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Controlling the Dengue Mosquito (Aedes aegypti): Assessment of the Effects of Metofluthrin, a Novel Vapor-Active Pyrethroid, on Mosquito Behavior in a Modeled Domestic Setting.

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Controlling the Dengue Mosquito (*Aedes aegypti*):
Assessment of the effects of metofluthrin, a novel vapor-active pyrethroid, on mosquito behavior in a modeled domestic setting.

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ABSTRACT:

Dengue fever is a potentially life-threatening illness that is endemic in over 100 countries and threatens approximately half of the world's population. The dengue virus is transmitted between humans by a mosquito vector, principally the dengue mosquito, *Aedes aegypti*. This mosquito prefers to live and feed in and around people's homes. As a result, preventing mosquito bites within a domestic setting is essential to controlling the dengue virus. Although several methods of bite prevention are currently available to the public, most of these methods only provide short-term protection from the dengue mosquito. In contrast, a recently developed synthetic pyrethroid called metofluthrin, when embedded in paper or plastic material, has been shown to effectively prevent *Ae. aegypti* from biting for several weeks. However, previous studies have disagreed over the ability of metofluthrin to spatially repel mosquitoes from a room. Furthermore, most metofluthrin studies have exposed large groups of mosquitoes to the chemical, and thus have not observed mosquito behavior on the individual level.

The aim of this study was to use a small-scale model of a domestic setting to observe the behavior of individual *Ae. aegypti* when exposed to metofluthrin. The model domestic setting consisted of a large container with a window, a dark harborage area, and an entrance port through which a blood meal could be offered to the mosquito. 34 female, laboratory-reared *Ae. aegypti* desiring a blood meal were released into the container one at a time, and their movements through the container were recorded during a five-minute control and five-minute metofluthrin period. Metofluthrin was found to dramatically reduce the number of human landings made by the mosquito, and appeared to spatially repel the mosquito to the window of the container. These observations indicate that metofluthrin has the potential to be an effective method of controlling *Ae. aegypti* in a domestic setting.

However, public acceptance and widespread use of metofluthrin is necessary for this mosquito control method to effectively prevent dengue outbreaks. To evaluate the efficacy of dengue education efforts in North Queensland, Australia and the likelihood that metofluthrin would be adopted as a mosquito control method in this region, 33 Cairns residents were interviewed. In general, Cairns residents were moderately knowledgeable about the dengue control techniques publicized by Queensland Health, and a high proportion expressed interest in using metofluthrin in their homes.

Key Words: *Aedes aegypti* behavior, dengue fever, metofluthrin, spatial repellency, domestic setting.

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1. INTRODUCTION

1.1 Dengue Fever in Tropical North Queensland

Dengue fever is an acute and potentially life-threatening illness characterized by fever, headaches, joint pain, muscle pain, and rash. Dengue can be caused by any of four closely related serotypes of the dengue virus, Dengue 1, 2, 3, and 4. These viruses belong to the family Flaviviridae, the same family as the viruses which cause yellow fever and Japanese encephalitis. The dengue virus is transmitted between humans by a mosquito vector, principally the dengue mosquito, *Aedes aegypti* (WHO 2009). Dengue is endemic in over 100 countries, and the disease threatens approximately half of the world's population (Gubler 2002). In 2005, the U.S. Centers for Disease Control classified dengue fever as "the most important mosquito-borne viral disease affecting humans," with millions of cases of dengue fever (DF) and thousands of cases of dengue hemorrhagic fever (DHF), a more severe form of dengue infection, occurring each year. The case fatality rate for dengue fever can be as high as 5%, but can be reduced to as low as 1% with early recognition and proper treatment (U.S. CDC 2009).

Over the past few years, dengue fever has become an increasingly significant public health issue in tropical North Queensland, Australia. Although dengue is not endemic in this region, six outbreaks of dengue fever occurred in Queensland in 2003 and 2004; one of these outbreaks brought about the first recorded death from dengue in Queensland in about 100 years (Queensland Government 2005). From November 2008 to September 2009, Queensland experienced its largest dengue outbreak in at least 50 years, with 931 confirmed cases of the disease. This outbreak claimed the life of an elderly Cairns woman and resulted in hundreds of people being admitted to the hospital (Bateman 2009).

On the global scale, the prevalence of dengue appears to be increasing as well. From 2000 to 2008, the World Health Organization recorded the presence of dengue fever in 15 new locations, including Hawaii, Nepal, and Bhutan. The widening geographical range of the dengue virus is likely a result of climate change, which has allowed the mosquito vectors of the dengue virus to expand their range (Isaac and Turton 2009). Clearly, effective management of the dengue virus is of utmost importance in North Queensland and globally.

1.2 Transmission of Dengue Fever by *Aedes aegypti*

On the mainland of North Queensland, the dengue mosquito *Aedes aegypti* is the only mosquito capable of transmitting the dengue virus. This domestic, day-biting mosquito is often dubbed the "cockroach of mosquitoes" because it prefers to live in and around people's homes, resting and hiding in dark areas and emerging to bite people around the feet and ankles. Furthermore, *Ae. aegypti* does not breed in swamps or drains, preferring to breed in man-made containers that hold water (such as cans, buckets, and tires) and natural containers such as bromeliads. Only the female mosquito bites humans, and can only become infected with the dengue virus after biting a human who has been infected with dengue for 3 to 4 days. 8 to 10 days after obtaining a blood meal from the infected human, the mosquito is capable of transmitting the virus to other people; thus, the cycle of dengue transmission takes about 11 to 14 days. Significantly, one dengue-infected female mosquito can bite and infect several people during her lifetime (Queensland Government 2009).

1.3 Current Methods of *Ae. aegypti* Control

Because the dengue virus requires a mosquito vector for transmission to humans, controlling *Ae. aegypti* in North Queensland allows for both prevention and control of dengue outbreaks. The dengue mosquito can be effectively controlled both by stopping the mosquito from breeding and by preventing the mosquito from biting humans.

To stop the dengue mosquito from breeding, North Queensland residents are encouraged to partake in "source reduction," the elimination of mosquito breeding sites. Water-holding containers in yards and houses that are potential breeding sites are located and removed or treated with insecticide. Mosquitoes are also killed before they can breed, mainly by discretely spraying insecticides in areas where *Ae. aegypti* likes to rest (Queensland Government 2005).

