Semi-Field Evaluation of Metofluthrin-Impregnated Nets on Host-Seeking Aedes aegypti and Anopheles dirus

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SEMI-FIELD EVALUATION OF METOFLUTHRIN-IMPREGNATED NETS ON HOST-SEEKING *AEDES AEGYPTI* AND *ANOPHELES DIRUS*

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ABSTRACT. The efficacy of a metofluthrin-impregnated net (MIN) known as the "Mushikonazu" on the house entry behavior of female *Aedes aegypti* and *Anopheles dirus* mosquitoes was evaluated using a semi-field 50-m tunnel setup. While the MIN is labeled for the control of chironomids and moth flies, this study determined the feasibility of using the device, given its current construction and metofluthrin formulation, as a spatial repellent against mosquitoes. Sentinel and cone bioassays were used to determine the insecticidal effect of the MIN. A spatial activity index (SAI) was calculated to evaluate responses of the mosquitoes. For the spatial repellent evaluation against *Ae. aegypti*, the overall mean of SAI was slightly less than 0 at wk 1 after the MIN application and then decreased for the last 4 wk showing a preference to treatment tent. For *An. dirus*, the mean SAI at wk 1 was positive, indicating a presumed repellent effect of the MIN against *An. dirus*. For the subsequent 4 wk, the SAI was negative, indicating a preference for the MIN. Results suggested that the MIN may not be a promising approach to repel *Ae. aegypti* and *An. dirus* under field conditions in Thailand. However, it remains probable that the MIN may be effective as a spatial repellent if modifications are made to the metofluthrin concentration or formulation and/or the construction of the device.

KEY WORDS *Aedes aegypti*, *Anopheles dirus*, metofluthrin, mosquito control, spatial repellent

INTRODUCTION

Malaria, dengue fever, and chikungunya are major international public health concerns, especially in developing countries. Mosquito control programs that are designed to reduce transmission of mosquito-borne diseases must incorporate some means of minimizing human-vector contact. Skin repellents (Yap et al. 2000, Barnard et al. 2002, Das et al. 2003, Katz et al. 2008), insecticide-treated nets (ITNs) (Guillet et al. 2001, Nahlen et al. 2003, Graham et al. 2005, N'Guessan et al. 2006), and insecticide-treated materials such as tarpaulins and tents (Hewitt et al. 1995, Graham et al. 2004) and clothing (Kimani et al. 2006) are just some approaches that were evaluated and implemented. However, they have drawbacks. The use of skin repellents requires consistent reapplication, and therefore such an approach is impractical in some situations, especially those involving refugee populations (N'Guessan et al. 2006). Coverage with ITNs is a highly effective method for preventing malaria in areas where malaria transmission is significant, but it is not practical for protecting humans from day-biting mosquitoes such as *Aedes aegypti* (L.) (Guillet et al. 2001, Nahlen et al. 2003, Graham et al. 2005). Insecticide-treated materials such as tarpaulins and tents may benefit the health of mostly transient communities, such as military and refugee populations, impacted by arthropod-borne diseases but are less viable in communities containing fixed structures (Hewitt et al. 1995, Graham et al. 2004, Kimani et al. 2006). Additionally, ITNs and other materials such as tarpaulins and tents that are toxic to the mosquito may actually enhance selective pressure for resistance (N'Guessan et al. 2006). While all of these measures are valuable tools to reduce transmission of disease by mosquitoes, spatial repellents with long-term efficacies may play a more practical and substantial role in minimizing vector-to-human contact. Spatial repellents act by preventing a vector from entering an area, home, or tent to feed (Kline et al. 2003, Bernier et al. 2005, Grieco et al. 2007).

The majority of mat and coil products found commercially require a heat source to vaporize and to release a pyrethroid to kill or repel mosquitoes. However, a novel pyrethroid metofluthrin (SumiOne®; Sumitomo Chemical Company Ltd., Osaka, Japan) is unique from most other pyrethroids in that it vaporizes at ambient temperature without requiring a heat source (Ujihara et al. 2004). The vapor pressure of metofluthrin is 100-fold greater than permethrin, another commonly used pyrethroid (Kawada et al. 2008). Such a characteristic has spawned the development of prototype metofluthrin-impregnated spatially repellent devices (Argueta et al. 2004a, 2004b; Kawada et al. 2004a, 2004b; Kawada et al. 2005a, 2005b; Kawada et al. 2006; Lucas et al. 2007; Kawada et al. 2008). Metofluthrin-treated paper strips of various dimensions and types were found to be effective at reducing densities

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of *Aedes albopictus* (Skuse) collected in Centers for Disease Control and Prevention traps (without light bulbs) in Japan (Argueta et al. 2004b), *Anopheles* spp. and *Culex* spp. in human-baited and cow-baited collections in Indonesia (Kawada et al. 2004b), and *Aedes vexans* (Meigen) and *Aedes* spp. in human-baited collections in the USA (Lucas et al. 2007). However, the effective duration of the paper strip devices ranged anywhere from 36 h for the study conducted in the USA to 6 wk for the studies in Asia (Argueta et al. 2004b; Kawada et al. 2004a, 2004b; Lucas et al. 2007). This extensive efficacy range likely arises from variation due to factors such as the structure and dimensions of the device, amount of metofluthrin, method of evaluation, species composition, air flow, and temperature.

