DEVELOPMENT AND EVALUATION OF A PYRIPROXYFEN-TREATED DEVICE TO CONTROL THE DENGUE VECTOR, *AEDES AEGYPTI* (L.) (DIPTERA:CULICIDAE)

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Abstract. The resurgence of dengue fever and the chikungunya epidemic make the control of *Aedes aegypti* mosquitoes, the vectors of these diseases, critically important. We developed and evaluated an *Ae. aegypti* control device that is visually-attractive to mosquitoes. This pyriproxyfen-treated device was evaluated for its impact on *Ae. aegypti* egg production and population dynamics in dengue-endemic areas in Thailand. The device consists of a "high rise" shaped ovitrap/resting station covered with black cotton cloth. The device is easily collapsible and transportable. *Ae. aegypti* are generally drawn towards darker, shadier areas making this device physically attractive as a resting station to mosquitoes of all physiological stages. The results show this device suppressed *Ae. aegypti* populations after it was introduced into a village. The observed effect was primarily the result of the *Ae. aegypti* exposure to pyriproxyfen shortly after adult emergence or after taking a blood meal resulting in decreased egg production. We believe the device may be further improved physically and the formulation should be replaced to provide even better efficacy for controlling *Ae. aegypti* mosquito populations.

Keywords: *Aedes aegypti*, pyriproxyfen, mosquito control, dengue, chikungunya

INTRODUCTION

Dengue and chikungunya (CHIK) viruses are two important arthropod-borne diseases, infecting millions of people annually (Jacob, 2000; Gubler, 2002; Pialoux *et al*, 2007). The vector for these two diseases is the *Aedes* mosquito. Since there are no vaccines or specific treatment for dengue or CHIK, the only effective methods available are vector control and the use of personal protective measures, such as repellent and treated clothing (Fradin and Day, 2002; Pennetier *et al*, 2005, 2010). Control of this vector is difficult. Control using methoprene, temephos, *Bacillus thuringiensis israelensis* (*Bti*) and various adulticides has proven effective in managing both larval and adult populations of *Ae. aegypti* (Gubler, 2004). However,

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chemical control is expensive and requires trained personnel. Given the explosive nature of the recent CHIK epidemic and the growing threat of dengue virus transmission worldwide (Gubler, 2004; Pialoux *et al*, 2007), it is important to explore new methods (or adapt existing ones) to control *Ae. aegypti*.

What is lacking is an expedient, low risk, sustainable approach to achieve *Ae. aegypti* population suppression. A preliminary study (Edman *et al*, 1997) demonstrated placing boxes lined with deltamethrin-impregnated cloth in houses (4 boxes per house) in Thailand reduced *Ae. aegypti* populations in those houses to a greater extent than houses without the resting boxes. Insecticide-treated ovitraps have been shown to measurably impact *Ae. aegypti* populations (Zeichner and Perich, 1999; Perich *et al*, 2003; Williams *et al*, 2007). *Ae. aegypti* females lay their eggs in many sites (Harrington and Edman, 2001; Reiter, 2007). This behavior improves likelihood of survival. Blood-fed *Ae. aegypti* exposed to a polyethylene terephthalate surface containing pyriproxyfen (1.0 g AI/m^2) transmitted this chemical from the treated surface to the water (Itoh *et al*, 1994). Mosquitoes and sand flies are capable of dispersing pyriproxyfen and *B. sphaericus* from treated sites to nearby larval habitats (Schlein and Pener, 1990; Itoh *et al*, 1994; Robert *et al*, 1997; Chism and Apperson, 2003; Sihuincha *et al*, 2005; Devine *et al*, 2009). Since *Ae. aegypti* tend to remain relatively close to their larval habitat, with maximum dispersal distances of about 100-200 meters (Harrington *et al*, 2005), control (or eradication) via this approach might be possible.

Pyriproxyfen is classified as a juvenile hormone (JH) analog and is a potent inhibitor of embryogenesis, metamorphosis and adult formation among insects (Ishaaya and Horowitz, 1992). It has also been shown to decrease the fertility and fecundity of adult *Ae. aegypti* that develop from sublethally exposed larvae (Dash and Ranjit, 1992) and adult mosquitoes not killed by contact with pyriproxyfen may act as vehicles of transmission of the chemical to uncontaminated environments. The tiny doses of pyriproxyfen transmitted may then negatively affect the development of susceptible larvae (Itoh, 1993; Itoh *et al*, 1994). This is advantageous for larval habitats that are logistically impenetrable. Pyriproxyfen has a favorable mammalian toxicity profile which suggests it is suitable for field use without deleterious effects to wildlife (Sihuincha *et al*, 2005).