To prevent *Ae. aegypti* from biting and transmitting dengue fever to humans, Queensland Health recommends wearing insect repellants containing DEET, using allethrin-based mosquito coils, and wearing loose-fitting and light-colored clothing (Queensland Government, Pamphlet). However, these methods of mosquito bite prevention can only provide temporary protection from the dengue mosquito, and must be re-applied after a few hours to ensure continued effectiveness.

1.4 Metofluthrin as a Novel Method of Mosquito Control

In contrast, a recently developed synthetic pyrethroid called metofluthrin may provide effective and long-lasting protection from the dengue mosquito. Research by Ujihara and others showed that metofluthrin is about 40 times as effective as the allethrin in mosquito coils at causing knockdown of the southern house mosquito *Culex quinquefasciatus* (Ujihara et al. 2004). Furthermore, metofluthrin possesses a relatively high vapor pressure, which means that it can vaporize and disperse quickly at room temperature without the need for heat or electricity (Kawada et al. 2008). Metofluthrin has been embedded in a range of materials for use in mosquito control, from multilayered plastic strips to a plastic latticework. In an indoor domestic setting, these products have been shown to maintain effectiveness against mosquitoes for an extended period, ranging from 6 weeks up to 18 weeks (Kawada et al. 2005a; Kawada et al. 2006; Kawada et al. 2008). Metofluthrin has even been shown to prevent mosquitoes from biting in outdoor shelters without walls (Kawada et al. 2004; Kawada et al. 2005b).

In addition, metofluthrin possesses low mammalian toxicity (Matsuo et al. 2005). Although high doses of metofluthrin have been shown to produce liver tumors in rats, the mode of action of the tumor formation is specific to rat liver cells; the chemical thus appears to be harmless to human liver cells (Hirose et al. 2009; Yamada et al. 2009). These characteristics make metofluthrin especially promising for use against the dengue mosquito.

1.5 Current Study

Recent research by Rapley and others has shown that in an indoor domestic setting, a sustained release metofluthrin emanator disorients *Ae. aegypti* and prevents them from biting, and subsequently kills the disoriented mosquitoes. However, this study did not observe the behavior of individual mosquitoes, and based its conclusions mainly on human landing counts and mortality of mosquitoes. As a result, no definitive conclusion on the ability of metofluthrin to spatially repel or expel mosquitoes could be drawn (Rapley et al. 2009).

To gain a greater understanding of how metofluthrin affects the behavior of *Ae. aegypti*, a small-scale model of a domestic setting was designed and a metofluthrin-impregnated plastic mesh sheet placed inside. With this setup, individual mosquitoes could be released one at a time into the model, allowing their behavior to be observed in detail. In this manner, the repellant effects of metofluthrin and its impact on mosquito-human biting and mosquito mortality could be evaluated on a case-by-case basis.

2. METHODS

2.1 Rearing and Maintaining a Colony of *Ae. aegypti*

Adult female *Aedes aegypti* mosquitoes were provided by the Cairns Public Health Unit. *Ae. aegypti* eggs were hatched in a yeast solution (0.02g yeast/L) that was heated to 31°C. Hatched larvae were placed in metal trays also heated to 31°C, fed "My Dog" dry pellet food at a rate of half a pellet per half a tray every three days. Larvae were monitored daily for metamorphosis to pupae. Pupae were transferred to a small cup and placed in a cage that protected emerging adults from desiccation. Adults were fed a diluted raw honey solution and a piece of apple. Females were blood fed approximately every five days by placing a forearm or leg over the feeding mesh of the cage; this was done to allow the females to develop eggs to continue perpetuation of the colony.

2.2 Experimental Setup

2.2.1 Components of Experimental Container

Experiments were conducted in an 80L clear plastic container (65cm x 45cm x 34cm). This container was modified to create a small-scale model of a domestic setting, the primary location where *Ae. aegypti* bite humans, in the following manner:

- The sides and bottom of the *container* were roughened using sand paper to provide surfaces on which a mosquito could land.
- An *entrance port* was made by attaching a sock to an opening in the side of the container. This port was used to release the mosquito into the container before the experiment and to capture the mosquito at the conclusion of the experiment. During the experiment, I placed my hand through this port to present the mosquito with a blood-meal source.
- A *window* was made by cutting a hole (48cm x21cm) in one side of the container and covering the hole with a fine black screen. To ensure that this window was perceived as such by the mosquitoes, all other surfaces of the container were covered with white paper. In this manner, the inside of the container was primarily illuminated by light passing through the window.
- A *harborage area* was made by placing two red boxes (each 16cm x 16cm x 14cm) side-to-side, opposite the window and touching the side opposite the entrance port, with the open end of the harborage boxes facing the window. The inside of the red boxes were roughened using sand paper to provide surfaces on which a mosquito could land. (Note that the outside of the red boxes were already sufficiently rough to allow mosquito landing.)

A piece white fiberboard was used to cover the top of the box. A small hole was drilled into the center of the fiberboard and a piece of wire was inserted to hold the metofluthrin mesh or control mesh during trials (Figure 1).

Figure 1. Experimental container, as viewed through the window. All sides of the container were covered with white paper to block out light and ensure that the most light entered the container through the window. Other components of the experimental container included a harborage area consisting of two red boxes (left side of container), an entrance port (right side of container), and a white fiberboard top with a piece of wire to hang the metofluthrin or control mesh.

2.2.2 Study Site

Experiments were conducted in the basement car park of the Cairns Public Health Unit (CPHU), located at 19 Aplin St., Cairns, QLD (Figure 2).