There is evidence to suggest that a metofluthrin-impregnated plastic strip may be a more effective alternative than a metofluthrin-treated paper strip as a spatial repellent against mosquitoes (Argueta et al. 2004a, 2004b; Kawada et al. 2004a, 2004b; Kawada et al. 2005a, 2005b; Kawada et al. 2006; Lucas et al. 2007; Kawada et al. 2008). The plastic strip device contains a polyethylene latticework structure that is designed to reduce the release rate of metofluthrin by approximately 50% that of earlier formulations (Argueta et al. 2004a, 2004b; Kawada et al. 2004a, 2004b; Kawada et al. 2005a, 2005b; Kawada et al. 2006; Lucas et al. 2007; Kawada et al. 2008).

In this study, we evaluated the long-term efficacy of a commercially-available metofluthrin-impregnated net (MIN) known as the "Mushikonazu" under semi-field conditions. Our test organisms were laboratory-reared *Ae. aegypti* and *Anopheles dirus* (Peyton and Harrison), principal vectors of dengue and malaria, respectively, in Thailand. The net appears to be characteristically similar (albeit with variations in the shape, rate of metofluthrin release, and formulation of metofluthrin) to the metofluthrin-impregnated plastic strips for which there is evidence of long-term efficacy against mosquitoes (Argueta et al. 2004a, 2004b; Kawada et al. 2004a, 2004b; Kawada et al. 2005a, 2005b; Kawada et al. 2006; Lucas et al. 2007; Kawada et al. 2008). While the MIN is labeled for the control of chironomid and moth flies, this study determined the feasibility of using the device, given its current construction and metofluthrin formulation/concentration, as a spatial repellent against malaria vectors in Asia or *Ae. aegypti* in Thailand. If the net does indeed exhibit spatial repellent properties that are long-lasting, the manufacturer could potentially commercialize this device as an expedient, sustainable, and low-risk approach to protecting vulnerable populations (refugees, deployed soldiers) from mosquitoes that vector harmful pathogens.

MATERIALS AND METHODS

Study location: The semi-field evaluations were conducted at Ban Nong Kwang, Kaeng Hang Maeo district, Chanthaburi province (13°0′30″N, 101°54′18″E), in eastern Thailand from October 2009 to April 2010 (Fig. 1). Temperature, relative humidity, and wind speed were measured throughout the evaluation using a HOBO data logger (Onset®, Bourne, MA) and Kestrel 4000 pocket weather meter (Nielsen-Kellerman, Boothwyn, PA).

Semi-field tunnels: Two outdoor tunnels (50 × 1 × 1.5 m each) were constructed at a field site using polyester 100 denier, 156-mesh netting. The netting was supported at a height of 1.5 m using arched wooden supports placed every 2 m. The floor of each tunnel was covered with a white plastic sheet. The entrances at either end of the tunnel were each blocked by a tent measuring 2.4 × 2.4 × 1.9 m high (Ital Siam Motors Co., Ltd., Thailand). Tents (1 control and 1 treatment tent) were positioned with the windows facing into the tunnel (Fig. 2). A box-shaped interception trap (0.6 × 0.6 × 0.6 m) made of polyester insect netting and PVC pipes was placed at 30 cm above the floor at the entrance to each tent (Grieco et al. 2000). All windows were opened in the 2 tents revealing only the screens. Two tunnels were placed parallel and approximately 100 m apart from each other.

Mosquito colony origin and maintenance: Laboratory-reared female *Ae. aegypti* and *An. dirus* (4–8 day post-eclosion) were used in this study. Both species were reared at 27 ± 2°C at 80% RH under a photo regime of 12:12 h (Light: Dark) provided by fluorescent lights (35 W). Larvae were fed with fish food ad libitum (C.P. Hi Pro®, Bangkok, Thailand). Adults were provided with 10% sucrose solution ad libitum for the energy source. *Aedes aegypti* and *An. dirus* were starved for 6 h prior to the experiments.

Metofluthrin-impregnated net (MIN): The MINs (Dainihon Jochugiku Co., Ltd., Japan) were obtained commercially in Japan under the product name "Mushikonazu plate, large, 60 days." The dimensions of the net measure 9 × 18 cm with a thickness of 0.2 cm and a weight of 5.0 g. The product contains 5% metofluthrin (w/w: SumiOne®) and 95% ethylene-methyl methacrylate copolymer (w/w). The suggested room size for using a MIN per the manufacturer's instructions is 14.4 × 7.2 m. The device is labeled for the control of chironomid flies and moth flies and the manufacturer claims the efficacy of the net to be approximately 60 days.

