THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Tropical Agriculture)

DEGREE

Tropical Agriculture

FIELD

Agriculture

FACULTY

TITLE: Characterization of the BG-Sentinel ™ Trap for Integration into an *Aedes aegypti* L. (Diptera:Culicidae) Push-Pull Control Strategy

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THESIS

CHARACTERIZATION OF THE BG-SENTINEL™ TRAP FOR INTEGRATION INTO AN *AEDES AEGYPTI* L.(DIPTERA: CULICIDAE) PUSH-PULL CONTROL STRATEGY

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Tropical Agriculture)
Graduate School, Kasetsart University,
2012

Ferdinand Villanueva Salazar 2012: Characterization of the BG-Sentinel™ Trap for Integration into an *Aedes aegypti* L. (Diptera:Culicidae) Push-Pull Control Strategy. Doctor of Philosophy (Tropical Agriculture), Major Field: Tropical Agriculture, Faculty of Agriculture. Thesis Advisor: Professor Theeraphap Chareonviriyaphap, Ph.D. 179 pages.

The study aimed at evaluating and characterizing a peridomestic trap (BG-Sentinel™) to understand its role as part of a novel push-push dengue vector control strategy. The process involved modelling the relationship between BG-Sentinel™ trap operation time and female *Ae. aegypti* capture rates and quantification of the impact of trap density on capture rates against varying *Ae. aegypti* adult population sizes using a semi field system built in Pu Teuy, Kanchanaburi, Thailand. Results showed a recapture range of 66-98% with 2-3 traps as statistically effective in recapturing mosquitoes as 4 traps for all mosquito release numbers (10, 25, 50, 100, 150, 200 and 250).

The effect of spatial repellents (DDT, metofluthrin and transfluthrin) exposure in experimental hut simulated home condition on BG-Sentinel™ *Ae. aegypti* recapture was quantified through screen house post exposure releases. Varying effects were observed with the use of three repellents from either without recovery period (immediate release population) or with recovery time (delayed release population) experiments. Both BGS recapture rates and data from interception traps from the experimental huts also showed best BGS location was opposite portals of entry at 0 m distance from the experimental huts.Using the best location and distance, BG-Sentinel™ functioned to collect *Ae. aegypti* populations under two different Thai household conditions, raised-wooden and non-raised-cemented house, documenting differences in densities between periods of monitoring (rainy and dry seasons), between times of collection and between locations around households. BGS collected not only *Ae. aegypti* females but also males and also the secondary vector *Ae. albopictus* in the presence of possible competing resting and breeding sites found within 0-3 m distance from local house. Implications of the results of this study to push-pull control strategy were discussed.

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Student Signature Thesis Advisor's Signature

ACKNOWLEDGMENTS

Heartfelt gratitude is due to the following:

- Dr. Theeraphap Chareonviriyaphap for giving me a chance to be part of his team, for believing and supporting in all my activities, for guiding and seeing me through the duration of the study,
- Dr. Nicole L. Achee for all the priceless leanings, the motivation, support and most of all the help in putting this work into perspective,
- Dr. John P. Grieco for the technical advise in protocol development, presentation and paper preparations,
- Ms. Suppaluck Polsomboon for facilitating all the field experiments and support in the conduct of the study,
- Dr. Atchariya Prabaripai, Dr. Pankhil Shah and Ms. Christine Dureza for their inputs in the statistical analysis,
- Drs. Wannapa Suwonkerd and Lars Eisen for all the technical advise.
- Kasetsart Team: Pi It, Gan, Ju, Nok,Thip, Oam, Oan, Vithi, Thong, Golf, Oui, Aoh, Neng, Kik, Yai, Na Mee, Na Aung, Ouan, Pi Noi, all the villagers from Pu Teuy that participated during the field works and Tolu (USUHS Team) for so many forms of help in the field, laboratory and computer works,
- Dr. Michael J Bangs through the NAMRU-2 grant that linked us to Professor Theeraphap that started this collaboration, and
- Thailand Research Fund, and Bill and Melinda Gates Foundation for the financial support.

For Prescy, especially to my Totoy, my extended family, friends and RITM (esp. Entomology Dept.)-DOH-Philippines colleagues.

Ferdinand V. Salazar

March 2012

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF ABBREVIATIONS

A	=	Area
Ae. aegypti	=	*Aedes aegypti*
ai	=	Active ingredient
ANOVA	=	Analysis of variance
BGS	=	BioGents Sentinel Trap
^{o}C	=	Degrees Centigrade
CDC-	=	Center for Disease Control, USA
CI	=	Contact Irritant
cm^{2}	=	Square centimeter
CRD	=	Completely Randomized Design
CTD	=	Center for Tropical Diseases
d	=	days
DDT	=	Dichloro-diethyl trichloroethane
DEN	=	Dengue Serotype
DR	=	Delayed Release
F	=	Generation (Mosquito Colony)
FAR	=	Field Application rate
h	=	Hour
HLC	=	Human landing Collections
IR	=	Immediate Release
KD	=	Knock Down
LD	=	Lethal Dose
lx/ft2	=	Lumex per square feet
m	=	meter
m^{2}	=	Square meter
nsH	=	Non-raised sentinel Household
PPS	=	Push-Pull Strategy

LIST OF ABBREVIATIONS (Continued)

RE	=	Reduction in Entry
RIDL	=	Release of Insect with Dominant Lethal
RN	=	Release Numbers
rsH	=	Raised sentinel household
SAC	=	Surface area coverage
SD	=	Standard Deviation
SE	=	Standard Error
SH	=	Sentinel Household
SR	=	Spatial Repellent
TP	=	Trap Position
TRF	=	Thailand Research Fund
µg	=	micro gram
V	=	Volume
WHO	=	World Health Organization

CHARACTERIZATION OF THE BG-SENTINEL™ TRAP FOR INTEGRATION INTO AN *AEDES AEGYPTI L.*(DIPTERA: CULICIDAE) PUSH-PULL CONTROL STRATEGY

INTRODUCTION

Dengue and dengue haemorrhagic fever occur in the tropics and subtropics with an estimated 2.5 billion people residing in areas where dengue is endemic (WHO, 2009). Dengue viruses are transmitted primarily by *Aedes aegypti*, a day biting mosquito that feeds and rests indoors and preferentially bites humans (Scott *et al.*, 1993, 2000; Gubler, 1998; Harrington *et al*., 2005). Despite years of public health efforts and research progress, an effective vaccine against dengue virus is not yet available. For this reason, disease prevention remains dependent on vector management and control strategies (Reiter and Gubler, 1997; WHO, 2009). However, controlling *Ae. aegypti* has proven difficult due to its strong association with domestic and peridomestic human environments which harbor and sustain development sites (artificial containers) for the immatures.

Historically, indices for measuring the abundance of the immature stages of the mosquito (e.g., Breteau Index and more recently pupae per person) have guided when and where control operations should be implemented (Reiter and Gubler, 1997; Focks, 2003; WHO, 2009). Although these indices can provide useful information, they are not consistently predictive of the abundance of adult mosquitoes or dengue incidence (Tun-Lin *et al*., 1996; Morrison *et al*., 2004). Adult traps have also been used for surveying the abundance of vectors (Rupp and Jobbins, 1969; Kline 2006) but most have been relatively ineffective, especially against a day-biting mosquito such as *Ae. aegypti* (Service, 1993; Scott and Morrison, 2003; Facchinelli *et al*., 2008). The development of new, improved traps for adults, such as the BG-Sentinel™ (BGS) and Zumba™ traps provides an opportunity for improved entomological surveillance and possibly also control of *Ae. aegypti* (Krockel *et al*., 2006, Maciel-de-Freitas *et al*., 2006, Williams *et al*., 2006, 2007; Ball and Ritchie, 2010a, b; Bhalala and Arias, 2009) and *Ae. albopictus* (Ritchie *et al*., 2006, Farajollahi *et al*., 2009). The combinations of attractant baits and insecticide treated traps have been used to effectively create "infestation barriers" for nuisance mosquito populations (Kline, 2006). Furthermore, trap and lure combinations have been successful in the control of several insects, including tsetse flies, the vector of African trypanosomiasis (Vale, 1993; Torr, 1994).

The BGS trap incorporates in its design the most important elements of *Ae. aegypti* host-seeking behavior by combining an olfactory cue (BGS Lure) with visual cues (black and white contrast) to attract the mosquito. This trap has proven to be an effective tool for surveillance of *Ae. aegypti* adults, out-performing other collection devices and traps such as the CDC backpack aspirator, the Fay-Prince trap, the Encephalitis Virus Surveillance trap and the commercially available Mosquito Magnet Liberty™ trap (Maciel-de-Freitas *et al*., 2006; Krockel *et al*., 2006; Williams *et al*., 2006). It has also been suggested that the BGS could be a possible replacement for human-landing catches of *Ae. aegypti* (Krockel *et al*., 2006). Based on these findings, the BGS was selected as the trapping device for integration into a push-pull control strategy for *Ae. aegypti* currently in the proof-of-concept stage.

Push-pull control strategies (PPS) have been proven effective in the control of agricultural pests (Miller and Cowles, 1990; Midega *et al*., 2006). The general concept of a push-pull system involves behavioral manipulation of the target pest population to repel or deter (push) them away from a source (in the case of an agricultural pest this would be crops) using stimuli that renders the source unsuitable or unattractive (Figure 1). The pests are simultaneously lured (pulled) to an attractant, for example a trap, through which they are removed from the location (Nielsen, 2001; Amudavi *et al*., 2007; Cook *et al*., 2007). The same strategy may prove to be effective in the control of pathogen-transmitting mosquitoes through the manipulation of naturally occurring differences in the attractiveness of host species (Hallem *et al*., 2004; Constantini *et al*., 2001) or through the use of repellents (Barnard and Xue, 2004; Fradin and Day, 2002) as push stimuli in combination with attracticides derived from host odors (Bhasin *et al*., 2001) or attractive pheromones

(Blackwell *et al*., 1994) as pull stimuli. In the current research program, the push component uses spatial repellent (SR) and/or contact irritant (CI) chemicals at sublethal doses (thus rendering them safer for human exposure) and minimized treatment coverage (for greater cost-effectivenessw) to reduce indoor densities of host-seeking *Ae. aegypti*. The BGS is to serve as the pull component to remove chemically repelled or irritated *Ae. aegypti* from the peridomestic environment thereby further reducing human-vector contact. As an added benefit, the BGS trap can facilitate the monitoring of mosquito movement between huts allowing for an evaluation of any potential diversion of mosquitoes to untreated locations.

Previous research has demonstrated that some pyrethroids can elicit repellent responses in *Ae. aegypti* at doses well below WHO field application rates (WHO, 2009; Grieco *et al*., 2007; Achee *et al*., 2009) but limited information exists regarding the effect on *Ae. aegypti* following exposure to repellent chemicals on altering host-seeking behavior. Indeed, Hao *et al*. (2008) described changes in both host-seeking and blood-feeding behaviors of *Ae. albopictus*, the secondary vector of dengue, upon exposure to plant volatiles under laboratory conditions. *Aedes albopictus* females surviving concentrations of geraniol, citral, eugenol, or anisaldehyde for 24 and 48 h all showed different degrees of reduction in host-seeking ability. After 48 h of exposure to 0.2.50 μg/cm^3 of anisaldehyde, 100% of the mosquitoes lost their host-seeking ability. Hao and colleagues noted that reduction of host-seeking ability was recovered after various times, The longest recovery time (144 h) was observed for geraniol after 24 h at 0.2.50 μg/cm^3. Anisaldehyde significantly interrupted the normal blood-feeding of mosquitoes in all stages (activation, orientation, probing, and engorgement) of behavior. Such knowledge regarding *Ae. aegypti* is also critical to determine the expected efficacy of the BGS in a repellent focused PPS and is one focus of the current study.

Most BGS trap evaluations have been conducted in unison with either CDC backpack aspirator or human landing collections (HLC) as the gold standard comparison. These evaluations performed in outdoors or indoors locations placed the BGS traps on the ground (Maciel-de-Freitas *et al*., 2006; Meeraus *et al*., 2008;

Kroeckel *et al*., 2006) following manufacturer's recommendations; i.e. placement in locations that are sheltered from wind, heavy rainfall, and direct sunlight, not too close to walls (min. distance of approx. 1 meter) and ensuring the space above the trap is clear by at least 1.5 meters to make it visible to patrolling mosquitoes. There have been no reports, however, of detailed BGS optimization for use in the peridomestic environment, to include optimal location and distance that will provide highest capture of females. This information is vital to guide PPS development trials.

This project was conducted in three phases: 1) screen house trials using known mosquito denominators (release numbers), 2) outdoor field trials using experimental huts and; 3) local home trials where *Ae. aegypti* total population densities were unknown. The study ascertained the effect of repellent exposure on BGS *Ae. aegypti* recapture rates. Further optimization determined if BGS *Ae. aegypti* recapture rates can be enhanced at particular location and distance from experimental huts (i.e., host-occupied structures). Finally, optimized conditions were tested in a dengue-endemic setting to assess performance of the BGS in the presence of competing *Ae. aegypti* resting sites.

OBJECTIVES

General Objective:

To characterize the BG-Sentinel^{TM} mosquito trap for integration into an *Aedes aegypti* push-pull control strategy.

Specific Objectives:

Phase I-Screen House Trials

1. Describe the relationship between BGS trap operation time and female *Ae. aegypti* capture rates.
2. Quantify the impact of trap density on capture rates against varying *Ae. aegypti* adult population sizes.
3. Correlate environmental variables (i.e., light, temperature and relative humidity) with BGS *Aedes aegypti* recapture rates.

Phase II-Experimental Hut Trials

1. Quantify the effect of *Ae. aegypti* exposure to spatial repellent chemicals on BGS recapture rates.
2. Quantify the change in *Ae. aegypti* BGS recapture at different locations and distances from a human-host occupied structure.

Phase III-Local Home Trials

1. Evaluate BGS trap efficacy against natural *Ae. aegypti* populations at sentinel households.
2. Identify potential *Ae. aegypti* resting sites that may compete with BGS trap capture.

3. Generate daily, monthly and seasonal BGS capture trends to guide sampling strategy for push-pull pilot trial.
4. Identify challenges to BGS implementation in a “real-life” setting.

LITERATURE REVIEW

1. Dengue

Dengue belongs to the list of neglected tropical diseases that needs immediate attention and containment (Chan, 1994; Gubler, 1998). It is a deadly mosquito-transmitted viral infection manifested in a spectrum of illness from its asymptomatic state to classic dengue fever to the severe, and sometimes fatal, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DEN-3 and DEN-4. Infection with one serotype provides lifelong immunity specific to that type but only transient cross protection against other serotypes. Secondary infection with a different serotype may lead to immunity enhancement resulting to the severe form of the disease (Halstead, 1989).

It is distributed in more than 100 countries worldwide consisting of more than 40% of the world population (2.5 billion) while close to 50-100 million new infections and 24,000 deaths are reported annually worldwide (WHO, 2002; 2009). Nearly 500,000 people were reported to be hospitalized with 90% consisting of children. (WHO, 2009).Transmission occurs even in the most developed countries in the tropical and subtropical parts of the globe; i.e. Singapore (Koh *et al*., 2007), Australia (Ritchie *et al*., 2002; Hanna *et al*., 2006) and the USA (Reiter *et al*., 2001). Dengue virus infection is caused by four distinct serotypes, dengue (DEN)-1, DEN-2,

In the Southeast Asian region, a total of 193, 890 dengue cases were reported in 2006, 283,705 in 2007 and a total of 212,123 cases as of September 2008 (WHO, 2009). In Thailand, a mean number of 56,205 dengue cases with a mean case fatality rate (CFR) of 0.135 was reported from 2006 to 2007. As of September 2008, there were about 76,059 cases, 91 deaths and a CFR of 0.12 (WHO, 2009).

The re-emergence and spread of dengue fever virus and its vectors is a result of several factors to include unanticipated worldwide increase in population and rapid creation of urban centers, coupled with high population mobility through airplanes and less effective mosquito control programme (Kindhauser, 2003), inadequate and irregular water supply and poor solid waste management practices (WHO-CTD, 1995), resistance of vectors and pathogens, decreasing number of new insecticides and drugs and finally expanding of vector habitats because of global warming (Hales, 2002; Reiter, 2001; Gubler *et al.*, 2001).

2. Dengue Vector and Virus Transmission

Dengue is principally vectored by the day-biting eusynanthropic mosquito, *Aedes aegypti*, which mates, feeds, rests, and lays eggs within human habitations. *Aedes aegypti* feed almost exclusively on human blood (Edman *et al*., 1992; Van Handel *et al*., 1994) and frequently take multiple partial blood meals during each gonotrophic cycle (Gubler, 1988; MacDonald, 1956; Platt *et al*., 1997; Scott *et al*., 1993b; Yasuno and Tonn, 1970). The incidence of multiple blood meals correlates with the DEN-1 transmission season in rural Thailand (Scott *et al*., 1993a). This effectively increases the speed of virus spread, as virus may be transmitted each time mosquito releases saliva into a susceptible host during feeding (Putnam and Scott, 1995). Multiple blood meals translate into more frequent human–mosquito contact, increased virus transmission, increased fecundity, and enhanced survival (Day *et al*., 1994; Scott *et al*., 1993a, 1993b) (i.e., enhanced vectorial capacity). This behaviour may be critical for the maintenance of dengue viral transmission at low but detectable levels during inter-epidemic periods.

Upon feeding on infected blood, *Ae. aegypti* females undergo a series of reproductive cycles, giving the chance for the virus to enter the egg and be passed on to the next generations (Monath, 1994). Laboratory experiments have shown that at certain titer the virus (Dengue 3) can be passed on to 7 generations of offspring (Joshi et al, 2002). Horizontal transmission can also occur during mating where males transfer virus to non-infected females, contaminating the eggs and so maintenance of the virus goes on (Woodring, 1996).

3. Status of Dengue Control

Currently, neither vaccines nor chemotherapeutic drugs are available for the prevention or treatment of dengue virus. The only proven strategy for controlling dengue transmission is through the reduction of *Ae. aegypti* densities. Suppression and surveillance of *Ae. aegypti* can be achieved through combinations of the following measures: 1) elimination of larval breeding habitats located in and around households (source reduction); 2) treatment of habitats with larvicides or insect growth regulators that prevent immature molting and/or eclosion to adulthood and; 3) the use of ULV and space sprays in intra- and peridomestic environments. All of these strategies have shown success either as stand-alone or integrated strategies (WHO, 2009).

With emphasis on larval control, legislative actions have shown significantly reduced transmission of dengue in the countries of Cuba and Singapore while the introduction of *Mesocyclops* in breeding containers is preventing dengue transmission in parts of Vietnam (WHO, 2009, Gubler and Clark, 1996). Source reduction of vector breeding sites, however is one strategy that needs to be re-structured with new competencies that must be developed for the method to significantly contribute to dengue prevention and control (Focks, 2003; Parks and Lloyd, 2004).

Indoor resting locations are commonly unaffected by ULV space-sprays and oviposition has been seen to be only temporarily reduced during and just after the application over five consecutive days using Dibrom 14, an organophosphate applied aerially (Clark *et al.,*1989). The ground ULV campaign against *Ae. aegypti* adults in Paramaribo, Suriname showed no sustained effect when the chemical was applied after dark when the target mosquito is resting (Hudson, 1986). In 2010, a systematic review of peridomestic space-spraying was published. This review concluded that more research is needed to come to a practical public health conclusion, either to recommend or to reject the use of peridomestic space spraying for dengue vector control and to provide clear guidelines for appropriate implementation and monitoring of effect (Esu *et al.,* 2010). A more recent review by Bonds, (2012) suggested that where the chemical is applied correctly under the required conditions, ULV space-spraying can be effective at controlling mosquito populations. The same review highlighted where ULV space sprays can falter, as applications should be conducted at the time of flight or activity of vectors with appropriate meteorological conditions (Conlon, 2011).

More importantly, sociobehavioral studies have shown that knowledge of dengue does not influence the practice of dengue control, and that lifestyle affects availability of mosquito habitats (Hairi *et al*., 2003). With these magnitude of problem in dealing with conventional control, it is not surprising to note that reports still show an increase in transmission, manifested in number of dengue cases and epidemics indicating that the high-cost and long-term dengue vector control programs generally ensued limited successes at prevention of disease.

4. A Novel Approach to Dengue Control

In 1987, a group of investigators (Pyke, Rice, Sabine and Zaluki) from Australia first conceived the term ‘push-pull’ as a strategy for insect pest management (Pyke *et al*., 1987). This approach was generally intended to reduce reliance to insecticides against *Heliocoverpa* spp. in cotton (Khan and Pickett, 2004) and for control of the onion fly; *Delia antique* (Miller and Cowles, 1990). The strategy requires a clear scientific understanding of the pest’s biology and the behavioural/chemical ecology of the interactions with its hosts. In this strategy, the pests are repelled or deterred away from the main crop (push) by using stimuli that mask host apparency or are repellent or deterrent (Khan and Pickett, 2004; Cook *et al*., 2007; Midega *et al*., 2006). The pests are simultaneously attracted (pull), using highly apparent and attractive stimuli, to other areas such as traps or trap crops where they are concentrated, facilitating their control. Currently, the most successful example of the push-pull strategy is used by farmers in Africa for the control of stemborers on cereal crops (Khan and Picket, 2004). After successful control of crop pests, the concept has been tried against vectors of cattle trypanosomiasis (Vale *et al*. 1988; Brightwell *et al*. 1991;Torr *et al*. 1996; Birkett *et al*., 2004). Identification of a potent repellent blend from waterbuck, *Kobus defassa* (Gikonyo *et al*., 2002, 2003), which is refractory to tsetse, has been shown to provide much better protection for cattle and produce an effective push component in the push–pull approach for faster and more effective suppression of tsetse populations, particularly where cattle are the dominant source of a blood meal for the flies. A preliminary experiment undertaken on the Kenyan coast, comparing the effects of protecting cattle with a synthetic repellent (push), baited traps (pull) and a combination of these two (push–pull), suggests a better performance of the push–pull approach in suppressing tsetse flies (Hassanali *et al*., 2012).

Similarly, insecticide resistance in human disease vectors is also a major problem as it is in agriculture. Reports have indicated the presence of insecticide resistance in *Ae. aegypti* populations from Thailand (Somboon *et al*., 2003; Ponlawat *et al*., 2005; Sathatriphop *et al*., 2006; Jirakanjanakit *et al*., 2007). As stated previously, adult control of *Ae. aegypti* utilizes indoor residual or space-spray techniques, such as thermal fogging and ultra-low volume (ULV) spraying (WHO, 2007; Gratz, 1991, 1999) for immediate kill effects to lower populations after declaration of an epidemic. Since the effective public health insecticide arsenal is continuously depleting due to resistance, the novel push-pull approach to dengue vector control could help in insecticide resistance management as it aims to use doses and coverage levels of chemicals that exploit spatial repellent (SR) and contact irritant (CI) actions with *minimal toxicity* to reduce insecticide resistance selection pressure as opposed to a direct chemical kill (Grieco *et al*., 2007; WHO, 2009; Achee *et al*., 2009). When applied at strategic positions in houses, spatial repellents will prevent entry of mosquitoes from homes while contact irritant chemicals will hasten exit of mosquitoes resulting to reduced human-vector contact and potentially reduced dengue transmission.

To ensure that those mosquitoes driven away from a treated home will not be diverted to non-protected households, a mechanical trap (pull) could be used to remove chemically repelled/irritated vectors from the peridomestic environment (Figure 1). The synergistic action of the PPS should lead to significant reduction in contact with human hosts resulting in lowered biting probabilities and therefore decrease in virus transmission. The use of animals to divert (pull) mosquitoes from feeding on and transmitting disease to human beings (zooprophylaxis) has been considered as a possible tool in reducing mosquito numbers and levels of malaria (WHO 1982). In this way, a push–pull approach may also find a useful application in controlling malaria vectors, particularly zoophilic species like *Anopheles arabiensis*.

Figure 1. Push-pull strategy (PPS) conceptual framework.

5. Use of Spatial Repellents as PPS Push Component

The use of topical repellents as personal protection measures has been advocated for several years. They are used extensively by military personnel (fighting troops) as protection against malaria and other tropical diseases (Kitchen *et al.,* 2008). The use of topical repellents, however, in communities where there is local transmission of mosquito-borne diseases is limited due to their typical high costs, the need for subsequent application according to its residual efficacy and unwanted side effects of some formulation (i.e., rashes). Topical repellents typically prevent biting only where the substance is applied or very close to the point of application. The concept of personal protection using a repellent has now evolved to a more encompassing approach of protecting a space rather than specific persons through the use of "spatial" repellents. Spatial repellents are chemicals that evaporate into the air affecting biting insects at a distance rather than by contact, inhibiting their ability to locate and track a host (Nolen *et a1*. 2002; Dogan and Rossignol, 1999). The vapor plume formed by the source of a spatial repellent creates an effective protective barrier so that not only direct users are protected but people within a certain radius of application who do not necessarily use them (Schreck *et al*., 1970). Household protection rather than personal protection is envisioned, thereby resulting to increase in the number of people protected. Moreover, continuous day and night protection can be provided through formulation that would allow continuous evaporation offering longer impact than the use of bed nets (for malaria) that work only at night while people are in bed. Such spatial repellents, when strategically placed (i.e. portals of entry), can effectively create a barrier of protection preventing entry of mosquitoes to households while keeping them open for normal ventilation, an advantage in tropical climates. Continuous use of spatial repellents is expected to result in prolonged searching for both host and shelter of vectors leading to increased vector mortality and thereby reduced pathogen transmission.

The overall goal of the PPS is to reduce indoor densities of *Ae. aegypti* through focal treatment of portals of entry (SR) or indoor resting sites (CI) using minimal chemical dose. The current control of adult *Ae. aegypti*, is focused on the toxic actions of chemical compounds. The larger part of this research program (push component) is focused on evaluating sublethal chemical approaches. The push component reports on the behavioral responses of female *Ae. aegypti* in response to SR/CI compounds. This is based on the premise that chemical actions that elicit contact irritancy (CI), and spatial repellency (SR) can cause vector movement away from a treatment source (i.e., a house) and also serve to reduce man-vector contact thereby potentially reducing pathogen transmission. Three target SR compounds used in the current study are reviewed briefly below.

DDT. DDT [1, 1, 1-trichloro-2,2-bis (4-chlorophenyl) ethane] belongs to the organochlorine group of synthetic insecticides known for its environmental persistence (Hodgson et al. 1998). DDT is classified as moderately hazardous (WHO, 2005) with broad-spectrum activity with low acute mammalian toxicity (Agency for Toxic Substances and Diseases Registry [ATSDR), 2002). DDT acts as neurotoxicant, specifically on nerve axon membranes that cause continued excitability leading to paralysis and eventually death. This action is a result of the increase sodium movement across nerve cell membranes by a direct interaction with sodium channel protein (Corbett *et al*., 1984). It has been used to control agricultural pests (crop, livestock and household pests) and vectors of human diseases such as malaria, typhus and other insect-borne illnesses (Mellanby, 1992; ATSDR, 2002). Through experimental hut evaluations of malaria vectors, DDT has been shown to prevent entry into the experimental huts by 95% (*Anopheles darlingi*) to 97% (*An. vestitipennis)* with behavioral actions documented for greater that 12 months (Roberts and Alecrim, 1991; Grieco *et al*., 2000).

Metofluthrin. Metofluthrin (2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (*E, Z*)(1*R*, 3*R*)-2,2-dimethyl-3-(prop-1-enyl) cyclopropanecarboxylate) (S-1264) is a fairly recent synthesized compound produced by Sumitomo Chemical Co. Ltd. ,Osaka, Japan (Ujihara *et al*., 2004, Shono *et al*., 2004). Metofluthrin has characteristically high vapor pressure (1.87 X 10^{-3} Pa at 25^{o}C), greater than 2 times that of *d*-allethrin and 100-times that of permethrin (Kawada *et al*., 2005). It vaporizes at normal temperature without heating, whereas the other conventional pyrethroids (permethrin etc.) require heating for evaporation. Metofluthrin has already been registered in several Asian countries such as Singapore, Indonesia, Myanmar and Vietnam. (Shono *et al*., 2004, Sugano *et al*., 2004). This compound has been the focus of several evaluations for toxicity and repellency. In a residential area in Hai Phong city, Vietnam, the use of metofluthrin-impregnated polyethylene plastic strips (one room irregardless of size was treated with one strip) against *Ae. aegypti* and *Culex quinquefasciatus* showed a rapid decrease in both mosquito populations immediately after the treatment and the treatment was effective for at least 6 weeks. (Kawada *et al*., 2004). Another field trial done in Lombok, Indonesia in shelters without walls (beruga) at rate of two metofluthrin strips/beruga showed a 60% reduction in human biting by *Culex quinquefasciatus* for at least 11 weeks (Kawada *et al*., 2005). Metofluthrin coil formulation has been documented to significantly reduce landing counts of *Ae. aegypti* while it is burning (Rapley *et al*., 2009). Metofluthrin impregnated paper strips also reduce human landing indoors and outdoors of *Anopheles balabacensis* and *Culex quinquefasciatus* (Kawada *et al*., 2004) while metofluthrin vaporizer at 0.6% also has effectively repelled *Armigeres subalbatus*, with a mean biting protection level of 71.8 and 73.5% on the legs and arms, respectively (Lee, 2007). The use of this technique was found to be a practical long-term solution for the prevention of mosquito bites without using electricity or heat to evaporate the metofluthrin.

Transfluthrin. Transfluthrin [cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-(2,3,5,6-tetrafluorophenyl)methyl ester, (1R,3S)] is a fast acting insecticide and exhibits high volatility (9.4 x 10^{-4} Pa at 20 ^{o}C) and knockdown activity (WHO, 2002). It is used in household and hygiene products, mainly against flying insects, such as mosquitoes and flies, but also against material pests, such as moths. In Dar es Salaam, Tanzania, the effectiveness of a cheap and easy method of household protection using kerosene-burning lamps (korobois) to vaporize transfluthrin against *Culex quinquefasciatus* Say and other mosquitoes (*Anopheles* and *Culex*) was investigated (Pates *et al*., 2002). The concentration of 0.5% transfluthrin in vegetable oil gave >90% reduction in biting protection compared to 50-75% reduction obtained from burning a mosquito coil containing a synthetic pyrethroid (0.25% d-allethrin). The use of this modified lamp may offer a more cost-effective alternative to the use of mosquito coil as means of personal protection, and a useful complement to the use of mosquito nets for the early part of the evening before bedtime. At the Ifakara Health Institute, Tanzania, the protective efficacy of a 4.0 x 0.3m strip of hessian sacks (natural fiber absorbent substrate) treated with 1% transfluthrin was evaluated in a 60m x 2m x 2.5m netting tunnel with laboratory grown *Anopheles arabiensis* (Ogoma *et al*., 2012). Results showed that freshly treated hessian strip reduced mosquito attack rate on human volunteers by >99% and consistently conferred >90% protective efficacy for a period of 6 months. Over the entire study period, only 22 out of 1400 released mosquitoes bit volunteers using the treated sacking strip while 894 out of 1400 mosquitoes released into cages containing volunteers using an untreated strip fed upon them. In residential houses in an urban squatter environment in Penang, Malaysia, the field performance of the three formulations of mosquito coils containing transfluthrin (0.018, 0.027 and 0.046% w/w) was compared with that of another formulation (d-allethrin 0.18% w/w) (Yap *et al*., 1996). Results showed that all the three formulations provided protection against *Culex quinquefasciatus* with >90% reduction in mosquito landing/biting activity. The use of transfluthrin (200 mg) impregnated paper strips showed reductions from 44 to 86% as recorded from CDC-back pack collections of *Ae. albopictus* for about four weeks (Argueta *et al*., 2004). In field tests, 0.6% transfluthrin effectively repelled *Armigeres subalbatus* and *Ae. albopictus*, with mean biting protection of 85.4 and 89.3% on exposed legs and arms, respectively, of the human volunteers (Lee, 2007).

6. Mosquito Behavior Towards Insecticides

Two types of responses of mosquitoes to chemical insecticides exist: physiological (toxicity/resistance) and behavioral . Combined, these responses can result in the insect being killed (toxic effect) upon contact, surviving exposure due to the development of insecticide resistance or simply evading exposure (behavioral avoidance). The impact of public health insecticides on vector populations is much more complex than toxicity alone. The potential effects of insecticides to modify normal behavioral responses of mosquitoes are critical in understanding the control of vector-borne diseases. Beyond the traditional emphasis on toxicity, understanding further chemical actions and the behavioral responses of vectors to insecticides will open new avenues and opportunities towards the development and use of many compounds and control strategies (novel or established) that could reduce vector-host interaction in public health (Achee *et al*., 2009). These sublethal chemical actions can prevent man-vector contact primarily by disruption of 'normal' patterns of host-seeking and blood feeding behavior (Muirhead-Thomson, 1951; Cullen and De Zulueta, 1962; Hamon *et al*. 1970; Elliott, 1972; Gillies, 1988; Chareonviriyaphap *et al*., 1997; Grieco *et al*., 2000, 2007). Two such actions are contact irritancy (CI) and spatial repellency (SR). Contact irritant response is defined as the oriented movement of vectors away from a chemical after tarsal contact (Dethier *et al*., 1960) and spatial repellent response as the oriented movement of vectors away from a chemical without making tarsal contact with chemical residue (Roberts *et al*., 2000). Indeed, past evidence suggests that some of the most effective insecticides ever used for vector (malaria) control as well as those that are currently being used, function mainly as repellents and only limitedly as toxic agents killing mosquitoes. DDT, perhaps the best-known example functions primarily as a spatial repellent, secondarily as a contact irritant and lastly as a toxicant (Taverne, 1999, Grieco *et al*., 2007). No other compound tested to date has exerted the same combination of actions or has been as effective as DDT to control malaria transmission and it is still the gold standard for the assays of these behavioral effects (Roberts *et al*., 2000, Achee *et al*., 2009). The behavioral impact of sub-lethal chemical residues that deter indoor vector feeding activities explains the continued effectiveness of some spraying programs, despite the presence of strong physiological resistance in the local *Anopheles* populations.

Thus spatial repellents can protect not only individuals but actually the entire households and perhaps neighbors too. These datasets, which have been generated from several studies worldwide suggest that compounds with spatial repellence effects do have a real chance of not only preventing direct contact between humans and disease transmitting mosquitoes, but they also can starve out mosquitoes by keeping them out of reach of hosts for longer time thereby being more likely to die from other environmental conditions such as predation, high or low outdoor temps, heat as well as mere lack of food for extended periods of time.

A number of assays allowed preliminary screening of the non-toxic chemical responses of the mosquitoes to insecticides. These assays include designs for identifying attraction/attraction inhibition (Kline *et al*., 2003, Bernier *et al*., 2005), contact irritancy (WHO 1970, Rutledge *et al*., 1999, Chareonviriyaphap *et al*., 2004), noncontact irritancy or excito-repellency (Roberts *et al*., 1997, Chareonviriyaphap *et al*., 2002), and anti-biting (Klun and Dubboun, 2000) responses of mosquito vectors under laboratory conditions. The latest addition to the list allows determination of three chemical actions, namely; contact irritancy, spatial repellency, and toxicity through the use of high-throughput screening system (HITSS). The same technique facilitates assay of large libraries of chemicals with the objective of identifying compounds that modify vector behavior, specifically those that could be implemented in insecticide residual spray (IRS) and insecticide treated net intervention strategies (Grieco *et al*., 2007).