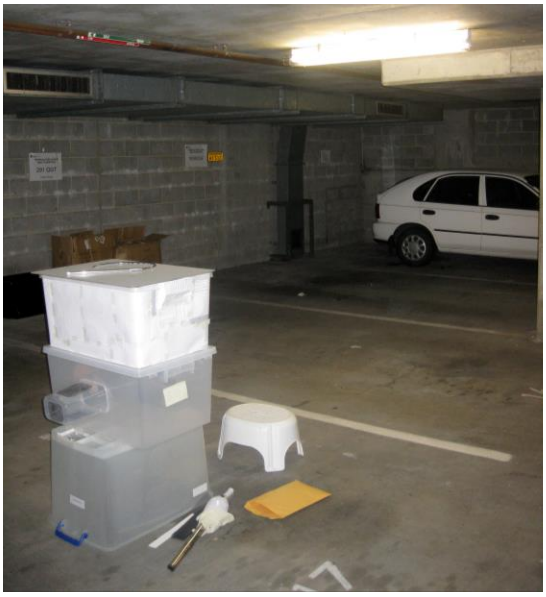

Figure 2. Study site, located in the basement of CPHU on 19 Aplin St., Cairns, QLD. The experimental container (top, white container) was elevated by placing it on top of two support containers in order to facilitate mosquito observation. The primary source of light at the study site was the overhead light above the experimental container; the container was positioned so that light would enter through the window (not visible). Some outside light also passed through windows in the carpark walls (not seen) to illuminate the study site.

The temperature and humidity of the study site were recorded using a Data Logger which was attached to the outside of the experimental container. The Data Logger took readings at five minute intervals from midday November 12, 2009 to midday November 26, 2009.

2.3 Container Site Preference Study

Prior to studying mosquito behavior during exposure to metofluthrin, it was necessary to evaluate how *Ae. aegypti* perceived the environment within the experimental container. The main purpose of this study was to determine if the container accurately modeled a domestic setting, namely whether the mosquitoes perceived the windows, walls, and harborage area of the container as such.

In order to evaluate *Ae. aegypti* perception of the container, four cohorts of five mosquitoes each were released into the container. Each mosquito's location was recorded after one minute and five minutes inside the container. In this manner, the landing sites preferred by *Ae. aegypti* initially and following acclimation could be determined.

2.4 Studying Metofluthrin Effects on *Ae. aegypti* Behavior

2.4.1 Determining Metofluthrin Dose

Mesh sheets embedded with metofluthrin (SumiOne Net 50/80B, Lot No. N-71026-1, *ai* cont. 5.1 wt%) were provided by Sumitomo Chemical Australia Pty Limited. All testing of the metofluthrin mesh sheets by Sumitomo used one sheet (150mm x 80mm, double sided) to treat a 30m^3 chamber (Davis, pers. comm. 2009). The amount of metofluthrin used in the experimental container in the present study was scaled accordingly to match this recommended dose. Thus, for the 0.1m^3 experimental container, 0.003 sheet (10x10mm, single sided), was used. Experiments were also conducted using double the recommended dose, 0.006 strip (10x20mm, single sided), to provide a comparison to the standard dose results (Figure 3). Metofluthrin mesh sheets were rated by Sumitomo to release the chemical for up to 30 days before needing to be replaced (Davis, personal comm. 2009). To ensure that the amount of metofluthrin released was consistent across experiments, however, new experimental sheets were cut from the source sheets weekly. When not in use, metofluthrin sheets were stored in an airtight bag in a dark location to prevent any possible degradation of the chemical.

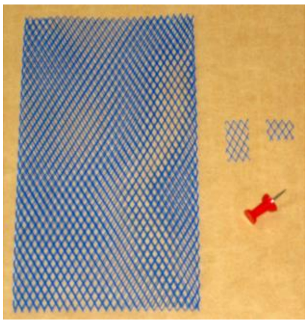

Figure 3. Metofluthrin mesh sheets (SumiOne Net 50/80B), provided by Sumitomo Chemical Australia Pty Limited. The large sheet (left) is a source sheet of the size provided by Sumitomo, and was designed to treat a 30m^3 room. The smallest sheet (right, 10x10mm) is the recommended dose for the 0.1m^3 experimental container, and the medium sized sheet (middle, 10x20mm) is double the recommended dose for the 0.1m^3 experimental container. Note that the source sheet is double sided, while the two smaller sheets are single sided.

2.4.2 Study Protocol: Acclimation, Control, Exposure to Metofluthrin

For each experiment, a non-blood fed female *Ae. aegypti* was selected from the colony. Females desiring a blood meal were attracted by placing a hand over the feeding mesh of the colony cage. A mouth aspirator was then used to capture a single female from the surface of the feeding mesh before any bites could occur. The antennae of the mosquito were visually inspected to confirm that the mosquito was female (Figure 4).

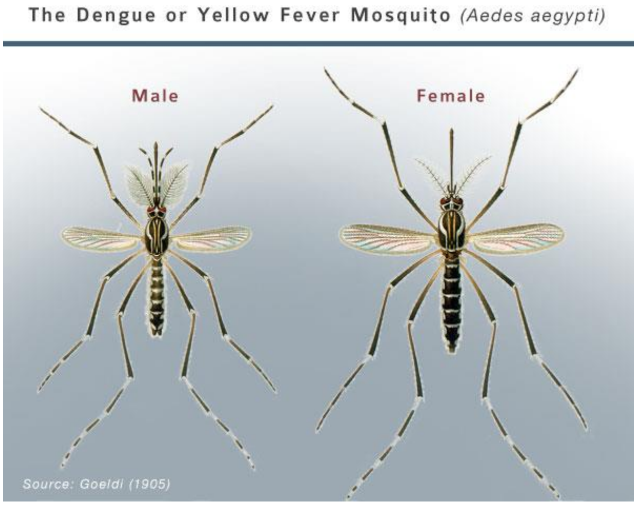

Figure 4. The morphology of male and female *Ae. aegypti*. Male antennae are much more "bushy" than female antennae, such that it is possible to distinguish the sex of a mosquito using the naked eye. Male *Ae. aegypti* are also typically smaller than female *Ae. aegypti*. Image courtesy of Biogents Mosquitaire (http://www.mosquitaire.it/cms/website.php?id=/en/tigermosquitoes.htm).

Once the female sex of the *Ae. aegypti* was confirmed, the mosquito was released into the experimental container for a five-minute "acclimation period." During this period, the mosquito was allowed to recover from any stress caused by transport from the colony, and to adjust to the environment of the experimental container without a blood-meal source present.