Calibration of semi-field assays: Two assays were performed to calibrate the tunnels (human versus human and presence versus absence of human "attractant" assay). In the human versus human assay, evaluations of the tunnel were

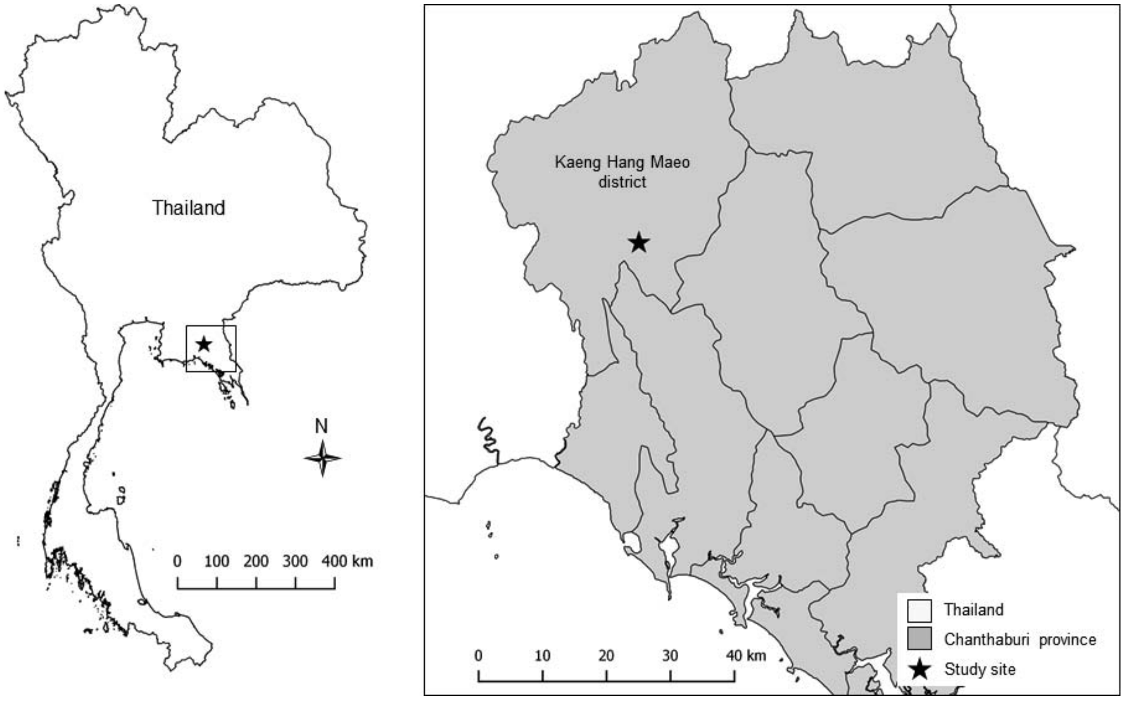

Fig. 1. Map of study site.

conducted in the absence of a candidate repellent to demonstrate that mosquitoes showed relatively equal preference for either tent (with a human attractant in both tents). One hundred and fifty host-seeking mosquitoes were released into the tunnel at its midpoint. *Aedes aegypti* were released into the tunnel at 0800 h, and the evaluation terminated at 1000 h. *Anopheles dirus* were released into the tunnel at 1800 h, and the evaluation terminated at 0600 h. Interception traps were emptied each hour using an aspirator by a human attractant positioned inside each tent. Prior to beginning a new trial, all mosquitoes left inside each tunnel were killed using electric mosquito bats. Human attractants were randomly rotated within and between tunnels after each replicate. The total of 18 and 22 replicates were conducted for *Ae. aegypti* and *An. dirus,* respectively (Table 1).

In the presence versus absence of human attractant assay, we evaluated host-seeking behavior of *Ae. aegypti* and *An. dirus* in the absence of a repellent and compared between presence and absence of human attractants in assigned tents to test the hypothesis that mosquitoes prefer tents containing human attractant over empty tents. The number of mosquitoes and procedures in this assay mimicked that of the human versus human assay except that there was only 1 human attractant per tunnel. Human attractants were rotated within and between tunnels after each replicate. We performed 8 replicates against each tested mosquito species.

Spatial repellency evaluation of MIN: After calibration assays were completed, 1 MIN was hung by a nylon string in the center of each treatment tent at a height of 10 cm from the tent ceiling, whereas there was no MIN in the control tents (Fig. 2). The MIN remained in the tents for the duration of the study. For the evaluations, the number of mosquitoes and the procedure were conducted as described earlier in the calibration assay. This procedure was replicated 4 times per wk for 5 consecutive wk.