To maximize mosquito exposure to treated ovitraps, we designed a device consisting of a pyriproxyfen-treated ovitrap partially enclosed by a pyriproxyfen-treated, collapsible, screened resting station. This was based on previous work showing *Ae. aegypti* resting on pyriproxyfen-treated netting subsequently transferred enough chemical to the larval habitat to reduce emergence (Itoh *et al*, 1994). We expanded upon the ovitrap concept using pyriproxyfen instead of an adulticide. The goal was to control adult emergence from treated ovitraps and to maximize transfer of pyriproxyfen to unreachable larval habitats in the surrounding area. We developed and conducted an initial evaluation of a prototype in the field and then evaluated the impact of this device on the *Ae. aegypti* population in a dengue-endemic village in Thailand.

MATERIALS AND METHODS

Evaluation of pyriproxyfen transfer from a treated device to a non-treated container

Mosquito rearing. Laboratory grown *Ae.*

aegypti maintained at the Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand were used for the study. These mosquitoes were reared in the laboratory at a temperature of 26(± 2)°C and a relative humidity of 75% under a light dark schedule of 12:12 hours (L:D). Fish food (C.P. Hi Pro®, Bangkok, Thailand) was used to feed the larvae. The mosquitoes were provided with cotton soaked in 10% multivitamin solution (Multilim Syrup, Atlantic Laboratories Corp, Bangkok, Thailand) until used in the study.

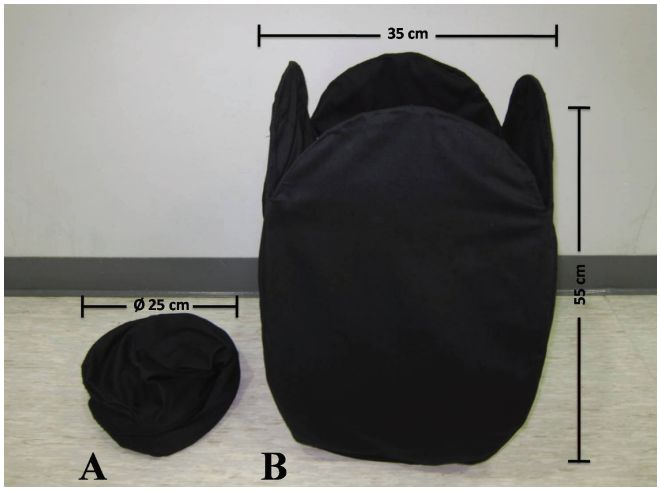

Fig 1–The prototype device used in this study, collapsed (A) and expanded (B).

Outdoor tunnels. Two large outdoor tunnels (each 50 m long x 1.0 m wide x 1.5 m high) were constructed using a 100 denier polyester 156-mesh netting. The netting was supported using arched wooden supports placed every 5 m. The tunnels were constructed along a vegetation-lined fence having a mean height of 2 m. One tunnel was designated as a control and the other tunnel a treatment tunnel.

Experimental design. The tunnels were used to evaluate whether gravid *Ae. aegypti* can transfer pyriproxyfen from a treated device to a water-holding container. The treated device consisted of a rectangular parallel piped "high rise" shape covered with black cotton cloth. It is collapsible and easy to transport and store. The device measured 35 cm x 35 cm x 55 cm. The device can be collapsed to a size of 25 cm diamiter and 4.5 cm in height with a weight of 450 g (Fig 1). The inner surface of the expended device (35 cm x 35 cm x 55 cm) was coated with pulverized pyriproxyfen (Sumilarv 0.5G®, Sumitomo Chemical Corporation, Osaka, Japan) at a dose of 0.05 g AI/m^2. This concentration was chosen since our previous study showed this dose affected the emergence rate of *Ae. aegypti* (Ponlawat *et al*, unpublished data). The treated device was placed inside a mosquito cage (60 cm x 60 cm x 120 cm) made from netting and the cage/device was subsequently positioned at one end of the treatment tunnel. An oviposition container (25 cm x 36 cm x 9 cm) containing 2 liters of tap water lined with paper towels was placed 50 m away at the opposite end of the treatment tunnel. The control tunnel was set-up in the same manner but with a non-treated device replacing the treated device. One hundred gravid *Ae. aegypti* (5-7 day old, 1 day post blood feed) were released into the mosquito cage and provided with 10% sugar solution for food. After 24 hours, the mosquitoes were released into the tunnel. After 6 days, the paper towel with any eggs was removed from the water container and air-dried. The number of eggs laid was counted and recorded.