Following the conclusion of the acclimation period, a gloved hand was used to place a small piece of untreated fine black mesh (either 10x10mm or 10x20mm) on the wire hanging from the fiberboard top; this black mesh served as a control for the metofluthrin mesh. The glove was removed and the hand was placed into the experimental container to offer the mosquito a blood-meal source. The following five minutes were termed the "control period." During this period, mosquito location, time spent at each location, and number of landings on the hand were recorded. Whenever mosquito landings occurred, the mosquito was shaken off one second after landing to prevent the mosquito from obtaining a blood meal.

If no human landings occurred during the control period, the mosquito was considered to not be interested in a blood meal and the experiment was discontinued. If human landings did occur during the control period, the experiment continued on to the "metofluthrin period." The untreated black mesh was removed from the experimental container, and a gloved hand was used to place a piece of metofluthrin mesh of equal size (either 10x10mm or 10x20mm) onto the wire hanging from the fiberboard top. Again, the glove was removed and the hand was placed into the experimental container to offer the mosquito a blood-meal source. During the following five-minute metofluthrin period, mosquito location, time spent at each location, and number of landings on the hand were recorded. Blood feeding was again prevented by shaking the hand one second after mosquito landing.

Following the conclusion of the five-minute metofluthrin period, the mosquito was monitored for the following ten minutes to determine if knockdown occurred within 15 minutes of exposure to metofluthrin. Once either knockdown occurred or the ten minutes passed, the mosquito and metofluthrin mesh were removed from the experimental container. The fiberboard top of the container was removed and a fan was used to remove any residual metofluthrin vapor from the container before the next repetition of the experiment. The experimental container was aired out for a minimum of 15 minutes between trials.

Trials alternated between the “standard” (10x10mm) and “double” (10x20mm) dose of metofluthrin mesh in order to control for temperature and humidity fluctuations over the course of the day. In total, 17 repetitions of the experiment were conducted for each metofluthrin dose.

3. RESULTS

3.1 Study Site Temperature and Humidity

From November 12, 2009 to November 26, 2009, the mean minimum and mean maximum temperatures at the study site were 25.4°C and 27.5°C, respectively. During that same time period, the mean minimum and mean maximum relative humidity at the study site were 64.7% and 80.9%, respectively.

3.2 Container Site Preference Study

Identifying the location of mosquitoes after one minute inside the experimental container provided insight into the landing sites initially preferred by *Ae. aegypti*. The harborage, top, and sides of the container were the most common landing sites at one minute, with 25% of mosquitoes observed at each of these locations. 15% of mosquitoes were not visible at one minute (most likely located in a blind spot of the box), and both the window and the bottom of the container contained 5% of mosquitoes (Figure 5).

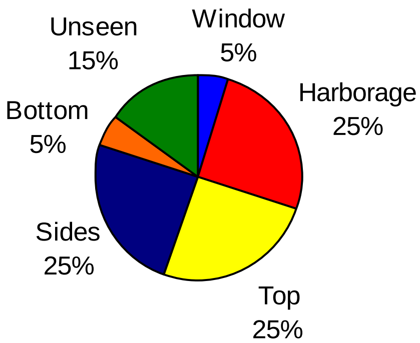

Figure 5. Percentage of *Ae. aegypti* found at various landing sites in the experimental container one minute after release. Mosquitoes were released in four cohorts of five mosquitoes each. At this time point, the harborage, sides, and top of the container were the most common landing sites, with 25% of mosquitoes located at each site.

The location of *Ae. aegypti* in the experimental container five minutes after release provided insight into the landing sites preferred by the mosquitoes once they became “acclimated” to the container. At this time point, the harborage was the most commonly occupied area of the container, with 35% of mosquitoes observed at that location.

25% of mosquitoes were unseen (most likely in the blind spot of the box); both the top and sides of the box contained 15% of mosquitoes, and 10% of mosquitoes were located at the window (Figure 6).

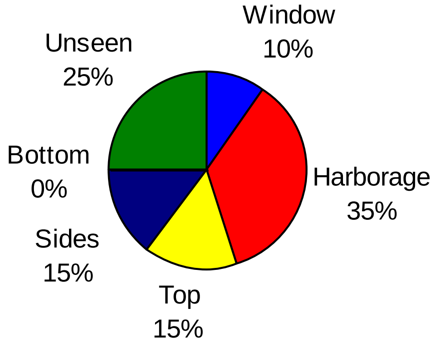

Figure 6. Percentage of *Ae. aegypti* found at various landing sites in the experimental container five minutes after release. Mosquitoes were released in four cohorts of five mosquitoes each. At this time point, the harborage was the most common landing site, with 35% of mosquitoes located at that site.

3.3 Metofluthrin Study

3.3.1 Standard Dose: Behavioral Effects on *Ae. aegypti*

The standard dose of metofluthrin for the experimental container (10x10mm single-sided mesh) was very effective at preventing *Ae. aegypti* from biting, reducing landing counts as compared to control by an average of 89.8%. 5.9% of mosquitoes were knocked down within 15 minutes of exposure to metofluthrin.

Clearly, introducing metofluthrin to the experimental container dramatically reduced the time the mosquito spent exhibiting biting behavior. Thus, in order to analyze the mosquito's other behaviors besides biting (i.e. trying to escape via the window, resting, flying around), it was necessary to recognize that, because the metofluthrin reduced the mosquito's interest in biting, the mosquito had more time to perform other behaviors during the metofluthrin period than during the control period. To control for this time difference, the mosquito's behaviors were analyzed as a percent of the time not spent exhibiting biting behavior.

In order to evaluate the spatial repellency of metofluthrin, the percent of time not biting that the mosquito spent on or near the window was determined. During the control period, the mosquito spent an average of 3.6% of the time on or hitting the window; when metofluthrin was introduced, an average of 16.1% of the time was spent on or hitting the window. This increase is statistically significant (p=0.0217) (Figure 7).