Sentinel assay: To determine spatial impact of the MIN, 6 sentinel cups containing 15 mosquitoes of the same test species in each cup were placed inside each tent and in the tunnels at 10 m intervals away from the tents (Fig. 2). Moreover, the temporal insecticidal effect was monitored for 5 consecutive wk. Sentinel cups were removed from the tents and tunnels and maintained at the field station. Mosquitoes in sentinel cups were provided with cotton balls soaked with 10% sucrose supplemented with multivitamin and kept for 24 h to observe mortality. Only sentinel cups located in the treatment tent and at 10 and 20 m were used to analyze the insecticidal effect overtime. Three replications per week were conducted against each tested mosquito species.

Cone bioassay: To determine whether metofluthrin accumulated on the tunnels and tents, mosquito net strips were hung every 10 m on the net of each tunnel and inside the tents at the beginning of the study (Fig. 2). A strip was

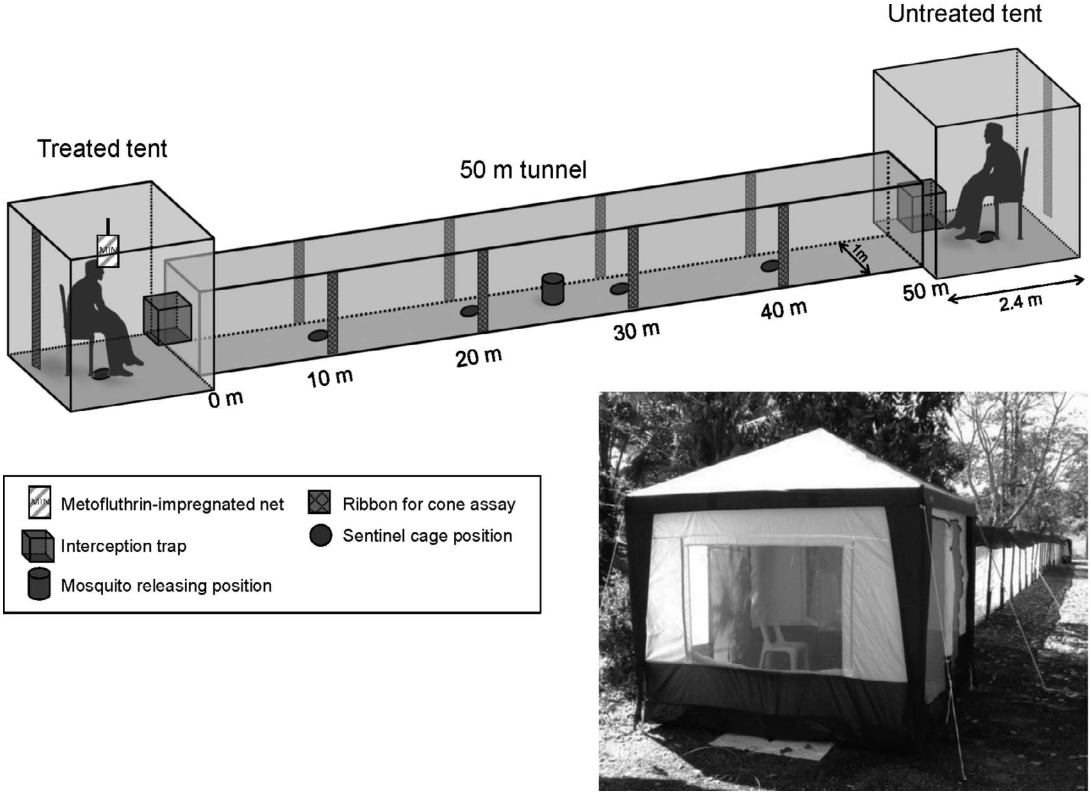

Fig. 2. Schematic of a 50-m tunnel (50 × 1 × 1.5 m) and components of spatial repellency evaluation. Entrances at both ends of each tunnel were blocked by a tent (2.4 × 2.4 × 1.9 m) with the entrances facing into the tunnel. Sentinel cups and ribbons were placed along the tunnels at 10 m intervals.

removed from each distance in each tunnel every week and tested for adult mortality by a World Health Organization (WHO) cone bioassay (Grieco et al. 2005). We performed 6 replicates each wk per tested mosquito species for 5 consecutive wk.

Susceptibility test: The effect of MIN on the mortality of *Ae. aegypti* and *An. dirus* was monitored each wk for 5 consecutive wk (during wk 6–10) using a WHO susceptibility test. In each wk of the experiment, the MIN used inside each tent was rolled into a cylinder tube. Fifteen mosquito females were gently introduced into the holding tubes. The number of knocked down mosquitoes at 30 min and mortalities at 24 h were monitored and recorded. Three replications were performed for each week. The control assays were conducted using untreated nets.

Data analysis: The spatial activity index (SAI) was calculated to evaluate responses of mosquitoes to either side of a tunnel in a calibration assay and MIN evaluation assay (Grieco et al. 2005). In each replicate, SAI was calculated using SAI = $(N_c - N_t)/(N_c + N_t)$, where N_c is the number of females collected from the interception trap on the control side and N_t is the number of females collected from the treated side. The SAI ranges from −1 to 1, with zero, negative, and positive values representing no preference

Table 1. Recapture rates of *Aedes aegypti* and *Anopheles dirus* released in the calibration of semi-field tunnel assays.