7. BG-Sentinel ™ (BGS) Trap

Continuous research on trap improvement should give way to greater collecting efficacy to allow integration of such tools as part of a control intervention. The development of new generation traps such as the BG-Sentinel™ (BGS) and Zumba™ traps, are two such examples for use against dengue vectors; *Ae. aegypti* (Krockel *et al*., 2006; Maciel-de-Freitas *et al*., 2006; Williams *et al*., 2006, 2007; Ball and Ritchie, 2010a, b; Bhalala and Arias, 2009) and *Ae. albopictus* (Ritchie *et al*., 2006; Farajollahi *et al*., 2009). The BGS has shown greater efficacy in collecting host-seeking *Ae. aegypti* adults compared to other collecting devices and traps such as the CDC backpack aspirator, the Fay-Prince trap, the Encephalitis Virus Surveillance trap and the commercially available Mosquito Magnet Liberty™ trap (Maciel-de-Freitas *et al*. 2006, Krockel *et al*. 2006, Williams *et al*. 2006). The performance of the BGS is explained by its comprehensive design combining the most important elements of *Ae. aegypti* host-seeking behavior by combining an olfactory cue (BG

Lure) with visual cues (black and white contrast) and convection currents mimicking respiring humans to attract the mosquito (Krockel *et al*., 2006).

Use of the BGS trap has been reported to sample *Ae eagypti* and other vectors belonging to subgenus *Stegomyia* including *Ae albopictus* (Ritchie *et al*., 2006, Meeraus *et al*., 2008) and *Ae polysiensis* (Schmaedick *et al*., 2008). Both male and female *Ae eagypti* across many physiological stages has been collected using this trap (Maciel-de-Freitas *et al*. 2006, Williams *et al*. 2006). A mark-release and recapture study done in Rio de Janeiro, Brazil showed that the BGS collected more *Ae. aegypti* compared with CDC back packnd concluded that it is an efficient tool for monitoring adult populations (Maciel-de-Freitas *et al*. 2006) and a good replacement for human landing collections (Krockel *et al*., 2006).

One potential dengue control advantage for the BGS could be development of an alternative *Ae. aegypti* abundance index to the traditional labor-intensive indices (immature, human landing collections and CDC backpack-aspiration) for assessing disease risk and the success of vector control programs. This however needs further understanding of the relationship between collections of adult mosquito vectors and the amount of disease transmission (Focks, 2003). It now remains for researchers to determine the relationship between BGS collections and dengue infection risk so that epidemiologically relevant *Ae. Aegypti* indices can be developed. The first step towards such goal is to interpret BGS collections in terms of the available vector population the field. Most recent work through screen house releases has shown that despite a significant bias detected in the BGS, whereby teneral nulliparous females were captured at a lower rate than all the other physiological groups (1-2 d teneral female, 8-9 days gravids, 15-16d parous females, 15-16d blood fed parous females and 3-4d males), the trap successfully captures all females and males despite their physiological status, body size and age (Ball, 2010). This makes BGS an effective and preferred tool for monitoring *Ae. aegypti* populations in Far North Queensland, Australia. Apart from that, the BGS has been evaluated as a tool to monitor RIDL^{TM} (release of Insects carrying a Dominant Lethal) males (Lacroix et al. 2009) and *w Mel*

(*Wohlbachia* infected) *Ae. aegypti* strains (Ritchie et al. 2011), two novel approaches currently being evaluated to control *Ae. aegypti* adult populations.

8. Mechanism of *Aedes aegypti* Host-seeking Behavior

Host-seeking mosquitoes use an array of combination of cues from visual, olfactory, gustatory to physical stimuli for host identification and location (Cork, 1996; Constantini, 1996). Vision plays a principal role in adult host seeking behavior. Adult mosquitoes possess both compound eyes and two ocelli that are used for detecting visual stimuli. Compound eyes are used primarily for navigation and sensing movement, patterns, contrast, and color while ocelli are believed to sense light levels (Allan *et al*., 1987). *Aedes aegypti,* for instance, has higher total eye sensitivity compared to other insects, which enables the species to function in low-light conditions (Muir *et al*., 1992). It has been reported that *Ae. aegypti* is most sensisitive at an effective range of 30-45% reflectance but can still discriminate objects from 30 and 34% reflectance (Muir *et al*., 1992). The species is active at a minimum of 1 lx/ft^2 amount of light as opposed to *Ae. albopictus* that needs >10 $lx.ft^2$ (Kawada *et al*., 2004). Using natural light from an open window, Brett (1938) determined color preference in *Ae. aegypti* by counting the number of *Ae. aegypti* landing on cloths stretched over a hand-enclosing box in a three-minute period. Each trial used either black or white as a standard and presented an equal area of the test color and the standard. Although the order of attractiveness for different colors was not the same when compared to black vs. white, the general order which emerged was black (most attractive); red (very attractive); grey and blue (neutral), khaki, green, light khaki, and yellow (less attractive).

Evidence has shown that host-seeking in mosquitoes is mediated by semiochemicals emanating from the host (Hallem *et al*., 2004; Takken, 1991; Takken and Knols,1999). Olfactory cues are detected through an intricate pathway, beginning with sensilla located on the antennae which detect odor, and palpi which detect carbon dioxide. Age and the physiological state of the mosquito determine whether the detection of olfactory cues results in a behavioral response (Takken, 1996). Blood feeding in mosquitoes has been shown to be initiated between 24 and 72 hours after a female emerges, this is the range of time required for receptors to mature and be responsive. Davis (1984) has shown that *Ae. aegypti* do not exhibit host-seeking behavior before 18-24 hours post emergence. However at 30 hours about 10% of the females tested began to exhibit host-seeking behavior. The 50% response level was reached at about 66 hours (nearly 3 days) post emergence and by 102 hours post-emergence 90% of the females were actively seeking a host. Females between 30 and 102 hours post-emergence are in a transitional condition during which their host-seeking behavior is clearly age dependent. The host-seeking behavior of virgin females of ages greater than 108 hours (4.5 days) post-emergence showed a consistent response rate of 94% for as long as 15 days post-emergence (Davis, 1984).

Laboratory olfactometers have been used to show attractiveness of compounds to mosquitoes (Clements, 1999). Almost all mosquito species use carbon dioxide, a major component in breath and on human skin as an alerting and attractive signal. Lactic acid, a component of human sweat, acts as an essential attractive synergist when combined with carbon dioxide as well as with other volatiles from the skin (Acree, 1968; Smith *et al.,* 1970; Eiras and Jepson, 1991; Geier *et al.,* 1996 (Constantini *et al*., 1996; Gillies, 1980; Khan, 1966). Samples of human sweat have been bio-assayed by many workers with varying results. Lactic acid was confirmed to be a major attractant for *Ae. aegypti* (Smith *et al*., 1970) and further studies showed that attractive effects of certain compounds are manifested only when they are in combination with lactic acid (Geier *et al*., 1999). Ammonia in combination with lactic acid have been shown to increase attractiveness to *Ae. aegypti* and in a range of haematophagous arthropods (Taneja and Guerin, 1997; Hribar *et al*., 1992). The same effect was reported with the combination of lactic acid and caproic acid (Williams *et al*., 2006). Physical stimuli like convection currents produced by the human hand in combination with host odours increase significantly attraction to *Ae. aegypti* (Eiras and Jepson, 1991). Combined, the above facts explain why the BGS trap has been proven effective in trapping host seeking *Ae aegypti*. Both visual properties, olfactory cues as well as convection currents are utilized within the BGS trap design

(Krockel *et al*., 2006). Visual cues of the BGS are effective over larger distances while odour cues (BG lure) consisting of lactic acid, ammonia and caproic acid function to attract mosquitoes when they are near. Convection currents generated when BGS trap is in operation mimic respiring humans causing additional attraction.

9. Estimating Adult Female *Aedes aegypti* Densities

There is a large body of literature on estimating *Ae. aegypti* population densities. These estimations are used to predict the risk of dengue virus transmission and therefore are important in guiding implementation of control strategies. However, most of the reports quantify vector populations in terms of immature stages: i.e. larvae (House Index, Container Index and Breteau Index), and pupae (Pupal Index). Very limited publications cite density in terms of adult *Ae. aegypti* females. Those publications that report adult density have used different units of expressing the magnitude of the population, from absolute numbers collected (human landing catches, back pack aspirator, adult traps), to adult female per person per hectare to derive population densities using different estimation models (Lincoln Index, Jolly, Fisher and Ford, Jackson and Baileys) (Trpis and Hauserman, 1986; Jolly-Seber, 1965, 1982; Bailey, 1952, 1952; Fisher and Ford, 1947; Jackson, 1933). To draw basis from these available data for the current study, the values expressed in the publications have been converted to *Ae. aegypti* female per area (m^2) (Table 1). The derived population numbers using a 40 m^2 assumed area (4m x 10 m) of a house approximates a similar area of the experimental huts and screen house used in the current study. These derivations have indicated that numbers of *Ae. aegypti* found inside households are generally low, approximating <2 females /m^2. This density value formed a benchmark standard throughout the study.

Table 1 Review of reported population densities of *Aedes aegypti* and derived densities expressed per square meter (m^2).

Estimated Density/ Unit Area	**Method Used To Estimate or Determine Density**	**Place of Study**	**References**
Data: There are 3,505 gravid females collected from *MosquiTrap* and 4,828 from backpack aspirator. Olaria had an estimated population of 62,509 inhabitants in an area of around 369 ha, with a density of 169.6 inhabitants/ha. ***Therefore, there were about 10 females /ha (Backpack) or (0.001 /m²) from MosquiTrap and 13 females/ha or (0.0013/m²) from backpack aspirators.***	Mark-release-recapture experiments 30 Households were sampled for backpack aspirators and 1 *MosquiTrap* was set per 104 participating household	Olaria (22º50'45" South; 43º15'39" West), Rio de Janeiro, Brazil	de-Freitas *et al*., 2008
Data: 1.1 to 43.3 adult female/ house ***Assuming that the average house area is about 40m² then, density per area could be (0.023 females/m²) to 1.08 females/m²). These did not include surrounding peridomestic area in the computation***	Nine quantitative entomologic surveys over 14 months of approximately 100 houses (randomly selected from the list of 611 houses each survey.	Tri Nguyen village (611 households) on Hon Mieu Island in Central Vietnam Village size: 0.2 km^2 (22 ha) in size.	Jeffery *et al*., 2009

Table 1 (Continued)

Estimated Density/ Unit Area	**Method Used To Estimate or Determine Density**	**Place of Study**	**References**
Data: The estimate of the mean daily population over the year, corrected for movement, was 1120, whilst that using the Jolly model was 1093. The area of the place was 5264m^2 (94 x 56 m) ***Therefore the computed number of females are 0.20/m^2 and and 0.21/m^2 for Fisher and Ford (1947) and of Jolly (1965) methods of population estimation.***	Mark-release-recapture techniques	Residential compound of a Buddhist temple, Wat Samphaya, in Bangkok	Sheppard *et al.*, 1969
Data: Numbers of adult *Ae. aegypti* females indoors before and after intervention were (3.6 vs. 6.8/house 3 months post-intervention) ***Assume 40 m^2 house area, the densities would be 0.09 females/m^2 and 0.17 females/m^2 before and after the intervention***	Adult females /house collected by aspirator indoors	Two Brazilian municipalities, Areia Branca in the State of Rio de Janeiro, Brazil	Perich *et al.*, 2003

MATERIALS AND METHODS

1. Phase I-Screen House Trials

1.1 Study area and experimental site.

Pu Teuy (14^{o}17'N, 99^o 11'E) is a small agricultural village of <1500 inhabitants, under Sai Yok district, Kanchanaburi province, some 150 km northwest of Bangkok (Figure 2). There was no reported dengue cases (Thongphaphum District Clinic 2010) in the area although high densities of *Aedes aegypti* are present, creating an environment prone for dengue transmission. The village is situated in a mountainous area (420 m above sea level) completely surrounded by dense primary forest, orchard and vegetable plantations. The experimental site is located within a radius of at least 800 m from the closest indigenous home, creating a sufficient buffer for mark-release-recapture mosquito behavioral studies (Reiter *et al*. 1995; Muir and Kay, 1998, Harrington *et al*., 2005). Weekly surveillance of immature *Ae aegypti* populations in the village are undertaken by the Thongpaphum District Clinic. Vector control interventions include distribution of organophosphate larvicide (temephos).

1.2 Study design.

A mark-release-recapture experiment in a completely randomized design (CRD) using varied numbers of BGS traps (1-4) and released *Ae. aegypti* female adults (10, 25, 50, 100, 150, 200, and 250) was conducted in a screen house located in Pu Teuy Village, Sai Yok District, Kanchanaburi Province, Thailand (14^{o}20′ N 98 ^{o}59′ E). The study was conducted from August 2009 to March 2010.

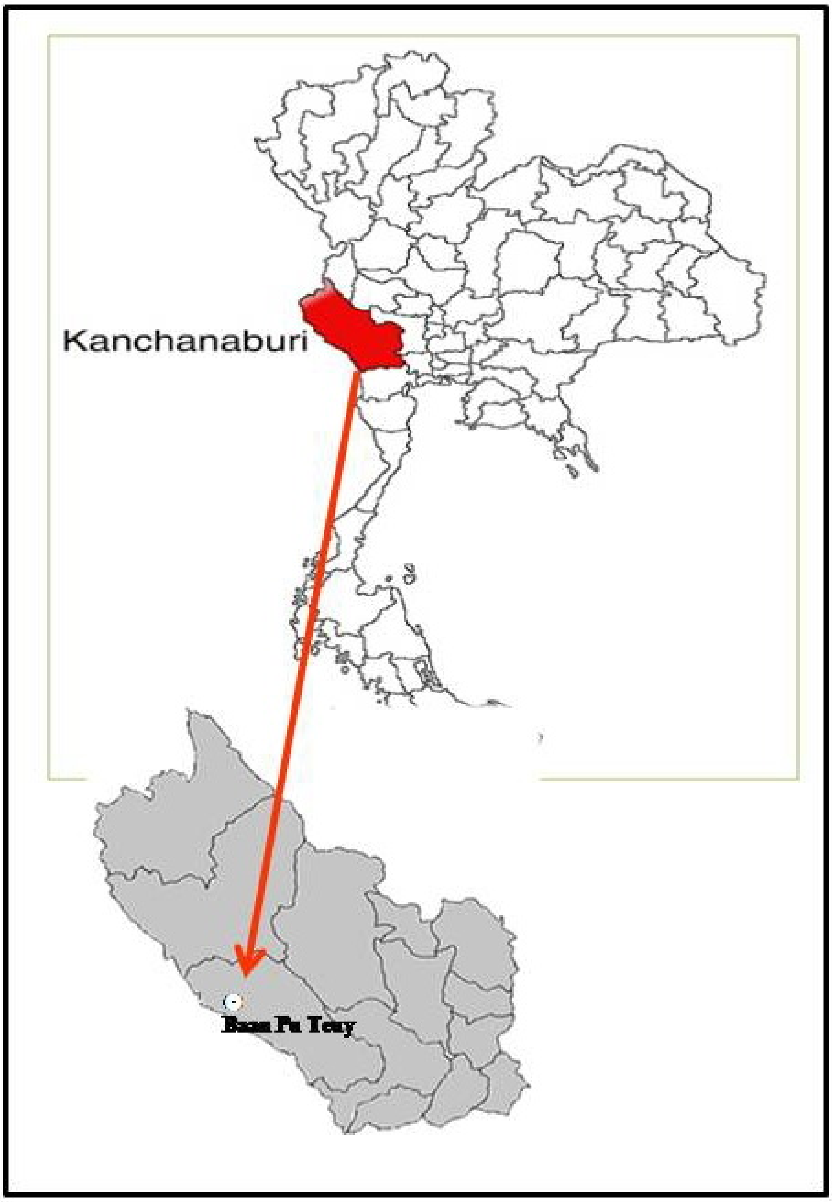

Figure 2 Study site in Baan Pu Teuy, Kanchanaburi, Thailand

1.3 Screen house

A screen house facility, measuring 4 m (width) x 3.5 m (height) x 40 m (length), was built to create a semi-field condition and facilitate the recapture of released *Ae. aegypti* (Figure 2). The screen house is subdivided into four 10 m long cubicles, each separated by folding metal screen partitions. The space volume per cubicle section is 140 m^3 (4 x 10 x 3.5 m). This is similar to the area of the experimental huts used in push-pull trials (Chareonviriyaphap *et al*., 2010) and the expected space volume that *Ae. aegypti* primarily would use in and around a typical home as observed from smaller dengue endemic villages (mean dispersal 28-93 m) in Thailand (Harrington *et al*., 2005). This area also took into consideration reports that *Ae. aegypti* responds directly to visual cues at a 10 m distance (Clements, 1999). The screen house floors are lined with white plastic sheeting to facilitate observation and recovery of knocked down mosquitoes. The screen house and BGS traps were cleaned regularly to remove predators that otherwise may consume knocked down or trapped mosquitoes. Environmental parameters (temperature, relative humidity and intensity) were measured for each cubicle section using HOBO Data Loggers computer Corporation, MA, 1997-2003). All the HOBO Data Loggers were calibrated prior to start of all experiments. A baseline study comparing the environmental variables (temperature, relative humidity and light intensity) in each cubicle was conducted.

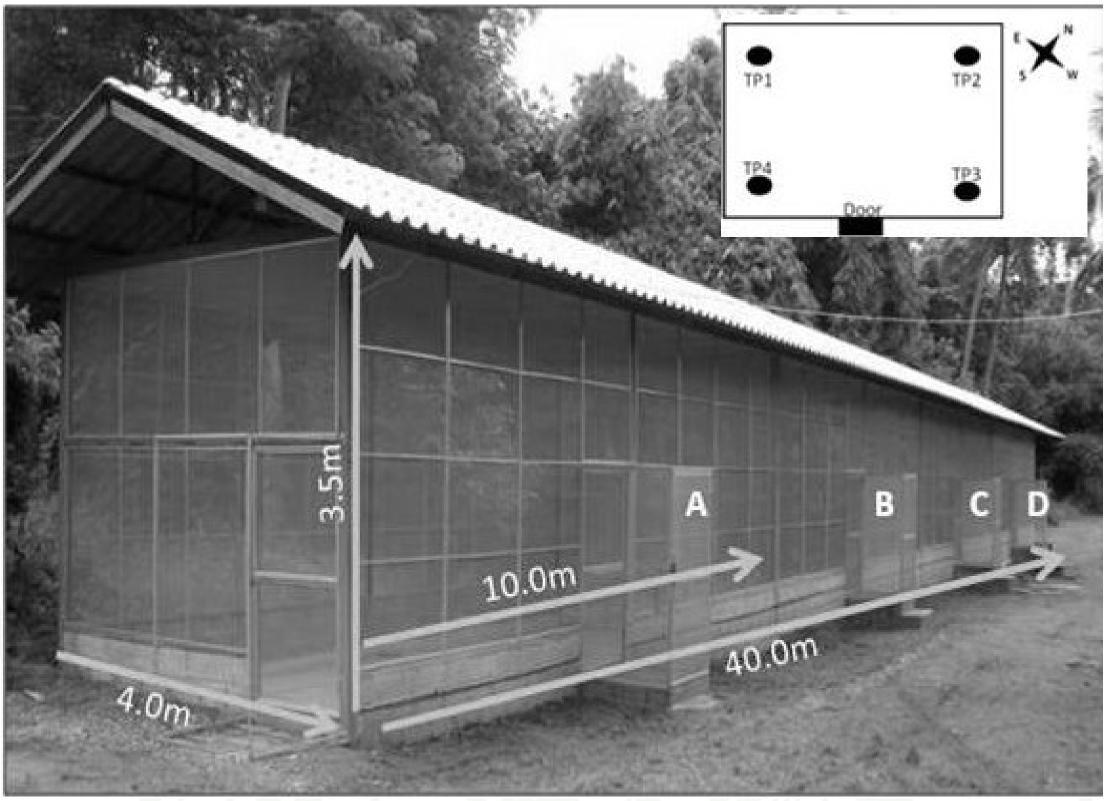

Figure 3 The screen house facility at Pu Teuy, Kanchanaburi, Thailand. All sides are screened and the cement floor is covered with white plastic to detect knocked down mosquitoes. Collapsible walls are used to partition the screen house into four separate 10m long cubicles (A-D). Top insert: designated BGS trap position within a single cubicle.

1.4 BG-Sentinel^{TM} (BGS) traps

BGS traps were baited with the BG-Lure (Biogents AG, Regensburg, Germany). The trap consists of a collapsible container made of white plastic sack material (Figure 3). The top of the container is covered with white gauze cloth surrounding a black plastic funnel. This funnel is connected to a mesh catch bag that collects trapped mosquitoes. A 12 volts suction fan below the base of the funnel creates downward suction after connection to an external power source (i.e., battery or electricity). The air is then pushed upwards passing through the gauze cover creating convection currents (Krockel *et al*., 2006). The contrasting black and white colors of the trap provide visual attraction.

The accompanying lure (BG-Lure) consists of lactic acid, ammonia and caproic acid, compounds that are found in human sweat (Geier *et al*., 2009, Bosch *et al*., 2000, Steib *et al*., 2001). When the trap fan is operating, the air current carries the lure volatiles out through the gauze cloth cover into the surrounding environment. The BG-Lures were used within 4 months after opening. The company claims that BG lure could lasts from 4-5 months under field conditions.

Figure 4 The BG- Sentinel (BGS)^{TM} trap, external view (A), inside view showing fan and lure pouch (B), the black funnel where mosquitoes enter with the catch bag (C) and the accompaying BG-Lure (D).

1.5 BGS Trap placement

Traps were placed in designated positions (i.e., position 1-4 depending on trap number being evaluated) in the four corners of each of the 140 m^3 cubicles on the floor (Figure 2). Specifically, traps were placed at the 1 m intersection from the adjacent corner walls per the manufacturer's suggestion to place the trap at least 1 meter away from walls. The insert in Figure 1 shows relative positions of BGS traps in each cubicle of the screen house. Upon entry into a specific cubicle, the first corner to the left was designated Trap Position 4 (TP4), traveling clockwise, the other corners were designated Trap Position 1 (TP1), Trap Position 2 (TP2) and Trap Position 3 (TP3). When only one trap was evaluated, the TP1 position was used. During the evaluation of two traps, both TP1 and TP2 were used while TP1, TP2 and TP3 were used to evaluate three traps and all four designated positions were used when evaluating four traps. Potential positional bias between all four traps (TP1-TP4) within individual cubicles and among cubicles was evaluated in separate a trial in which 1 trap was rotated through all four trap positions over the course of 16 days (4 replicates per trap position).

1.6 Mosquitoes

Ae. aegypti mosquitoes were reared at the Pu Teuy field insectary, Sai Yok District, Kanchanaburi Province following previously described standard methodology (Kongmee et al. 2004). Adults were from the F_2-F_5 generation and all originated from immatures collected monthly in Pu Teuy village. Test populations were non-blood fed, 3-5 d old nulliparous females. Mosquito Release Numbers (RN) were grouped into three categories: Low (10, 25, 50), medium (100, 150) and high (200, 250). The Low RNs were based on reported *Ae. aegypti* numbers from homes in dengue-endemic areas (Jeffery *et al*., 2009, Sheppard *et al*., 1969, Perich *et al*., 2003, Maciel de-Freitas et al. 2008) and represent the most common densities of *Aedes aegypti* to occur in real-life situations. Medium and high RNs were used to assess the

Table 2 *Aedes aegypti* releases numbers (RN) per screen house cubicle (A=40 m^2; V=140 m^3).

Aedes aegypti Release Numbers	Density per m^2	25% of Release Numbers[1]	Numbers of *Ae aegypti* females used for control cups[2]
10	0.25	2.50	10
25	0.625	6.25	15
50	1.250	12.50	25
100	2.50	25.00	25
150	3.75	37.50	40
200	5.00	50.00	50
250	6.25	63.50	65

[1]Basis for determining number of mosquitoes used in the control cups

[2]Actual numbers used for control cups

feasibility of BGS applications if high *Ae. aegypti* populations were encountered in the field. Table 2 lists mosquito test densities evaluated and number of control mosquitoes used in each set-up. Mosquitoes in control cups were used to monitor for the mortality while the trial is being conducted. Mortality of >20% requires repeat of the experimental set-up.

Mosquitoes were marked with fluorescent dust (BioQuip Products, Rancho Dominquez, CA) 12 h prior to release, following the method of Achee *et al*. (2005), to facilitate detection of knocked down individuals and distinguish them from wild mosquitoes that may have entered the screen house during trials. Marked specimens were sugar-starved approximately 24 hrs prior to testing to encourage and elicit host-seeking behavior but provided water-soaked cotton pads until time of release at 0530h. A new release population was used for each treatment replicate.

An assessment of the dusting application method was performed to validate 100% coverage of mosquitoes and confirm that no negative effect of the marking procedure was seen for BGS recapture rates.

1.6 Recapture monitoring

Individual mosquito test populations were released inside screen house cubicles on Day 1 at 0530 h. Initial baseline trials were conducted using 1 trap and 100 *Ae. aegypti* RN to determine the length of the monitoring period needed to observe maximum cumulative recapture. This time period was considered the "Impact Period" or time of peak recapture and was used to guide future sampling in the study. Based on these initial trials, monitoring of trap recapture in subsequent experiments was conducted at 0930, 1330, and 1730 hours on Day 1 and at 0530 at 0930, 1330, and 1730 hours on Day 2. At each sampling interval, the BGS collection bags were removed and replaced with a clean bag. In addition, the knock down response, defined as the insect lying on its side and not being able to right itself after gently prodding (Grieco *et al*., 2007), were recorded by systematic observation of the flooring of each cubicle. Collection bags were immediately placed at -20ºC to kill captured mosquitoes. Recaptured mosquitoes were recorded according to marking color, time of collection, trap number and the cubicle they were collected from. Four replicates were performed for each treatment trial.

1.8 Knock down quantification

Each of the screen house cubicles was divided into four equal parts by drawing longitudinal lines at 1 m distance from the side walls (Figure 4). Each 1 m swath was traversed upon entry into the cubicles before collection of the BGS catch bags. Knock down was recorded per collection interval in designated cubicles.

1.9 Preventing and minimizing predation

After each experimental trial, or approximately every week, the ceilings, walls and floors of the screenhouse were cleaned and checked for the presence of possible predators; i.e. spiders, etc. This decreased the probability of predation on knocked down and flying mosquitoes inside the screen house during subsequent trials.

1.10 Ethical considerations

Mark-release-recapture experiment protocols were submitted to and approved by the Kasetsart Ethical Review Board. As a counterpart, we committed ourselves to suspend mosquito release if any dengue cases were registered in the study area before or even during the experiment.

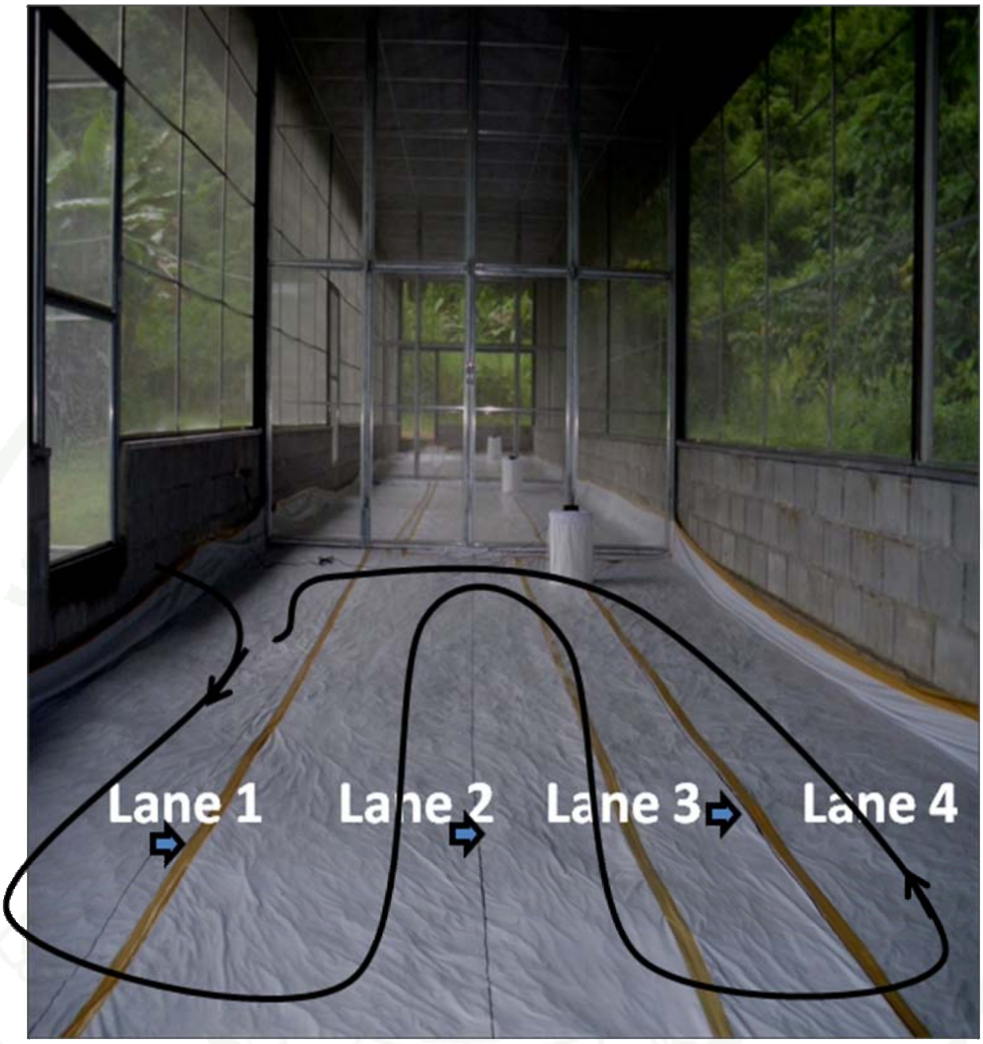

Figure 5 Schematic diagram of direction of movement in recording knocked down mosquitoes during screen house evaluations.

1.11 Data analysis

Percentage recaptured mosquitoes for each trial was corrected by adjusting for the number of knocked down mosquitoes. The percentage of recaptured mosquitoes was quantified according to recapture time points (day 1 – 0930 hours, 1330 hours, 1730 hours and day 2- 0530 hours). Cumulative recapture rate was transformed using arcsine square root values for analysis of variance (PROC ANOVA). One way ANOVA was performed on transformed values of cumulative recapture rate to determine the effect of number of BGS traps (1, 2, 3 or 4) and density of releasing number (low, medium or high) at different recapture times, and multiple comparison was done using Scheffe's test at P = 0.05 (SAS institute Inc. 2002-2008). One way ANOVA (using PROC ANOVA in SAS 9.2) was performed on transformed value of cumulative recapture rate at different recapture times to determine the effect of number of BGS traps at varying mosquito release numbers. Multiple comparison of means was done using Scheffe's test ($\alpha = 0.05$). Mean cumulative percentage recapture +/- SE of untransformed data are reported.

2. Phase II-Experimental Hut Trials

2.1 Experimental Huts

The experimental huts (Chareonviriyphap *et al*., 2010) used in the study have been previously described. The huts mimicked indigenous Thai homes in materials and dimensions and are the field assay employed to evaluate *Ae. aegypti* exiting and entering behaviors following exposure to irritant and repellent chemicals within the larger research program (Manda *et al*., 2011).

2.2 Screen house

A screen house facility, measuring 4 m (width) x 3.5 m (height) x 10 m (length) located on site was used in semi-field experiments to facilitate the recapture of released *Ae. aegypti* (Salazar *et al*., 2011 in press). The screen house is subdivided into four 10 m long cubicles with a space volume 140 m^3 (4m width, 10 m length and 3.5 m height) per cubicle section. This encompasses a combined area of the experimental huts (4m x 5m with additional 1m platform on each side) used for vector behavior studies at the field site (Chareonviriyaphap *et al*., 2010) and the expected space volume that *Ae. aegypti* primarily would use in and around a typical home in a dengue-endemic environment in Thailand (Harrington *et al*., 2005, Scott *et al.,* 2000). Based from the screen house and experimental hut dimensions, the space volumes calculated for distance evaluations were as follows: 0m (105 m^3), 3m (252m^3) and 10m (840 m^3).

2.3 Climatic data.

Temperature (^oC), relative humidity (%) and light intensity (lx/ft^2) were measured during experimental trials using Data Loggers (HOBO U12-012 Model, Onset Computer Corporation, Pocasset, MA).

2.4 BG Sentinel^{TM} (BGS) traps.

The BGS trap (Biogents AG, Regensburg, Germany) was selected for evaluation as the pull component based on previous descriptions of the trap being an effective tool for the monitoring and surveillance of dengue vectors (Krockel *et al*., 2006, Maciel-de-Freitas *et al*., 2006, Williams *et al*., 2006). A previous study evaluated varying BGS efficacy based on traps density and *Ae. aegypti* release numbers (Salazar *et al*., 2012 in press). The BGS trap has been calibrated for monitoring *Ae. aegypti* populations (Ball and Ritchie, 2010) and evaluated as a potential tool for monitoring RIDL^{TM} (Lacroix *et al*., 2009) and *w Mel* (*Wohlbachia* infected) *Ae. aegypti* strains (Ritchie *et al*. 2011). All BGS traps used in the current study were baited with the BG-Lure (used within 4 months according to package labeling) and operated according to manufacturer's instructions.

2.5 Mosquitoes.

Immature stages of local *Aedes aegypti* populations were collected from Pu Teuy village, Sai Yok District, Kanchanaburi, Thailand weekly. Immatures were reared to adults at the on-site field insectary. Nulliparous, 3-5 days old sugar-starved females were used for all experimental trials (post-exposure and optimization of trap location and distance). Mosquito test cohorts (control/treatment) were marked with a unique fluorescent powder colour prior to use in studies (Achee *et al*., 2005).

2.6 Chemical Exposure

Repellent compounds included DDT, metofluthrin and transfluthrin . These chemicals were selected due to their spatial repellent characteristics and evaluation in push-pull trials. Cohorts of female *Ae. aegypti* mosquitoes (50) were exposed to repellent compounds inside experimental huts either as treated fabric (DDT and transfluthrin) or a standard mosquito coil (metofluthrin and transfluthrin). Sentinel cages were placed at the inside center of each hut (treated and chemical-free) (Figure 6A). For each repellent treatment, two separate cohorts were used: 1) an Immediate Release (IR) exposed 0600h -1200h and released into the screen house at 1200h and 2) a Delayed Release (DR) exposed 1200h - 1800h and released at 0530h the following day having a recovery period of 12h provided with water soaked cotton pads.