The presence of pyriproxyfen in the

oviposition container was indirectly determined using a larval bioassay. Twenty laboratory-reared late third to early fourth instar larvae were added to a 200-ml cup containing 100 ml of "contaminated" water from the oviposition container after 6 days of exposure to presumably "contaminated" mosquitoes. Five replicates for each treatment were conducted. Each day, emerged adults in each cup were removed, sorted by sex and counted. Productivity was defined as the number of adult mosquitoes that emerged from each cup. Our hypothesis was that a biologically-effective dose of pyriproxyfen could be transferred by gravid mosquitoes from a treated device to a non-treated oviposition container inhibiting larval development and adult emergence in the "contaminated" container.

Evaluation of pyriproxyfen-treated devices on *Ae. aegypti* populations within a dengue-endemic village

Study area. Field experiments were carried out in two villages (Ban Chon and Neon Mayom) of the same area size (158,962 m^2) located 1.3 km apart in Tapong Subdistrict, Mueang District, Rayong Province, Thailand (Ban Chon: 12°37'38" N, 101°22'39" E; Neon Mayom: 12°37'5" N, 101°23'1" E). There were 65 and 71 houses with similar populations (160 and 171) in Ban Chon and Neon Mayom villages, respectively. Inner and outer geographical circles were designated for each village (Fig 2). The diameter of the inner circle for each village was 150 m. The area of the inner circle was approximately 17,662 m^2. The distance between the inner and outer circles was 150 m and the area of the outer circle was 141,300 m^2. The field study was conducted continuously over a 30 week period from September 2009 to April 2010.

Experimental design. To determine if the device could significantly suppress the *Ae. aegypti* population within and beyond the treatment area, we deployed 4 pyriproxyfen-treated prototype devices at each house located within the inner circle in Ban Chon (BC) village (treatment site, 12 houses). No devices were deployed in Neon Mayom (MY) village (control site). A device somewhat similar to that described above in the previous experiment was used for the village study; it also contained pyriproxyfen at a concentration of 0.05 g AI/m^2, but inside the device we placed a black plastic bucket (28 cm x 28 cm x 17 cm) containing 3 liters of pyriproxyfen-treated water.

Adult densities. Adult mosquito densities were determined using BG-Sentinel traps (Williams *et al*, 2006) and a CDC backpack aspirator (Model 1012, John W Hock, Gainesville, FL) (Clark *et al*, 1994). The BG-Sentinel trap (with BG Lure) has been shown to be useful for rapid assessment and routine monitoring of local *Ae. aegypti* populations (Williams *et al*, 2007). Four houses in the inner circle and 8 houses in the outer circle of each village were randomly selected. One BG-Sentinel trap was placed inside each selected house. The traps were operated for 8 hours each day (08:00 AM to 04:00 PM) for 3 consecutive days every two weeks for 6 weeks prior to deployment of resting stations and every two weeks thereafter for three months. The CDC backpack aspirator collections were performed with a 2-person team as described previously (Clark *et al*, 1994). Briefly, the aspirator operator sampled under, inside, and behind furniture and among clothing, bedding and other structures within the house. Mosquitoes inside and/or around resting stations were not aspirated. Sampling lasted for 15 minutes and was performed once every two weeks for a month prior to deployment

of the study device and every two weeks thereafter for three months. Aspirator collections were performed in all the houses located within the inner cluster that had not already been randomly selected for placement of BG-Sentinel collections. Ten to 15 additional houses in the outer circle of each village were randomly selected for aspirator collection. Houses used for BG-Sentinel collection were not used for backpack aspirator collections.