Tunnel	Species	Treatment	Replications	Total no. released	Total no. recaptured	% recaptured
1	*Ae. aegypti*	Human vs. human	7	1050	987	94.00
		Absence vs. presence	5	750	655	87.33
	An. dirus	Human vs. human	10	1500	918	61.20
		Absence vs. presence	4	600	452	75.33
2	*Ae. aegypti*	Human vs. human	11	1650	1541	93.39
		Absence vs. Presence	3	450	377	83.78
	An. dirus	Human vs. human	12	1800	1296	72.00
		Absence vs. presence	4	600	449	74.83

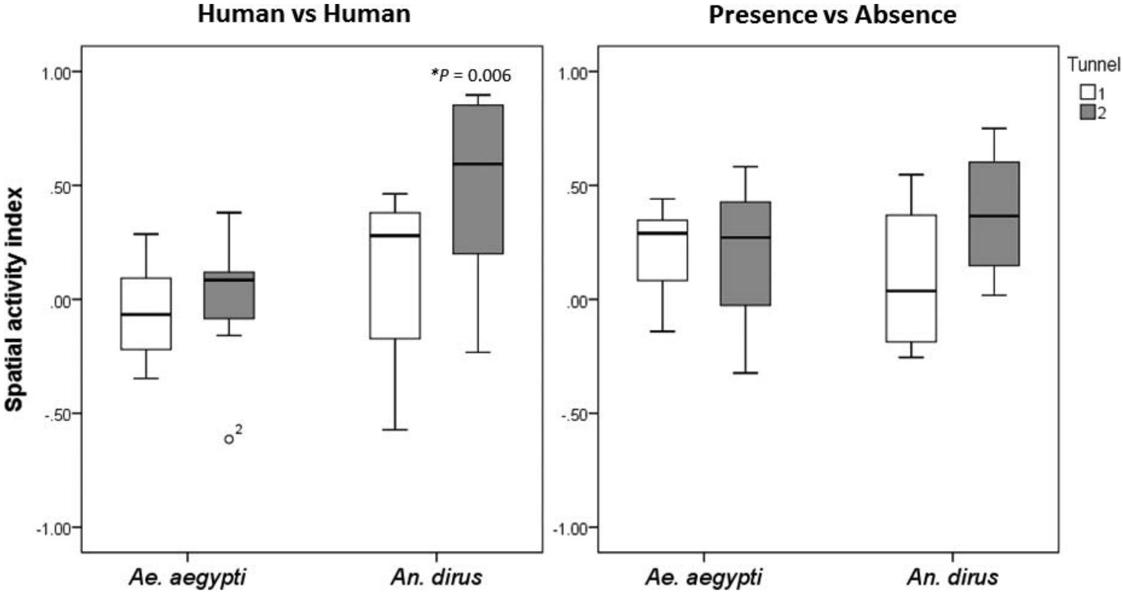

Fig. 3. Box plots of SAI for *Ae. aegypti* and *An. dirus* calculated from the calibration of semi-field tunnels. Two assays were performed to calibrate the tunnels; human versus human (left) and presence versus absence of human attractant assay (right), $^*P < 0.05$, Wilcoxon signed rank test. Boxes denote the median and interquartile range (IQR). Whiskers extend 1.5 IQR from box ends, with outliers denoted "o²."

to either control or treatment tents, preference to treated tents, and preference to control tents, respectively. A Wilcoxon signed rank test was used to determine whether the SAI in each study was different from zero. In the MIN evaluation assay, differences of SAI between each of the 5 wk of the experiment were analyzed using Kruskal-Wallis test. The homogeneous subset was tested by the Stepwise step-down method. For the sentinel and cone bioassays, binary logistic regression was performed to evaluate whether distance (spatial) and wk of the experiment (temporal) related to mosquito mortalities. All analyses were performed with SPSS Statistics version 22 (IBM Corp., Armonk, NY).

RESULTS

Weather data: The mean temperature was 28.1°C (20.8°C to 40.2°C) with 59.5% RH (29.8–98.2% RH), and a mean wind speed of 2.4 km/h. The climatic indices remained constant throughout the 5 wk of evaluation. We assumed the evaporation rate of metofluthrin in the tunnels to be similar to that found in a typical Thai house.

Calibration of semi-field assays: Overall recapture rates for *Ae. aegypti* and *An. dirus* were 91.3% and 69.2%, respectively (Table 1). Recapture rates for *Ae. aegypti* in the human versus human assay and in the presence versus absence of human attractant assay were 93.6% and 86.0%, respectively. Recapture rates for *An. dirus* in the human versus human assay and in the presence versus absence of human attractant assay were 67.1% and 75.1%, respectively. In the human versus human assay, the SAI values for *Ae. aegypti* were equal to zero for both tunnels ($P = 0.40$ and 0.48, Fig. 3). Therefore, there was no position effect indicated for *Ae. aegypti*. The SAI value for *An. dirus* from tunnel no. 1 revealed no position effect ($P = 0.29$). However, we did detect a position effect on *An. dirus* tested in tunnel no. 2 ($P = 0.006$), suggesting that there were factors affecting flight behavior of *An. dirus* in tunnel no. 2. Consequently, spatial repellency evaluations of the MIN against *An. dirus* were performed only in tunnel no. 1.