Screen house cubicles were designated as control - for release of unexposed cohorts (those placed into chemical free huts) - or treatment cubicles (those placed into repellent-treated huts) (Figure 6B). Within each cubicle, 4 BGS traps were operated simultaneously with collection bags monitored following sampling periods from earlier studies (Salazar *et al*., in press). This includes Day1 (IR) at 1330 h and 1730 h and Day 2 (DR) at 0530 h, 0930 h, 1330 h, and 1730 h.

For DDT, exposure was conducted using field application rate (FAR; 2g *ai*/m^2) at 75% and 50% surface area coverages [SAC]). Assessments were also performed using 25% SAC against FAR and ½ FAR (1g *ai*/m^2). For metofluthrin, high (0.00625% *ai*) and low (0.00312% *ai*) dose coils were evaluated. Transfluthrin evaluations included 1.0 (40 µg *ai*/cm^2), 0.50 (20 µg *ai*/cm^2), 0.125 (5 µg *ai*/cm^2), and 0.0625 FAR (=2.5 µg *ai*/cm^2) using 25% surface area coverage.

Experiments were conducted during the following time periods: 1) DDT at FAR and 75% May 31-June 4, 2010; 2) DDT at FAR and 50% SAC October 6-10, 2010; 2) DDT using 25% SAC at 1.0 and 0.5 FAR March 9-12, 2011. For metofluthrin exposures were carried out January 19-25, 2010 while tranfluthrin exposures were conducted May 7-11, 2011.

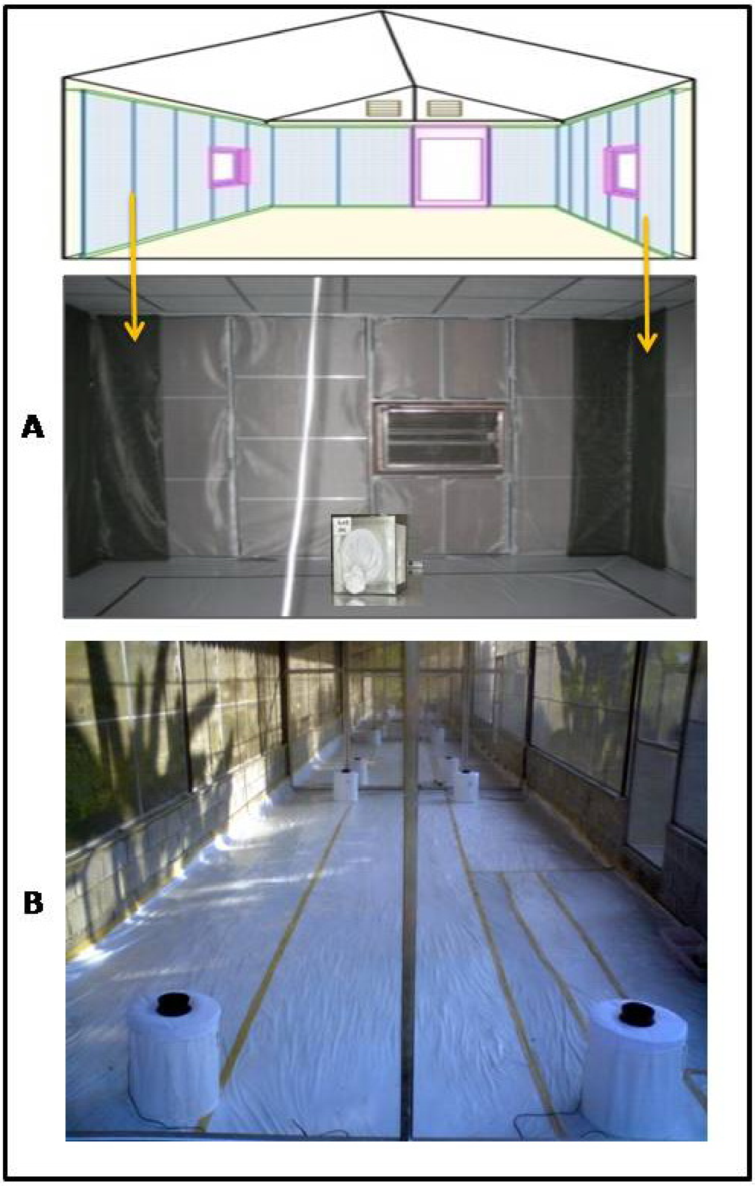

Figure 6 Exposure of 3-5 day old *Aedes aegypti* in experimental huts to spatial repellents -either as coils or treated material (A). B-Monitoring of *Aedes aegypti* recaptures within the screen house using four BG-Sentinel™ traps (B).

2.7 Location and Distance Effects

2.7.a BGS Recaptures

Individual mosquito test populations (100 3-5 day old female *Ae. aegypti*) were released at designated release points for each experimental hut on Day 1 at 0530 h. BGS recapture was monitored at 0930 h, 1330 h, and 1730 h on the day of mosquito release and 0500 h, 0930 h, 1330 h, and 1730 h on subsequent testing days. These monitoring intervals were based on the earlier quantification of recaptures from varying trap densities and *Ae. aegypti* release numbers. At each sampling interval, BGS collection bags were removed and replaced. Recaptured mosquitoes were recorded according to marking color, time of collection, BGS trap number and collection hut.

2.7.b Interception Traps (IT) Recaptures

Collections from IT followed established protocols (Grieco et al. 2007). Three test populations of one hundred female mosquitoes each were marked with unique colors and released at a designated point 10 m outside each experimental huts 30 min before sunrise (Figure 7). Collections from IT fixed on the interior of windows and doors of the hut (to capture entering mosquitoes) were conducted for 1 min during 20 min intervals from 0600-1800 h (Figure 8). Temperature and relative humidity were recorded for each sampling period. Two persons inside each hut served as collectors and generated host cues.. Collectors rotated among huts every six hours during each sampling day to control for collector bias and host attractiveness.

Recaptured mosquitoes were placed within individually labeled containers and recorded according to marking color (i.e., hut origination), time of collection, and trap number (i.e., window 1-3 or door). Three replicates were performed for each treatment trial. IT collections are used to compare reduction in *Ae. aegypti* entry for each of the treatments on location and distance effects.

2.7.c Location Effects

Two locations were evaluated using four BGS traps: 1) vertices (corners) outside the experimental hut and 2) opposite each portal of entry (3 windows and the door) (Figure 9). Vertex location was chosen to reflect the position of BGS in the screenhouse trials while portals of entry (windows and doors) were considered as they reflect direct mosquito entry points into the experimental huts. The use of four BGS traps was based on previous screen house evaluations (Salazar et al, in press). For both of the locations, BGS traps were placed on the hut platform (30 cm above ground; Figure 7). A total of three huts were used in the experiment, one hut served as the control with no BGS and two others as treatment. The three treatments: two positions and control were rotated among the huts following a Latin Square study design for three consecutive days/replications.

2.7.d Distance Effects

Three distances from a host-occupied structure were evaluated: 0m, 3m, and 10m (Figure 10). Three huts were used simultaneously in separate trials: 1) Trial 1: BGS at 0m, 3m and 10m (July 5-8, 2010) , 2) Trial 2: No BGS and BGS at 3m and 10 m (July 31 to August 2, 2010) and; 3) Trial 3: BGS at 0m, 3m and 10m (December 15-17, 2010). Temporary shelters were used to protect BGS traps from rain and direct sunlight when evaluating 3 m and 10 m distances (Figure 11). The shelter was raised 10 cm above ground with a galvanized iron roofing 1.5 m above the platform. This provided a 6 inch space around the BGS following BGS manufacturer's specification (Figure 11). BGS sampling followed similar protocol as that described for location effects (see above).

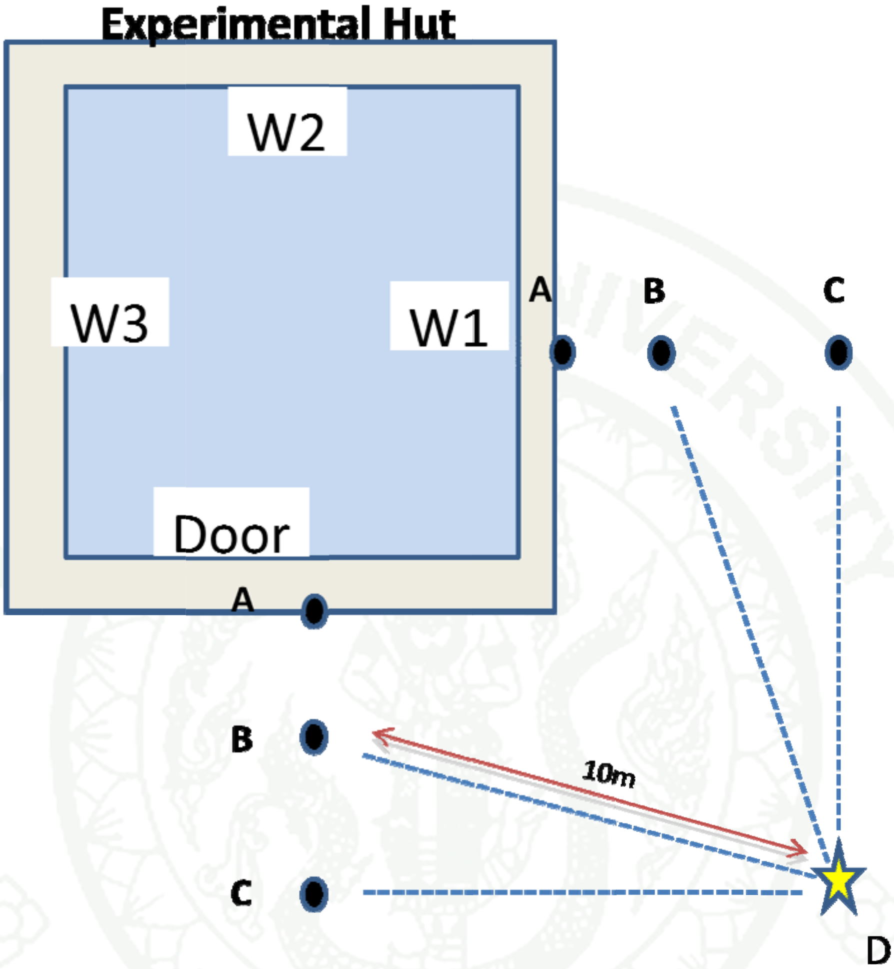

Figure 7 Diagram of the release point (yellow star) relative to BG-Sentinel™ trap positioning at experimental huts (black circle): A-0m, B-3m and C-10m. Windows designated W1-W3 counterclockwise with the door as reference point.

Figure 8 Mosquitoes collected from interception traps through the opening pouches at the windows (A) and doors (B-upper and C-lower). Treated facbric placed surrounding portals of entry (windows and doors).

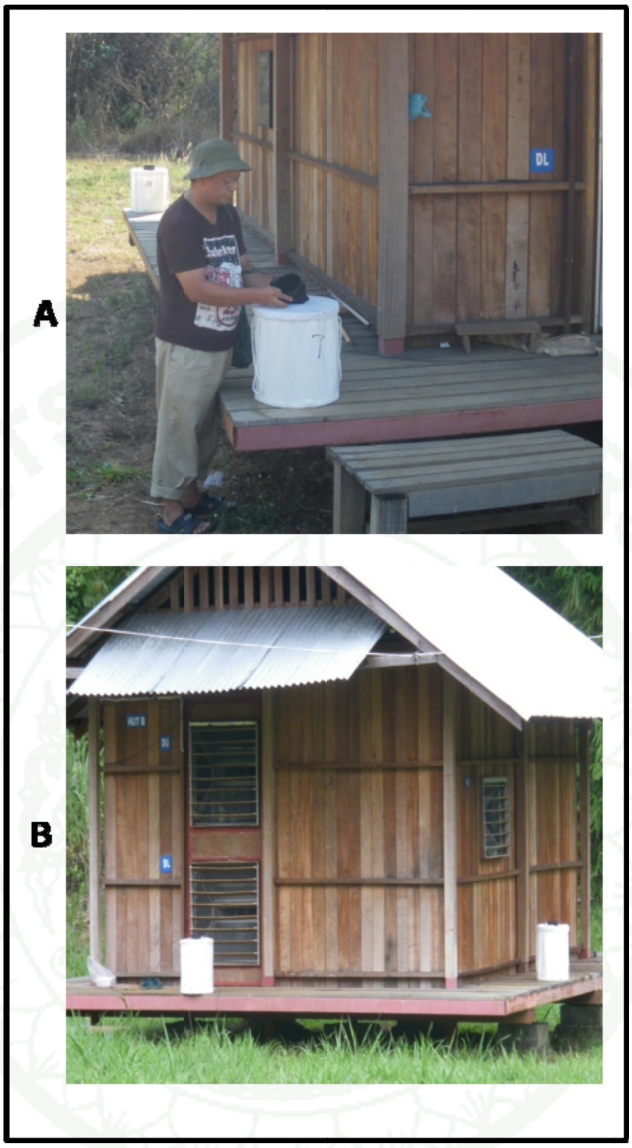

Figure 9 Evaluation of location effects of BG-Sentinel™ *Aedes aegypti* recapturerates: A-vertices and B- opposite portals of entry (windows and doors).

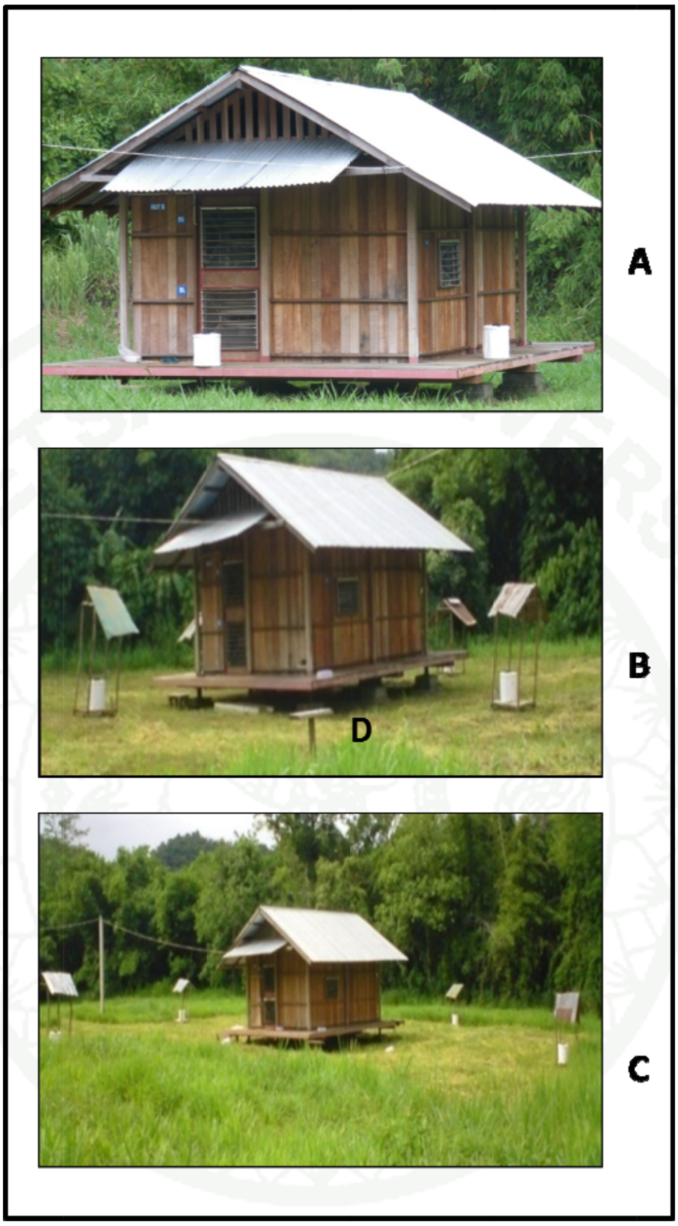

Figure 10 Evaluation of distance effects of BG-Sentinel™ *Aedes aegypti* recapture rates at A) 0m, B) 3m and C) 10m distances from experimental huts. D-platform for releasing mosquitoes.

Figure 11 Temporary shelter constructed to protect BG-Sentinel ™ (BGS) traps from rain and direct sunlight during distance effects trials. The shelter was elevated 10 cm above ground with 6 in space around the BGS. A galvanized metal roof was positioned 1.5 m above each BGS.

2.8 BGS Trap Contribution to Push-Pull Strategy under Experimental Conditions.

Push-pull strategy (PPS) evaluations were conducted using four experimental huts with treatments as follows: control (Hut A), repellent (Hut B), repellent + BGS (Hut C) and BGS only (Hut D) (Figure 12). Repellents evaluated included metofluthrin coils (0.00975% *ai*) and transfluthrin treated polyester material (25% SAC at 0.125FAR (5 µg *ai*/cm^2). Transfluthrin treated material was fixed to interior hut walls around portals of entries as previously described (Grieco *et al*. 2007, Chareonviriyaphap *et al*. 2010; (Figure 8). Treatment of fabric consisted of pipetting a predetermined volume of diluent based on individual material panel absorption rates mixed with the desired amount of active ingredient (*ai*). Metofluthrin coils were positioned on top of a metal dish and positioned in the center of the corresponding hut. Coils were lit 15 min prior to first IT sampling period (0615 h) and remained lit until1800 h during a 12 h observation period each day. BGS traps were positioned opposite portals of entry (windows/doors) at 0 m from the exterior house wall based on findings from location and distance effects evaluations (see above).

Evaluation of *Ae. aegypti* hut entry rates and BGS capture densities were conducted using similar protocols as that described for location and distance effects (see above). The push-pull evaluations for each of the repellent formulation were conducted in the following time periods: 1) metofluthirn coils (0.01%) in July 26-29, 2011; 2) transfluthrin treated polyester fabric (0.125 FAR (5 µg *ai*/cm^2) at 25%SAC) in June 14-17, 2011; 3) transfluthrin coils (0.003%) in July 11-14, 2011 and; 4) transfluthrin treated lace fabric (0.125 FAR, 25% SAC) in September 21-23, 2011.

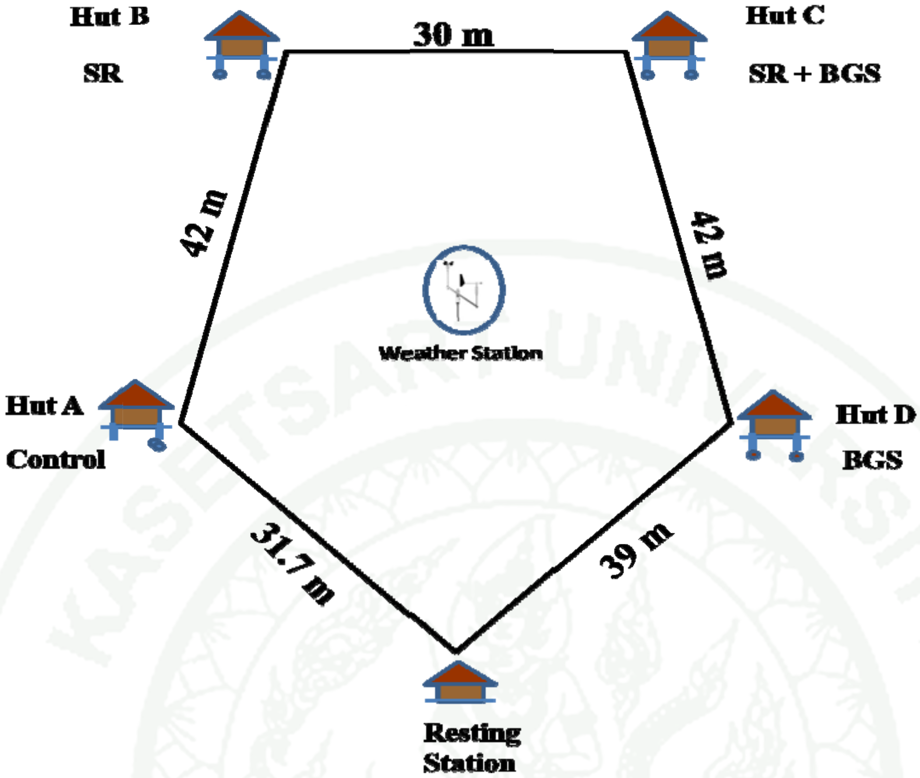

Figure 12 Schematic diagram of the positions of experimental huts and treatment allocations (control, spatial repellent (SR), SR+ BG-Sentinel™ (BGS), BGS) during push-pull evaluations.

Recaptured mosquitoes were placed within individually-labeled cartones and recorded according to marking color (i.e., hut origination), time of collection, and trap number [i.e., window 1-3 or door (upper and lower)]. Four replicates were performed for each treatment trial.

2.9 Data Analyses

For all experimental trials in Phase II studies (post exposure, location and distance), the percentage of recaptured mosquitoes was quantified cumulatively according to recapture time points after release (day 1 – 0930 hours, 1330 hours, 1730 hours and day 2- 0530 hours). Percentage recaptured mosquitoes from post-exposure studies were corrected by adjusting for the number of knocked down mosquitoes after exposure (IR) and before releasing them into the screen house (DR). Kruskal Wallis test was used to compare the effects of chemical repellent exposure on BGS *Ae. aegypti* recapture percentages between treatments and control cohorts and the comparison of cumulative BGS recapture rates from immediate (IR) and delayed (DR) release/cohorts of *Ae. Aegypti* was performed using Mann-Whitney test. Kruskal Wallis test was also performed to compare the BGS recapture percentages of *Ae. aegypti* between location points (vertices and portals of entry) and distances (0, 3, 10 m) during these trials. Percentage reduction in entry ***(%RE***) from interception traps for each treatment during push-pull trials was computed using the following formula: ***%RE*** = 1- [recaptures from treatments divided by (÷) recaptures from control] x 100. Control huts served as bases for computation with ***0% RE*** assumed for the controls [1- (recaptures from control ÷ recaptures from control)]. For all the tests, a p-value of 0.05 or less was considered for statistical significance. All statistical analyses were performed in STATA 11.2 using the ranksum and kwallis syntax for Mann-Whitney test and Kruskal Wallis test, respectively. Mean cumulative percentage recapture ± SD are presented in Tables.

3. Phase III-Local Home Trials

3.1 Local Home Survey of BGS Competing Resting Sites.

A survey of available outdoor *Ae. aegypti* mosquito resting and breeding sites was conducted from twenty households in the village of Pu Teuy , Kanchanaburi, Thailand in January, 2011 (Figure 13). Water containers (jars, drums, basins, vegetation) and density of potential BGS competing resting sites at 0, 3 and 10m surrounding the houses were quantified. Survey parameters included container type, relative size and distance from house exterior. The dimensions of containers were recorded to determine approximate volumes of water they could contain, and was used to classify containers into relative sizes (small -<250L), medium (250-≤500L), large (>500-≤1000L) and extra large (≥1000L). For vegetation, presence and relative location from the household was recorded using three categories: 1) trees (having woody stem >1m height), 2) shrubs (having woody stem <1m height) or 3) herbs (having non-woody stem). Other probable resting sites were also recorded to include: clothes, storage shanties and other structures within 10 m diameter surrounding the house.

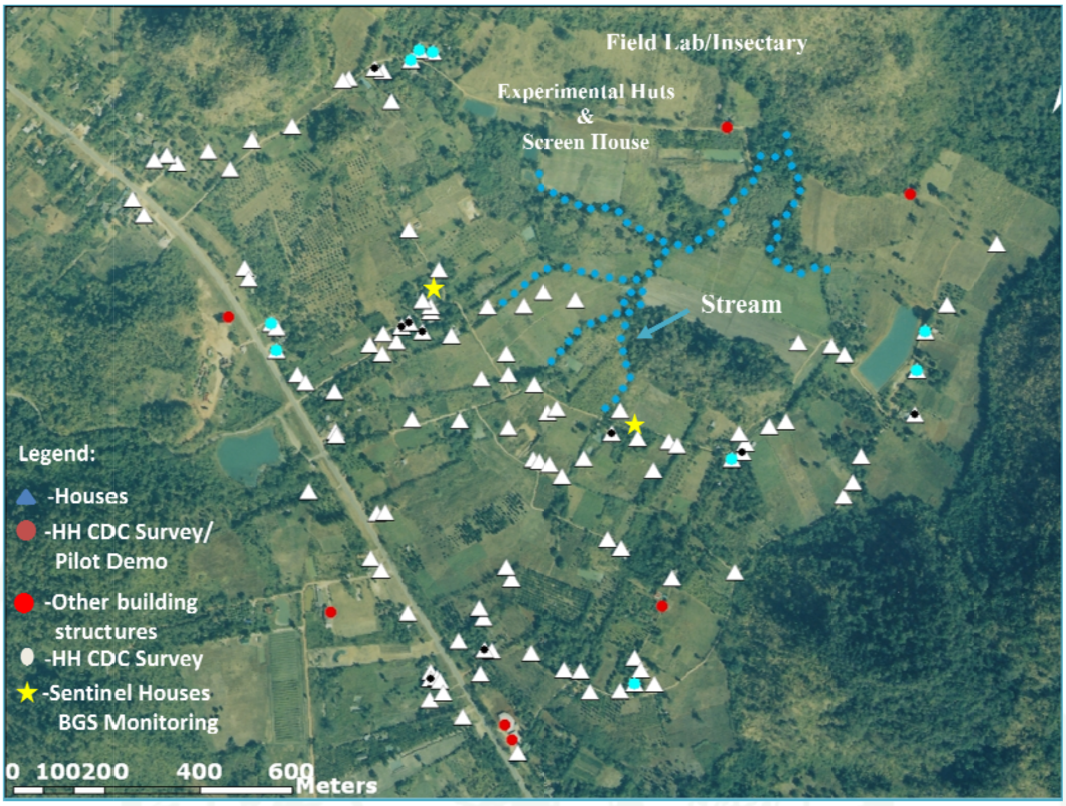

Figure 13 Field site in Pu Teuy village, Thongphphum, Kanchanaburi, Thailand. used to survey *Ae. aegypti* resting and breeding sites as well as monitor BG-Sentinel™ trap efficacy.

3.2 Monitoring of natural *Aedes aegypti* populations in a local village setting

3.2.a BGS trap.

Two sentinel households (SH) were selected to verify if the optimized BGS conditions defined under screen house and experimental hut trials would be applicable to a "real-life "setting (Figure 13). These were the households with the highest *Ae. aegypti* density (2 female/20 minute/month) from the CDC-back pack aspirations. The first SH was made of cement and not raised or one storey type house (Figure 14) while the other was made of wood and raised 2m above ground (Figure 15). Four BGS traps each with a unique identification code were placed at each SH with one BGS positioned opposite portals of entry (eves, windows, doors) on all four sides. For the non-raised SH, BGS traps were placed on the ground (Figure 14). For the raised SH, three BGS traps were placed at ground level (windows 1and 2 and door) and one BGS was located on a platform 2 m above the ground (designated as Window-3) (Figure 15). BGS traps were operated from 0530 - 1730 h with collection bags monitored at three sampling intervals (0930 h, 1330 h, 1730 h) for five days every second week of the month from February -September 2011. Dengue vectors collected were identified and recorded by house, trap number, time of collection, species and gender.

3.2.b CDC-Back Pack Aspiration.

Monthly CDC-back pack collections were conducted from January – September 2011 in the 20 houses randomly selected for resting and breeding site surveys to determine natural indoor *Ae. aegypti* densities to serve as baseline for a PPS demonstration trial and to help interpret BGS collection data (Figure 13). A total

Figure 14 The non-raised and cemented local house used as a sentinel household for BG-Sentinel™ monitoring of *Ae. aegypti* populations showing the most productive trapping position (door).

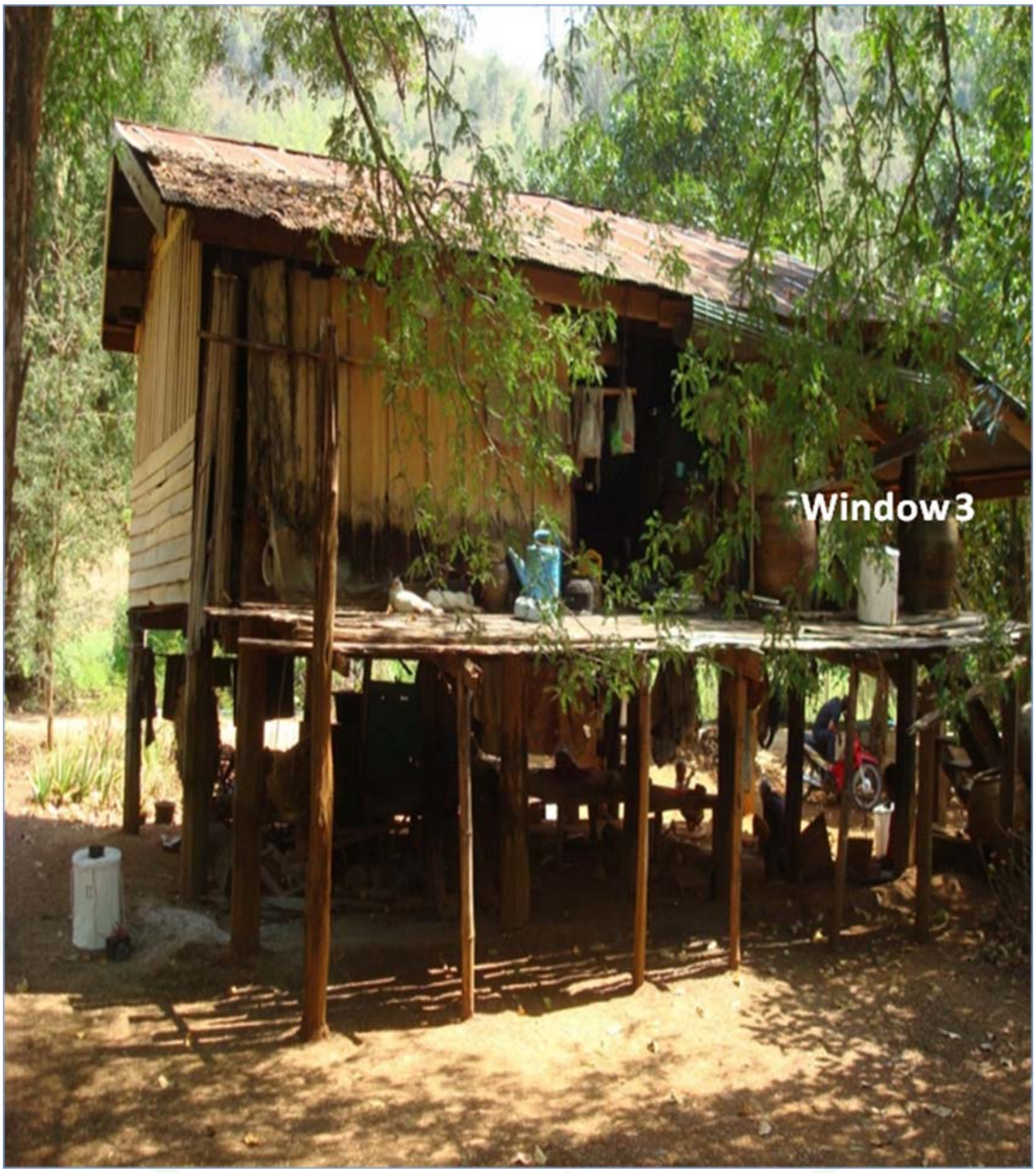

Figure 15 The raised and wooden local house used as a sentinel household for BG-Sentinel™ monitoring of *Ae. aegypti* populations showing the most productive trapping position (window 3).

of 20 min was allocated per household to sample from insides of the living room, bedroom, kitchen and bathroom.

CDC-back pack collections were performed simultaneously at each of the 20 houses one day during same week as the five-day BGS monitoring period (February-September 2011). .

3.3 Data Analysis.

Containers were classified and grouped according to distance from surveyed houses and relative container size. Monthly BGS and CDC back pack collections trends were generated to show differences in densities of *Ae. aegypti* and *Ae. albopictus* from SH at the households surveyed. Overall peak time of BGS collections was determined by tallying numbers per sampling interval (0530-0930; >0930-1330; >1330-1730). Overall percentage contribution of *Ae. aegypti* capture from BGS at all locations were calculated using total proportion collected at an individual SH for the entire survey period.

RESULTS AND DISCUSSION

Results

1. Phase I-Screen House Trials

1.1 Baseline experiments.

Results from baseline trials indicated no significant difference among cubicles for mean temperature (A=24.8, B=24.7, C=24.3 and D=24.8^{o}C) (Figure 16) or relative humidity (A=75.4, B=75.4, C=77.0, D=76.0%) (Figure17). Baseline light intensities were: A=83.33; B=70.93; C=21.11 and D=33.03 lx/ft^2 (Figure 18) with average light intensity of cubicle A being significantly higher than that of cubicle C ($F_{(3,92)}$ =4.11, $p<0.01$). Despite inter-trial variability amongst cubicles, these values were all greater than previously reported for thresholds of *Ae. aegypti* host-seeking activity of 0.1 lx (0.01 foot candle = 1 lumex (lx)/ft^2(Kawada *et al*., 2005). Combined, these results justified the use of a completely randomized study design (CRD).

There were no differences in the mean recaptures of marked or dyed (84.00%) and un marked (82.90%) mosquitoes in the preliminary experiments conducted Results showed that the "Impact Period" occurred on Day 1 with an overall recapture of 84% and peak recaptures (28-35%) occurring between the time points 0930 hours and 1330 hours (Figure 19). Total recapture on Day 2 during these trials was 11.3%. Experiments evaluating positional bias of trap placement indicated no significant difference in trap recapture among the T1-T4 cubicle positions (Table 3).

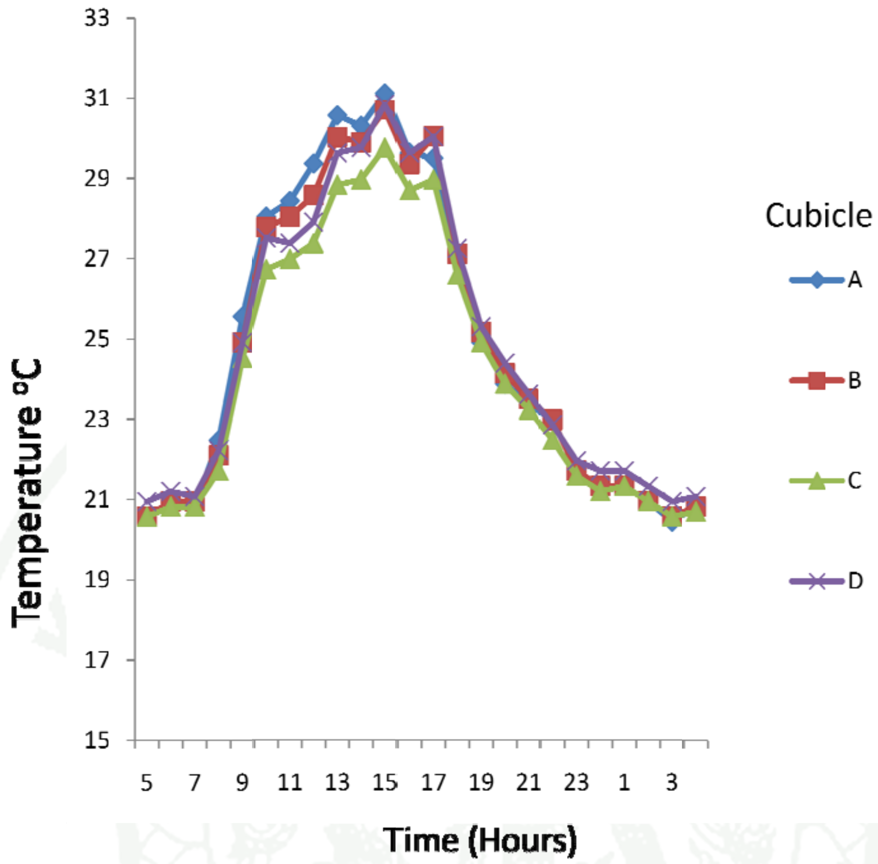

Figure 16 Temperature (°C) readings every 20 minute interval from the four screen house cubicles.