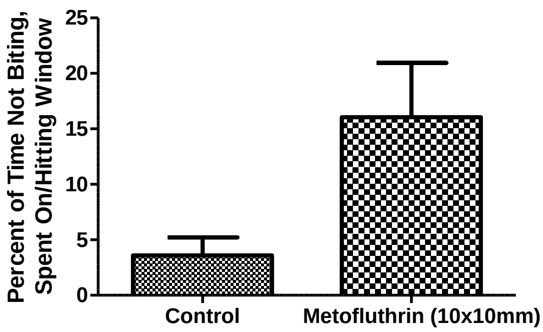

Figure 7. Spatial repellency of metofluthrin on *Ae. aegypti*. When no metofluthrin was present, *Ae. aegypti* (N=17) were on or hitting the window an average of 3.6% of the time not biting. When metofluthrin was introduced, the mosquitoes were on or hitting the window an average of 16.1% of the time not biting. This increase is statistically significant (p=0.0217).

To further evaluate the effect of metofluthrin on mosquito behavior, the percent of time not biting that the mosquito spent resting and flying around was determined. The mosquito rested (on the harborage, sides, or top of the container) for an average of 47.7% of the time not spent biting during control and 48.0% of the time when metofluthrin was introduced; this difference was not statistically significant (p=0.9733) (Figure 8a). The mosquito flew around for an average of 48.6% of the time not biting during control and 35.3% of the time when metofluthrin was introduced; this difference was not statistically significant (p=0.1643) (Figure 8b).

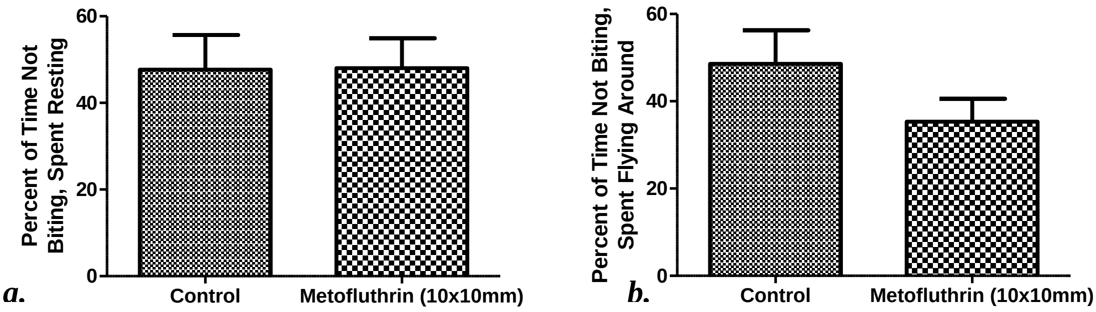

Figure 8. Comparison of *Ae. aegypti* behaviors during control and metofluthrin (10x10mm) periods. ***a.*** Of the time not spent biting, mosquitoes (N=17) rested on the harborage, sides, or top of the container for 47.7% of the time during control and 48.0% of the time when metofluthrin was introduced; this difference was not statistically significant (p=0.9733). ***b.*** During control, mosquitoes flew around for an average of 48.6% of the time not biting; when metofluthrin was introduced, flying time dropped to an average of 35.3%. This decrease was not statistically significant (p=0.1643).

3.3.2 Double Dose: Behavioral Effects on *Ae. aegypti*

Doubling the dose of metofluthrin (to a 10x20mm mesh) was even more effective at preventing *Ae. aegypti* from biting, reducing landing counts as compared to control by an average of 93.5%. Furthermore, 47.1% of mosquitoes were knocked down within 15 minutes of exposure to the double dose of metofluthrin.

The spatial repellency of the 10x20mm metofluthrin mesh was evaluated by determining the percent of time not biting that the mosquito spent at the window. During the control period, the mosquito spent an average of 1.93% of the time not biting on or hitting the window; when metofluthrin was introduced, an average of 9.83% of the time not biting was spent on or hitting the window. This increase is statistically significant (p= 0.0207) (Figure 9).

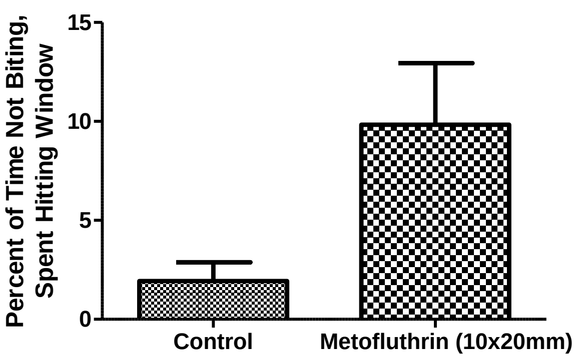

Figure 9. Spatial repellency of metofluthrin (double dose) on *Ae. aegypti*. During the control period, *Ae. aegypti* (N=17) were on or hitting the window an average of 1.93% of the time not biting. Introduction of metofluthrin increased the time spent on or hitting the window to an average of 9.83%. This increase is statistically significant (p=0.0207).

The percent of time not biting that the mosquito spent resting and flying around was also determined for the double dose of metofluthrin. The mosquito rested for an average of 62.5% of the time not biting during control and 60.1% of the time when metofluthrin was introduced; this difference was not statistically significant (p= 0.7716) (Figure 10a). The mosquito flew around for an average of 35.4% of the time not biting during control and 25.5% of the time when metofluthrin was introduced; this difference was not statistically significant (p= 0.1615) (Figure 10b).

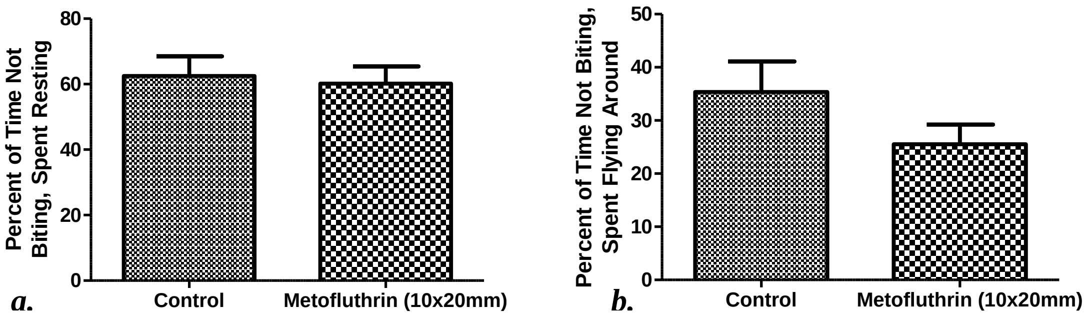

Figure 10. Comparison of *Ae. aegypti* behaviors during control and metofluthrin (10x20mm) periods. ***a.*** Of the time not spent biting, mosquitoes (N=17) rested on the harborage, sides, or top of the container for 62.5% of the time during control and 60.1% of the time when metofluthrin was introduced; this difference was not statistically significant (p=0.7716). ***b.*** During control, mosquitoes flew around for an average of 35.4% of the time not biting; when metofluthrin was introduced, flying time dropped to an average of 25.5%. This decrease was not statistically significant (p=0.1615).