In the presence versus absence of human attractant assay, there were no significant differences between the SAI values for *Ae. aegypti* and zero for both tunnels ($P = 0.14$ and 0.59, respectively, Fig. 3). Additionally, the SAI values for *An. dirus* were equal to zero for both tunnels ($P = 0.72$ and 0.07, respectively). This demonstrates that the presence of human attractants had no measurable effect on *Ae. aegypti* and *An. dirus* flight patterns in the tunnels.

Spatial repellency evaluation of MIN: Spatial repellency evaluation of the MIN was performed for 5 consecutive wk to determine long-term efficacy. The SAI was calculated weekly. Overall, the SAI values for *Ae. aegypti* (ranging from −0.3 to 0.2) and *An. dirus* (ranging from −0.6 to 0.4) were not significantly different from zero in both tunnels (Wilcoxon signed rank test, $P > 0.05$; Fig. 3). These results indicate that there was no

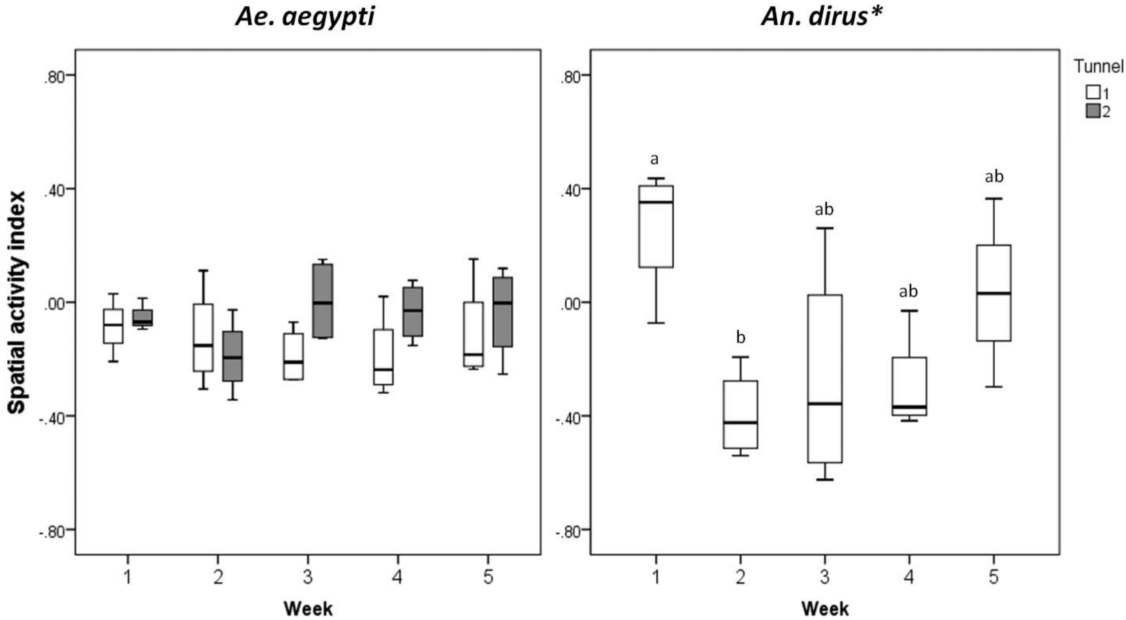

Fig. 4. Box plots of SAI for *Ae. aegypti* (left) and *An. dirus* (right) from spatial repellency evaluations of MIN. SAI values were calculated from the number of mosquitoes recaptured weekly in the tunnel assay (*$P < 0.05$, Kruskal-Wallis). Boxes denote the median and IQR. Whiskers extend 1.5 IQR from box ends. Data points with the same letter are not significantly different from each other.

spatial repellency effect of the MIN on *Ae. aegypti* and *An. dirus* throughout the 5-wk period.

The SAI values for *Ae. aegypti* among 5 wk were not significantly different (Kruskal-Wallis, $\mathcal{X}^2 = 2.268$, df 4, $P = 0.69$; Fig. 4). However, we detected the difference of SAI values for *An. dirus* at wk 1 compared to the value at wk 2 (Kruskal-Wallis, $\mathcal{X}^2 = 10.286$, df 4, $P = 0.04$). Therefore, the spatial repellency of the MIN against *An. dirus* was more effective at wk 1 when the MIN was newly opened (Fig. 4).