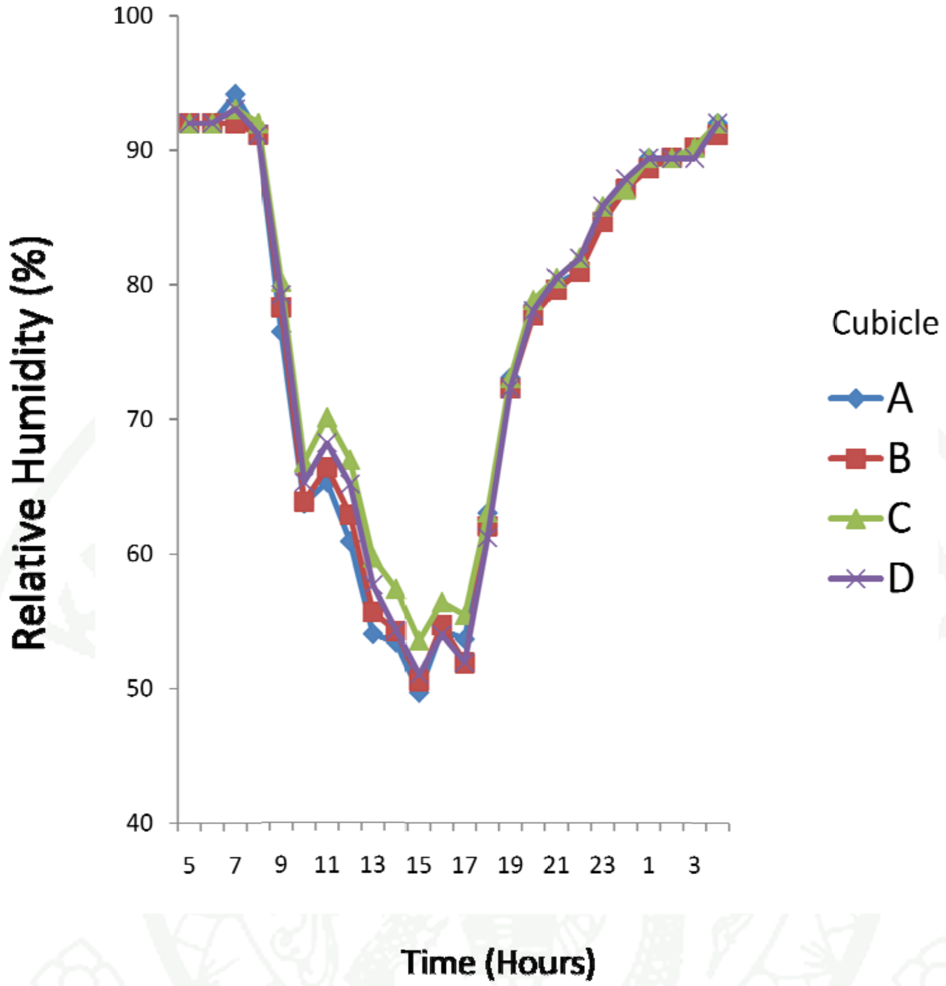

Figure 17 Relative humidity (%RH) readings every 20 minute interval from the four screen house cubicles.

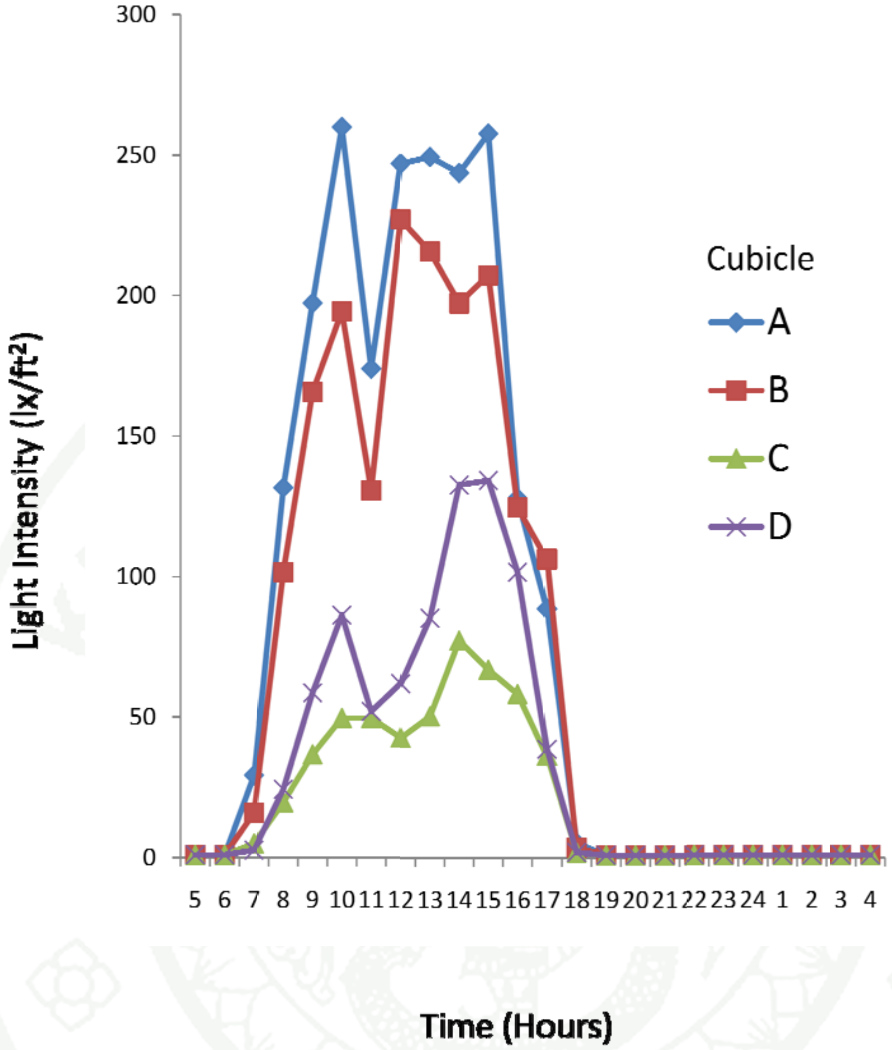

Figure 18 Light intensity (lx/ft^2) readings every 20 minute interval from the four screen house cubicles.

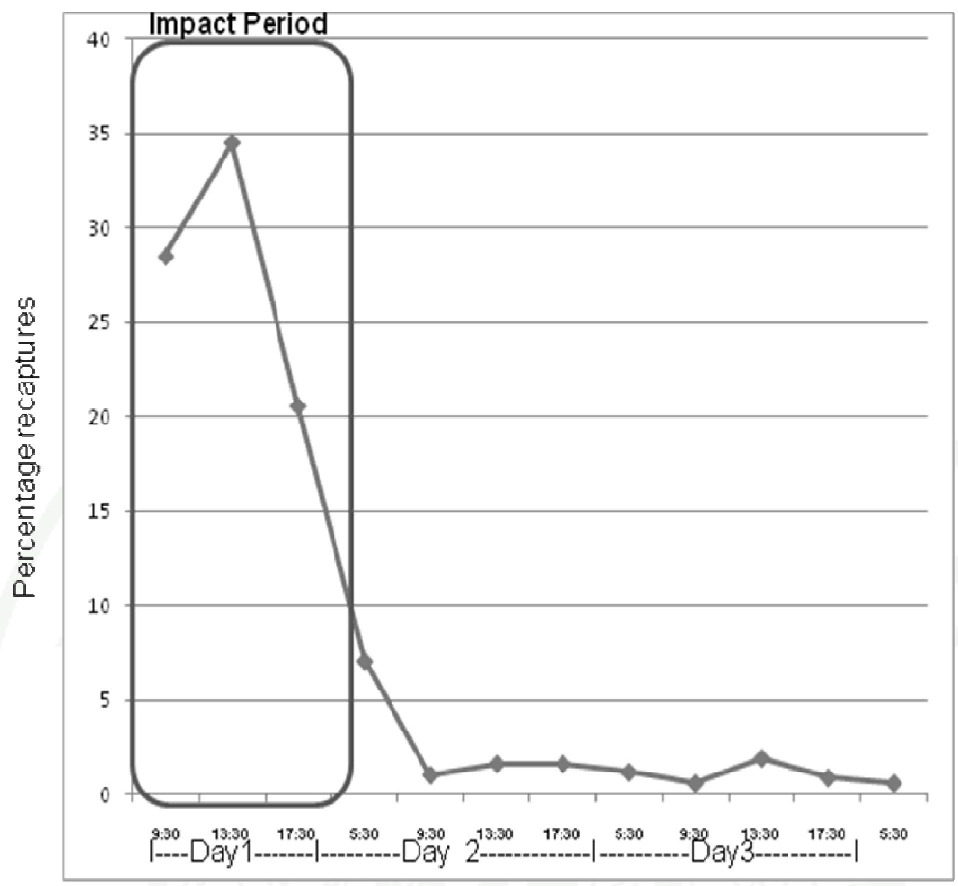

Figure 19 Outcome of baseline experiment (cumulative percentage of recovered mosquitoes) with 1 BG-Sentinel™ trap and 100 *Aedes aegypti* released showing peak of recapture (impact period).

Table 3 Comparison of BG-Sentinel™ *Aedes aegypti* recaptures from the four trapping positions in four screen house cubicles.

Position[2]	Mean total recaptures from screen house cubicles[1]			
	A	B	C	D
TP1a	346/400 (86.5)	343/400 (85.75)	342/400 (85.50)	369/400 (92.50)
TP2a	343/400 (85.75)	343/400 (85.75)	360/400 (90.00)	353/400 (88.25)
TP3a	332/400 (83.00)	340/400 (85.00)	352/400 (88.00)	356/400 (89.00)
TP4a	354/398 (88.94)	358/400 (89.50)	359/400 (87.50)	360/400 (90.50)

[1]Total recaptures after two days of monitoring

[2]Same lowercase letters indicates no statistically significant difference between positions (ANOVA, $p>0.05$)

1.2 *Ae aegypti* Recapture success in relation to number of BGS traps used.

Cumulative percentage recapture on Day 1 for all RNs evaluated ranged from 41-92% for one trap, 66-92 % for two traps, 69-95% for three traps, and 72-98% for four traps (Tables 6-8). When including Day 2, the cumulative percentage recapture increased to 66-92%, 80-94%, 86-95%, and 82 to 98% for 1-4 traps, respectively (Tables 6-8).Going from one trap to 2-4 traps added a range of 10-18% increase in the total recapture rate across RNs.

Analysis of grouped RN categorical data (low, medium, high) revealed an overall trap and RN relationship (Table 4). Significantly lower BGS recapture success of *Ae. aegypti* resulted when a single trap ($F_{(3,\ 108)}$=5.96, $p=0.0008$) was used (82.7%) whereas there were no significant differences between recapture percentages when using 2-4 traps; with means of 86.3%, 90.2% and 91.3%, respectively, at the end of Day 2 (Table 4). The cumulative percent recaptures observed at 1330 hours ($F_{=(3.108)}$=4.46, $p=0.0054$)and 1730 hours ($F_{(3,\ 108)}$=6.51, $p=0.0004$) on Day 1 showed statistically similar trend compared to the combined totals from Days1-2. (Table 4). Overall, the highest mean cumulative recapture (91.30%) was recorded from the use of 4 traps.

1.3 BGS recapture success in relation to *Ae aegypti* release numbers

Overall, analyses from combined data for BGS trap density showed that except for the first collection period (0930 hours), significantly fewer mosquitoes were recaptured on Day 1 from the low RN category compared when compared to the medium and high RN categories at 1330 hours ($F_{(3,109)}$=7.11, $p=0.0010$), 1730 hours ($F_{(3,109)}$=8.90, $p=0.0003$) and at the end of Day 2 ($F_{(3,109)}$=7.30, $p=0.0010$) (Table 2). However no significant difference was observed between percentages of mosquitoes collected from medium and high RNs when comparing all trap densities (Table 5).

1.4 Cumulative recapture trends between RNs and BGS trap density.

Combined Day 1 and Day 2 cumulative percentage recapture rates when four BGS were used was >91% for RNs of 100, 150, 200 and 250, and >80% for RNs of 10, 25 and 50 (Tables 6-8). Using two or three traps resulted in cumulative percentage recapture rates >80% for all RNs. The use of one trap at the RN of 25 resulted in a combined Day 1 and Day 2 percentage recapture rate below 80% (Table 6).

The low RN category consistently resulted in fewer recaptured mosquitoes when using 1- 4 traps (Figures 20-23) as compared to medium and high RNs. As expected, with the use of one (Figure 20) and two (Figure 21) traps, recapture rates increased as the RNs increased. When three (Figure 22) and four (Figure 23) traps were used, higher recapture rates were observed from medium RNs compared to the high category RNs but the differences were not statistically significant (Tables 5, 6-8).

1.5 Comparison of recapture rates from the low RN category.

Overall, cumulative recapture rates at the end of Day 2 were not significantly different when using 1-4 traps and the low RNs (10, 25, and 50) (Table 6). However, significant differences were observed with the release of 10 mosquitoes at 0930 hours ($F_{(3,12)}$=4.11, $p=0.0320$), when using one (67.5%) versus four traps (95.0%). With the RN of 25, significant differences were indicated from recaptures made at 1330h ($F_{(3,12)}$=3.94, $p<0.0360$) and at the end of Day 1 ($F_{(3,15)}$=4.94, $p=0.0184$). The highest recapture rates for the release of 25 mosquitoes occurred at 1730 hours (71.5%). At the RN of 50, no significant differences were observed based on cumulative percent recapture rates (Table 6) at the end of Day 1 and Day 2.

1.6 Comparison of recaptures from medium RN category.

The highest overall cumulative recapture rates for Day 2 were recorded when using four BGS in combination with RNs of 100 and 150 (Table 7). At the RN of 100, the use of three (94.8%) or four (97.0%) traps resulted in a significantly higher recapture of females compared to when one (85.5%) or two traps (83.2%) were used ($F_{(3,12)}$=13.80, *p=0.0003*). Significant differences were also observed at 1730 hours on Day 1 with the use of four traps resulting in higher numbers of marked females being recapturing than when 1-3 traps were used (Table 7). Perhaps more importantly, the use of two traps did not significantly increase the recapture rates over what was found when using one trap. The use of two traps was also no different from the use of three traps and use of three was no different from the use of four traps ($F_{(3,12)}$=11.58, *p=0.0007*). However, by Day 2, the use of three and four traps did result in significantly higher recapture rates as compared to when two traps were used. With the RN of 150, the use of four traps (97.8%) showed the highest cumulative mean percent recapture, significantly higher than when using one (89.1%), two (88.8%), or three traps (91.8%) ($F_{(3,12)}$=16.49, *p=0.0001*). Consistently lower numbers of females from the 150 RN were caught on Day 1 from the use of only one trap compared to the use of 2-4 traps at 0930 ($F_{(3,12)}$=9.15, *p=0.0020*) and 1330 hours ($F_{(3,12)}$=19.09, *p=0.0001*) (Table 4).

1.7 Comparison of recaptures from high RN category.

With the RN of 200, consistently lower recapture rates were recorded on Day 1 with the use of one trap compared to the use of two-four traps at 1330 hours ($F_{(3,12)}$=7.73, *p<0.0039*) and Day 1 ($F_{(3,12)}$=9.97, *p<0.0014*) sampling intervals (Table 8). This was true through Day 2 ($F_{(3,12)}$ =9.41, *p<0.0018*). The greatest overall recapture from the high RN category occurred from the use of four traps though rates were not significantly different from those recorded when using two or three traps (Table 8). Analysis of overall percent cumulative recaptures with the RN of 250 did not indicate significant differences with the use of 1-4 traps.

1.8 Environmental associations among RN and BGS recapture rates.

No correlations were found between the environmental variables measured and recapture rates for any of the RN and BGS trap density combinations.

1.9 Recapture from BGS traps based on monitoring interval.

Across BGS trap densities and release numbers, the highest mean recapture (overall mean recapture of marked *Ae. aegypti*) of 49% was from the 0930 hours sampling period. The 1330 hours sampling point resulted in the next highest overall mean recapture (28%) across all RNs and another 4% recaptured from the last collection period at 1730 hours (Table 4) validating Day 1 as the “impact period”.

Table 4 Cumulative BG-Sentinel™ trap recapture rates for combined *Ae. aegypti*[1] release numbers (RNs)[2] by number of traps in performance.

BGS trap	Mean (S.E.) percentage of *Ae. aegypti* recaptured[3]			
	Cumulative percentage recapture by recovery time point during the first day (Day 1)			Cumulative percentage recapture for Days 1-2
	0930 hours	1330 hours	1730 hours	
1	32.7b (0.04)	65.8b (4.25)	74.5b (4.49)	82.7b (2.46)
2	59.8a (5.07)	78.1ab (3.45)	83.5ab (0.14)	86.3ab (1.32)
3	60.4a (4.99)	82.3a (2.99)	87.3a (0.14)	90.2a (1.45)
4	42.1ab (5.82)	79.1a (3.28)	87.4a (0.14)	91.3a (1.51)

[1] 3-5 days old starved females

[2] Release numbers categories: Low (10, 25, 50), medium (100, 150), high (200, 250)

[3] Different lowercase letters in the same column indicate significant differences between mean recapture percentages (ANOVA, 95% confidence limit)

Table 5 Cumulative BG-Sentinel™ trap recapture rates by individual *Ae. aegypti*[1] release numbers (RNs)[2].

RN	Mean (S.E.) percentage of *Ae. aegypti* recaptured[3]			
	Cumulative percentage recapture by recovery time point during the first day (Day 1)			Cumulative percentage recapture for Days 1-2
	0930 hours	1330 hours	1730 hours	
Low	40.8a	67.8b	76.4b	83.1b
(10, 25, 50)	(5.16)	(3.39)	(0.14)	(1.73)
Medium	58.4a	82.7a	87.8a	90.9a
(100, 150)	(3.63)	(2.47)	(0.14)	(1.02)
High	51.2a	82.7a	88.8a	91.0a
(200, 250)	(3.97)	(1.98)	(0.14)	(0.99)

[1] 3-5 days old starved females

[2] Release numbers categories: Low (10, 25, 50), medium (100, 150), high (200, 250)

[3] Different lowercase letters in the same column indicate significant differences between mean recapture percentages (ANOVA 95% confidence limit)

Table 6 Cumulative mean percentage recapture of *Ae. aegypti*[1] low Release Number (RN) category by mosquito release density, number of BG-Sentinel™ traps performing and monitoring interval.

Release Density	Number of traps	Cumulative percentage recapture by recovery time point during the first day (Day 1)[3]			Cumulative percentage recapture for Days 1-2	Mean Day-Time (12hr) Environmental Data during the conduct of the releases		
		0930 hours	1330 hours	1730 hours		Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
10	1	67.5b	70.0a	70.0 a **(28/40)**	72.5a	32.5	52.5	208.6
	2	87.5ab	87.5a	87.5a **(35/40)**	87.5a	35.4	51.4	259.8
	3	82.5ab	87.5a	87.5a **(35/40)**	87.5a	31.5	39.2	246.1
	4	95.0a	92.5a	92.5a **(37/40)**	92.5a	30.3	42.0	231.9

[1] 3-5 days old starved females

[2] Different lowercase letters in the same column within the same release number indicate significant differences between mean recapture percentages (ANOVA at 95% confidence limit)

[3] Values in parentheses are total recaptures/total release-knockdown

Table 6 (Continued)

Release Density	Number of traps	Cumulative percentage recapture by recovery time point during the first day (Day 1)[3]			Cumulative percentage recapture for Days 1-2	Mean Day-Time (12hr) Environmental Data during the conduct of the releases		
		0930 hours	1330 hours	1730 hours		Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
25	1	3.0a	24.0b	41.0b **(41/100)**	66.0a	25.5	64.3	145.6
	2	7.0a	40.0ab	66.0ab **(66/100)**	80.0a	25.1	63.9	179.9
	3	7.2a	48.0ab	69ab **(66/96)**	88.5a	23.6	64.8	131.1
	4	8.1a	55.0a	71.5a **(71/99)**	81.7a	26.7	63.5	175.9

[1] 3-5 days old starved females

[2] Different lowercase letters in the same column within the same release number indicate significant differences between mean recapture percentages (ANOVA at 95% confidence limit)

[3] Values in parentheses are total recaptures/total release-knockdown

Table 6 (Continued)

Release Density	Number of traps	Cumulative percentage recapture by recovery time point during the first day (Day 1)[3]			Cumulative percentage recapture for Days 1-2	Mean Day-Time (12hr) Environmental Data during the conduct of the releases		
		0930 hours	1330 hours	1730 hours		Temperature (^{o}C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
50	1	22.0bc	90.4a	92.0a **(183/199)**	92.5a	23.2	77.6	175.4
	2	41.0ab	72.8bc	79.0a **(156/200)**	80.8a	28.8	79.7	263.1
	3	60.5a	84.4ab	86.0a **(171/199)**	85.9a	28.0	74.4	235.1
	4	8.0c	61.8c	75.0a **(149/199)**	81.9a	25.2	68.4	157.6

[1] 3-5 days old starved females

[2] Different lowercase letters in the same column within the same release number indicate significant differences between mean recapture percentages (ANOVA at 95% confidence limit)

[3] Values in parentheses are total recaptures/total release-knockdown

Table 7 Cumulative mean percentage recapture of *Ae. aegypti*[1] medium Release Number (RN) category by mosquito release density, number of BG-Sentinel™ traps performing and monitoring interval.

Medium Release Numbers	Number of traps	Cumulative percentage recapture by recovery time point during the first day (Day 1)			Cumulative percentage recapture for Days 1-2	Mean Day-Time (12hr) Environmental Data during the conduct of the releases		
		0930 hours	1330 hours	1730 hours		Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
100	1	43.3a	68.8a	77.0c **(308/400)**	85.5b	27.2	58.4	285.8
	2	66.3a	81.9a	83.0bc **(331/400)**	83.2b	28.5	80.3	249.4
	3	59.3a	88.5a	93.5ab **(374/400)**	94.8a	27.4	73.8	205.5
	4	51.5a	89.3a	96.0a **(388/400)**	97.0a	26.2	67.9	143.9

[1] 3-5 days old starved females

[2] Different lowercase letters in the same column within the same release number indicate significant differences between mean recapture percentages (ANOVA at 95% confidence limit)

[3] Values in parentheses are total recaptures/total release-knockdown

Table 7 (Continued)

Medium Release Numbers	Number of traps	Cumulative percentage recapture by recovery time point during the first day (Day 1)			Cumulative percentage recapture for Days 1-2	Mean Day-Time (12hr) Environmental Data during the conduct of the releases		
		0930 hours	1330 hours	1730 hours		Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
150	1	27.6b	60.2b	75.8c **(454/600)**	89.1b	27.2	79.8	186.2
	2	74.1a	85.6a	86.8bc **(517/598)**	88.8b	28.3	81.8	188.2
	3	80.3a	90.5a	91.8ab **(549/599)**	91.8b	28.8	73.9	250.8
	4	64.8a	97.2a	97.8a **(585/599)**	97.8a	26.2	71.4	129.4

[1] 3-5 days old starved females

[2] Different lowercase letters in the same column within the same release number indicate significant differences between mean recapture percentages (ANOVA at 95% confidence limit)

[3] Values in parentheses are total recaptures/total release-knockdown

Table 8. Cumulative mean percentage recapture of *Ae. aegypti*[1] high Release Number (RN) category by mosquito release density, number of BG-Sentinel™ traps performing and monitoring interval.

High Release Numbers	Number of traps	Cumulative percentage recapture by recovery time point during the first day (Day 1)			Cumulative percentage recapture for Days 1-2	Mean Day-Time (12hr) Climatic Data during the conduct of the releases		
		0930 hours	1330 hours	1730 hours		Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft²)
200	1	26.3b	64.1b	77.0b **(627/800)**	82.0b	26.7	81.2	138.3
	2	72.1a	87.4a	89.5a **(713/796)**	90.3ab	28.8	77.6	222.6
	3	73.8a	87.7a	88.8a **(707/799)**	88.5ab	29.3	73.7	265.2
	4	43.6b	87.0a	94.8a **(756/800)**	96.4a	26.8	70.5	132.3

[1] 3-5 days old starved females

[2] Different lowercase letters in the same column within the same release number indicate significant differences between mean recapture percentages (ANOVA at 95% confidence limit)

[3] Values in parentheses are total recaptures/total release-knockdown

Table 8 (Continued)

High Release Numbers	Number of traps	Cumulative percentage recapture by recovery time point during the first day (Day 1)			Cumulative percentage recapture for Days 1-2	Mean Day-Time (12hr) Climatic Data during the conduct of the releases		
		0930 hours	1330 hours	1730 hours		Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
250	1	39.4bc	83.2ab	89.0a **(886/1000)**	91.2a	28.8	70.7	131.3
	2	76.1a	91.5a	92.5a **(922/997)**	93.6a	28.9	77.8	242.9
	3	54.2ab	89.8a	94.8a **(945/998)**	94.7a	26.6	77.6	208.2
	4	23.8c	71.2b	84.5b **(844/1000)**	91.6a	26.8	69.2	120.1

[1] 3-5 days old starved females

[2] Different lowercase letters in the same column within the same release number indicate significant differences between mean recapture percentages (ANOVA at 95% confidence limit)

[3] Values in parentheses are total recaptures/total release-knockdown

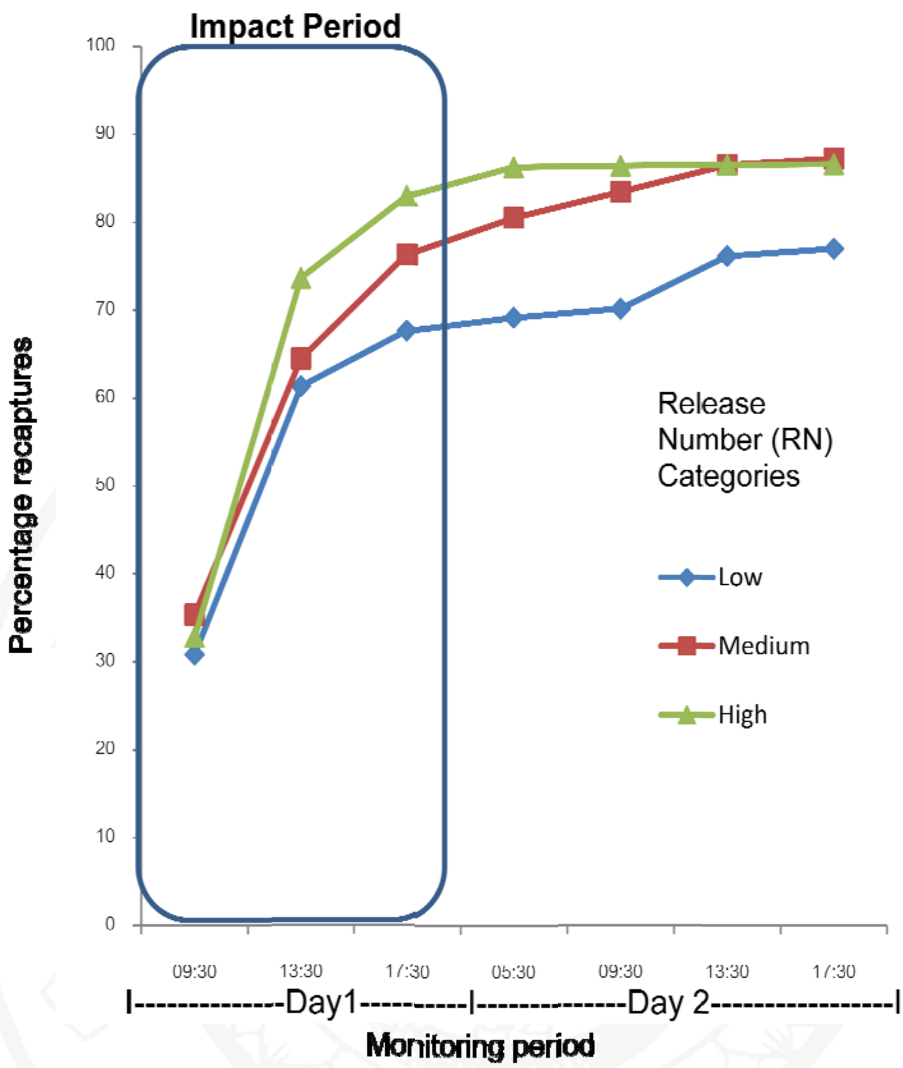

Figure 20 Cumulative percentage recpature of marked *Ae. aegypti* females using one BG-Sentinel™ traps against low, medium and high mosquito release densities . The Impact Period designates the monitoring interval when the greatest recapture occurred.

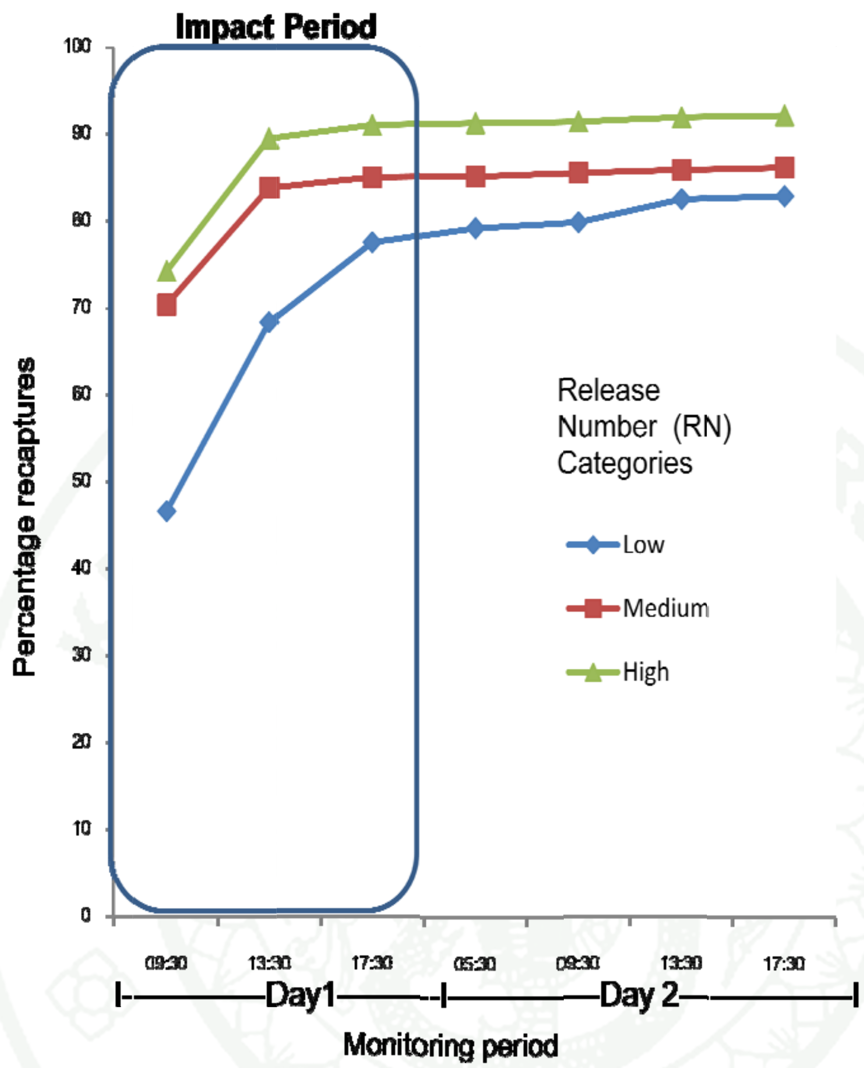

Figure 21 Cumulative percentage recpature of marked *Ae. aegypti* females using BG-Sentinel™ traps against low, medium and high mosquito release densities . The Impact Period designates the monitoring interval when the greatest recapture occurred.

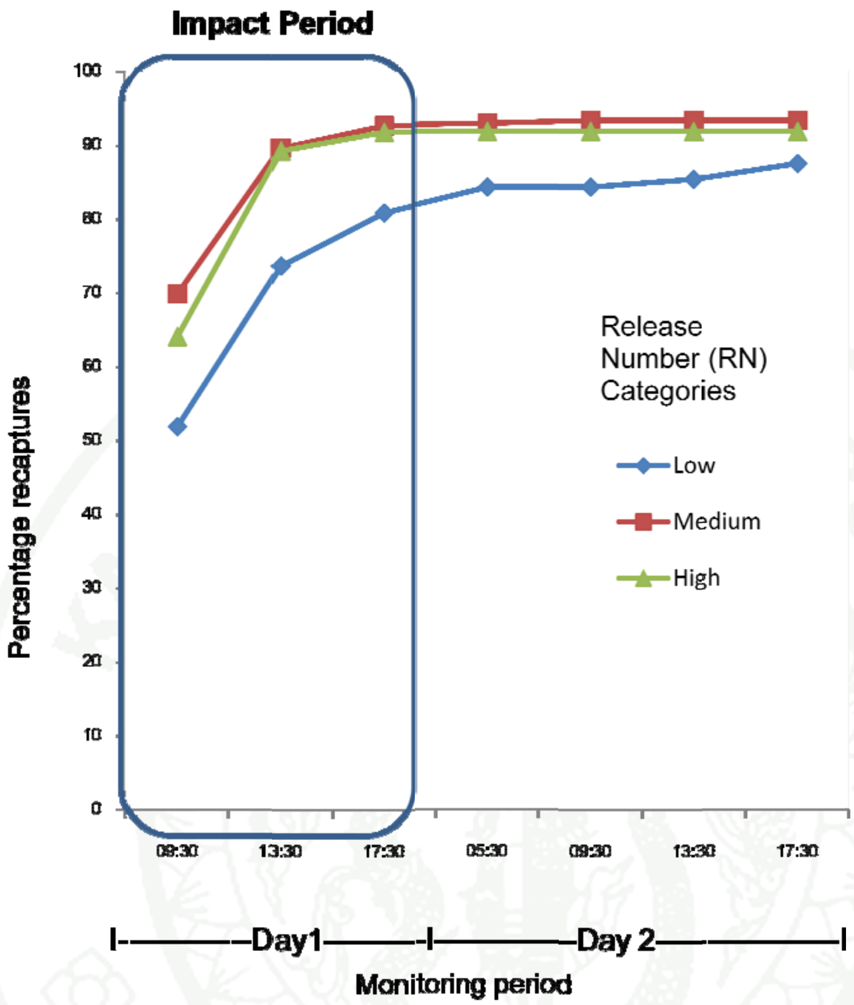

Figure 22 Cumulative percentage recpature of marked *Ae. aegypti* females using three BG-Sentinel™ traps against low, medium and high mosquito release densities . The Impact Period designates the monitoring interval when the greatest recapture occurred.