3.3.3 Comparing Standard to Double Dose Results

In order to evaluate whether the mosquito behavior observed upon exposure to the small-scale metofluthrin mesh used in this experiment accurately represents the mosquito behavior that would be observed in a life-sized domestic setting, the results of the metofluthrin standard dose and double dose experiments were compared. If both the standard and double dose of metofluthrin used in this experiment generated similar behavior patterns, the mosquito behavior observed could be considered a "typical" metofluthrin response; a similar response would thus be expected if metofluthrin was used in a life-sized domestic setting.

Thus, the standard and double dose results were compared using a two-tailed t-test. A comparison between the two doses was made for the percent of time not biting spent at the window, resting, and flying during the metofluthrin period. None of the differences in the time spent at each location were statistically significant (Figure 11).

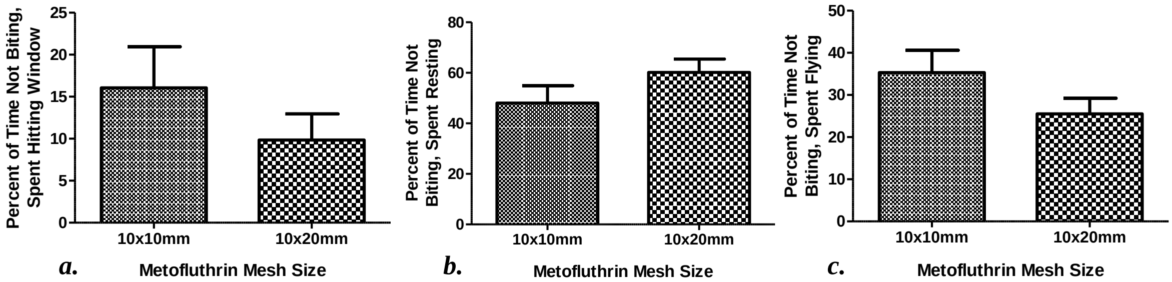

Figure 11. Comparison of the standard metofluthrin dose (10x10mm) results to the double metofluthrin dose (10x20mm) results. ***a.*** The percent of time not biting spent hitting the window was smaller for the double dose of metofluthrin than for the standard dose, but this difference was not statistically significant (p=0.2915). The differences in time spent resting (***b***) and flying (***c***) were also not statistically significant (p=0.1719 and p=0.1384, respectively).

4. DISCUSSION

4.1 Effectiveness of Experimental Container at Modeling a Domestic Setting

The container site preference study, in which the location of 20 mosquitoes was recorded at one and five minutes after release into the container, indicated that the experimental container effectively modeled a domestic setting. This study showed that the top, sides, and harborage—the most prominent areas in the container—were the sites most preferred by *Ae. aegypti*. Likewise, in a life-sized domestic setting, the walls, ceiling, and harborage areas around furniture are the most prominent surfaces, and thus are the areas where mosquitoes are most likely to land. Furthermore, *Ae. aegypti* prefer to rest and hide in dark areas (Queensland Government 2009); the fact that the greatest percentage of mosquitoes was located in the harborage after five minutes in the container thus indicates that the harborage was perceived as such by the mosquitoes. The low affinity of the mosquitoes for the window also suggests that the mosquitoes perceived it as a window; *Ae. aegypti* are domestic mosquitoes that prefer to live in people's houses, and thus are unlikely to want to escape from a modeled domestic setting (Queensland Government 2009). Overall, these results suggest that the experimental container was effectively perceived as a domestic setting by the *Ae. aegypti*. Furthermore, the relatively high temperatures (mean daily temperature in the high 20°C) of the study site corresponded with the temperatures expected during the hot summer months when dengue outbreaks usually occur in North Queensland (Rapley et al. 2009).

4.2 Effects of Metofluthrin on Behavior of *Ae. aegypti*

At both the standard and double dose, the metofluthrin mesh was shown to effectively reduce the biting behavior of *Ae. aegypti*. This finding concurs with previous research on other metofluthrin-impregnated materials used in and around domestic settings, where landing counts were also dramatically reduced (Lucas et al. 2007; Rapley et al. 2009). Thus, data from this study and past research indicate that metofluthrin is an effective mosquito bite inhibitor.

Previous research has argued that metofluthrin causes a reduction in human biting both by knocking down mosquitoes and by disrupting mosquitoes' orientation toward the host (Kawada et al. 2006). In the present study, only about 6% of *Ae. aegypti* were knocked down within 15 minutes of exposure to the standard dose of metofluthrin. This low knockdown rate most likely reflects the short length of time that the mosquitoes were exposed to the metofluthrin. Research by Kawada and others (2004) in the laboratory and Rapley and others (2009) in a domestic setting observed nearly 100% mosquito mortality at 24-hours post-treatment with metofluthrin. Thus, had the trials in the present study been allowed to continue for a longer period, it is likely that a greater knockdown rate would have been observed. The ability of metofluthrin to induce mosquito knockdown is further supported by the fact that, when the double dose of metofluthrin was used in the present study, nearly 50% of mosquitoes were knocked down within 15 minutes.