Physiological state determination. All collected mosquitoes were identified to species. *Ae. aegypti* were dissected under a microscope on site to determine ovarian development and classified as either nulliparous (ovaries tightly coiled into skeins) or parous (ovaries stretched and uncoiled) (Service, 1993). Blood-fed, gravid females were not dissected but classified as parous.

Sentinel containers. Black plastic buckets containing 3 liters of tap water were placed randomly in ten selected houses in both the inner and outer circles of each village. The number of pupae in each container was recorded every two days and then the pupae were removed from the containers. We did this to determine the mean number of pupae produced weekly.

Larval bioassay. To investigate pyriproxyfen transfer from treated prototype devices to sentinel containers, 100 ml of water taken from the sentinel containers was used for the larval bioassay. Twenty third-instar laboratory-reared *Ae. aegypti* were added to each cup and the fraction mortality (the number of larvae tested - the number of adults that emerged/ 20 larvae) was determined. Tap water was added to each sentinel container to replace the volume removed. Larval bioassays were conducted once prior to deployment of resting stations and every two weeks thereafter for 13 weeks. Control mortality was corrected using Abbott's formula.

Data analysis

Data from each experiment were tested for conformation to the assumptions of normality and homoscedasticity. The mean numbers of eggs laid in the treated and control tunnel and the larval bioassay results were compared with the Student's *t*-test (MINITAB 16, Minitab, State College, PA). The densities of *Ae. aegypti* in the studied areas prior to using the device were compared using the Kruskal-Wallis test.

Generalized estimating equations (GEE) were used for this analysis to put the modeling emphasis on planned treatments while still accounting for temporal correlations due to repeated sampling of village houses (Fitzmaurice *et al*, 2011). Similarities between GEE and generalized linear models (GLM) included specifying a link function and conditional variance structure with the GEE including an association structure defining the correlation between sample times. However, unlike the GLM, the GEE were considered semi-parametric since no assumed distribution was imposed on the response counts.

Treatment efficacy was expressed as a marginal model for the expected counts:

$$\log E(Y_{ij}) = \beta_1+\beta_2 \text{Period}_i+\beta_3 \text{Loc}_2+\beta_4 \text{Loc}_3+\beta_5 \text{Period}_i \text{ x } \text{Loc}_2+\beta_6 \text{Period}_i \text{ x } \text{Loc}_3,$$

where Y_{ij} is the count for the j^{th} treatment in the j^{th} period of observation (i = 1,2). The coefficient, β_1, represents the expected count in the Neon Mayom during the pre-treatment period. The variables Loc_2 and Loc_3 are indicator variables for the inner and outer rings, respectively, at Ban Chon. The binary variable, Period, denotes the baseline and post-treatment follow-up periods, with Period = 0 as the baseline period and Period = 1 as the

post-treatment follow-up period. A successful control effect was inferred when a significant interaction occured between the trap location and pre/post-treatment counts, *ie*, $\beta_5 \neq 0$ or $\beta_6 \neq 0$.

For our models, we assumed a logarithmic link function with a conditional variance given by:

$$var(Y_{ij}|X_{ij}) = \phi \times u^2_{ij},$$

where ϕ was a scale parameter to estimate overdispersion. We used a first-order auto-regressive correlation structure:

$$\text{cor}\,(Y_{is}, Y_{it}) = \alpha^{|s-t|},\ s \neq t,$$

to model the within-subject relationship between observations, in our case, weekly samples in a single house. Hypotheses were tested using the Wald statistic:

$$W^2 = (L\hat{\beta})'\{L\widehat{COV}(\hat{B})L'\}^{-1}\,(L\hat{\beta}),$$

where $\hat{\sum}_l$ was the estimated conditional covariance matrix, where W^2 was compared to the χ^2 distribution. Statistical testing was done using R, version 2.14.0 (R Development Core Team, Vienna, Austria).

The parity rate was the number of parous *Ae. aegypti* females per the total number dissected. The chi-square (χ^2) test (MINITAB 16, Minitab, State College, PA) and the Cochran-Mantel-Haenszel test (R Development Core Team, Vienna, Austria) were used to analyze the physiological state data.