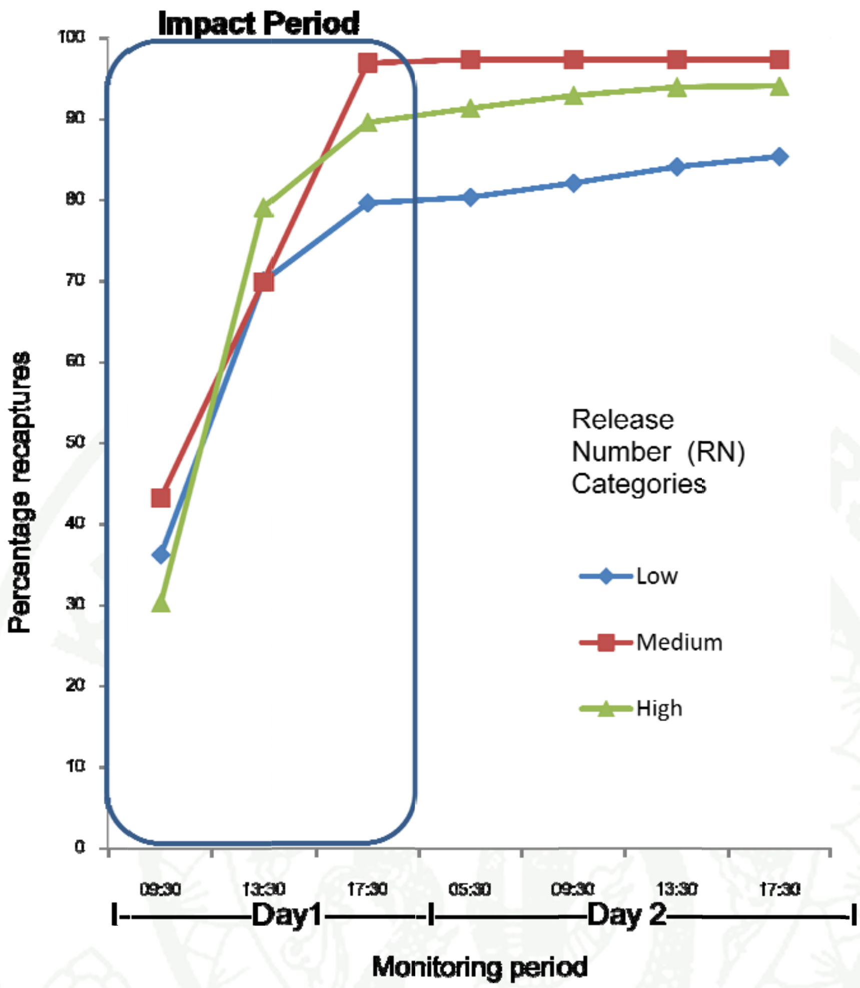

Figure 23 Cumulative percentage recpature of marked *Ae. aegypti* females using four BG-Sentinel™ traps against low, medium and high mosquito release densities . The Impact Period designates the monitoring interval when the greatest recapture occurred.

2. Phase II-Experimental Hut Trials

2.1 Effect of Chemical Exposure on BGS Recaptures

2.1.a DDT

Evaluations using DDT at 1.0 FAR (2 g ai/m^2) and 75%SAC (Figure 24, Table 9) or 50% SAC (Figure 25, Table 10) indicate no significant difference in BGS recapture rates between control (no chemical exposure) and treatment cohorts (exposed to chemical) for either IR or DR populations. Total cumulative BGS recapture ranged from 90-93% for the control cohorts and 92% from treatment cohorts using 75% SAC (Figure 24, Table 9), while recaptures ranged from 85-86% and 86-90% from control and treatment cohorts using 50% SAC, respectively (Figure 25, Table 10). Using 25% SAC, BGS cumulative recaptures ranged from 91-96%, 92-95%, 93-94% for control, 1.0 and 1/2 FAR (1g *ai*/m^2) treatment cohorts, respectively (Figure 26, Table 11). There was no signifant difference ($p>0.05$) between environmental variables between control and treatment set-ups. Mean daily temperature, relative humidity and light intensity in the screen house are also shown in respective tables.

2.1.b Metofluthrin

Results from metofluthrin experiments indicate no significant differences between control and treatments cohorts (blank and chemical) using high dose (0.00625%) and low (0.00312%) dose coils for either IR or DR exposure populations (Figures 27-28, Tables 12-13). Significantly higher recaptures, however, were obtained from DR populations as compared to IR populations using both low and high dose coils (Figure 27-28, Tables 12-13). Cumulative recaptures ranged from 77-93%, 73-90% and 80-85% for control, blank and metofluthrin low dose exposure populations; respectively (Figure 27, Table 12). Cumulative recaptures ranged from 87-93%, 83-85%, and 92 -93% for control, blank and high dose metofluthrin exposure populations, respectively (Figure 28, Table 13).

2.1.c Transfluthrin

The use of 1.0 FAR (40 µg *ai*/cm^2) transfluthrin at 100, 50 and 25% SAC showed very high mortality rates ranging from 95 (143/150) -100% (150/150) upon exposure to treated huts which prevented post-exposure BGS recapture evaluation. Trials using the lower doses of 0.125 FAR (5 µg *ai*/cm^2) and 0.0625 FAR (2.5 µg *ai*/cm^2) using 25% SAC did not have the same effect. Results from these trials indicate that IR populations had significantly lower recaptures compared to control cohorts (Figure 29, Table 14). In addition, significantly lower *Ae. aegypti* BGS recapture was recorded from 0.125 FAR (45%) compared to 0.0625 FAR (76%) (Kruskal Wallis, $p=0.01$). Like metofluthrin coil treatments, significantly higher recaptures were obtained from DR exposed cohorts as compared to IR populations (Figure 29, Table 14). However, there was no significant difference between DR controls (94%) and treatment populations for either 0.125 (71%) or 0.0625 FAR (90%) exposure levels (Table 14).

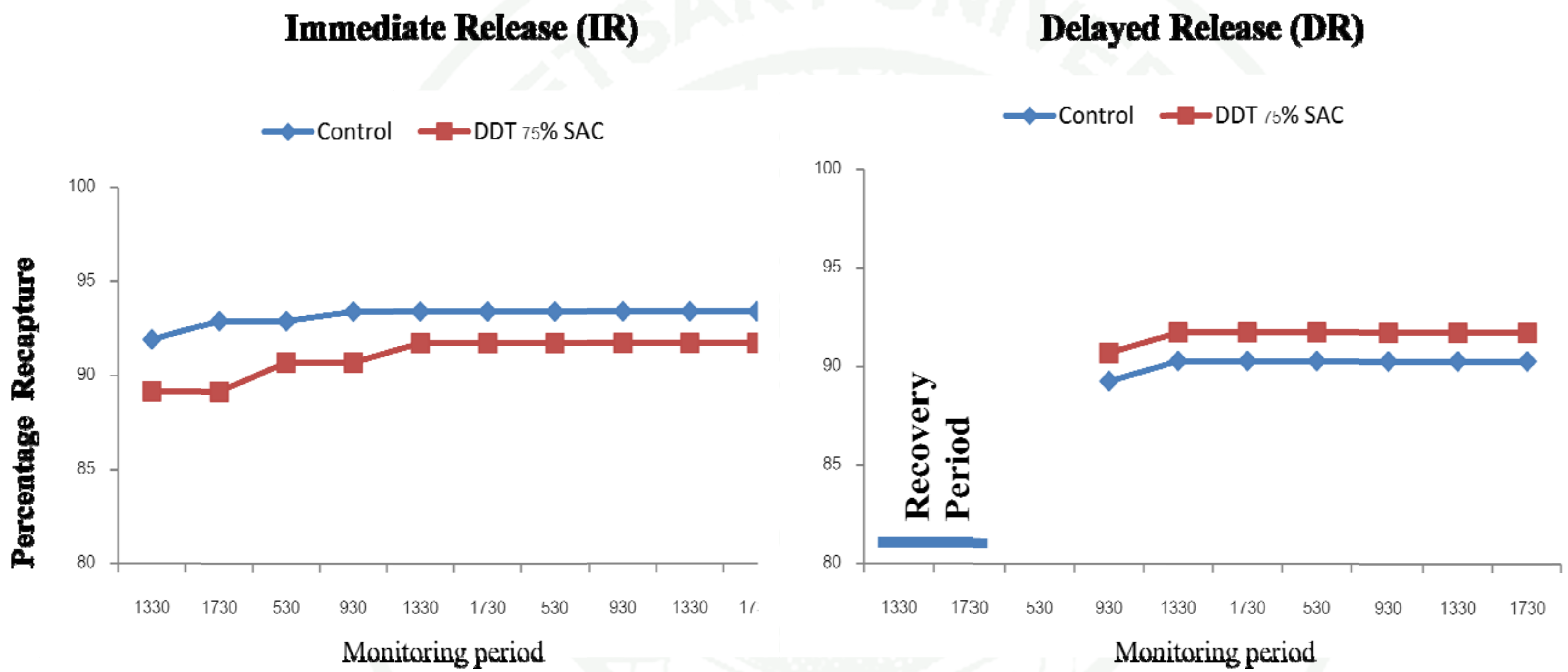

Figure 24 *Aedes aegypti* BG-Sentinel™ trap recaptures rates from immediate (IR) and delayed release (DR) populations following chemical exposure to DDT at 1.0 field application rate (2g/m^2) and 75% surface area coverage.

Table 9 Cumulative BG-Sentinel™ trap recapture rates from immediate (IR)[1] and delayed (DR)[2] releases of *Ae. aegypti*[3] exposed to 75% surface area coverage of 1.0 FAR (2g *ai*/m^2) DDT in treatment and control experimental huts.

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]											Mean Day-Time (12hr) Climatic Data		
	←-----Day 1-----→		←---------------Day 2-----------------→				←-------------------Day 3------------------→							
	1330h	1730h	530h	930h	1330h	1730h	530h	930h	1330h	1730h	N[6]	Temperature (^{o}C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
IRa														
Control	91.9a (±4.5)	92.9a (±3.5)	92.9a (±3.5)	93.4a (±3.5)	93.4a (±3.5)	93.4a (±3.5)	-	-	-	-	185/198	33.4	25.9	382.1
DDT (2g *ai*/m^2)	89.2a (±5.3)	89.2a (±5.3)	90.7a (±4.1)	90.7a (±4.1)	91.8a (±3.8)	91.8a (±3.8)	-	-	-	-	178/194	31.9	28.6	331.6

[1] Cohort exposed from 0600-1200 hours, released immediately afterwards
[2] Cohort exposed from 1200-1800 hours, release after a total holding period of 12 hours
[3] 3-5 days old starved females
[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

Table 9 (Continued)

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]											Mean Day-Time (12hr) Climatic Data		
	←-----Day 1-----→		←---------------Day 2-----------------→				←-------------------Day 3------------------→							
	1330h	1730h	530h	930h	1330h	1730h	530h	930h	1330h	1730h	N[6]	Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
DRa														
Control				89.3a	90.3a	90.3a	90.3a	90.3a	90.3a	90.3a	177/196	33.5	25.9	371.7
	-	-	-	(±4.8)	(±3.7)	(±3.7)	(±3.7)	(±3.7)	(±3.7)	(±3.7)				
DDT				90.7a	91.8a	91.8a	91.8a	91.8a	91.8a	91.8a	178/194	31.9	28.4	318.8
(2g *ai*/m^2)	-	-	-	(±5.2)	(±3.8)	(±3.8)	(±3.8)	(±3.8)	(±3.8)	(±3.8)				

[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

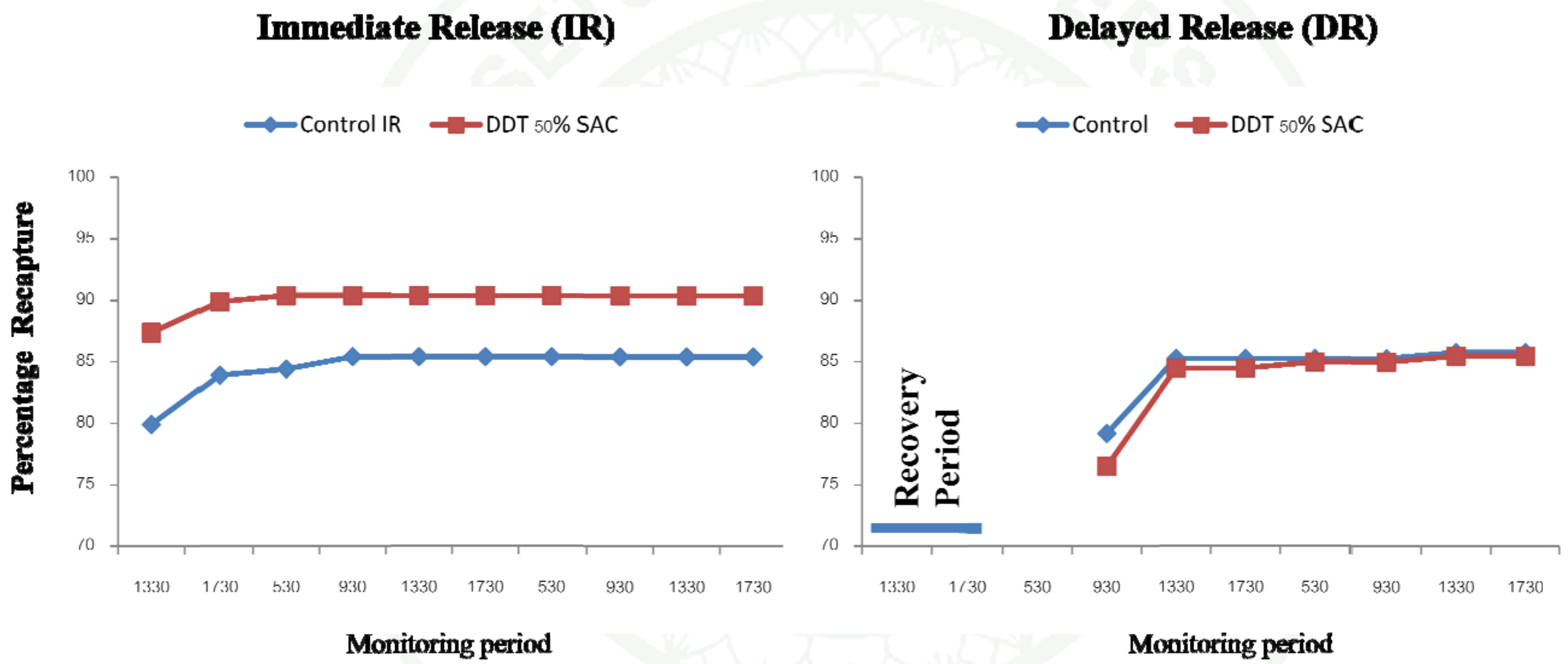

Figure 25 *Aedes aegypti* BG-Sentinel^{TM} trap recaptures rates from immediate (IR) and delayed releases (DR) populations following chemical exposure to DDT at 1.0 field application rate (2g/m^2) and 50% surface area coverage.

Table 10 Cumulative BG-Sentinel™ trap recapture rates from immediate (IR)[1] and delayed (DR)[2] releases of *Ae. aegypti*[3] exposed to 50% surface area coverages of 1.0 FAR (2g *ai*/m^2) DDT in treatment and control experimental huts.

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]											Mean Day-Time (12hr) Climatic Data		
	←-----Day 1-----→		←---------------Day 2-----------------→				←---------------Day 3----------------→							
	1330h	1730h	530h	930h	1330h	1730h	530h	930h	1330h	1730h	N[6]	Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
IRa														
Control	79.9a (±10.6)	83.9a (±4.9)	84.4a (±5.9)	85.4a (±5.5)	85.4a (±5.5)	85.4a (±5.5)	-	-	-	-	170/199	28.7	31.9	479.0
DDT (2g *ai*/m^2)	87.4a (±9.6)	89.9a (±7.7)	90.4a (±7.7)	90.4a (±7.7)	90.4a (±7.7)	90.4a (±7.7)	-	-	-	-	178/198	29.7	69.1	454.5

[1] Cohort exposed from 0600-1200 hours, released immediately afterwards
[2] Cohort exposed from 1200-1800 hours, release after a total holding period of 12 hours
[3] 3-5 days old starved females
[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

Table 10 (Continued)

Release[5] /Treatments	←-----Day 1-----→ 1330h	1730h	←-------Day 2-------→ 530h	930h	1330h	1730h	←-------Day 3-------→ 530h	930h	1330h	1730h	N[6]	Mean Day-Time (12hr) Climatic Data: Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]													
DRa														
Control	-	-	-	79.2a (±10.8)	85.3a (±5.9)	85.3a (±5.9)	85.3a (±5.9)	85.3a (±5.9)	85.8a (±6.3)	85.8a (±6.3)	169/197	30.8	66.7	494.2
DDT (2g *ai*/m^2)	-	-	-	76.5a (±11.1)	84.5a (±3.4)	84.5a (±3.4)	85.0a (±3.5)	85.0a (±3.5)	85.5a (±3.5)	85.5a (±3.5)	171/200	29.2	71.8	501.2

[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

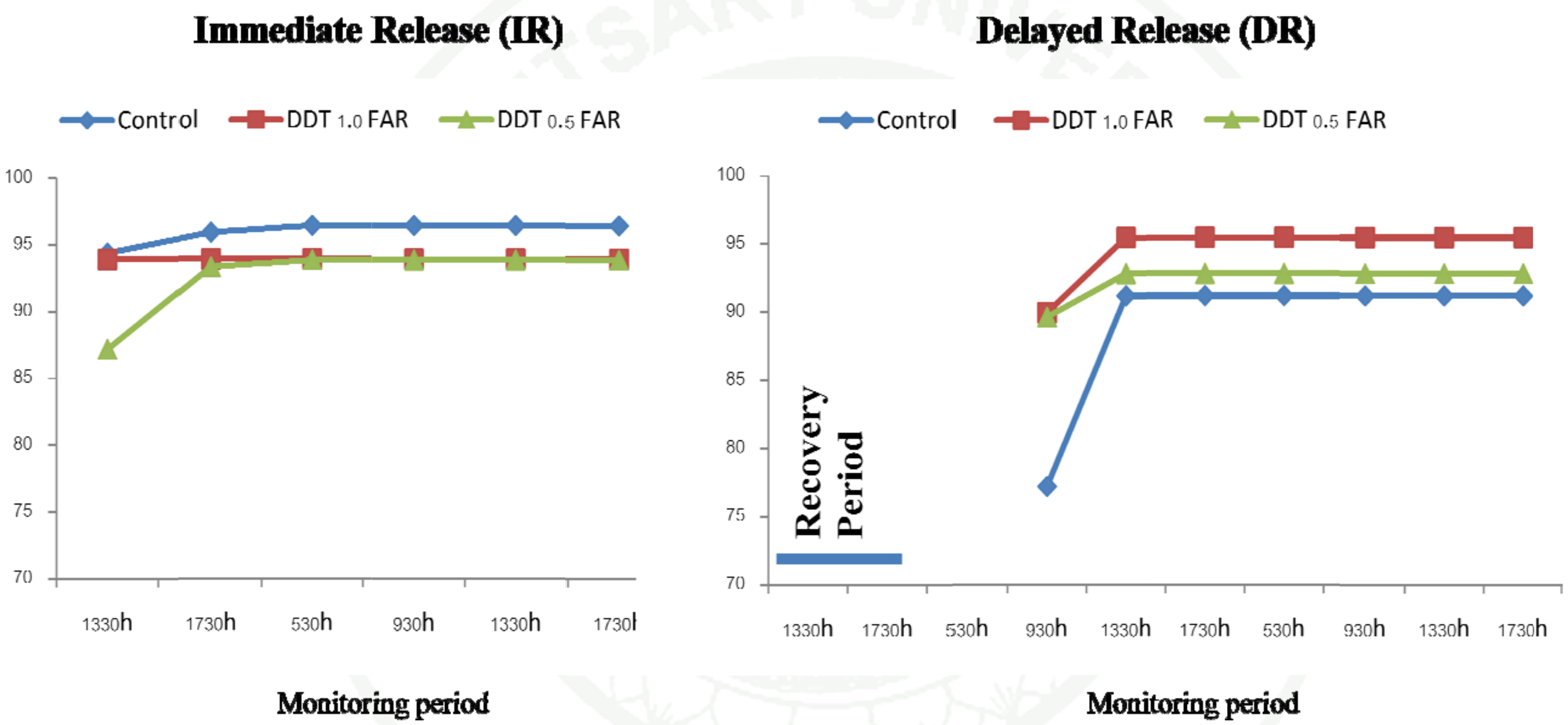

Figure 26 *Aedes aegypti* BG-Sentinel™ trap recaptures rates from immediate (IR) and delayed releases (DR) populations following chemical exposure to 25% surface area coverage of DDT at DDT at 1.0 (2g/m^2) and 0.5 (1g/m^2) field application rates.

Table 11 Cumulative BG-Sentinel™ trap recapture rates from immediate (IR)[1] and delayed (DR)[2] releases of *Ae. aegypti*[3] exposed to 25% surface area DDT in treatment in varying field application rates (FAR) and control experimental huts.

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]											Mean Day-Time (12hr) Climatic Data		
	←-----Day 1-----→		←---------------Day 2-----------------→				←-------------------Day 3------------------→							
	1330h	1730h	530h	930h	1330h	1730h	530h	930h	1330h	1730h	N[6]	Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
IRa														
Control	94.4a (±4.7)	95.9a (±4.5)	96.4a (±4.6)	96.4a (±4.6)	96.4a (±4.6)	96.4a (±4.6)	-	-	-	-	189/196	28.1	24.4	192.16
1.00 FAR (2g *ai*/m^2)	93.9a (±3.0)	93.9a (±3.0)	93.9a (±3.0)	93.9a (±2.9)	93.9a (±3.0)	93.9a (±3.0)	-	-	-	-	185/197	27.4	26.0	126.2
0.50FAR (1g *ai*/m^2)	87.2a (±14.6)	93.3a (±3.9)	93.9a (±3.6)	93.9a (±3.6)	93.9a (±3.6)	93.9a (±3.6)					183/195	27.1	31.7	76.4

[1] Cohort exposed from 0600-1200 hours, released immediately afterwards
[2] Cohort exposed from 1200-1800 hours, release after a total holding period of 12 hours
[3] 3-5 days old starved females
[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

Table 11 (Continued)

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]										N[6]	Mean Day-Time (12hr) Climatic Data		
	←-----Day 1-----→		←---------------Day 2----------------→				←------------------Day 3------------------→							
	1330h	1730h	530h	930h	1330h	1730h	530h	930h	1330h	1730h		Tempe rature (°C)	Relative Humi dity (%)	Light Intensity (lx/ft^2)
DRa														
Control	-	-	-	77.2a (±8.9)	91.2a (±3.7)	91.2a (±3.7)	91.2a (±3.7)	91.2a (±3.7)	91.2a (±3.7)	91.2a (±3.7)	176/193	28.4	25.3	205.5
1.00 FAR (2g *ai*/m^2)	-	-	-	90.0a (±5.4)	95.5a (±2.6)	95.5a (±2.6)	95.5a (±2.6)	95.5a (±2.6)	95.5a (±2.6)	95.5a (±2.6)	190/199	27.8	26.6	135.2
0.50FAR (1g *ai*/m^2)	-	-	-	89.6a (±4.7)	92.8a (±4.1)	92.8a (±4.1)	92.8a (±4.1)	92.8a (±4.1)	92.8a (±4.1)	92.8a (±4.1)	181/195	27.5	32.8	85.6

[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

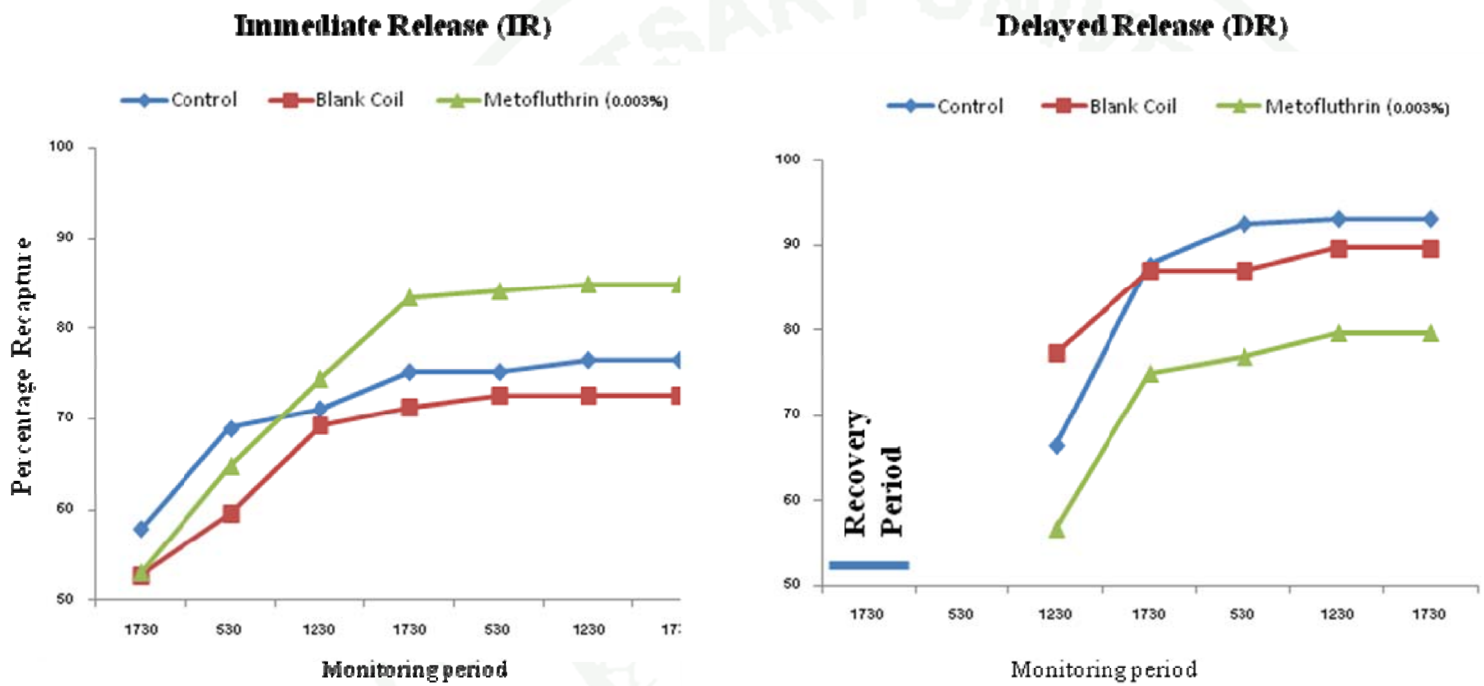

Figure 27 *Aedes aegypti* BG-Sentinel™ trap recaptures rates from immediate (IR) and delayed releases (DR) populations following chemical exposure to low dose (0.0031%) metofluthrin coils and blank coils.

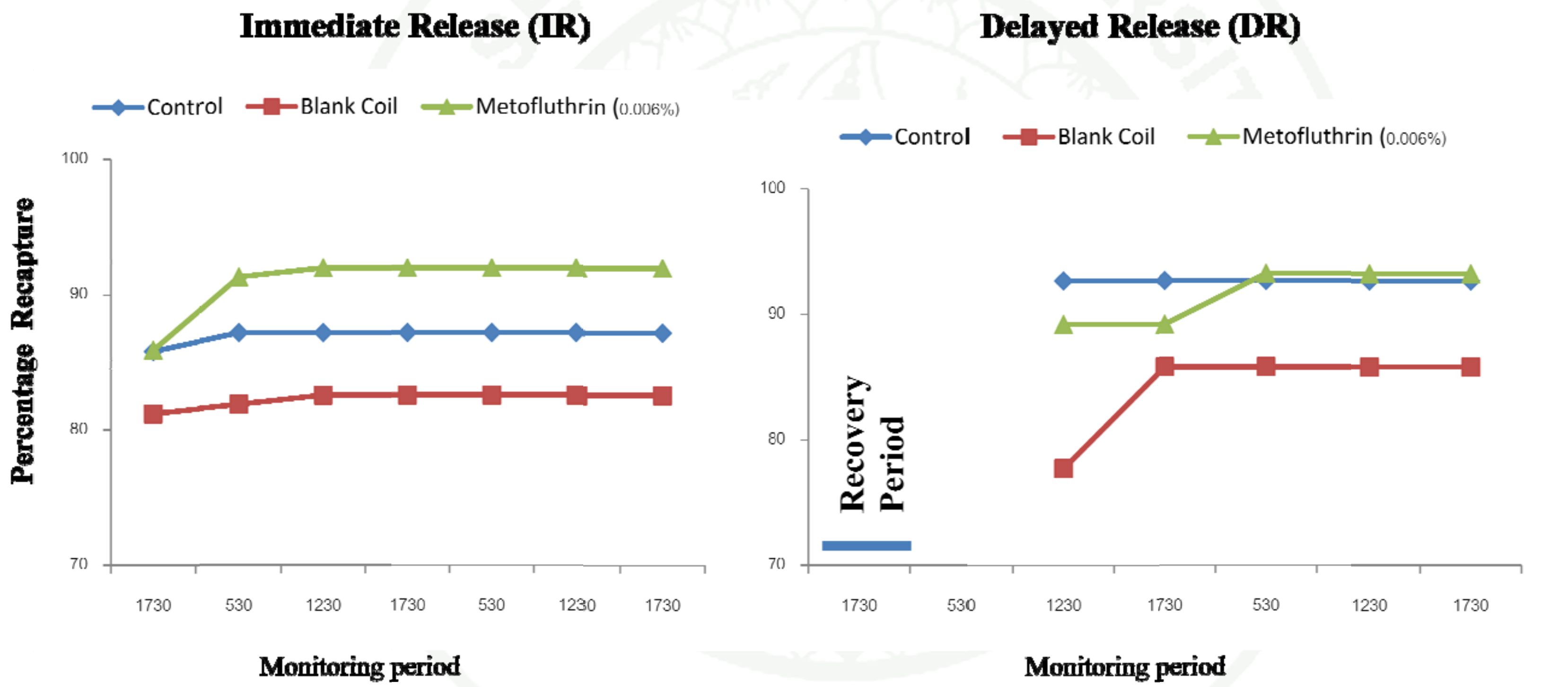

Figure 28 *Aedes aegypti* BG-Sentinel^{TM} trap recaptures rates from immediate (IR) and delayed releases (DR) populations following chemical exposure to high dose (0.006%) metofluthrin coils and blank coils.

Table 12 Cumulative BG-Sentinel™ trap recapture rates from immediate (IR)[1] and delayed (DR)[2] releases of *Ae. aegypti*[3] exposed to Metofluthrin low dose (0.003%) coil treatment and control experimental huts.

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]: Day 1 1730h	Day 2 0530h	Day 2 1230h	Day 2 1730h	Day 3 0530h	Day 3 1230h	Day 3 1730h	N[6]	Mean Day-Time (12hr) Climatic Data: Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft²)
IRa											
Control	57.9a (±5.6)	69.0a (±6.5)	71.0a (±6.3)	75.2a (±7.9)	75.2a (±7.9)	76.6a (±7.9)	76.6a (±7.9)	111/145	24.9	60.2	167.6
Blank Coil	52.7a (±7.9)	59.6a (±13.6)	69.2a (±18.7)	71.2a (±6.3)	72.6a (±8.7)	72.6a (±8.7)	72.6a (±8.7)	106/146	25.2	59.9	177.9
Metofluthrin	53.1a (±11.0)	64.8a (±8.2)	74.2a (±8.9)	83.5a (±1.3)	84.2a (±0.3)	84.8a (±1.1)	84.8a (±1.1)	123/145	24.1	61.8	66.6

[1] Cohort exposed from 0600-1200 hours, released immediately afterwards
[2] Cohort exposed from 1200-1800 hours, release after a total holding period of 12 hours
[3] 3-5 days old starved females
[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

Table 12 (Continued)

Release[5] /Treatments	Day 1	←Day 2		→	←Day 3		→		Mean Day-Time (12hr) Climatic Data		
	1730h	0530h	1230h	1730h	0530h	1230h	1730h	N[6]	Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
DRb											
Control	-	-	66.4a (±18.8)	87.7a (±9.5)	92.5a (±2.6)	93.2a (±3.4)	93.2a (±3.4)	136/146	26.2	63.0	178.5
Blank Coil	-	-	77.2a (±14.6)	86.9a (±3.7)	86.9a (±3.7)	89.9a (±1.7)	89.9a (±1.7)	130/145	26.3	62.7	173.9
Metofluthrin	-	-	56.6a (±7.4)	74.8a (±10.3)	76.9a (±11.5)	79.7a (±13.8)	79.7a (±13.9)	113/143	25.2	65.7	61.5

Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]

[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

Table 13 Cumulative BG-Sentinel™ trap recapture rates from immediate (IR)[1] and delayed (DR)[2] releases of *Ae. aegypti*[3] exposed to Metofluthrin high dose (0.006%) coil treatment and control experimental huts.