Sentinel assay: Determination of the insecticidal effect of the MIN (the distance from the tent that metofluthrin affected adult mortality) was performed concurrently with the spatial repellency evaluation. There were significant interactions between mortality and both space (distance from MIN) and time (week of MIN applied) for *Ae. aegypti* and *An. dirus* ($P < 0.05$, binary logistic regression). Mosquito mortality data were statistically analyzed until wk 4 of the study due to the few mosquitoes that died at wk 5. A percentage mortality comparison among sentinel cups in the tents and tunnels showed significantly higher mortality of *Ae. aegypti* in sentinel cups placed in the treatment tent and at 10 and 20 m away from the treatment tent (Table 2). Mortality percentages were high for the first 4 wk after MIN application with peak mortality at wk 3. Mortality was significantly reduced in wk 5 indicating there were no effects of metofluthrin on mortality in wk 5 (Table 2).

Percentage mortality comparison for *An. dirus* between sentinel cups in control and treatment tents showed higher mortality in the latter group. Mortalities were high for mosquitoes in sentinel cups placed in the treatment tent and at 10 and 20 m. Percentage mortality and odds ratios of *An. dirus* in sentinel cups are shown in Table 2. Mortality percentages were high for the first 3 wk after MIN application with peak mortality at wk 3.

Cone bioassay: For measurement of metofluthrin accumulation on the net over time, a WHO cone bioassay was performed for 4 consecutive wk of the evaluation. We divided distances into 3 groups (control side, midtunnel, and treatment side). Net strips placed inside the control tent and at 40 m from the treatment tent were grouped to the control side. The midtunnel group included the nets located at 20 and 30 m away from the treatment tent. Strips inside the treatment tent and at 10 m from the treatment tent were assigned to the treatment side. Among 2 independent variables (distance and week) in the model, there was a significant interaction between mortality and week for *Ae. aegypti*, while logistic regression revealed both independent variables affected the mortality rate of *An. dirus* ($P < 0.05$, Table 3) indicating that there were substantial accumulations of metofluthrin on the mosquito net strips. For *Ae. aegypti*, the mortality rate was significantly increased at wk 3, and then dropped at wk 4, mimicking the result found with the

Table 2. Comparison of percentage mortalities of *Aedes aegypti* and *Anopheles dirus* from sentinel assays at different distances and weeks using an odds ratio (OR) with 95% confidence interval (CI).

	Ae. aegypti				*An. dirus*			
	n	% mortality	OR (95% CI)	*P*	*n*	% mortality	OR (95% CI)	*P*
Distance								
Untreated tent	420	1.19	Ref.	Ref.	390	3.84	Ref.	Ref.
40 m	450	3.11	2.67 (0.95–7.47)	0.062	450	10.44	2.97 (1.60–5.30)	0.000
30 m	450	2.44	2.10 (0.72–6.04)	0.178	450	6.22	1.66 (0.87–3.15)	0.123
20 m	450	5.33	4.68 (1.77–12.37)	0.002	450	6.89	1.85 (0.98–3.48)	0.056
10 m	450	4.44	3.86 (1.44–10.38)	0.007	450	7.56	2.04 (1.09–3.81)	0.025
Treated tent	420	5.71	5.03 (1.90–13.31)	0.001	390	9.23	2.54 (1.37–4.72)	0.003
Week								
1	240	6.67	Ref.	Ref.	210	5.71	Ref.	Ref.
2	270	5.56	0.82 (0.40–1.70)	0.601	270	5.19	0.90 (0.41–1.99)	0.800
3	270	8.89	1.37 (0.71–2.63)	0.353	270	21.11	4.42 (2.30–8.47)	0.000
4	270	3.33	0.48 (0.21–1.11)	0.088	270	2.59	0.44 (0.17–1.14)	0.090
5	270	1.48	0.21 (0.07–0.64)	0.006	270	4.07	0.70 (0.30–1.62)	0.406

sentinel assay (Table 3). Similar to *Ae. aegypti* result, mortality rate of *An. dirus* was highest at wk 3. Therefore, the accumulation of metofluthrin on the net highly induced mortality of both species of mosquitoes at wk 3.

Susceptibility test: Results from WHO susceptibility assay revealed that, in general, both *Ae. aegypti* and *An. dirus* were susceptible to metofluthrin contained in MIN with 100% knock-down rates at 30 min after exposure of mosquitoes to MIN. After 24 h post-exposure, the percentage mortalities ranged from 64.44 to 100 for *Ae. aegypti*, and 66.67 to 95.56 for *An. dirus* (Table 4).

DISCUSSION

At a minimum, our semi-field tunnel assay is a valuable tool for evaluating the spatial repellent properties of a candidate device on *Ae. aegypti.* There was no tunnel effect on *Ae. aegypti* response, but there was a tunnel effect on that of *An. dirus.* We hypothesized that light from a neighboring house influenced the observed effect in tunnel no. 2 for *An. dirus*. For the presence and absence of human attractant assay, the number of mosquitoes collected from each trap within and between tunnels was also not significantly different for either species. We postulate that the human attractant in this assay was too far from the release point of the mosquitoes to elicit a response, and thus trap capture results were due to random flying behavior.