RESULTS

Evaluation of pyriproxyfen transfer to a non-treated water-filled oviposition container

Egg production was significantly reduced in females exposed to pyriproxyfen early in their development (1 day post-blood feed) (t = -3.86; df = 8; p < 0.05). The mean numbers of eggs laid were 486.6 and 2,604.4 in the treated and control tunnels, respectively. Evaluation of the "non-treated" container showed a significant difference (t = -4.33; df = 38; p < 0.01) between the percent adult emergence in the treated (74.5) and control (90.5) tunnels.

Table 1
Mean number of female *Ae. aegypti* per house over 6 weeks during pre-treatment at the two study villages.

Village	*n*	Mean	SE
Treatment village			
Inner circle	26	3.58	0.74
Outer circle	33	3.18	0.53
Control village	29	3.28	0.58

n, number of sampled houses

Table 2
Multivariate Wald test of model interaction by surveillance method.

Surveillance method	DF	Wald W^2	$p(>\|\chi^2\|)$
BG sentinel trap	2	31.8	<0.001
CDC backpack aspirator	2	4.3	0.12
Sentinel containers	2	2.0	0.36

Evaluation of pyriproxyfen-treated devices on *Ae. aegypti* populations in the village

Pre-treatment. Baseline data (Table 1) collected during May and June 2009, showed the mean numbers (±SE) of *Ae. aegypti* per house in the inner and outer circles of the treatment village were 3.58 ± 0.74 and 3.18 ± 0.53, respectively, and in the control village was 3.28 ± 0.58 which is not significantly different (p = 0.99).

Post-treatment. Of the three surveillance methods, only the trap counts from the BG-sentinel traps showed a significant

Table 3
Log rate ratio estimates and standard errors (based on sandwich variance estimator) from the GEE model for BG Sentinal adult surveillance. Estimates represent differences from β_1.

Variable	Estimate	SE
Intercept (β_1)	2.2482	0.1688
Period (β_2)	0.1547	0.2490
Loc_2 (β_3)	-0.1951	0.2193
Loc_3 (β_4)	-0.5154	0.3073
Period x Loc_2 (β_5)	-0.9072	0.2561
Period x Loc_3 (β_6)	-0.0303	0.3067

Estimated dispersion parameter: $\hat{\phi} = 2.22$. Estimated working correlation: $\hat{\alpha} = 0.478$.

change (Table 2), the trap counts were different in each of the 3 areas (Fig 3). The post-treatment rate ratio for the inner circle of the treatment village was 0.40 [95% confidence interval (CI), 0.24 - 0.67] (or $e^{-0.9072}$), indicating the presence of the pyriproxyfen-treated device significantly reduced adult counts during the study period (Table 3). There was a non-significant reduction in the rate ratio in the outer circle of the treatment village ($e^{-0.0303}$ = 0.97; 95% CI 0.37-1.57). The counts in the control village increased during the study period ($e^{0.1547}$= 1.17; 95% CI 0.68-1.66), but not significantly.

The mean numbers of adults in the treatment village (inner and outer circles) remained generally lower than in the control village (Fig 4). However, there were no significant differences ($F = 0.10$; df = 3, 24; $p = 0.96$) in the fraction mortality between the treated and control villages (Fig 5).

Physiological state determination

A total of 2,937 *Ae. aegypti* mosquitoes were dissected (1,718 from the control village and 1,219 from the treatment village)

Table 4
Physiological state of *Ae. aegypti* collected using BG-sentinel traps and CDC backpack aspirators from the study areas.

Collection method	Village	Treatment	#Nulliparous	#Parous	Total	%Parity	χ^2	*p*-value
BG-Sentinel trap	Control	Pre	9	30	39	76.92	0.90	0.34
		Post	227	1,091	1,318	82.78		
	Treated	Pre	16	232	248	93.55	11.04	0.00[a]
		Post	83	480	563	85.26		
CDC backpack	Control	Pre	5	25	30	83.33	0.45	0.50
		Post	41	290	331	87.61		
	Treated	Pre	11	134	145	92.41	1.79	0.18
		Post	31	232	263	88.21		
		Total	423	2,514	2,937			

[a]Indicates significant difference ($p < 0.05$)

Fig 2–The inner and outer geographical circles in the study village.

to determine their physiological state. In the treatment village, the frequencies of parous and nulliparous females collected were significantly different pre- and post-treatment ($\chi^2 = 11.04$, $p = 0.00$) (Table 4). There were no significant differences in parity rates pre- and post-treatment in the control village. There were no significant differences in the frequencies of parous and nulliparous females collected with the CDC backpack aspirators pre- and post-treatment in either village (Table 4). Pre-treatment, the parity odds ratio from the treatment village was significantly higher than the control village ($p < 0.05$, Cochran-Mantel-Haenszel test). However, there were no significant differences in parity odds ratios between the treatment and control villages post-treatment.