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]: Day 1 1730h	Day 2 0530h	Day 2 1230h	Day 2 1730h	Day 3 0530h	Day 3 1230h	Day 3 1730h	N[6]	Mean Day-Time (12hr) Climatic Data: Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
IRa											
Control	85.8a (±5.3)	87.2a (±5.8)	87.2a (±5.8)	87.2a (±5.8)	87.2a (±5.8)	87.2a (±5.8)	87.2a (±5.8)	123/148	28.1	65.6	167.6
Blank Coil	81.2a (±8.9)	81.9a (±7.9)	82.6a (±6.9)	82.55a (±7.0)	82.6a (±6.9)	82.6a (±6.9)	82.6a (±6.9)	123/149	28.0	65.8	185.1
Metofluthrin	85.9a (±6.0)	91.3a (±1.1)	92.0a (±1.9)	92.0a (±1.9)	92.0a (±1.9)	92.0a (±1.9)	92.0a (±1.9)	137/149	26.7	69.2	63.7

[1] Cohort exposed from 0600-1200 hours, released immediately afterwards
[2] Cohort exposed from 1200-1800 hours, release after a total holding period of 12 hours
[3] 3-5 days old starved females
[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

Table 13 (Continued)

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]							N[6]	Mean Day-Time (12hr) Climatic Data		
	Day 1	←Day 2		→	←Day 3		→				
	1730h	0530h	1230h	1730h	0530h	1230h	1730h		Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft²)
DRb											
Control	-	-	92.7a (±4.2)	92.7a (±4.2)	92.7a (±4.2)	92.7a (±4.2)	92.7a (±4.2)	144/150	28.0	65.8	194.8
Blank Coil	-	-	77.7a (±1.6)	85.8a (±5.4)	85.8a (±5.4)	85.8a (±5.4)	85.8a (±5.4)	126/148	29.7	65.9	186.8
Metofluthrin	-	-	89.2a (±5.4)	89.2a (±5.4)	93.2a (±3.0)	93.2a (±3.0)	93.2a (±3.0)	138/148	26.7	69.4	61.7

[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)

[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)

[6] Total recaptured /total released minus total knock down

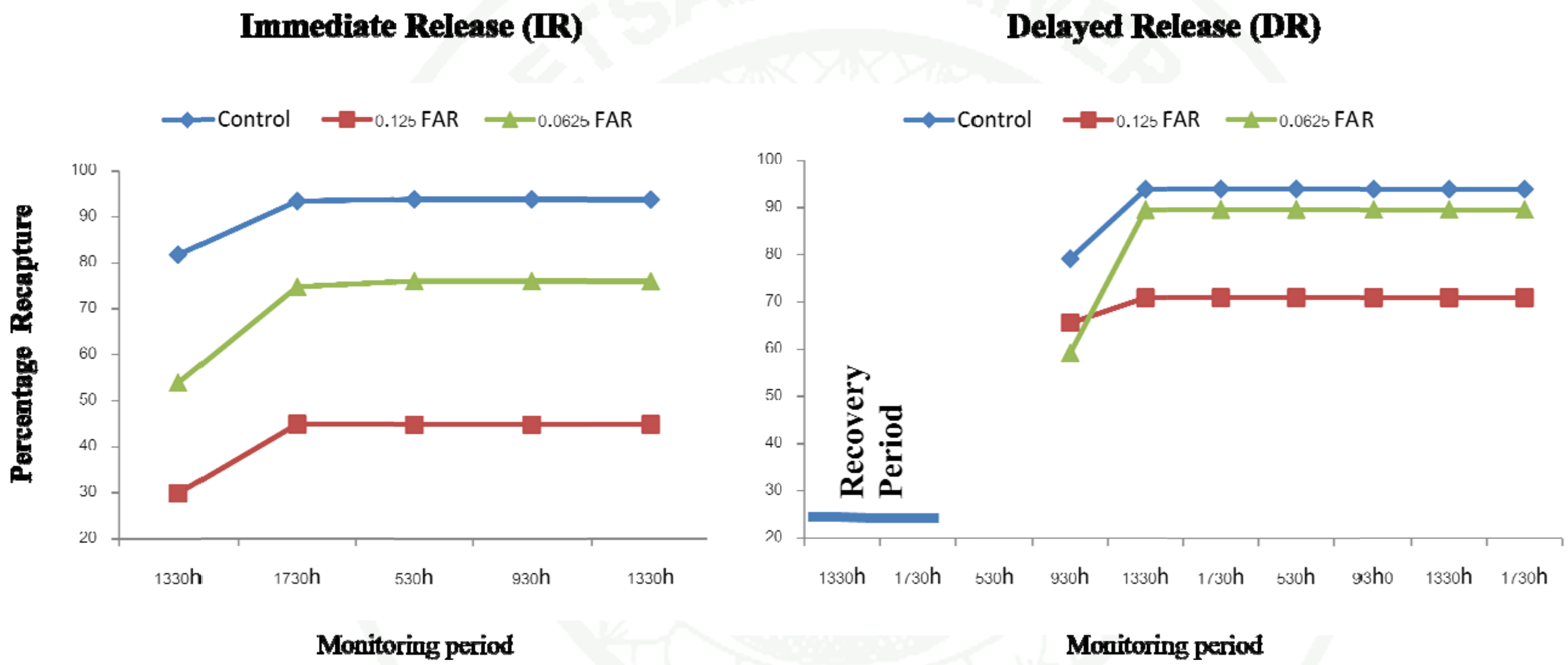

Figure 29 *Aedes aegypti* BG-Sentinel™ trap recaptures rates from immediate (IR) and delayed releases (DR) populations following chemical exposure to 0.125 and 0.625 field application rates of transfluthrin at 25% surface area coverage.

Table 14 Cumulative BG-Sentinel™ trap recapture rates from immediate (IR)[1] and delayed (DR)[2] releases of *Ae. aegypti*[3] exposed to transfluthrin treated with 0.125 (5 µg *ai*/cm^2), and 0.0625 FAR (2.5 µg *ai*/cm^2) and control huts.

Release[5] /Treatments	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]											Mean Day-Time (12hr) Climatic Data		
	←-----Day 1-----→		←---------------Day 2-----------------→				←-----------Day 3----------→							
	1330h	1730h	530h	930h	1330h	1730h	530h	93h0	1330h	1730h	N[6]	Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
IR$_a$														
Control	81.8a (±11.1)	93.3a (±8.6)	93.8a (±7.6)	93.8a (±7.6)	93.8a (±7.6)	93.8a (±7.6)	-	-	-	-	180/192	28.7	77.1	314.7
Transfluthrin (5 µg *ai*/cm^2)	29.9c (±11.8)	44.8c (±18.7)	44.8c (±18.7)	44.8c (±18.7)	44.8c (±18.7)	44.8c (±18.7)	-	-	-	-	39/87	27.2	83.4	97.1
Transfluthrin (2.5 µg *ai*/cm^2)	53.9b (±16.0)	74.7b (±9.3)	76.0b (±9.3)	76.0b (±9.3)	76.0b (±9.3)	76.0b (±9.3)					117/154	26.1	37.1	120.3

[1] Cohort exposed from 0600-1200 hours, released immediately afterwards
[2] Cohort exposed from 1200-1800 hours, release after a total holding period of 12 hours
[3] 3-5 days old starved females
[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)
[6] Total recaptured /total released minus total knock down

Table 14 (Continued)

Release[5] /Treatments	Day 1 1330h	Day 1 1730h	Day 2 530h	Day 2 930h	Day 2 1330h	Day 2 1730h	Day 3 530h	Day 3 93h0	Day 3 1330h	Day 3 1730h	N[6]	Temperature (°C)	Relative Humidity (%)	Light Intensity (lx/ft^2)
	Cumulative mean percentage (±SD) of *Ae. aegypti* recaptured by time point[4]											Mean Day-Time (12hr) Climatic Data		
DR$_b$														
Control	-	-	-	79.2 (±9.4)	93.9 (±4.3)	93.9 (±4.3)	93.9 (±4.3)	93.9 (±4.3)	93.9 (±4.3)	93.9 (±4.3)	185/197	28.0	78.6	308.3
Transfluthrin (5 µg *ai*/cm^2)	-	-	-	65.6 (±18.3)	70.9 (±16.4)	70.9 (±16.4)	70.9 (±16.4)	70.9 (±16.4)	70.9 (±16.4)	70.9 (±16.4)	43/61	26.8	85.8	98.0
Transfluthrin (2.5 µg *ai*/cm^2)	-	-	-	59.2 (±28.1)	89.6 (±4.6)	89.6 (±4.6)	89.6 (±4.6)	89.6 (±4.6)	89.6 (±4.6)	89.6 (±4.6)	92/138	25.7	37.7	126.8

[4] Different lowercase letters in the same column indicate significant differences between mean recapture percentages within exposures either IR or DR (Kruskal-Wallis 95% confidence limit) and between IR and DR (Mann-U Whitney Test, 95% confidence limit)

[5] Different lowercase letters in the same column indicate significant differences between mean recapture percentages between IR and DR (Mann-U Whitney Test, 95% confidence limit)

[6] Total recaptured /total released minus total knock down

2.2. Location and Distance Effects

2.2.1 Location.

Results indicate 39% greater recapture of *Ae. aegypti* females from BGS traps placed opposite portals of entry (windows/doors) compared to those BGS traps positioned at vertices of experimental huts (Table 15). A mean recapture of 38.7% (116/300) was recorded from the four BGS placed at portals of entry as compared to 23.67% (71/300) from the BGS on vertices. No significant differences however were found between recaptures from the two locations. Individual BGS contributions based on specific locations among the portals of entries are as follows: Window 1=38% (44/116), Door=38% (44/116), Window 2=20% (23/116) and Window 4=4% (5/116) (Figure 7). Highest BGS recapture was recorded from the 0930 h sampling period for all trials (Table 15). Corresponding data from IT collections showed greater reduction in *Ae. aegypti* entry into the experimental huts as compared to a control when BGS traps were located opposite portals of entry (69%, 37/300) compared to when BGS were located at vertices (31%) 82/300 (Table 16).

2.2.2 Distance.

Overall, highest BGS recapture of 18.5% (111/600) and 14.2% (128/900) were obtained from 0 m and 10 m distances, respectively (Table 17). Lowest recapture of 7.89% (71/900) was recorded from the 3 m distance. Interception trap data from experimental huts showed that BGS traps positioned at a 0m distance ensued highest percentage reduction in *Ae. aegypti* entry (65.6%) as compared to the control versus that observed at the 3 m (17.19%) and 10 m (14.59%) distance trials (Table 18). Combining data from optimum location (portals of entry) and distance (0m) identified, BGS recapture of *Ae. aegypti* at specific locations are as follows : Window 1=40% (90/227), Door=32% (72/227), Window 2=20% (46/227) and Window 3=8% (19/227) (Figure 7 and 30).

Table 15 Total *Ae. aegypti* [1] BG-Sentinel™ trap recapture rates from vertices and opposite portals of entry (window/door) of chemical free experimental huts located in Pu Teuy, Kanchanaburi, Thailand.

Treatment/ LOCATION	Time	DAY1	DAY2	DAY3	MEAN % RECAPTURE[2] (N=300)
Portals of Entry (Windows and Door)	0530	-	0	0	0.0 (0)
	0930	37	21	54	37.3 (112)
	1330	0	2	0	0.7 (2)
	1730	1	0	1	0.7 (2)
	Total	38/100	23/100	55/100	38.7a (116)
Vertices	0530	-	0	0	0.0 (0)
	0930	13	23	34	23.3 (70)
	1330	1	0	0	0.3 (1)
	1730	0	0	0	0.0(0)
	Total	14/100	23/100	34/100	23.7a (71)

[1] 3-5 days old starved females

[2] Different lowercase letters among the total recaptures rates between locations indicate significant differences between mean recapture percentages [1] (Kruskal Wallis, 95% confidence limit)

Table 16 Recapture rates of *Aedes aegypti*[1] marked populations from interception traps (IT) fitted onto chemical-free experimental huts in conjunction with BG-Sentinel™ trap collections positioned at varying locations from the host-occupied space.

Treatment	No. of marked mosquitoes			Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	No BGS	BGS (vertices)	BGS (portals of entry)								
No BGS	108/300 36.0%	6/300 2.0%	5/300 1.7%	119/900 13.2%	11/600 1.8%	1-(108/108) = 0.0%	1-(119/119) 100-100 = 0.0%	29.9 ± 0.5	31.1 ±0.7	67.6 ±2.3	62.4 ±2.8
BGS (vertices)	10/300 3.3%	61/300 20.3%	11/300 3.7%	82/900 9.1%	22/600 3.7%	1-(61/108) =43.5%	1-(82/119) =31.1%	30.1 ± 0.5	31.0 ± 0.7	67.1 ±2.3	62.4 ±2.8
BGS (portals of entry)	5/300 1.7%	3/300 1.0%	29/300 9.7%	37/900 4.1%	8/600 1.3%	1-(29/108) =73.2%	1-(37/119) =68.9%	29.9 ±0.5	31.1 ± 0.7	67.7 ±2.2	62.4 ±2.8

[1]3-5 days old starved females

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps was computed by: ***%RE*** = 1- [recaptures from (location treatments) divided by (÷) recaptures from control] x 100.

Table 17 Summary of *Ae. aegypti* BG-Sentinel™ trap recapture rates from varying distances away from the portals of entry of chemical free experimental huts. Pu Teuy, Kanchanaburi, Thailand.

Distance	Time	Trial 1	Trial 2	Trial 3	MEAN % RECAPTURE[2] (n/N)[3]
Om	0530	0	-	3	0.50(3/600)
	0930	83	-	7	15.0 (90/600)
	1330	2	-	11	2.2 (13/600)
	1730	2	-	3	0.8 (5/600)
	Total	**87/300**		**24/300**	**18.5 a (111/600)**
3.0m	0530	0	3	0	0.3(3/900)
	0930	26	31	1	6.4(58/900)
	1330	3	2	2	0.8(7/900)
	1730	1	0	2	0.3(3/900)
	Total	**30/300**	**36/300**	**5/300**	**7.9a (71/900)**
10.0m	0530	0	0	1	0.1(1/900)
	0930	63	34	3	11.1(100/900)
	1330	3	6	3	1.3(12/900)
	1730	5	2	8	1.7(15/900)
	Total	**71/300**	**42/300**	**15/300**	**14.2a(128/900)**

[1] 3-5 days old starved females

[2] Different lowercase letters among the total recaptures rates between locations indicate significant differences between mean recapture percentages [1] (Kruskal Wallis, 95% confidence limit) (n=total number recaptured/N=total number released)

[3] n=total number captured/N=total number released

Table 18 Recapture rates of *Aedes aegypti*[1] marked population from interception trap (IT) fitted onto chemical-free experimental huts in conjunction withwhen BG-Sentinel™ traps are placed at varying distance away from the portals of entry.

Treatment	No. of Marked Mosquitoes [4]			Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Trial 1	Trial 2	Trial 3								
NO BGS	-	91/300 30.3%	-	91/300 30.3%	10/600 1.7%	1-(30.33/ 30.33) = 0.0%	1-(32/32) = 0.0%	28.46 ± 0.5	27.31 ±0.5	75.54 ±2.1	84.97 ±2.3
BGS 0 m.	49/300 16.3%	-	11/300 3.7%	60/600= 10.0%	12/1200= 1.0%	1-(10/30.33) = 67.0%	1-(11/32) =65.6%	29.1 ± 0.5	28.0 ±0.5	74.0 ±2.4	80.5 ±2.7

[1]3-5 days old starved females

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps was computed by: ***%RE*** = 1- [recaptures from (distance treatments) divided by (÷) recaptures from control] x 100.

Table 18 (Continued)

Treatment	No. of Marked Mosquitoes [4] Trial 1	Trial 2	Trial 3	Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
BGS 3 m.	101/300 33.7%	85/300 28.3%	24/300 8.00%	210/900= 23.3%	57/1800= 3.2%	1- (23.33/30.33) .=23.1%	1- (26.5/32) =17.2%	29.05 ± 0.52	28.0 ±0.5	73.4 ±2.3	80.5 ±2.7
BGS 10 m.	88/300 29.3%	116/300 38.7%	16/300 5.3%	220/900= 24.4%	52/1800= 2.9%	1- (24.44/30.33) =19.4%	1 -(27.33/32) =14.6%	29.0 ±0.5	28.0 ±0.51	74.5 ±2.4	80.5 ±2.7

[2] Total number of marked mosquitoes coming from other huts; Total diversion from all trials

[3] Total number recaptured from the control served as basis for computation of reduction in entry (RE) where ***0% RE*** is assumed= [1- (%recaptures from control ÷ **%**recaptures from control)]. Percentage reduction in entry ***(%RE)*** from interception traps for each treatments (0m, 3m and 10m distances) was computed by: ***%RE*** = 1- [%recaptures from (0m, 3m and 10m distance treatments) divided by (÷) %recaptures from control] x 100.

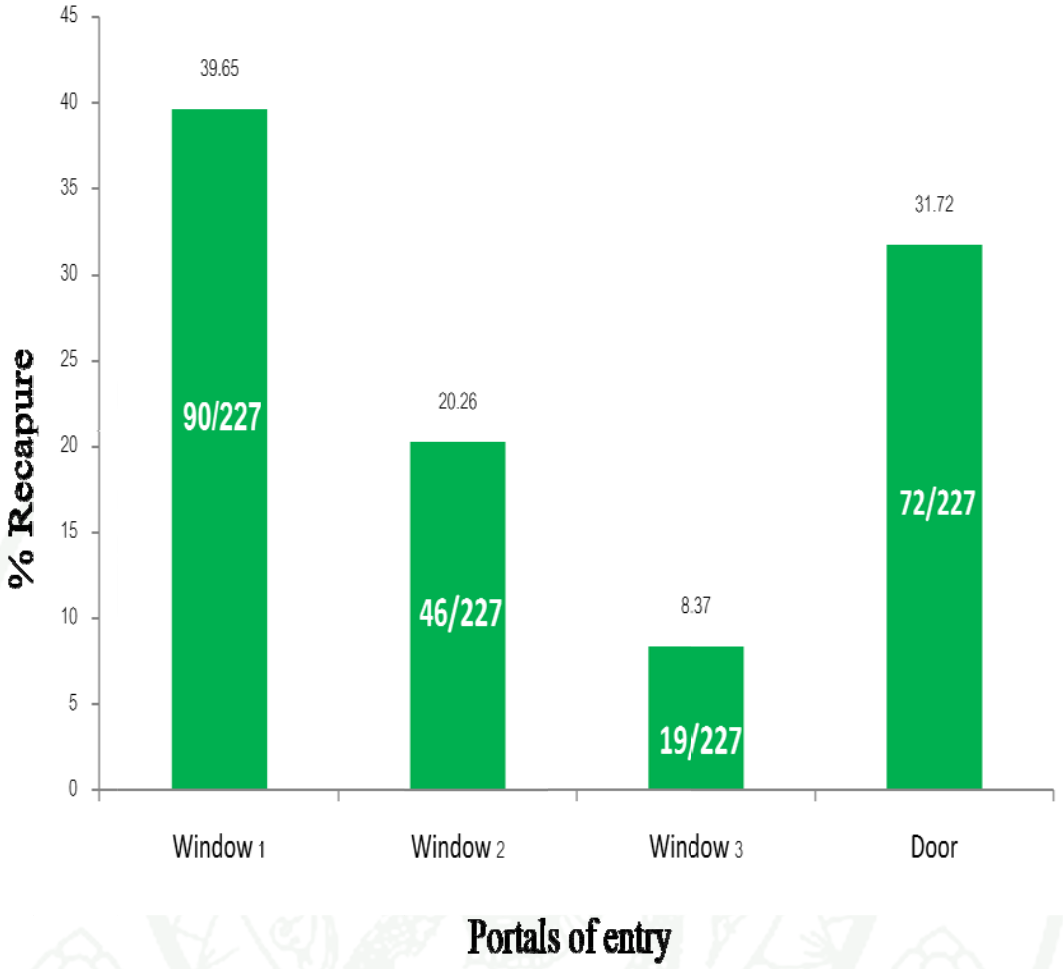

Figure 30. *Aedes aegpti* BG-sentinel ™ trap recapture rates from different portals of entry location during experimental hut trials at 0m distance.

2.3. Contribution of BGS Traps to PPS Experimental Hut Studies.

Overall, the use of BGS traps alone recaptured a range of 27.0% (109/400) to 40.0% (160/400) with a mean of 32.7% (490/1500) (Table 19, Figure 31, Table 24). A BGS mean recapture rates of 32.7% was recorded when BGS traps were used alone and 28.1% when used in combination with a SR (Table 19). There was no statistically significant difference observed from *Ae. agypti* BGS mean recapture rates from the two treatment conditions (*p.>0.05*). The highest BGS recapture (33.0%) was recorded during the BGS-metofluthrin coil (PPS) combination trial (Table 19 and Table 24). Highest recapture from BGS traps alone treatment occurred during transfluthrin 0.125 FAR polyester fabric trials.

For all PPS evaluations (combined for both metofluthrin and transfluthrin trials) , the overall mean percent total reduction in *Ae. agypti* entry into the experimental huts (i.e., IT data) was as follows: 1) 48.5% when BGS were used alone, 2) 42.8% when SR was used alone and, 3) 76.5% from a combination treatment (BGS+SR) (Table 24). The overall means for transfluthrin PPS evaluations specifically were: 1) 51.6% BGS alone; 2) 50.9% for SR alone and, 3) 78.9% for a combined SR and BGS treatment (Table 24).

Data from IT collection showed consistently greater reduction in *Ae. aegypti* entry into the experimental huts when using the combination of BGS with a SR compared to when each tool was used separately (Tables 20-23, Figure 32). The percentage total reduction in entry from SR (push) treatment alone ranged from 17-79%, with transfluthrin 0.125 FAR pink lace fabric eliciting the strongest effects (78.6%) and metofluthrin coil 0.01% the lowest effect on reduction (16.5%). With the use of BGS (pull) alone, the total reduction in entry ranged from 41-59% again with transfluthrin (polyester) having a greatest impact (58.9%) and metofluthrin coil at 0.01% the lowest (Table 24, Figure 32). For PPS (SR+BGS) combination treatment, the percentage reduction in entry ranged from 66 -87% with transfluthrin treated lace having the strongest impact (87.0%) but transfluthrin coil at 0.03% eliciting the weakest effect (65.8%) (Tables 20-23, Table 24, Figure 32). Overall, SR coil formulations showed percent reduction in *Ae. aegypti* entry ranging from 65-69% while fabric treatment applications showed reduction in entry from 83-87% (Figure 32). Use of same spatial repellent compounds, such as transfluthrin, at similar concentrations (i.e., 0.0125 FAR) but different treatment substrates (polyester vs. lace) showed differences in hut entry of *Ae. aegypti* (Table 24, Figure 32).

Table 19 Summary of BG-Sentinel™ (BGS) trap recapture of *Ae. aegypti*[1] from push-pull experimental hut evaluations using representative spatial repellents.

Treatment	Time	SPATIAL REPELLENTS USED				
		Metofluthrin Coil (0.01%) (N=400)	Transfluthrin Coil (0.03%) (N=400)	Transfluthrin (polyester) 0.125 FAR, 25% SAC (N=400)	Transfluthrin (lace) 0.125 FAR, 25% SAC (N=300)	OVERALL TOTAL N=1500
BGS + Repellent	0500	2(0.50%)	6(1.5%)	0(0.00%)	0(0.00%)	\8 (0.53%)
	0930	121(30.3%)	88(22.0%)	91(22.75%)	44(14.67%)	344 (22.93%)
	1330	7(1.8%)	18(4.5%)	13(3.25%)	14(4.67%)	52 (3.47%)
	1730	2 (0.5%)	5 (1.3%)	3 (0.75%)	8 (2.67%)	18 (1.20%)
	Total	**132(33.0%)**	**117(29.3%)**	**107(26.75%)**	**66(22.00%)**	**422 (28.13%)**

[1]3-5 days old starved females

Table 19 (Continued)

Treatment	Time	SPATIAL REPELLENTS USED				
		Metofluthrin Coil (0.01%) (N=400)	Transfluthrin Coil (0.03%) (N=400)	Transfluthrin (polyester) 0.125 FAR, 25% SAC (N=400)	Transfluthrin (lace) 0.125 FAR, 25% SAC (N=300)	OVERALL TOTAL N=1500
BGS Only	0500	0(0.00%)	5(0.25%)	1(0.25%)	2(0.67%)	8 (0.53%)
	0930	105(26.25%)	109(27.25%)	132(33.00%)	77(25.67%)	423 (28.20%)
	1330	4(1.00%)	16(4.00%)	24(6.00%)	7(2.33%)	51 (0.34%)
	1730	0(0.00%)	3 (0.75%)	3 (0.75%)	2(0.67%)	8 (0.53%)
	Total	**109(27.25%)**	**133(32.25%)**	**160(40.00%)**	**88(29.33%)**	**490 (32.67 %)**

Table 20 Recapture rates of *Aedes aegypti*[1] from interception traps during push-pull experiments using 0.01% metofluthirn coils and BG-Sentinel™ traps in Pu Tuey, Kanchanaburi, Thailand.

Treat-ment	No. of Mosquitoes				Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Hut A Control	Hut B MT Coil (Push)	Hut C MT Coil + BGS (Push-pull)	Hut D BGS (Pull)								
Hut A Control	163/400 40.8%	23/400 5.8%	4/400 1.0%	22/400 5.5%	212/1600 13.3%	49/1200 4.1%	1-(163/163) =0.0%	1-(212/212) =0.0%	27.9 ± 0.38	27.0 ± 0.36	75.68 ±1.82	66.36 ±1.72
Hut B MT Coil (Push)	25/400 6.3%	127/400 31.8%	9/400 2.3%	16/400 4.0%	177/1600 11.1%	50/1200 4.2%	1-(127/163) =22.1%	1- (177/212) =16.5%	28.3 ±0.4	27.0 ± 0.4	73.6 ± 1.8	66.4 ±1.7

[1]3-5 days old starved females

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps for each treatment (MT, BGS and BGS+MT) was computed by:

%RE = 1- [recaptures from (MT, BGS, BGS+MT treatments) divided by (÷) recaptures from control] x 100.

Table 20 (Continued)

Treatment	No. of Mosquitoes				Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Hut A Control	Hut B MT Coil (Push)	Hut C MT Coil + BGS (Push-pull)	Hut D BGS (Pull)								
Hut C MT Coil + BGS (Push-pull)	3/400 0.8%	14/400 3.5%	37/400 9.3%	11/400 2.8%	65/1600 4.1%	28/1200 2.3 %	1-(37/163) =77.3%	1 - (65/212) =69.3%	28.6 ±0.4	27.0 ± 0.4	71.6 ± 1.7	66.4 ±1.7
Hut D BGS (Pull)	9/400 2.3%	9/400 2.3%	10/400 2.5%	97/400 24.3%	125/1600 7.8%	28/1200 2.3%	1-(97/163) =40.5%	1-(125/212) =41.0%	28.6 ±0.4	27.0 ± 0.4	71.1 ± 1.7	66.4 ±1.7

[1]3-5 days old starved females

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps for each treatment (MT, BGS and BGS+MT) was computed by:

%RE = 1- [recaptures from (MT, BGS, BGS+MT treatments) divided by (÷) recaptures from control] x 100.

Table 21 Recapture rates of *Aedes aegypti* from Push-Pull experiment using 0.03% Transfluthrin (TF) coils and BG-Sentinel™ trap in Pu Tuey, Kanchanaburi.

Treatment	No. of Mosquitoes				Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (^{o}C) ±SE	Avg. Temp Out (^{o}C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Hut A Control	Hut B TF Coil (Push)	Hut C TF Coil + BGS (Push-pull)	Hut D BGS (Pull)								
Hut A Control	129/400 32.3%	17/400 4.3%	2/400 0.5%	13/400 3.3%	161/1600 10.1%	32/1200 2.7%	1-(129/129) =0.0%	1-(161/161) =0.0%	27.6 ±0.3	26.4 ± 0.3	80.9 ± 1.5	73.6 ±1.8
Hut B TF Coil (Push)	13/400 3.3%	95/400 23.8%	8/400 2.0%	10/400 2.5%	126/1600 7.9%	31/1200 2.6%	1-(95/129) =26.4%	1-(126/161) =21.7 %	27.5 ±0.3	26.4 ± 0.3	80.9 ± 1.4	73.6 ±1.8

[1] 3-5 days old starved females

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps for each treatment (TF, BGS and BGS+TF) was computed by:

%RE = 1- [recaptures from (TF, BGS, BGS+TF treatments) divided by (÷) recaptures from control] x 100.

Table 21 (Continued)

Treatment	No. of Mosquitoes				Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Hut A Control	Hut B TF Coil (Push)	Hut C TF Coil + BGS (Push-pull)	Hut D BGS (Pull)								
Hut C TF Coil + BGS (Push-pull)	2/400 0.5%	5/400 1.3%	37/400 9.3%	11/400 2.8%	55/1600 3.4%	18/1200 1.5%	1- (37/129) =71.3%	1 – (55/161) =65.8%	27.8 ±0.3	26.4 ± 0.3	75.5 ± 1.4	73.6 ±1.8
Hut D BGS (Pull)	0/400 0.0%	8/400 2.0%	8/400 2.0%	67/400 16.8%	83/1600 5.2%	16/1200 1.3%	1- (67/129) =48.1%	1- (83/161) =48.5%	28.8 ±0.4	26.4 ± 0.3	74.0 ± 1.4	73.6 ±1.8

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps for each treatment (TF, BGS and BGS+TF) was computed by:

%RE = 1- [recaptures from (TF, BGS, BGS+TF treatments) divided by (÷) recaptures from control] x 100.

Table 22 Recapture rates of *Aedes aegypti* from Push-Pull experiment using 25% SAC Transfluthrin treated polyester (P) fabric at a dose of 0.125 field application rate[5] and BG-Sentinel™ Trap in Pu Tuey, Kanchanaburi, Thailand.

Treatment	No. of Mosquitoes				Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Color from Hut A	Hut B TFP (Push)	Hut C TFP + BGS (Push-pull)	Hut D BGS (Pull)								
Hut A Control	193/400 48.3%	31/400 7.8%	13/400 3.3%	4/400 1.0%	241/1600 15.1%	48/1200 4.0%	1-(193/193) =0.0%	1-(241/241) =0.0%	27.5 ±0.3	26.6 ± 0.3	81.4 ± 1.4	72.4 ±1.6
Hut B TFP (Push)	12/400 3.0%	86/400 21.5%	9/400 2.3%	3/400 0.%	110/1600 27.5%	24/1200 2.0%	1-(86/193) =55.4%	1-(110/241) =54.4 %	27.0 ±0.3	26.6 ± 0.3	81.0 ± 1.5	72.4 ±1.6

[1]3-5 days old starved females

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); *0% **RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE***) from interception traps for each treatment (TF, BGS and BGS+TF) was computed by:

%RE = 1- [recaptures from (TF, BGS, BGS+TF treatments) divided by (÷) recaptures from control] x 100.

[5] Application rate at 5 µg *ai*/cm^2

Table 22 (Continued)

Treatment	No. of Mosquitoes				Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Color from Hut A	Hut B TFP (Push)	Hut C TF P + BGS (Push-pull)	Hut D BGS (Pull)								
Hut C TFP + BGS (Push-pull)	4/400 1.0%	8/400 2.0%	21/400 5.3%	6/400 1.5%	39/1600 2.4%	18/1200 1.5%	1-(21/193) =89.1%	1 – (39/241) =83.8%	27.2 ±0.3	26.6 ± 0.3	77.3 ± 1.4	72.4 ±1.6
Hut D BGS (Pull)	11/400 2.8%	4/400 1.0%	4/400 1.0%	80/400 20.0%	99/1600 6.2%	19/1200 1.6%	1-(80/193) =58.6%	1- (99/241) =58.9%	28.0 ±0.4	26.6 ± 0.3	74.3 ± 1.6	72.4 ±1.6

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps for each treatment (TF, BGS and BGS+TF) was computed by:

%RE = 1- [recaptures from (TF, BGS, BGS+TF treatments) divided by (÷) recaptures from control] x 100.

Table 23 Recapture rates of *Aedes aegypti*[1] from push-pull experiments using 25% SAC of Transfluthrin (TF) treated pink lace polyester

at 0.125 field application rate[5] and BG-Sentinel™ trap in Pu Tuey, Kanchanaburi, Thailand.

Treatment	No. of Mosquitoes				Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
	Hut A Control	Hut B TF Lace (Push)	Hut C TF Lace + BGS (Push-pull)	Hut D BGS (Pull)								
Hut A Control	117/300 39.0%	23/300 7.7%	4/300 1.3%	10/300 3.3%	154/1200 12.8%	37/900 4.1%	1-(117/117) =0.0%	1-(154/154) =0.0%	26.3 ± 0.3	28.2 ± 0.4	83.0 ± 1.4	73.1 ± 1.9
Hut B TF Lace (Push)	3/300 1.0%	24/300 8.0%	3/300 1.0%	3/300 1.0%	33/1200 2.8%	9/900 1.0%	1-(24/117) =79.5%	1-(33/154) =78.6%	26.5 ± 0.2	28.2 ± 0.4	80.9 ± 1.3	73.1 ± 1.9

[1]3-5 days old starved females

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry ***(%RE)*** from interception traps for each treatment (TF, BGS and BGS+TF) was computed by:

%RE = 1- [recaptures from (TF, BGS, BGS+TF treatments) divided by (÷) recaptures from control] x 100.

[5] Application rate at 5 μg *ai*/cm^2

Table 23 (Continued)

Treatment	No. of Mosquitoes: Hut A Control	Hut B TF Lace (Push)	Hut C TF Lace + BGS (Push-pull)	Hut D BGS (Pull)	Total IT Recapture	Total IT Diversion[2]	Within Hut % Reduction[3]	Total % Reduction[4]	Avg. Temp In (°C) ±SE	Avg. Temp Out (°C) ±SE	Avg. Hum. In (%RH) ±SE	Avg. Hum. Out (%RH) ±SE
Hut C TF Lace + BGS (Push-pull)	0/300 0.0%	5/300 1.7%	11/300 3.7%	4/300 1.3%	20/1200 1.7%	9/900 1.0 %	1- (11/117) =90.6%	1 – (20/154) =87.0%	26.7 ± 0.3	28.2 ± 0.4	79.6 ±1.4	73.1 ± 1.9
Hut D BGS (Pull)	3/300 1.0%	4/300 1.3%	19/300 6.3%	58/300 19.3%	84/1200 7.0%	26/900 2.9%	1- (58/117) =50.4%	1- (84/154) =45.5%	26.9 ± 0.3	28.2 ± 0.4	78.1 ± 1.3	73.1 ± 1.9

[2] Total number of marked mosquitoes coming from other huts

[3] % Reduction in entry (RE); ***0% RE*** is assumed from controls [1- (recaptures from control ÷ recaptures from control)].

[4] Total % reduction in entry (***%RE***) from interception traps for each treatment (TF, BGS and BGS+TF) was computed by:

%RE = 1- [recaptures from (TF, BGS, BGS+TF treatments) divided by (÷) recaptures from control] x 100.

Table 24 Summary of percent *Ae. aegypti* [1]reduction into host-occupied spaces in push-pull strategy experimental huts trials using varying spatial repellents and treatment formulations.