Our results revealed that the MIN device showed no real capability to repel both *Ae. aegypti* and *An. dirus* in this assay under semi-field conditions in Thailand. Other formulations of metofluthrin appear to be more effective. Kawada et al. (2005b) found that metofluthrin-impregnated plastic strips placed inside houses located in Vietnam significantly reduced density indices for *Ae. aegypti* and *Culex. quinquefasciatus* (Say) over a 6-wk period. Another study from Vietnam showed that 1 plastic strip reduced mechanical aspirator collections of *Ae. aegypti* inside homes for at least an 8-wk period (Kawada et al. 2006). Metofluthrin coils significantly reduced the landing rate of *An. sundaicus* (Rodenwaldt) and house entry behavior of *Ae. aegypti* (Achee et al. 2012, Syafruddin et al. 2014). Emanators containing 5% and 10% metofluthrin suppressed the biting rate of *Ae. aegypti* in domestic settings (Ritchie and Devine 2013).

Table 3. Comparison of percentage mortalities for *Aedes aegypti* and *Anopheles dirus* from WHO cone bioassays across different locations (untreated side, midtunnel, and treatment side) and weeks using an odds ratio (OR) with 95% confidence interval (CI).

	Ae. aegypti				*An. dirus*			
	n	% mortality	OR (95% CI)	*P*	*n*	% mortality	OR (95% CI)	*P*
Distance								
Control side	570	1.58	Ref.	Ref.	605	16.36	Ref.	Ref.
Midtunnel	660	3.03	1.95 (0.88–4.31)	0.100	630	10.48	0.60 (0.43–0.84)	0.003
Treatment side	570	2.81	1.80 (0.79–4.11)	0.163	615	15.61	0.95 (0.70–1.28)	0.719
Week (treatment side)								
1+2	210	1.90	Ref.	Ref.	255	2.75	Ref.	Ref.
3	180	6.11	3.35 (1.05–10.72)	0.041	180	36.67	7.38 (2.27–24.03)	0.001
4	180	0.56	0.29 (0.03–2.60)	0.267	180	12.78	4.25 (1.24–14.55)	0.021

Table 4. Overall percentage mortalities of *Aedes aegypti* and *Anopheles dirus* at wk 6 thru wk 10 from WHO susceptibility test of MINs after hung in the treated tent at both tunnels for 5 wk ($n = 675$ per each tested mosquito species).

	Ae. aegypti mortality (%)			*An. dirus* mortality (%)		
Week	Tunnel 1	Tunnel 2	Control	Tunnel 1	Tunnel 2	Control
6	91.11	95.56	0.00	71.11	84.44	0.00
7	100.00	97.78	0.00	77.78	95.56	2.22
8	97.78	97.78	4.44	84.44	77.78	2.22
9	100.00	64.44	0.00	86.67	66.67	0.00
10	100.00	86.67	0.00	84.44	84.44	0.00

In shelters without walls (berugas) in Indonesia, 2 metofluthrin-impregnated plastic strips repelled more than 60% of *Cx. quinquefasciatus* for at least 11 wk while 4 strips repelled >60% of the mosquitoes for more than 15 wk (Kawada et al. 2005a). In Tanzania, significant reductions in *An. gambiae* (Giles) densities were found in houses treated with metofluthrin-impregnated plastic strips versus untreated houses over a 124-day period (Kawada et al. 2008). In addition to the formulation of the repellent devices, the concentration of metofluthrin plays an important role in reducing contact between humans and mosquito vectors. The OFF! Clip-on with 31.2% metofluthrin significantly affected the mosquito collection rate (Xue et al. 2012, Revay et al. 2013, Dame et al. 2014).

In this study, percentage mortality of *Ae. aegypti* and *An. dirus* in sentinel cups placed inside the treatment tent was higher than in the control tent, indicating that the MIN does have an effect on adult mortality at close range. From the WHO cone bioassay, significant knockdown was not found from net strips in any tent or at any distance away from the treatment tent, indicating that metofluthrin did not readily accumulate in the net's fabric. However, we found 100% knock-down rates and high mortalities for both *Ae. aegypti* and *An. dirus* from the WHO susceptible assay.

Results suggested that the MIN, as the device is currently constructed and formulated, may not be a promising product to reduce house entry behavior dengue, chikungunya, and malaria vectors under field conditions in Thailand. However, it remains probable that the MIN may be effective as a spatial repellent if modifications are made to the metofluthrin concentration or formulation and/or the construction of the device. Further investigation is warranted to determine the effect of MIN on human landing rates. Additionally, such a device, if modified to be effective as a spatial repellent device against mosquitoes, would complement other existing control/preventive measures (ITNs, LLINs, skin repellents, tents) where protection may be compromised with time via dissipation of the insecticide, holes in nets, and ambient conditions that negatively impact the performance of the product.

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