J.S. and T.P.Y. Sander. 2002. Aerial application for control of public health pests. *Asp. Appl. Biol.* 66: 1-8.
- Cooperband, M.F., F. Golden, G. Clark, W. Jany, and S.A. Allan. 2010. Prallethrin-induced excitation increases contact between sprayed ultra-low-volume droplets and flying mosquitoes (Diptera: Culicidae) in a wind tunnel. *J. Med. Entomol.* 46: 292-306.
- Cox, R.L. and T.W. Wilson. 1984. Effects of permethrin on the behavior of individually tagged honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Environ. Entomol.* 13: 375-378.
- Delpuech, J.M., B. Legallet, and P. Fouillet. 2001. Partial compensation of the sublethal effect of deltamethrin on sex pheromonal communication of *Trichogramma brassicae*. *Chemosphere* 42: 985-991.
- Desneux, N., A. Decourtye, and J.M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52: 81-106.
- Desneux, N., M.H.P. Delegue, and L. Kaiser. 2003. Effects of sub-lethal and lethal doses of lambda-cyhalothrin on oviposition experience and host-searching behavior of a parasitic wasp, *Aphidius ervi*. *Pest Manag. Sci.* 60: 381-389.
- Elliott, M., N.F. Janes, and C. Potter. 1978. The future of pyrethroids in insect control. *Annu. Rev. Entomol.* 23: 443-469.
- Foster, W.A. and E.D. Walker. 2002. Mosquitoes (Culicidae). In: G. Mullen and L. Durden (eds.), *Medical and Veterinary Entomology*. pp. 203-262. Elsevier Science, San Diego, California.
- Geier, M., O.J. Bosch, and J. Boeckh. 1999. Influence of odour plume structure on upwind flight of mosquitoes towards hosts. *J. Exp. Biol.* 202: 1639-1648.
- Gerberg, E.J., D.R. Barnard, and R.A. Ward. 1994. *Manual for Mosquito Rearing and Experimental Techniques*. American Mosquito Control Association, Lake Charles, LA.
- Gillies, M.T. 1980. The role of carbon dioxide in host finding by mosquitoes (Diptera: Culicidae): a review. *Bull. Entomol. Res.* 70: 525-532.
- Gillies, M.T., M.D.R. Jones, and T.J. Wilkes. 1978. Evaluation of a new technique for recording the direction of flight of mosquitoes (Diptera: Culicidae) in the field. *Bull. Entomol. Res.* 68:145-152.
- Grieco, J.P., N.L. Achee, T. Chareonviriyaphap, W. Suwonkerd, K. Chauhan, M.R. Sardelis, and D.R. Roberts. 2007. A new classification system for the actions of IRS chemicals traditionally used for malaria control. *PlosOne* 8:e716.
- Haynes, K.F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. *Annu. Rev. Entomol.* 33: 149-168.
- Klempner, M.S., T.R. Unnasch, and L.T. Hu. 2007. Taking a bite out of vector-transmitted infectious disease. *New Engl. J. Med.* 356: 2567-2569.
- Mamood, A.N. and G.D. Waller. 1990. Recovery of learning responses by honeybees following sublethal exposure to permethrin. *Physiol. Entomol.* 15: 5-60.
- Mann, R.S., P.E. Kaufman, and J.F. Butler. 2009. *Lutzomyia* spp. (Diptera: Psychodidae) response to olfactory attractant- and light emitting diode-modified Mosquito Magnet X (MM-X) Traps. *J. Med. Entomol.* 46: 1052-1061.
- Miller, J.R., P.Y Siegert, F.A. Amimo, and E.D. Walker. 2009. Designation of chemicals in terms of the locomotor responses they elicit from insects: An update of Dethier et al. (1960). *J. Econ. Entomol.* 102: 2056-2060.
- Newsom, L.D. 1967. Consequences of insecticide use on nontarget organisms. *Annu. Rev. Entomol.* 21: 257-286.
- Newton, E.A.C. and P. Reiter. 1992. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. *Am. J. Trop. Med. Hyg.* 47: 709-720.
- Pile, M.M., M.S.J. Simmonds, and W.M. Blaney. 2008. Odour-mediated upwind flight of *Culex quinquefasciatus* mosquitoes elicited by a synthetic attractant. *Physiol. Entomol.* 16: 77-85.
- Prasifka, J. R., M.D. Lopez, R.L. Hellmich, and P.L. Prasifka. 2007. Effects of insecticide exposure on movement and population size estimates of predatory ground beetles (Coleoptera: Carabidae). *Pest Manag. Sci.* 64: 30-36.

- Rieth, J.P. and M.D. Levin. 2008. The repellent effect of two pyrethroid insecticides on the honey bee. *Physiol. Entomol.* 13: 213-218.
- Robert, L.L. and J.K. Olson. 1989. Susceptibility of female *Aedes albopictus* from Texas to commonly used adulticides. *J. Am. Mosq. Contr. Assoc.* 5: 251-253.
- Schreck, C.E. and D.L. Kline. 1989. Personal protection afforded by controlled-release topical repellents and permethrin-treated clothing against natural populations of *Aedes taeniorhynchus*. *J. Am. Mosq. Contr. Assoc.* 5: 77-80.
- Schricker, B. and W.P. Stephen. 1970. The effect of sublethal doses of parathion on honeybee behavior. I. Oral administration and the communication dance. *J. Apic. Res.* 9: 141-153.
- Soderlund, D.M. and J.R. Bloomquist. 1989. Neurotoxic actions of pyrethroid insecticides. *Annu. Rev. Entomol.* 34: 77-96.
- Stephen, W.P. and B. Schricker. 1970. The effect of sublethal doses of parathion II site of parathion activity, and signal integration. *J. Apic. Res.* 9: 155-164.
- Taylor, K.S., G.D. Waller, and L.A. Crowder. 1987. Impairment of a classical conditioned response of the honey bee (*Apis mellifera* L.) by sublethal doses of synthetic pyrethroid insecticides. *Apidol.* 18: 243-252.
- Thompson, H.M. 2003. Behavioural effects of pesticides in bees – Their potential for use in risk assessment. *Ecotoxicol.* 12: 317-330.
- Turell, M.J., M.R. Sardelis, D.J. Dohm, and M.L. O'Guinn. 2001. Potential North American vectors of West Nile Virus. *Ann. N.Y. Acad. Sci.* 951: 317-324.
- Vandame, R , M. Meled, M.E. Colin, and L.P. Belzunces. 1995. Alteration of the homing-flight in the honey bee *Apis mellifera* L. exposed to sublethal dose of deltamethrin. *Environ. Toxicol. Chem.* 14: 855-860.
- Vijverberg, H.P.M. and J.V. Bercken 1990. Neurotoxicological effects and the mode of action of pyrethroid insecticides. *Crit. Rev. Toxicol.* 21: 105-126.
- Wonham, M. J., M. A. Lewis, J. Renclawowicz, and P. van den Driessche. 2006. Transmission assumptions generate conflicting predictions in host-vector disease models: a case study in West Nile virus. *Ecol. Lett.* 9: 706-725.