Treatments	*Ae. aegypti* BG-Sentinel™ (BGS) Recapture Rates (%)		*Ae. aegypti* entry into huts (% Reduction)		
	BGS only	BGS + SR[2]	BGS only	SR only	BGS + SR
Metofluthrin Coil (0.009%)	29.8	33.0	41.0	16.5	69.3
Transfluthrin Coil (0.030%)	32.3	21.7	48.5	21.7	65.8
Transfluthrin (Polyester)[3]	40.0	26.8	58.9	54.4	83.8
Transfluthrin (Lace)[3]	29.3	22.0	45.5	78.6	87.0
Mean Transfluthrin	33.9	23.5	50.9	51.6	78.9
Mean Overall	32.7	28.1	48.5	42.8	76.5

[1]3-5 days old starved females

[2] Spatial Repellents

[3] 0.125 field application rate (5 µg *ai*/cm^2)

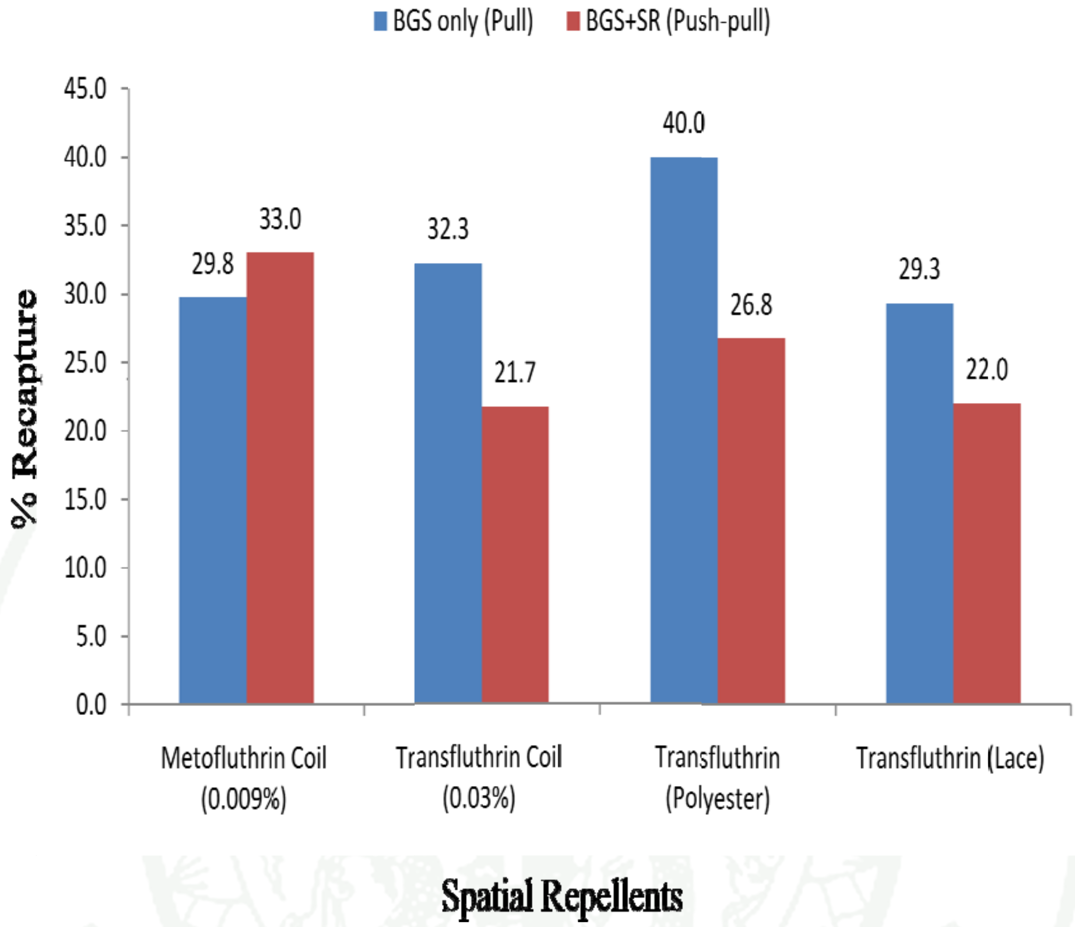

Figure 31 *Aedes aegpti* BG-sentinel ™ trap recapture rates during push-pull evaluations of different spatial repellents.

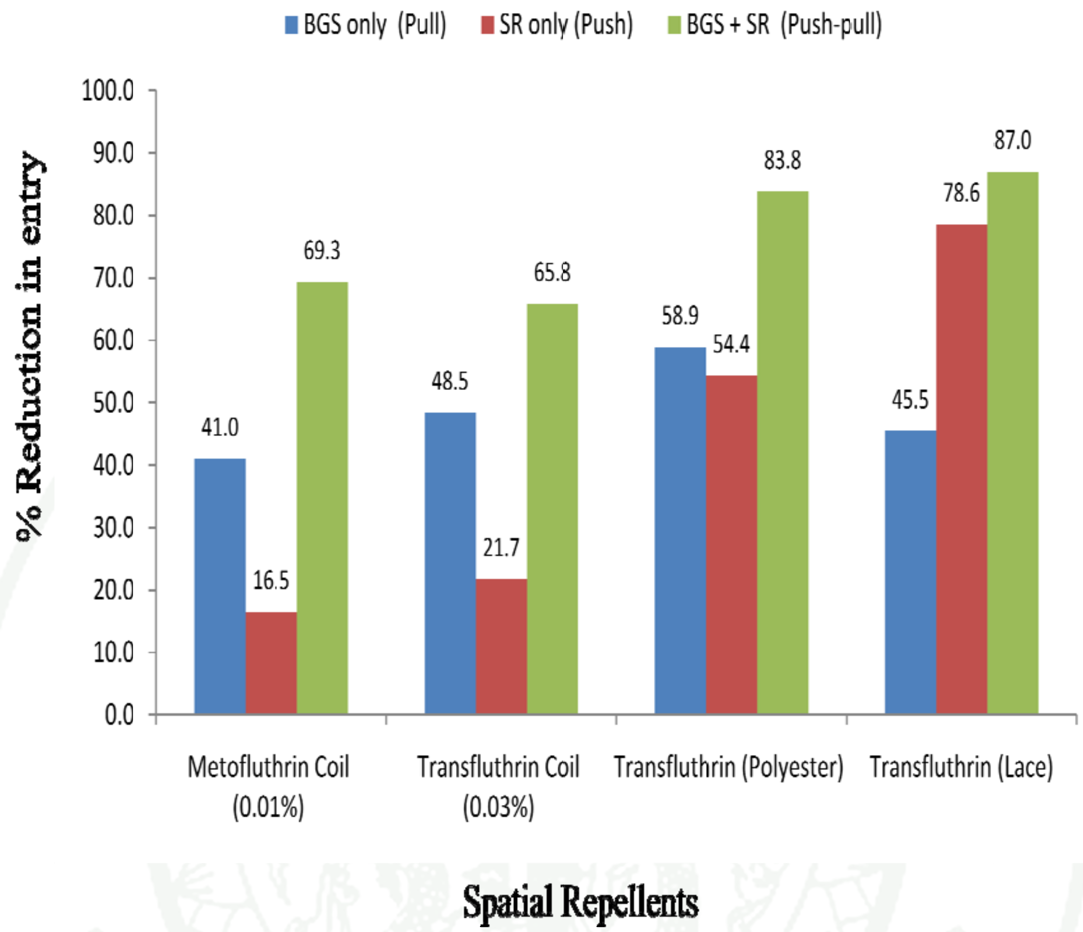

Figure 32 *Aedes aegpti* % reduction in entry based on collections from interception traps the experimental huts during push-pull evaluations of different spatial repellents.

3. Phase III- Local Home Trials

3.1. Local Home Survey of BGS Competing Resting Sites.

Overall, a total of 221 water-holding containers were recorded from the 20 households surveyed (Table 25-26). Predominant containers were jars 54% (119/221), drums/tanks 22.0% (48/221), basins 9.00% (20/221), pails 7.23% (16/221) and tyres 5.89% (13/221) (Table 25). The overall container size distributions were as follows: small 72.0% (159/221), medium 11.0% (25/221), large 2.0% (4/221) and extra large 15.0% (33/221) (Table 26). About 90.0% (199/221) of all water containers were found within 0-3 m distance of surveyed homes (Table 25) with extra large containers comprising 13% (28/221) of all potential breeding sites in the same distance (Table 26). Other containers such as sprinklers, plastic boxes made up the remaining 2.3% (5/221) habitats quantified. Other types of resting/breeding sites included: fish ponds, bamboo crates, storage shanties, chicken coop, old clothes hanging and Bhuddist's altar. For the two sentinel households (SH) monitored using BGS traps, 96.0% (26/27) of water containers were jars found within 0-3 m. The remaining 4% (1/27) consisted of drums (Table 25). Generally, more vegetation (tree, shrubs, or herbs) were found as the distance from house walls increased from 0 to 10 m (Table 27). Farther beyond 10 m were orchards (mango etc) and plantations of trees (palm oil), and vegetables (eggplants, vines etc.)

3.2. Monitoring of *Aedes aegypti* population: CDC-back pack vs. BGS Collections.

Monthly *Ae. aegypti* capture trends for both BGS and CDC-back pack collections from the sentinel household (SH) are shown in Figure 33. Highest population densities of *Ae. aegypti* using CDC-back pack collections were captured from March –August, 2011. However, BGS trap monitoring showed peak *Ae. aegypti* populations from March -May with a spike occurring in August (75 females and 14 males) and lowest densities occurring in February (6 females and 4 males) (Figure 33). BGS traps captured both males and females of the two dengue vector species, *Ae. aegypti* and *Ae. albopictus*, with their most productive trapping sites in proximity to competing man-made resting objects (Figure 34; Table 28).

The most productive BG-Sentinel™ trapping locations were window 3 (having 42.5% (102/240) overall contribution to *Ae. aegypti* females collected) from the raised wooden sentinel house (Figure 15), and opposite the door (with 34.4% (44/128) overall contribution to *Ae. aegypti* females collected) from the non-raised cemented sentinel house (Figure 14). The most productive BG-Sentinel™ trapping location for female *Ae. albopictus* was opposite the door from the raised SH and opposite window 2 of the non-raised SH (Table 27). Male *Ae. albopictus* were collected from BGS traps located opposite doors of the two SH (Table 28).

Capture time trends indicate the most productive BGS sampling period for collecting *Ae. aegypti* was from 1330-1730h while that for *Ae. albopictus* occurred during both morning (0530-0930 h) and afternoon (1330-1730 h) sampling points (Figure 35). Total mean mosquito collection densities for dry (Feb-May) and rainy (Jun-Sep) seasons were 48 and 45 females using BGS monitoring, respectively and 5.2 and 0.5 females using CDC-back pack collections, respectively from the SH (Figure 36). Total mean number of *Ae. aegypti* for dry and rainy seasons were 7.2 and 2.8 females for all the households (SH and others) monitored using CDC-back pack (Figure 36).

Table 25 Summary of man-made water holding containers that represent potential BG-sentinel ™ competing sites quantified during a local home survey in Pu Teuy, Thongphaphum, Kanchanaburi.Thailand January 19, 2011.

Distance[1]	Households[2]	Container Types[3]							Total (%)
		Jar	Drums/Tanks (plastic, metal and cement)	Basins	Pails	Tyres	Sprinklers	Plastic Box	
0 m	All	55 (24.9)	13 (5.9)	5 (2.3)	11 (5.0)	6 (2.7)	0 (0)	0(0)	90 (40.7)
	Sentinel	18 (66)	0 (0)	0 (0)	-	-	-	-	-
3 m	All	58 (26.2)	27 (12.2)	12 (5.4)	5 (2.3)	2 (0.9)	4 (1.8)	1 (0.4)	109 (49.3)
	Sentinel	8 (30)	1 (4)	0 (0)	-	-	-	-	-
10 m	All	6 (2.7)	8 (3.6)	3 (1.4)	0 (0)	5 (2.3)	0 (0)	0 (0)	22 (10.0)
	Sentinel	0 (0)	0 (0)	0 (0)	-	-	-	-	-
Total (%)	All	119 (53.8)	48 (21.7)	20 (9.1)	16 (7.2)	13 (5.9)	4 (1.8)	1 (0.4)	221(100.0)
	Sentinel	26 (96%)	1 (4%)	0 (0%)	-	-	-	-	-

[1] Location of containers based on the roof of household, 0m located immediately or within the roof, 3m distance and 10 m distance from edge of roof

[2] All = 20 households selected for survey; Sentinel = two sentinel houses selcted from the original 20 used in BGS monitoring

[3] Numbers in parenthesis are corresponding percentages of containers from the total number of containers found

Table 26 Size distribution of man-made water holding containers that represent potential BG-Sentinel™ competing sites quantified during a local home survey in Pu Teuy, Thongphaphum, Kanchanaburi.Thailand January 19, 2010.

Distance	Size[2]	Jar	Drum/tanks (Plastic/Metal and Cement)	Basin	Pail	Tyre	Sprinklers	Plastic Box	Total
0 m	small	36	12	5	11	5	0	0	69
	medium	9	0	0	0	1	0	0	10
	extra large	10	0	0	0	0	0	0	10
	extra large	10	0	0	0	0	0	0	10
3 m	small	30	24	12	5	2	4	1	78
	medium	12	1	0	0	0	0	0	13
	large	0	0	0	0	0	0	0	0
	extra large	16	2	0	0	0	0	0	18
10 m	small	1	5	3	0	3	0	0	12
	medium	0	0	0	0	2	0	0	2
	large	0	3	0	0	0	0	0	3
	extra large	5	0	0	0	0	0	0	5
Overall Total		119	48	20	16	13	4	1	221
% Contribution		53.8	21.7	9.0	7.2	5.9	1.8	0.4	100.0

[1] Location of containers based on the roof of household, 0m located immediately or within the roof, 3m distance and 10 m distance from edge of roof

[2] Size categories: small (-<250L), medium (-250-≤500L), large (>500-≤1000L) and extra large(-≥1000L).

Table 27 Number of houses and corresponding distances where vegetations were found, Pu Teuy, Thongphaphum, Kanchanaburi Thailand. January 19, 2010.

Distance	Households	Trees[1]	Shrubs[2]	Herbs[3]
0 m	All	1	1	2
	Sentinel	0	1	1
3 m	All	8	6	11
	Sentinel	2	2	2
10 m	All	9	13	11
	Sentinel	2	2	2

[1] Having woody stem >1m height

[2] Having woody stem <1m height

[3] Having non-woody stem <1m height

Table 28 Summary of BG-Sentinel™ (BGS) trap outdoor and CDC-back pack indoor collections of natural dengue vector populations from two sentinel households in Pu Teuy, Kanchanaburi, Thailand, January to September, 2011.

House	Species	Total Collected BGS	Total Collected CDC[1]	Most Productive BGS site[2]	Competing Sites[3]
Raised (wood)	*Ae. aegypti* ♀	240	27	W3 (43%)	2 M Jars, vegetation, clothes
	Ae. aegypti ♂	36	32	W3 (53%)	2 M Jars vegetation, clothes
	Ae. albopictus ♀	11	-	Door (36%)	1 L Jar Vegetation
	Ae. albopictus ♂	1	-	Door (100%)	1 L Jar Vegetation
Non-Raised (cement)	*Ae. aegypti* ♀	128	1	Door (34%)	2 M Jar, Table, vegetation
	Ae. aegypti ♂	7	0	Door (43%)	2 M Jar, Table, vegetation
	Ae. albopictus ♀	15	-	W2 (40%)	Vegetation
	Ae. albopictus ♂	7	-	Door (71%)	2 M Jar, Table, vegetation

[1] '-' Denotes density not recorded, no data

[2] Location of BGS in peridomestic area opposite portals of entry; W3 (window 3); W2 (window 2) and percentages contribution to overall captures from Jan-Sep, 2012.

[3] Describes number and size (M-medium, L-large) of adjacent water containers found and other resting sites

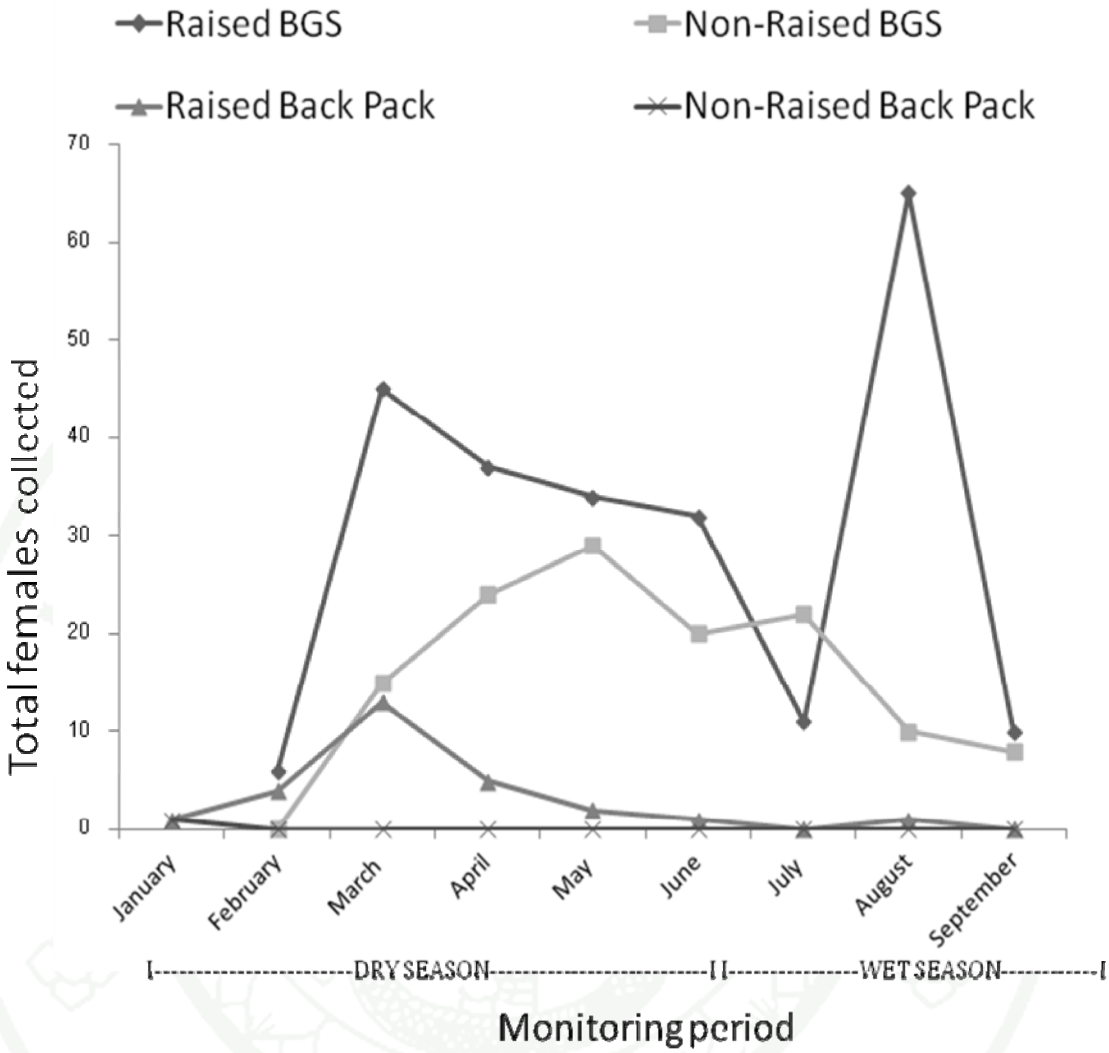

Figure 33 BG-Sentinel™ trap and CDC-back pack collections of *Ae. aegypti* females collected from raised-wood and non-raised cement sentinel households from January - September, 2011 in Pu Teuy village, Kanchanaburi, Thailand.

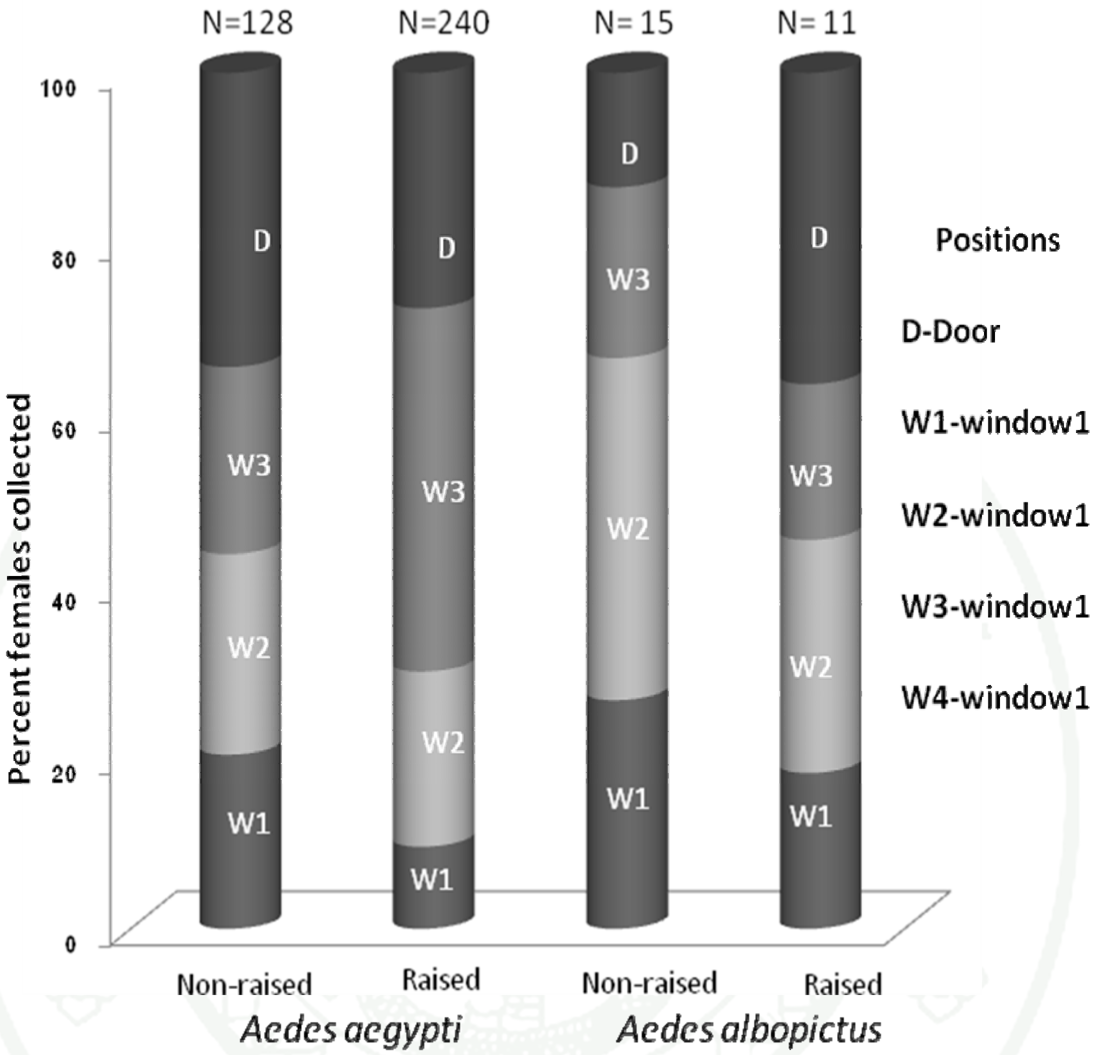

Figure 34 Density of female dengue vectors collected from BG-Sentinel™ traps from four different positions at two sentinel households in Pu Teuy village, Kanchanaburi, Thailand from January - September, 2011.

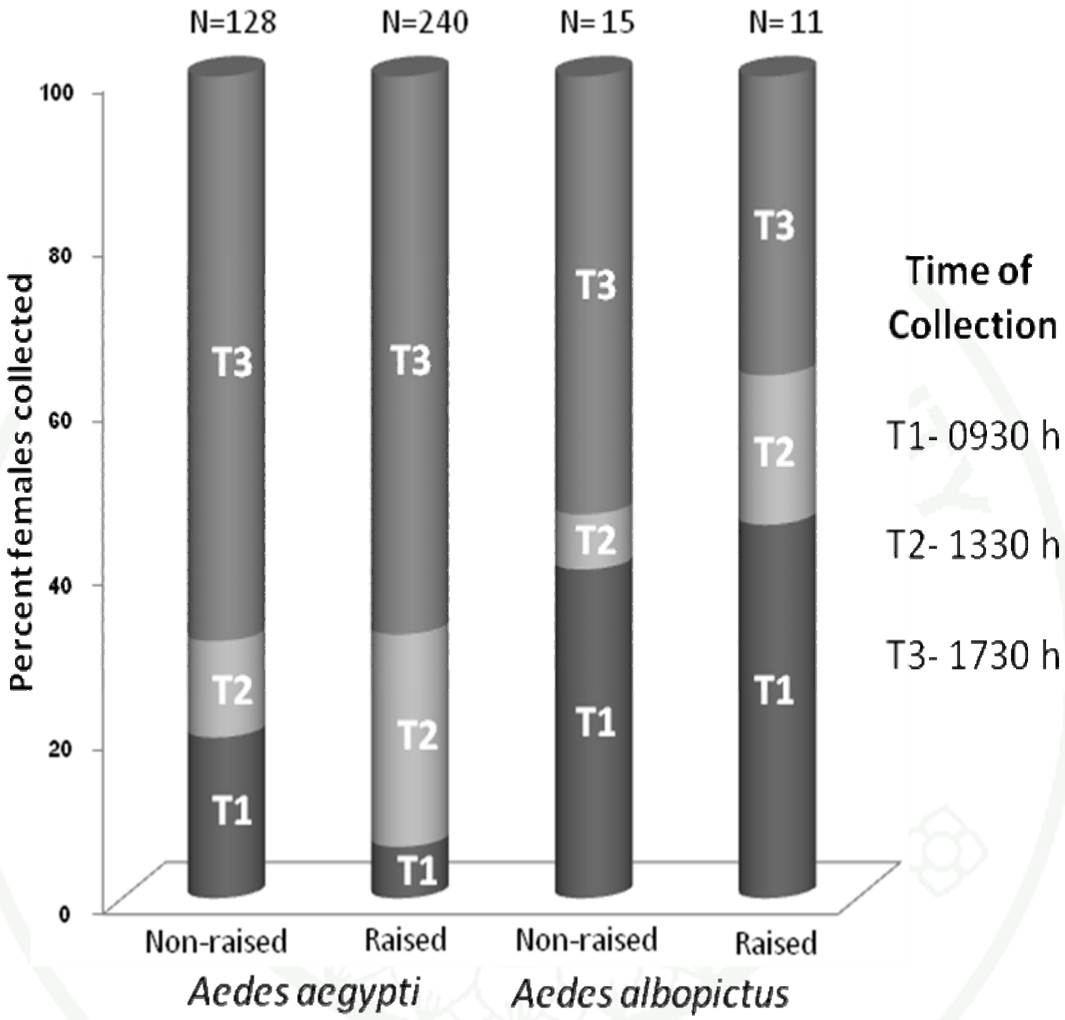

Figure 35 Abundance of female dengue vectors by time as collected from BG-Sentinel™ trap from the two sentinel households in Pu Teuy village, Kanchanaburi, Thailand from January to September, 2011.

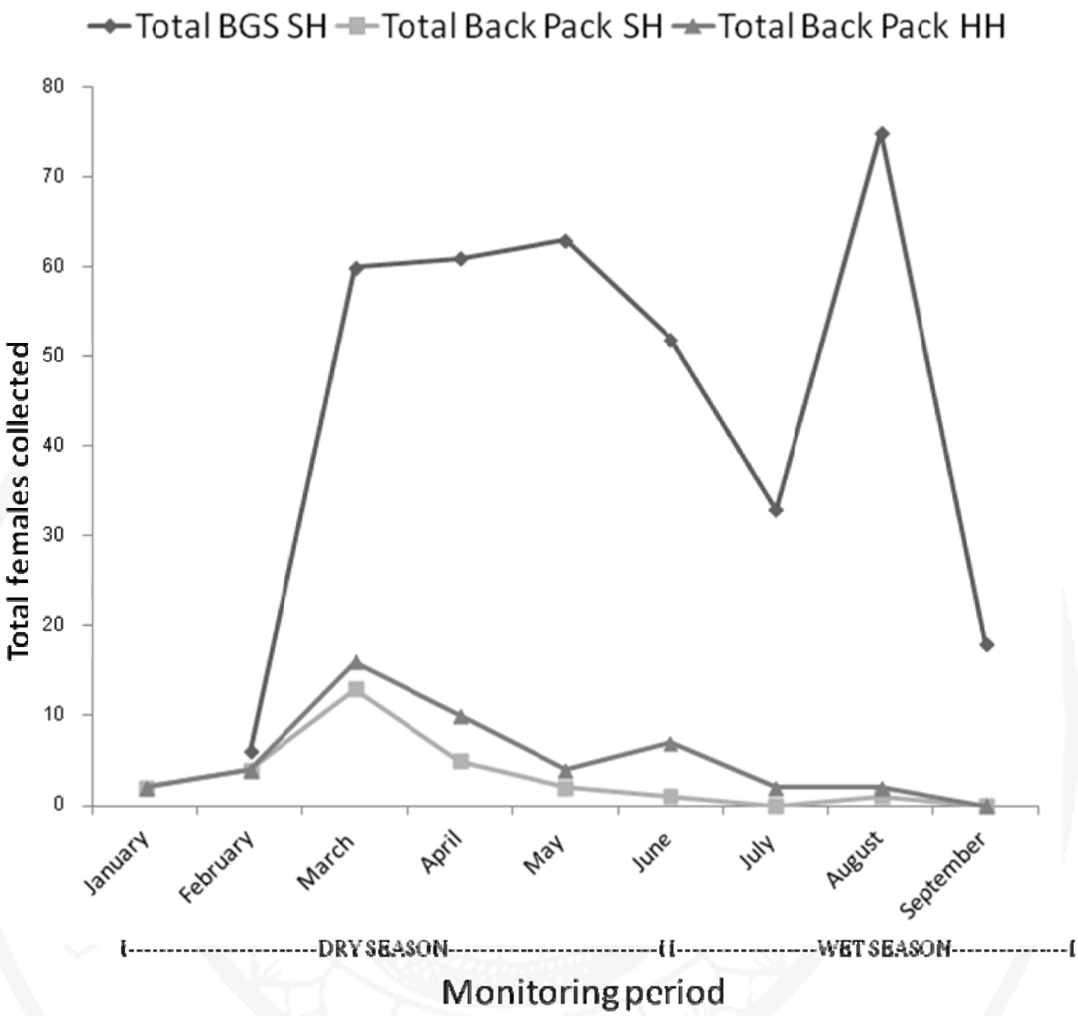

Figure 36 Total BG-Sentinel™ trap and CDC-backpack *Aedes aegypti* females monthly densities from the two sentinel households (SH) and CDC-back pack collection from all the households (HH) surveyed in Pu Teuy village, Kanchanaburi, Thailand from January to September, 2011.

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Discussion

The overall goal of this study was to characterize a peridomestic trap (BG-Sentinel™) for integration into an *Aedes aegypti* PPS. The process involved evaluating the relationship between BGS trap operation time and female *Ae. aegypti* capture rates and quantification of the impact of trap density on capture rates against varying *Ae. aegypti* adult population sizes using a semi field system. With the use of experimental huts, the effect of chemical (spatial repellent) exposure on BGS *Ae. aegypti* recapture was quantified as well as the evaluation of optimal location and distance for maximum BGS recapture. Using the the optimized conditions, BGS trap efficacy was evaluated against natural *Ae. aegypti* populations at sentinel households to validate findings from a controlled field setting to a "real-life" setting.

1. Phase I -Screen House Trials

Previous studies established that the BG-Sentinel™ mosquito trap (BGS) is, compared to other traps or active collection methods, an effective tool for capturing adult *Ae. aegypti* in the outdoor environment (Krockel et al. 2006, Maciel-de-Freitas et al. 2006, Williams et al. 2006). Based on this information, the BGS is being evaluated as a component in a spatial repellent push-pull strategy to reduce the abundance of host-seeking *Ae. aegypti* inside homes and in the peridomestic environment thereby reducing the probability of human-vector contact and dengue virus transmission. The current study was designed to quantify recapture success under controlled screen house conditions and based on varying *Ae. aegypti* release numbers and BGS trap densities in order to guide data interpretation from push-pull experimental trials. The efficacy of the BGS trap was quantified in previous studies (Krockel *et al*., 2006, Maciel-de-Freitas *et al*., 2006, Williams *et al*., 2006, 2007; Ball and Ritchie, 2010a, b; Bhalala and Arias, 2009); the objective of this study was rather to determine *changes* in BGS recapture rates of *Ae. aegypti* under varying mosquito population densities to identify optimum trap numbers to use in a natural setting. Such information was not previously generated and represents new scientific knowledge important for mosquito surveillance and control implementations.

Overall, the results of this screen house study agree with previously published reports that the BGS trap is effective in recapturing 3-5 d old *Ae. aegypti* (Maciel-de-Freitas *et al*. 2006, Krockel et al. 2006, Williams *et al*. 2006). As expected, recapture rates varied based on BGS trap density and mosquito RN, with the highest cumulative percentage recapture over a 24 h period reaching 98%. The "Impact Period" during which the highest recapture success was observed, was the first day of release with about 84% of the released mosquitoes recaptured by 1730 hours or 12 h post-release. The peak collection time for all trials occurred during the 0530 to 0930 hours interval indicating that test specimens were more likely to be host-seeking (i.e., actively searching for a blood source) in the hours shortly after release, which is consistent with the peak time for landing collections recorded (Thavara *et al*. 2001) and experimental hut studies done in Thailand (Suwannachote *et al*. 2009, Chareonviriyaphap *et al*. 2010). Statistical analysis of grouped *Ae. aegypti* RN consistently showed that recapture rates using 2, 3, or 4 traps did not differ significantly at Day1 (1330 and 1730 hours) and from cumulative percentage recapture for Days 1-2. The use of only one trap however consistently showed significantly lower recapture rates. When examining the data by individual RN category, the use of a single BGS trap at the RN of 10, an *Ae. aegypti* adult density that better reflects what occurs naturally in the field, resulted in a cumulative recapture rate of 72.5% from Day 1 and Day 2, with 68% being recaptured by the end of the peak capture period(0530-0930 hours). This recapture rate was higher than any of the other RN categories using 1 BGS. Understandably, this experiment was done under controlled conditions and may not represent what is occurring in a natural field setting. The impacts this result may have on dengue transmission is unknown but at 72.5% recapture it is likely to contribute to reduced human-vector contact should the trap perform equally well outside of the restricted screen house setting. The facts that 2 BGS traps collected similar densities as 4 traps in a space volume of 140 m^3, and that 1 BGS trap can recapture more than 50% of the *Ae. aegypti* females in this same given space and time may have operational significance for both *Ae. aegypti* surveillance and control activities.

Interestingly, the low *Ae. aegypti* RN categories (10, 25, 50 mosquitoes) showed reduced Day 1 and 2 cumulative percentage recapture compared to both medium (100 and 150) and high *Ae. aegypti* RN (200 and 250) categories, while the percentage recapture among medium and high RN did not differ significantly across all BGS trap densities evaluated. Theoretically, higher recapture was expected at lower RN categories due to mosquito to trap ratio; our results do not support this. This could be a factor of "observation loss" such that mosquitoes may have escaped the screen house when trap monitoring was being conducted and/or that knocked down mosquitoes in the non-captured test population were not completely recovered. Additionally, because the mosquito test population in the lower RN categories was small relative to the medium and high RN categories, this could have resulted to reduce the overall percent total recapture.