DISCUSSION

The tunnel and field trials showed the pyriproxyfen-treated device can reduce fecundity and change the age structure of the *Ae. aegypti* population in the field. The adult density and the parity rates obtained from BG-sentinel surveillance shows the device may suppress increases in *Ae. aegypti* populations. This effect is primarily the result of *Ae. aegypti* exposure to a pyriproxyfen-treated device shortly after taking a blood meal resulting in decreased egg deposition. Ishaaya and Horowitz (1992) found newly deposited eggs (0-1 day old) from female sweet-

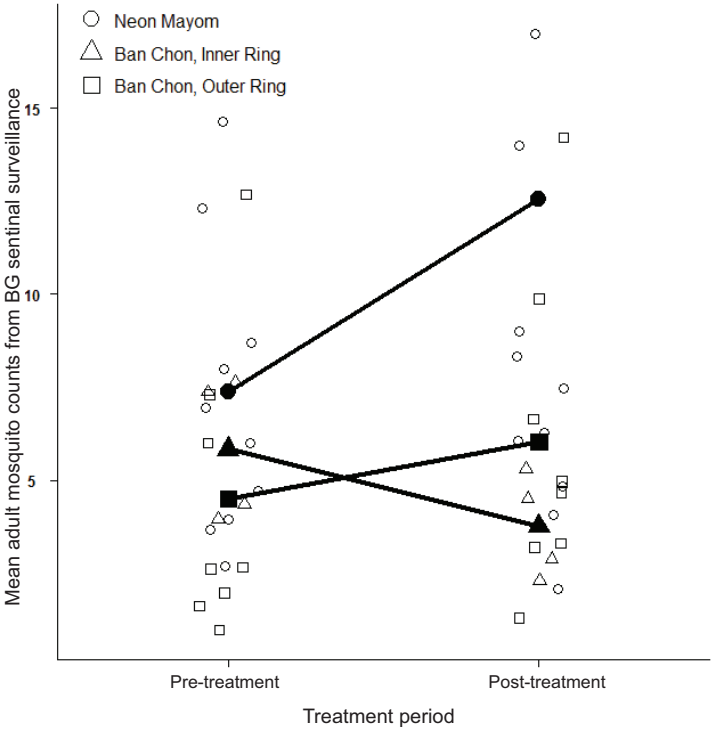

Fig 3–Average adult counts of each village (solid point) and average counts collected from houses (open point) pre- and post-treatment.

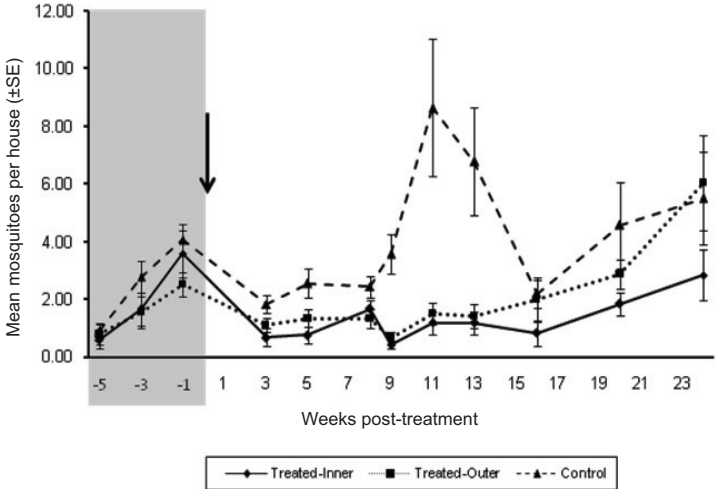

Fig 4–Spatial and temporal trends in the number of female *Ae. aegypti* pre- and post-treatment (arrow shows the time of device application).

potato whiteflies exposed to pyriproxyfen were less likely to hatch. The same result was seen in California red scale females treated with pyriproxyfen (Eliahu *et al*, 2007). Among mosquitoes, Itoh *et al* (1994) determined the number of *Ae. aegypti* eggs laid decreased with exposure to pyriproxyfen. The fewest number of eggs laid resulted from females being exposed to pyriproxyfen on the day of a blood meal. Our tunnel study results demonstrate pyriproxyfen has a significant effect on egg production and deposition (and hence adult productivity) when females are exposed shortly after a blood meal.