The reduction in mosquito landing counts observed in the present study also suggests that metofluthrin disrupted the mosquitoes' orientation toward the host. This finding leads to the question: if mosquitoes exposed to metofluthrin are disoriented and do not bite humans, what do they do? When both the standard and double dose of metofluthrin were used in the present study, I found no significant difference between the control and metofluthrin periods in the time not biting that the mosquitoes spent resting or flying around the container. These findings suggest that metofluthrin does not influence the likelihood that mosquitoes will seek harborage or fly around. However, mosquitoes did spend a statistically significantly greater amount of time on or hitting the window during the metofluthrin period as compared to the control period for both the standard and double metofluthrin dose. Although the percent of time not biting spent at the window was never above 20% even when metofluthrin was present, this finding suggests that the disoriented mosquitoes identified the window as a potential exit from the metofluthrin-filled container, and attempted to escape to the outside environment. As such, the metofluthrin mesh appeared to spatially repel the *Ae. aegypti*, as shown with other metofluthrin products in past research (Kawada et al. 2005a; Kawada et al. 2008).

When metofluthrin is used in a life-sized domestic setting, however, the spatial repellency of metofluthrin may be more difficult to observe. In the present study, the window occupied a large area of the experimental container and was relatively close to the mosquito's location at all times. Thus, the mosquito could easily recognize the window as an escape route, and attempt to exit through the window once the metofluthrin was introduced. In contrast, mosquitoes released in a life-sized domestic setting can be farther away from the windows or other potential escape routes when metofluthrin-induced disorientation begins. As a result, the mosquitoes may be unable to locate potential escape routes, and may have no choice but to seek harborage or fly around aimlessly. For this reason, the spatial repellency of metofluthrin could have been observed in this study where it was not in other studies set in a life-sized domestic setting (i.e. Rapley et al. 2009).

To further evaluate whether the mosquito behavior observed in this study would be likely to be seen in a domestic setting, a comparison of standard and double metofluthrin doses can be used. Although scaling down a domestic setting to the size of the experimental container facilitated accurate recording of individual mosquito behavior, scaling down the size of the metofluthrin mesh used in the container presented the risk that not enough chemical was present to impact mosquito behavior. However, both the standard and double dose of metofluthrin used in this experiment generated similar mosquito behavior patterns; no statistically significant difference was observed between the two doses for the time the mosquito spent at the window, resting, or flying around. This result indicates that the standard dose of metofluthrin used in the experimental container was sufficient to generate a "typical" metofluthrin response, and that similar behavior would be expected in a life-sized domestic setting.

4.3 Suggestions for Further Inquiry

Several improvements to the design of the present study could generate results that are more similar to those that would be expected in a domestic setting. One such improvement would involve redesigning the experimental container so that the window dimensions more accurately reflect those of a domestic setting. In this manner, the spatial repellency of metofluthrin could be assessed when the windows are not as easy for the mosquito to find. In such an experiment, the spatial repellency of metofluthrin could be compared to a chemical that is known to spatially repel *Ae. aegypti*. One such chemical is the metofluthrin relative transfluthrin (Ritchie, pers. comm. 2009). Additionally, previous studies have compared the effectiveness of metofluthrin to allethrin-based mosquito coils, a common method of mosquito control used by North Queensland residents (Rapley et al. 2009). Comparing the effectiveness of metofluthrin and an allethrin-based mosquito coil in the small-scale domestic setting used in the present study could provide a detailed analysis of which bite prevention strategy is more effective.

Another potential improvement to the design of the present study would involve visually recording or mapping the flight path of disoriented *Ae. aegypti* in order to characterize the behavioral effects of metofluthrin in greater detail. In the present study, disoriented mosquitoes exposed to metofluthrin appeared to change location in the experimental container with greater frequency than during control. However, no interpretable measurement of this tendency was taken. Recording the flight path of disoriented mosquitoes, including where and how many times a mosquito landed on a given area in the container, would provide a greater understanding of how metofluthrin influences mosquito behavior.

Increasing the length of time that *Ae. aegypti* are exposed to metofluthrin in the experimental container would also have several benefits. Exposing mosquitoes to metofluthrin for a 24 hour period would allow a more accurate small-scale metofluthrin knockdown rate to be determined. An even longer-term experiment (i.e. over several weeks) could be used to determine the length of time that the metofluthrin mesh remains effective against *Ae. aegypti*. As the concentration of metofluthrin in the experimental container decreases, are newly-introduced mosquitoes still knocked down, or are they only disoriented? Is disorientation of mosquitoes sufficient to inhibit human biting? Clearly, if the metofluthrin mesh becomes widely used by the public, it will be important to know how mosquito behavior is affected over the course of the product's life, as well as how often the mesh should be replaced.

5. MOSQUITO CONTROL IN PRACTICE

5.1 Public Survey of Dengue Control Measures and Interest in Metofluthrin Use

Because dengue fever is spread by a mosquito vector, public knowledge of and involvement in dengue management is essential for effective control of the disease. For this reason, mosquito control methods such as metofluthrin must not only be effective, but must also obtain public approval and widespread use in order to make a difference in practice. Queensland Health uses several approaches to disseminate information about dengue control methods throughout the state. These approaches include promotional materials such as brochures and posters, training sessions, information sheets, and a web site (Queensland Government 2005). In general, educational materials describe the causes and symptoms of dengue fever, advocate using mosquito repellant and seeking medical advice if a diagnosis of dengue is possible, and inform the public about how to survey their homes for breeding sites (Queensland Government, Pamphlet). Education programs target both the general community as well as specific settings, such as schools, work sites, and travelers' hostels (Queensland Government 2005).

In order to evaluate how effective these dengue education efforts have been, and to evaluate interest in metofluthrin as a mosquito control method, 33 Cairns residents were interviewed during the first two weeks of November 2009. 31 interviews were given orally and two were given via email. The nature of my project was fully disclosed to all participants, no information was collected from participants if there was any possibility of the survey harming them in any way, and no surveys were administered to minors.

The demographics of the 33 Cairns residents who took the dengue fever management and metofluthrin interest survey are summarized in Table 1.

	Mean	Maximum	Minimum
Age	39.5	66	18
Time in Cairns (Years)	14.3	56	1
Gender	13 Male	20 Female	
Race	31 Caucasian	2 Other	

Table 1. Demographic information of the 33 Cairns residents interviewed. Mean participant age was 39.5 years; mean time in Cairns was 14.3 years. 13 males and 20 females were interviewed, and the majority of subjects (31) identified themselves as Caucasian.