We did not measure pyriproxyfen levels in "non-treated" water suspected of contamination with pyriproxyfen nor do we have visual evidence pyriproxyfen was "carried" from the device to the non-treated water source. Devine *et al* (2009) found pyriproxyfen can be physically carried from a treated surface to an oviposition substrate by *Ae. aegypti*. In our study, indirect evidence, primarily from our larval study suggests contamination of non-treated water by mosquitoes is possible (Ponlawat *et al*, unpublished data).

We used pulverized pyriproxyfen granules to treat our device, but this formulation is not ideal, since pulver-

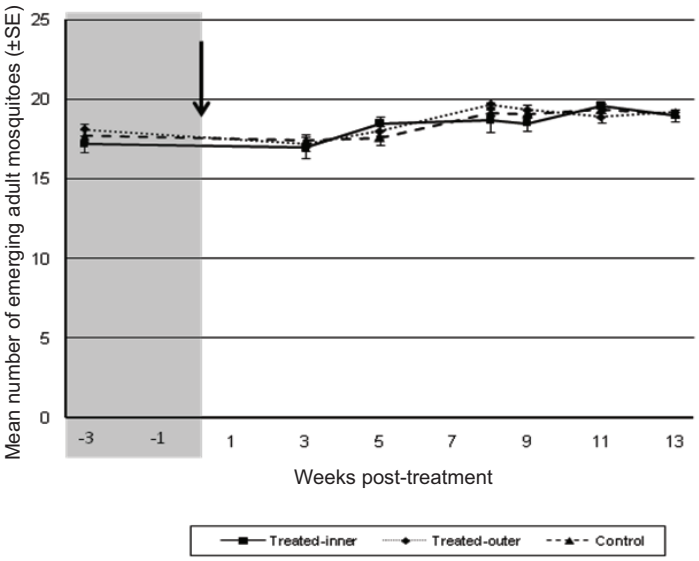

Fig 5–Number of **e**merged adults pre- and post-treatment from larval bioassay (arrow shows the time of device application).

ized granules accumulate on the bottom over time if not disturbed or shaken. We propose treatment with a more soluble formulation would suppress *Ae. aegypti* populations to a greater degree than in our study. In this study, we were able to confirm the effectiveness of our two different prototype devices against *Ae. aegypti* in semi-field and field conditions in Thailand.

Our devices are easily collapsible, transportable and effective. Further studies are needed to determine which device (with or without a bucket containing periproxyfen treated water) is most effective in the village. We believe improving the physical characteristics of the device and replacing the formulation can result in a more effective device for suppressing populations of container-breeding mosquitoes. Since *Ae. aegypti* are drawn toward darker areas, this device is physically attractive as a resting station, and can result in exposure of mosquitoes to pyriproxyfen early in their development leading to reductions in mosquito productivity. This pyriproxyfen-treated device should be tested to determine if it may be used in efforts to contain outbreaks of dengue or chikungunya infection.

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REFERENCES

Chism BD, Apperson CS. Horizontal transfer of the insect growth regulator pyriproxyfen to larval microcosms by gravid *Aedes albopictus* and *Ochlerotatus triseriatus* mosquitoes in the laboratory. *Med Vet Entomol*

2003; 17: 211-20.

Clark GG, Seda H, Gubler DJ. Use of the «CDC backpack aspirator» for surveillance of *Aedes aegypti* in San Juan, Puerto Rico. *J Am Mosq Control Assoc* 1994; 10: 119-24.

Dash AP, Ranjit MR. Comparative efficacy of aphid extracts and some juvenoids against the development of mosquitoes. *J Am Mosq Control Assoc* 1992; 8: 247-51.