To evaluate how familiar participants were with dengue fever in their personal lives, participants were asked if they or someone they knew had contracted dengue fever in the past; 64% of those surveyed answered in the affirmative.

Participants were asked a series of questions to evaluate their knowledge of dengue mosquito control measures. The first question read, "What measures do you take to eliminate the breeding sites of dengue mosquitoes in and around your home?" 88% of those surveyed responded that they empty out water-holding containers, 9% said they spray insecticides, and 42% said they use some other control method, such as mosquito traps, disposing of palm fronds, or having a pond with fish that eat mosquitoes.

After controlling mosquito breeding, preventing mosquitoes from biting is the next most effective means of preventing dengue fever from spreading (Queensland Government, Pamphlet). To evaluate the extent of bite prevention used by Cairns residents, survey participants were asked to list the actions they take to prevent mosquito bites. 73% of participants said they use mosquito repellant to prevent bites, 9% said they don't go outside when the mosquitoes are bad, 9% said they use mosquito coils, and 30% said they use another method of bite prevention, such as candles or wearing light-colored clothing.

Participants were also asked who they believe is responsible for managing dengue fever in North Queensland. 36% of those interviewed identified the local council or government as exclusively responsible for managing dengue, and 64% believed that everyone is responsible. When asked to rate the effectiveness of dengue control in the Cairns area, 33% of participants ranked dengue management as very effective, 33% believed that management is somewhat effective, 21% ranked management as poor, and 12% said they did not know.

Clearly, dengue management strategies are most effective when the public is interested and willing to be involved. To evaluate if metofluthrin mesh sheets are likely to be widely used by the public, and thus to be an effective mosquito control strategy, the characteristics of the metofluthrin mesh were described to survey participants. 76% of those interviewed expressed interest in using metofluthrin, 15% said they were unsure, and 9% said they would not be interested in using metofluthrin. Participants were also asked if they had any questions or concerns about metofluthrin use. 54.5% percent of those interviewed wanted to know if metofluthrin can be harmful to animals, kids, or in general; 6% said that their likelihood to use metofluthrin would be dependent on the cost of the chemical; and 36% said they have no concerns about metofluthrin.

5.2 Analysis of Survey Responses: Dengue Knowledge and Prospects for Metofluthrin

In general, Cairns residents were moderately knowledgeable about dengue control techniques. The fact that 88% of those surveyed said that they empty out water-holding containers in their yards is an encouraging figure, as this is the main tactic advocated by Queensland Health to prevent *Ae. aegypti* from breeding (Queensland Government, Pamphlet). The fact that 73% of those surveyed use mosquito repellant to prevent mosquitoes from biting is also encouraging; stopping mosquitoes from biting not only prevents the mosquito from passing the dengue virus on to a new host, but also deprives female *Ae. aegypti* of the blood meal necessary for her to develop and lay eggs (Queensland Government 2009).

However, of concern is the fact that 36% of Cairns residents identify the government as being responsible for dengue control. These individuals are less likely to partake in efforts to prevent *Ae. aegypti* from breeding, and therefore put others in their community at greater risk. This delegation of responsibility to the government level may be a reflection of the unusually large dengue outbreak that occurred from November 2008 through September 2009. During this time period, the Cairns Dengue Action Response

Team performed an extensive amount of dengue control work in the Cairns area, including interior and exterior spraying and the removal of potential mosquito breeding sites from yards (Marshall, pers. comm. 2009). The fact that extensive government effort was exerted recently may have led many Cairns residents to believe that dengue control is the job of the government. To encourage more people to view dengue management as everyone's responsibility, future information disseminated by the Queensland government should emphasize that dengue control is most effective when every individual does their part.

Also of concern is the fact that survey responses contained a considerable amount of "conventional wisdom" which is actually incorrect. 9% of those surveyed stated that they avoid going outside to prevent mosquito bites. However, because *Ae. aegypti* is an indoor, day-biting mosquito, this method of dengue prevention is unlikely to be effective (Queensland Government 2009). Furthermore, a small percentage of those surveyed said that they stop dengue mosquitoes from biting by using "mozzie zappers," which attract and kill mosquitoes using an electric light. In fact, these traps are not intended to target the dark-loving *Ae. aegypti* (Marshall, pers. comm. 2009). Overall, these responses show that some people make the easy mistake of misunderstanding the characteristics of *Ae. aegypti*. Thus, additional educational materials should be produced to inform the public about the specifics of the dengue mosquito.

Encouragingly, 76% of those interviewed expressed interest in using metofluthrin; this suggests that the chemical has potential for extensive use by the public. The fact that 54.5% percent of those interviewed wanted to know if metofluthrin can be harmful to kids or animals makes it clear that information on the low toxicity and high effectiveness of the chemical must be disseminated through the population.

6. CONCLUSIONS

Because approximately 40% of the world's population resides in tropical areas where dengue transmission occurs, effective management of the mosquito vectors of the dengue virus is vitally important (Queensland Government 2005). Mosquito control programs in the tropical regions of the world and Australia use insecticides and other methods to reduce *Ae. aegypti* populations during dengue outbreaks, thus limiting the spread of the disease (Ritchie et al. 2002). However, excessive use of insecticides, especially in year-long continuous larval and adult control programs, has the potential to select for mosquito resistance to the insecticides (Luz et al. 2009). Furthermore, expansive population growth has overwhelmed the public health structure in many countries where dengue is endemic, resulting in government-based mosquito control programs becoming ineffective (U.S. CDC 2009).

The metofluthrin mesh sheet has great potential to supplement stressed government-based mosquito control programs by providing a localized, effective means of preventing mosquito biting. This study used a small-scale domestic setting to demonstrate that the metofluthrin mesh disorients *Ae. aegypti* and prevents them from biting humans. The metofluthrin mesh also appears to spatially repel the dengue mosquito, at least when a possible escape route (i.e. a window) is nearby and is easily recognized by the mosquito. A survey of Cairns residents also suggested that metofluthrin is likely to be widely accepted by the public, potentially improving the effectiveness of dengue control in North Queensland. In conclusion, metofluthrin holds considerable promise as a dengue control method both in North Queensland and globally.

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