Devine GJ, Perea EZ, Killeen GF, Stancil JD, Clark SJ, Morrison AC. Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *Proc Natl Acad Sci* 2009; 106: 11530-4.

Edman JD, Kittayapong P, Linthicum KJ, Scott TW. Attractant resting boxes for rapid collection and surveillance of *Aedes aegypti* (L.) inside houses. *J Am Mosq Control Assoc* 1997; 13: 24-7.

Eliahu M, Blumberg D, Horowitz AR, Ishaaya I. Effect of pyriproxyfen on developing stages and embryogenesis of California red scale (CRS). *Pest Manag Sci* 2007; 63: 743-6.

Fitzmaurice GM, Laird NM, Ware JH. Applied longitudinal analysis. 2nd ed. New Jersey: John Wiley & Sons, 2011.

Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med* 2002; 347: 13-8.

Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol* 2002; 10: 100-3.

Gubler DJ. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? *Comp Immunol Microbiol Infect Dis* 2004; 27: 319-30.

Harrington LC, Edman JD. Indirect evidence against "skip-oviposition" behavior by wild *Aedes aegypti* (Diptera: Culicidae) from Thailand. *J Med Entomol* 2001; 38: 641-5.

Harrington LC, Scott TW, Lerdthusnee K, *et al*. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *Am J Trop Med Hyg* 2005; 72: 209-20.

Ishaaya I, Horowitz AR. Novel phenoxy juvenile-hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweet-potato whitefly (Homoptera, Aleyrodidae). *J Econ Entomol* 1992; 85: 2113-7.

Itoh T. Control of DF/DHF vector, Aedes mosquito, with insecticides. *Trop Med* 1993; 35: 259-67.

Itoh T, Kawada H, Abe A, Eshita Y, Rongsriyam Y, Igarashi A. Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats. *J Am Mosq Control Assoc* 1994; 10: 344-7.

Jacob M. Dengue: emergence as a global public health problem and prospects for control. *Trans R Soc Trop Med Hyg* 2000; 94: 7-8.

Pennetier C, Corbel V, Hougard JM. Combination of a non-pyrethroid insecticide and a repellent: a new approach for controlling knockdown-resistant mosquitoes. *Am J Trop Med Hyg* 2005; 72: 739-44.

Pennetier C, Chabi J, Martin T, *et al*. New protective battle-dress impregnated against mosquito vector bites. *Parasites Vectors* 2010; 3: 81.

Perich MJ, Kardec A, Braga IA, *et al*. Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Med Vet Entomol* 2003; 17: 205-10.

Pialoux G, Gaüzère BA, Jauréguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis* 2007; 7: 319-27.

Reiter P. Oviposition, dispersal, and survival in *Aedes aegypti*: implications for the efficacy of control strategies. *Vector-Borne Zoon Dis* 2007; 7: 261-73.

Robert LL, Perich MJ, Schlein Y, *et al*. Phlebotomine sand fly control using bait-fed adults to carry the larvicide *Bacillus sphaericus* to the larval habitat. *J Am Mosq Control Assoc* 1997; 13: 140-4.

Schlein Y, Pener H. Bait-fed adult *Culex pipiens* carry the larvicide *Bacillus sphaericus* to

the larval habitat. *Med Vet Entomol* 1990; 4: 283-8.

Service MW. Mosquito ecology: Field sampling methods, 2nd ed. Liverpool: Elsevier Applied Science, 1993.

Sihuincha M, Zamora-Perea E, Oellana-Rios W, *et al*. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera : Culicidae) in Iquitos, Peru. *J Med Entomol* 2005; 42: 620-30.

Williams CR, Long SA, Russell RC, Ritchie SA. Field efficacy of the BG-Sentinel compared with CDC Backpack Aspirators and CO_2-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *J Am Mosq Control Assoc* 2006; 22: 296-300.

Williams CR, Long SA, Webb CE, *et al*. *Aedes aegypti* population sampling using BG-Sentinel traps in north Queensland Australia: statistical considerations for trap deployment and sampling strategy. *J Med Entomol* 2007; 44: 345-50.

Zeichner BC, Perich MJ. Laboratory testing of a lethal ovitrap for *Aedes aegypti*. *Med Vet Entomol* 1999; 13: 234-8.