The opposite scenario may be expected using 1 BGS trap versus 4 traps, i.e., the lower the density of BGS traps, the lower the recapture. However, recapture percentages using the 250 *Ae. aegypti* RN, for example, did not differ significantly among the varying BGS trap densities; in other words, 1 trap recaptured similar numbers as compared to 4 traps. This might be the result of 1 BGS being the only "host" available in the immediate environment. Another possible explanation could be that the collection of females in the 1 trap increased the attractiveness of that trap due to an increased level of aggregation pheromone/s when female *Ae. aegypti* are in groups thereby causing attraction of more females. Therefore, individual female mosquito behaviors of flight and response to host cues may not be independent under the study design and setting presented herein. Pheromone mediated swarming has been reported in *Ae. aegypti* for both males and females (Cabrera and Jaffe, 2007). Laboratory olfactometry experiments using *Aedes sierrensis* strongly suggests female-mediated attraction of other females to host, meaning presence of other female mosquitoes at the host significantly enhances the host-seeking response, most likely by a chemical stimulus released by the females (Ahmadi and McClelland, 1985). A related phenomenon has been observed in other haematopahgous insects wherein an ’invitation pheromone’ a special class of assembly pheromones, is emitted to assist in harborage and mate finding rather than host finding function. The presence of this assembly pheromeones have been documented in bedbugs (Levinson and Bar Ilan, 1971), ticks (Rechav *et al*., 1977; George, 1981) and sandflies (Schlein *et al*., 1984). This phenomenon could be further explored in future BGS studies.

The semi field system (screen house) generates data to bridge the gap between the laboratory and field condition, understandably it does not match a real-life situation. Since this phase represents the first component in optimizing the BGS for a push-pull strategy, this requires quantifying BGS recapture rates against varying mosquito numbers prior to performing outdoor trials in which total population densities are not known. Such controlled experiments provide the ability to model efficacy rates based on known mosquito denominators in the screen house releases. The screen house experiments provide critical information before moving to a more realistic setting, as to how many traps to use per house for maximum recapture given certain expected mosquito population densities. Results from this experiment will also be used to interpret potential changes in BGS trap recapture rates when used in conjunction with experimental huts during outdoor push-pull trials and further evaluation of BGS in real life setting.

Some inherent limiting factors in the current study design may have contributed to artificially high recapture success as compared to a natural setting. For example, in Queensland, Australia, BGS traps collected a mean of 1.92 ± 0.39 female *Ae. aegypti* per continuous 24 hour period (Williams *et al*. 2006). In Brazil, a study reported a mean *Ae. aegypti* collection of 1.5 female/trap/3 hr (Krockel *et al*. 2006). The high recapture values from our experiments are likely a result of the confined space volume of the screen house cubicle under which evaluations were performed. By restricting full free movement and choice, female mosquitoes may in effect have been ‘forced’ into contact with the BGS trap more frequently than in a natural setting containing alternative and competing stimuli and cues. It should be noted, however, that the overall 'available' natural population size in the studies from Brazil and Australia were unknown. Furthermore, the screen house did not present competing (confounding) host sources or resting places for the mosquito, which again may have led to artificially elevated levels of mosquito contact with the trap.

Future screen house tests could be modified to include items typically found in the peridomestic environment that could compete with or alter trap attractiveness. For example, a recent study by Ball and Ritchie (2010a) indicate that presence of low-reflectance (black) containers significantly reduces recapture rates by BGS traps of male and female *Ae. aegypti*. Another potential bias of this screenhouse study is the exclusive use of non-blood fed, 3-5 d old, assumed mated and nulliparous females. Ball and Ritchie (2010b) found no difference in recapture rate for *Ae. aegypti* females with respect to age (10-12 versus 24-26 and 32-34 d) but reported that physiologically, nulliparous females were recaptured at lower rates compared to parous or gravid ones. However, I purposefully selected the physiological conditions for the release population to match those of the *Ae. aegypti* test populations used in evaluating the "push" component in experimental huts located in the same study locality; therefore allowing use of the screen house data to help interpret findings during outdoor field trials. In addition, because the screen house studies were conducted at the same locality where the push-pull trials are being performed, the climatic conditions during this study were expected to be similar to when the BGS are used in the outdoors.

Data from the current study has revealed several potential field applicable pieces of information. The use of the individual screen house cubicles with an approximated space (140 m^3) equal to the area of experimental huts used for related field trials and the immediate peridomestic area typically surrounding local households (i.e., backyard, gardens etc.) may help bridge the gap between semi-field and field studies to ascertain expected recaptures from BGS traps using varying *Ae. aegypti* release densities. The current study also revealed a peak BGS recapture interval between 0530-1330 hours, which is similar to the time period when *Ae. aegypti* most commonly enters experimental huts under field conditions (Grieco *et al*. 2007, Suwannachote *et al*. 2009). Although releases were made at 0530h alone, this time trend still reflects the normal host-seeking activity pattern of *Ae. aegypti* females and has potential operational significance to when BGS traps would be most effective in removing flight-active host(blood)-seeking mosquitoes in a push-pull strategy. Considering that the BGS trap requires an external power source (i.e., battery pack or direct electricity), this information is specifically important for informing when the trap may be most effective thereby how long it should run and the possibility of cost-effective operations if the traps can be turned off to conserve battery/electricity power supplies. Being able to maximize trapping efficiency and minimize cost is just one important consideration to provide a cost-effective and sustainable control method. If the push-pull strategy proves successful, it could be integrated into a consumer-implemented control method to augment organized vector control measures. Knowledge as to when home-owners should operate the peridomestic trap would be important for ensuring maximum benefit. Further studies are necessary though to substantiate such claims and datasets from studies similar to the current report could prove useful in modeling these outcomes.

2. Phase II-Experimental Hut Trials

Push-pull evaluations have shown that the use of either a spatial repellent or BGS trap contribute to reduction in *Ae. aegypti* entry into experimental huts as compared to a control but that a combination of both tools resulted in greater reduction as compared to when the tools are used separately. Spatial repellents are expected to function in disease prevention by preventing entry of vectors into human occupied spaces thus reducing man-vector contact. However, the use of a mechanical trap can also prevent entry and catch exiting repelled vectors in the peridomestic environment thus preventing diversion to non-treated sites. There are a number of questions underlying this component of the study: 1) under controlled conditions, will *Ae. aegypti* exposed to SR chemicals in experimental huts (simulated home condition) alter their host-seeking behvior and therefore cause adverse effects in BGS recapture efficacy, 2) if mosquitoes are adversely affected, will they recover after some time, 3) will spatial repellents exert latent/delayed effects on *Ae. aegypti* host-seeking behavior that could affect BGS recapture rates, 4) is there an optimum BGS location and distance where greatest *Ae. aegypti* recaptures can be obtained to guide placement at local houses and, 5) will BGS traps work in the presence of competing resting sites.

2.1 Effect of Chemical Exposure on BGS Recapture

Exposure studies to DDT at all chemical concentrations and SAC evaluated resulted in no significant difference between BGS recapture rates between control (no chemical exposure) and treatment cohorts (exposed to chemical) either immediately or following a delayed time (12 h) post-exposure. These findings suggest no immediate and/or latent effect. This aspect of DDT is an ideal property for spatial repellent to be paired with BGS in push-pull strategy.

Similarly, metofluthrin high and low dose coils did not show significant differences in BGS recaptures between control and treatments populations. However, significantly higher recaptures were obtained from DR as compared to IR for both low and high dose metofluthrin coil experiments. Higher DR BGS recaptures may indicate better recovery after the 12 h holding period.

This current study showed that exposed *Ae. aegypti* to concentration of DDT and metofluthrin that indicate 17 to 56% repellency action in experimental huts are still recaptured by BGS at similar rates. These recapture rates are comparable to earlier screen house BGS recaptures (Salazar *et al*., in press). These findings suggest that these products in push component may not negatively affect the efficacy of BGS and could be integrated together to target indoor and outdoor *Ae. aegypti* population.

On the other hand, post exposure to transfluthrin at 0.125 and 0.0625 FAR using 25% SAC showed significantly lower recaptures from transfluthrin treatments compared to controls from IR. This is most likely due to higher mortalities that occurred from the exposure. From DR trials, however, there was no significant difference between controls (94%) and treatments populations for either the 0.125 (71%) or 0.0625 FAR exposures (90%).

The results from these spatial repellent exposure trials suggest that there may probably be an effect of host seeking behavior by transfluthrin and slightly by metofluthrin exposures. Results suggest however that recovery is possible after within 12 h after exposure. Analyses showed that from the three compounds tested (using varying application rates and surface area coverages) DDT did not affect BGS recaptures either immediately or in delayed releases. For metofluthrin, treatments and controls did not show significant difference between each other for either release however BGS recaptures were significantly higher from delayed release compared to immediate release. This suggest probable recovery or increased host seeking activity of the recovered (delayed release) population. Contrary to the results of push-pull trials, there is no discernible effect observed as BGS recaptures still follow the regular trend from which highest collections occur at 0530 to 1330h, and there is no marked increase in collection on the succeeding days or later time (1730 h).

The higher recovery from delayed releases of metofluthrin and transfluthrin treatments can be explained through the effect of chemicals on host seeking behavior of *Ae. aegypti*. Spatial repellency is manifested through either biting inhibition or disruption of orientation toward the human host, for these experiments towards attraction to BGS . Repellents generally may induce changes in responses of olfactory receptor neurons to putative kairomones of female mosquitoes. Researches findings points to the grooved peg sensilla and sensilla trichodea located on the mosquito antennae to be the ones involved in host seeking behavior (Meijerink *et al*., 2001; Qiu, 2005). The grooved peg sensilla were found reactive to polar compounds such as ammonia, amines, lactic acid, and short-chain carboxylic acids (van den Broek *et al*., 2000. Meijerink *et al*. 2001, Diehl *et al*. 2003) that are all host-seeking kairomones, components of human sweat and BG lure. The decline in sensitivity of grooved peg sensilla or sensilla trichodea located on the antennae of *Ae. aegypti* to human odor as a result of repellent exposure might be a mechanism for the temporary suppression of host-seeking behavior (Fox *et al*., 2001).

Our experimental trial though showed transfluthrin to significantly reduce recapture, further studies are needed to ascertain basis of reduction of either directly due to toxicity or due to disorientation in host seeking behavior. Further research is needed look for possible concentration that will show maximum spatial repellency effect with minimal or negligible affect of BGS recapture rates. More than that, the efficacy of these repellents showed be evaluated against natural populations of mosquitoes as in pilot trials or demonstrations.

2.2 Location and Distance Effects

Location and distance experiments demonstrate the importance of these parameters to BGS recapture efficacy. This is based on our results showing 39% [(recapture from portals of entry – recapture from vertices)/ recaptures from portals of entry x 100%] greater recaptures *Ae. aegypti* females from BGS traps placed opposite portals of entry (windows/doors) -38.7% (116/300) compared to BGS traps placed at vertices of the experimental huts- 23.67% (71/300). Vertices of the experimental huts are relatively disadvantageous due to exposure to turbulent air current from two opposite directions. Overall, the 0m and 10m distances showed highest recapture of 18.50% (111/600) and 14.22% (128/900); respectively. Lower recapture of 7.89% (71/900) was recorded from the distance of 3m. Interception trap data from experimental huts showed that BGS traps placed at 0m distance ensued highest percentage reduction in *Ae. aegypti* entry of 65.6% compared to the 3m (17.19%) and 10m (14.59%) distances. Therefore based on experimental hut trials, the best locations are opposite portals of entry and most productive distance at 0m. Extrapolating finding from screen house experiments, the optimum location (portals of entry) and distance (0m) showed greater recaptures since they are still within the confines of the assessed screen house volume (140m^3). When BGS are at 0m distance, the space volume surrounding them is about 105 m^3 (5m x 6m x 3.5 m) smaller than that of the screen house. Moving farther away from the hut by 3m would have an overall larger space volume of 252 m^3 (8m x 9m x 3.5), much bigger when BGS were placed at 10m having a space volume of 840 m^3 (15m x 16m x 3.5m).

Distance effects evaluation showed close *Ae. aegypti* recapture values for 0m and 10m distance showed very close recapture values. The actual reduction in entry differed based on IT collections however differed, the highest was when BGS was at 0m. This may simply reflect the strong anthropophily of *Ae. aegypti* (Edman *et al*., 1992; Van Handel *et al*., 1994). The presence of BGS close to human host competes highly with its attraction to human host cues as indicated by high BGS recapture and low hut entry. Highest recaptures were recorded from BGS traps at the portals of entry possibly due to stronger host cues at these sites that may direct the mosquitoes to that location. It is a common knowledge that female mosquitoes use host odors to find their hosts (Takken 1991, Takken and Knols 1999) and that the combination of lactic acid, CO_2, ammonia, caproic acid and several fatty acids released from all warm-blooded vertebrate animals seems to play an important role in the host-seeking behavior of the mosquito (Geier *et al*. 1996, 1999; Costantini *et al*. 1998; Bosch *et al*. 2000, Williams *et al*., 2006). Thus, putting BGS near human host; in experimental huts trials when BGS is at 0m distance opposite portals of entry increase the concentration of those compounds around (lactic acid, CO_2, ammonia, caproic acid) having them as the basic composition of BG lure (Krockel *et. al*., 2006).

The appearance of productive locations (: Window 1=40% (90/227), Door=32% (72/227), Window 2=20% (46/227) and Window 3=8% (19/227) maybe due to the wind current/direction, how the air enters and circulates within the hut and exit outside portals of mosquito entry bringing host cues (sweat odours) at the peak recapture time (0530h to m1330 h). It has been known for long that odours guide insects in their flights seeking for hosts (Clements, 1999) while final approach is guided by visual cues for day biters as *Ae aegypti*. Alighting is once again stimulated by sweat volatiles (human host) and BG lure (lactic acid, ammonia and caproic acid) derived from human sweat. Host cues is a factor absent from screen house study (only cues from BG Lure and probably females *Ae. aegypti*).

Earlier studies have shown that BGS collections were statistically similar to standard human landing catches (Krockel *et al*., 2006), thus was suggested to serve as replacement of HLC to avoid risk of pathogen transmission. The result from the location and distance trial is very important in guiding the push-pull pilot trial to be able to determine how the presence of BGS traps in proximity to the house affects reduction in *Ae. aegypti* entry into houses from within the peridomestic environment. Placement at local homes could be prioritized given a limited number of BGS available by locating them at the most productive sites. In addition, BGS at 0m distance in most cases may not need additional shelter from sunlight and rain as they within the roof of most houses.

2.3 Contribution of BGS Traps to PPS Experimental Hut Studies.

BGS trap showed relative consistency in terms of recapture rates when used alone (30-40%) or when in combination with spatial repellents (22-33%). There is somehow consistency in terms of contribution to the % reduction in entry to the huts (41 to 59%). Coil formulation showed reduction in entries from 65-69% while fabric treatment applications showed reduction entry from 83-87%. (Figure 32) Use of same spatial repellent compound, example transfluthrin at similar concentration but different treatment substrates showed difference in the reduction in entry (polyester-83%. lace-87%) probably due to affinity with which insecticide particle bind with the substrate. There a limited ways by which interaction of repellents and BGS could be discussed using this available data. Future studies are necessary to determine the mechanism in which the pair work, whether mosquitoes are pulled initially by BGS that resulted to prevention in entry or that the mosquitoes are being pushed by spatial repellents and ending up being pulled by BGS, is there synergism or antagonism happening, can not be determined at this point. The relationship between push and pull may vary with compounds, formulations, concentration used, treatment substrates and placement inside huts. These are all to be addressed in the push component. This part of the work fulfill the needed gap on characterizing BGS as pair to spatial repellents from screen house defining trap density over *Ae. aegypti* population density, to chemical exposure effects and distance and location effects and their efficacy in local home.

3. Phase III-Local Home Trials

Survey of potential resting/breeding sites at the Pu Teuy study site were similar to the typical and commonly used type of containers found in Thailand (Koenraadt *et al*., 2008, Gratz 1993, Tonn *et al*., 1970). Monitoring dengue vector populations showed minimal densities from either all households or specific to the two SH using CDC-back pack. However, BGS continued to indicate varying monthly vector populations in peridomestic environment. BGS traps collected both *Ae. aegypti* and *Ae. albopictus* in the presence of natural resting sites at SH with most productive BGS sites in proximity to competing man-made resting objects. Two most productive BGS locations were identified from each of the SH, window 3 (with highest numbers of female and male *Ae. aegypti*) and door (with highest numbers of female and male *Ae, albopictus*) from the raised SH while door (with highest numbers of females and males *Ae aegypti* and male *Ae. albopictus*) and window 2 (where highest numbers of female *Ae. albopictus*) locations from the non raised. Results further showed that highest trap captures in local homes occur at points (window 3 at wooded raised house and at the door in non-raised cement house) where there is highest probable interaction between human and mosquitoes where there would be more odor cues, and vibration or movement where there is highest probable interaction between human and mosquitoes where there would be more odor cues, and vibration or movement that attract mosquitoes which is one of the working principles in some traps (Dennett *et al*, 2006). These favored sites were earlier documented in the experimental hut trials where window1 and door locations showed highest BGS recaptures, will have operational bearing on the use of BGS as logistics may allow the use of only one, two, three or four BGS. BGS at 0m opposite portals of entry works, as earlier documented from experimental huts.

Table 29 Summary of *Aedes aegypti* BG-Sentinel^{TM} (BGS) trap recapture rates from screen house, experimental huts and local home evaluation in Pu Teuy village, Kanchanaburi, Thailand.

No. of BGS	SCREEN HOUSE		EXPERIMENTAL HUTS[3]	LOCAL HOME TRIAL[4]		
	Over-all[1]	RN 100[2]	Optimum location (portals of entry) and distance (0m)	Non-Raised SH	Raised SH	Total of two SH
1	83b	85b	15 (90/600) W1	34.4 (44/128) D	42.5 (102/240) W3	39.7 (146/368)
2	86ab	83b	27 (162/600) W1+D	57.8 (74/128) D+W2	70.0 (168/240) W3+D	65.8 (242/368)
3	90a	94.8a	35 (208/600) W1+D+W2	79.7 (102/128) D+W2+W3	90.4 (217/240) W3+D+W2	86.7 (319/368)
4	91a	97a	38 (227/600) W1+D+W2+W3	100.0 (128/128) D+W2+W3+W1	100.0 (240/240) W3+D+W2+W1	100.0 (368/368)

[1] Across trap density (1-4 BGS) and release populations (10, 25, 50, 100, 150, 200 and 250) in 140m3 cubicles

[2] Analysis of BGS density contribution at RN100, the population density used experimental hut trials

[3] Based on consolidated data for location and distance effects in chemical free experimental huts (marked recaptured/marked released) and locations (going counterclockwise facing the door-window 1 (W1), window 2, and window 3) used at 0m on hut platform

[4] BGS contributions added from most productive (highest recapture) to the least productive (lowest recapture) sites, (females recaptured /total recapture) of natural population (density not known) from sentinel households (SH)

Modeling recapture using semi field (screen house) system showed that the use of 2 traps is as statistically efficacious as the use of three to four traps (Table 28). Using release population of 100, the use of 3 traps recaptured same efficacy as four. Unlike screen house areas where we tried to prevent bias from placement positions, experimental hut studies showed that there were favored locations where higher BGS recaptures can be obtained (Table 28). Experimental hut data showed the locations in decreasing order of recaptures: Window 1 (15%, 90/600) > Door (12%, 72/600) > Window 2 98% , 46/600) > Window 3 (3%, 19/600). These varying degrees of BGS productivity in different locations from the experimental is also witnessed from two sentinel houses during the local home trial. The productive locations however vary with household (SH1:Door (34%, 44/128) > Window 2 (23%, 30/128) > Window 3 (22%, 28/128) >Window 1(20%, 26/128) while in SH2: Window 3 (43%, 102/240) > Door (28%, 66/240) > Window 2 (20%, 49/240)>Window 1(10%, 23/240) probably due to surrounding competing resting and breeding sites and proximity to humans. The use of 3 BGs traps in screen house trial showed 90 and 95% recaptures across release populations and using 100 release numbers. In the experimental huts the use of 3 BGS traps in most productive location contributed 35% (208/600) recaptures, and 92.5% (208/227) of all marked recaptured mosquitoes (Table 28). The use of 3 BGS in their most productive sites from local homes contributed 87% (319/368) of captured natural population from 8 months (Feb-Sep 2011) monitoring period, 12h/5d/4BGS trapping. The use of 3 or 4 traps in the future will depend on the wanted disease impact as probably would be quantified in the future push-pull demonstration. Depending on available resources, several pieces of information can be considered for BGS deployment to local homes;1) number of BGS to be used, 2) placement (location and distance), 3) peak time of collection and 4) seasonal abundance which were all generated using BGS monitoring for 8 months.

As a corollary to the findings synthesized above, a recent report documented that between households, BGS collections varied significantly, however very little variation was observed from collections within a premise over a few days (Williams et al., 2007). This outcome could be explained in two ways: 1) location of BGS in each household is not standardized, therefore trap efficacy varies between houses based on trap locations (e.g., veranda vs. carport) and the absolute adult population varies within each household. Our results verified this contention that BGS location affect numbers collected, or in one house, BGS can collect different densities at different positions, as there are locations more productive than others (affected by productivity of breeding/resting places in proximity and availability of enhancing human host cues). These suggest that there is a need to standardize trap location and assess the environment to which the trap is set, probably to include the amount of clutter in the immediate surroundings, to which for our case, major clutter could be presence of competing breeding/resting sites which we have documented.

Another point that was noted by the same study (Williams et al., 2007) was that there were limitations from which BGS can deployed in private premises. These would be areas where the trap are easily accessed, locations granted by the house owners. The distribution of BGS in their study area (Cairns, Far North Queensland, Australia) was not randomly or evenly distributed. BGS distribution was rather determined by the access granted by the owners (basically outside-peridomestic area) (Ball, 2010). This finding can impact our future push-pull activities and should be taken seriously, though by principle we intend to use BGS only within peridomestic area. Our preliminary studies in Thailand and Peru however showed high probability of future acceptance of this intervention in our target household (Paz-Soldan *et al*., 2011).

Capture time trends indicate most productive period for collecting *Ae. aegypti* using the BGS is 1330-1730h while *Ae. albopictus* were in high numbers during both morning and afternoon. Both the productive location of BGS and peak time of collection may have operational significance as they relate to BGS availability and duration of how long should BGS be operated in a day. Peak time of female *Ae aegypti* BGS collection coincides with the afternoon peak biting times in Thailand earlier reported at 15.00 hours, with diurnally sub periodic resting cycle peaking at 10.00 h (Sucharit *et al.*, 1993). In Bangkok, the rhythms of biting activity changed slightly from season to season (Yasuno and Tonn, 1970). Like the afternoon peak in the cool season was recorded between 14.00 and 15.00 hours, and then it shifted an one hour later in the hot season and later still in the rainy season. Nevertheless, the peaks were found mostly 2-3 hours before sunset, and thus the biting activity appears to be controlled by the cycle of day and night. The time of the afternoon peak of biting activity in Bangkok is earlier than that of East African mosquitoes, which were most active just before sunset (Chadee and Martinez, 2000). In the application of push-pull strategy with BGS as component should be supported by the biology and distribution of *Ae aegypti* in specific locality.

Total monthly numbers of *Ae. aegypti* during dry (February-May 2011) and rainy (June to September 2011) seasons from SH were: 48 and 45 females using BGS while 5.2 females and 0.5 females from CDC-BP collections. Total mean number of *Ae. aegypti* for dry and rainy seasons were 7.2 and 2.8 females, respectively; from all the households monitored using CDC-back pack. Results showed that BGS works under two different Thai household conditions, raised and non-raised, cement and wooden, documenting differences in densities between periods of monitoring (rainy and dry seasons); collecting not only *Ae. aegypti* females but also males and the secondary vector *Ae. albopictus* from their productive sites.

Based on our results, BGS appeared to be an excellent tool for detecting rises and falls in absolute abundance. This result agrees with the another study by Williams *et al*., (2008) where BGS compared to CIMSiM, appeared to be more sensitive in detecting changes in adult absolute abundance. The container inhabiting mosquito simulation model (CIMSiM) is a weather driven life table simulation model designed to estimate abundance of container breeding mosquitoes such as *Ae. aegypti* (Focks *et al*., 1993). It is considered to be the most detailed tool available for understanding population dynamics of *Ae. aegypti* (Magori *et al.*, 2009). Recently Williams *et al*., (2008) validated CIMSIM in Cairns, North Queensland and found that it can accurately estimate pupal crop from which adult estimates were generated.

This program was earlier reported to be used in New Orleans, Louisiana, USA to estimate adult densities based on pupal period, sex ratio and survival rates (Focks *et al*., 1981). This is another important study where the data generated by BGS was valued highly over sophisticated monitoring system as CIMSiM .

These results also show the potential role of BGS traps in preventing / picking up mosquitoes drawn away by using repellents and may also prevent entry of females from the outside population. In the future activities, it will be interesting to determine if the lower *Ae. aegypti* numbers found inside the sentinel houses (SH) are due to removal by BGS prior to entry. The presence of the vector indoors and outdoors strengthens argument for combined PPS strategy attacking both indoor and outdoor spaces.

The use of BGS as part of PPS control strategy is in principle may prove to be compatible with the rest of the currently recommended methods for control; environmental management (i.e., improvement of water supply and water-storage systems, mosquito-proofing of water-storage containers, solid waste management, street cleansing), chemical control (i.e., larvicides, adulticides), individual and household protection, biological control, insecticide treated materials and other traps (i.e., lethal ovitraps) and probably for both use of RIDL and *Wolbachia* which are currently in the pipeline.

Looking beyond optimistically, highly efficient traps is going to come out soon, and would really out grow its customary role as part of surveillance and monitoring programs. The tsetse fly success was instrumentally brought by the development of effective targets that combine visual and olfactory attractants to locate their hosts (Vale 1993, Jordan 1995) showing that the trap technology can be successful against a haematophagous pest. Our study however with the use of BGS trap, having both olfactory and visual components showed that trap can be made part of a control strategy in combination with spatial repellents to increase protection-contribution to control. Having those into consideration, the use of BGS needs knowledge of the spatial distribution of *Ae aegypti.* The results of our study agree with earlier suggested approaches for trapping. Day and Sjogren (1994) describe four approaches to the deployment of traps or targets: 1) to attract mosquitoes away from where protection is desired (this is the 0m distance where greatest interaction between human host and mosquito happens), 2) to situate traps around the protection area as a perimeter barrier (peridomestic area of houses), 3) to place traps or targets individually within the protection area (combination of the two) , and 4*)*) to intercept mosquitoes during dispersal from breeding sites or resting sites (the same concept why survey of breeding/resting site was made and also with their relative distances from the house). The argument that low collections from CDC-back pack aspirations could be due to removal prior to entry, wherein *Ae. aegypti* females were intercepted during dispersal from breeding sites or resting sites, a good argument to place them near productive breeding sites which were mostly within 0-3m distance in the case of our study area.

The presence of low reflective harbourage sites nearby the traps affected trapping efficacy on both males and females, more pronounced in males (Ball, 2010). However, untidiness may ultimately be one of the variables the attracts *Ae aegypti* to the premise (Ball, 2010). This is an argument that could be explored in the future in terms of placement if clutter is found near portals of entry and ist possible effect on BGS recapture rates.

Knowledge of basic population parameters and dynamics is also essential to determine the extent of trapping required to attain a certain level of population control. Weidhaas and Haile (1978) estimated that, depending on the biotic potential of the mosquito species, the trapping requirement could be as high as 40% per day to achieve a substantial reduction in the population. Our artificial releases showed that about 68-98% of females can be recaptured from the screen house and bout 20-40% from the experimental huts. Service (1995) theorized that the immense biotic potential and population densities of mosquitoes make it unlikely that traps or targets alone could reduce mosquito populations to an acceptable level. The idea of combination approaches like PPS increases the possibility of the strategy to work. This has to be quantified in the future studies on how BGS really affects population of *Ae. aegypti*. The overall results of characterizing BGS supports the final goal of integrating its use in push-pull strategy.

CONCLUSION AND RECOMMENDATION

It is the goal of this study to understand the role of a peridomestic trap (BG-Sentinel ^{TM}) as part of a novel push-push dengue vector control strategy. From its initial stage of modeling efficacy rates in a screen house setting generating number of traps required in varying field populations to the use experimental hut in determining appropriate location and distance for maximum trap recaptures and finally in local homes to show efficacy in the presence of competing *Ae. aegypti* resting sites. BGS traps show promise as part of a control strategy that could probably provide contribution beyond monitoring and surveillance function by lowering man-mosquito contact through prevention of vector entry into homes and diversion non-protected areas and in lowering peridomestic populations of vectors.

The screen house study determined changes in BGS recapture rates of *Ae. aegypti* under varying mosquito population densities to identify optimum trap numbers to use in a natural setting. This included evaluating varying numbers of traps (1-4) and mosquito release numbers (10, 25, 50, 100, 150, 200, and 250) on recapture rates under screen house conditions. Based on these variations in trap and mosquito numbers, release intervals were rotated through a completely randomized design with environmental factors (temperature, relative humidity and light intensity) monitored throughout each experiment. Data from four sampling time points (0530, 0930, 1330 and 1730 hours) indicate a recapture range among treatments of 66-98%. Furthermore, 2-3 traps were as statistically effective in recapturing mosquitoes as 4 traps for all mosquito release numbers. Time trends indicate Day 1 (the day the mosquitoes were released) as the “impact period” for recapture with peak numbers of marked mosquitoes collected at 0930 hours or 4 h post-release. Such information was not previously generated and represents new scientific knowledge important for mosquito surveillance and control implementations using BGs traps.

Second phase dealt with post exposure studies that determined the effect of chemical repellent exposure on BGS *Ae. aegypti* recapture rates under field conditions using experimental hut and screen house evaluation methods. From varying DDT field application rates (FAR) and surface area coverages (SAC) tested, results indicate no significant differences on BGS recapture rates between control (no chemical exposure) and treatment cohorts (exposed to chemical) for either IR or DR populations. Similarly, results from metofluthrin experiments showed no significant differences between controls and treatments using high dose (0.00625%) coils and low (0.00312%) dose coils for both IR and DR. Significantly higher recaptures however were obtained from DR as compared to IR for both low and high dose metofluthrin coils experiments. The use of both 0.125 (=5 µg *ai*/cm^2) and 0.0625 FAR (=2.5 µg *ai*/cm^2) transfluthrin at 25% SAC showed significantly lower recaptures compared to controls from IR, but no significant difference from DR. DR transfluthrin releases showed significantly higher BGS recaptures than IR.

Location trials revealed higher trap recapture rates obtained when the BGS were positioned opposite portals of entry (38.67 %; 116/300) (windows and doors) of experimental huts as compared to vertices (23.67 %; 71/300). Data from interception traps also showed that higher reduction in entry from BGS located opposite portals of entry (69%) 37/300 compared to those located on vertices (31%) 82/300. Highest recaptures of 18.50% (111/600) and 14.22% (128/900) were obtained from 0m and 10m distances, respectively from distance optimization trials. Lower recapture of 7.89% was recorded from 3m. Interception traps data however supports the use of 0m location opposite portals of entry as indicated by highest percentage reduction in entry at 0m (65.62%) compared to 3m (17.19%) and 10m (14.59%) distances.

The use of either spatial repellent (17-79%) or BGS (41-59%) contributes to the reduction in entry to the experimental huts, highest reduction however were recorded when they are combined in push-pull (65-87%) set-up.

Survey of competing resting sites showed about 90% (199/221) of all water containers were found within 0-3 m distance. Predominant container were jars =54%

(119/221), drums/tanks =22% (48/221), basins=9% (20/221), pail=7.23% (16/221) and tyres =5.89% (13/221).

BGS monitoring showed peak *Ae. aegypti* population from Mar-May with a spike in August (75 females and 14 males/12h-5d/4 BGS) and lowest in February (6 females and 4 males/12h-5d/4 BGS). Two most productive BGS locations were identified from each of the sentinel household, window 3 (with highest numbers of female and male *Ae. aegypti*) and door (with highest numbers of female and male *Ae, albopictus*) from the raised SH while door (with highest numbers of females and males *Ae aegypti* and male *Ae. albopictus*) and window 2(where highest numbers of female *Ae. albopictus*) locations from the non raised. Capture time trends indicate most productive period for collecting *Ae. aegypti* using the BGS is 1330-1730h (Figure 35) while *Ae. albopictus* were in high numbers during both morning (0530-0930 h) and afternoon (1330-1730 h). Total mean numbers for dry (Feb-May) season and rainy (Jun-Sep) season from SH were 48 and 45 females/12h-5d/4 BGS/month with 5.2 and 0.5 females/20 min/month for CDC-back pack collections in the same time period, respectively. Total mean number of *Ae. aegypti* for dry and rainy seasons were 7.2 and 2.8 females/20 min/month for all the households (including the sentinel houses) monitored using CDC-back pack aspirator.

Using optimized conditions, BGS functioned to collect populations at both raised wooden /non-raised cement household structures representing different complex environments. Although *Ae. aegypti* indoor collections were low using CDC-back pack aspiration; the BGS continued to indicate vector population fluctuation in peridomestic environment. This further strengthens the argument for combined PPS strategy, to attack both indoor and outdoor spaces. These information leads to a pilot PPS demonstration under a “real-life” scenario. Future studies including a pilot demonstration trial will attempt to determine if the low *Ae. aegypti* indoor density is due to removal by BGS that prevented entry. Low natural indoor *Ae. aegypti* population poses a challenge to monitor them in the pilot demonstration.

For larger push-pull startegy application, there is a need to establish of the spatial distribution of vectors for an early risk assessment for a more targeted BGS placement and distribution. The study further pointed out that the number of BGs to be used can be further streamlined as supported by presence of productive sites, by the peak of collection when and how long BGS should operate and the species of vector present. Regular updates on vector biology and behavior is required to provide basis for interpretation of trap catch that will enable the linking of monitoring programs with prevention and control options. Sensitivity of traps can also be explored as they relate to specific physiological stages of *Ae. aegypti*.

A continuing evaluation of candidate traps with new mechanisms and lures can also be done as push-pull strategy progresses. Alternative studies on the use of lures derived from the mosquito itself maybe worth pursuing into. The currently available BG Lure is derived from human sweat components; it will be rather interesting to try insect derived ones. In agricultural pests, studies are being done to evaluate action of some compounds as male-produced volatiles that may function as sex pheromone or aggregation pheromone (Bryning *et al.* , 2005; Olsson *et al*. 2006; Tanaka *et al*., 1986; Chambers *et al*., 1996). These substances may assist the development of lures and may provide insights for novel control mechanisms if methods to interfere with the behavioral responses can be identified. The same principle could be applied in *Ae aegypti* when there are compounds synthesized functioning as such. As earlier cited, presence of females can increase attractiveness of host to attract more females, so mosquito derived substances may show promise in trapping. The development/improvement of lure dispensers that can release volatiles consistently over a period for several months and over a broad temperature range will increase capacity of traps.

In the future it may be possible that molecular approaches could provide a means to initially screen for or rapidly determine whether an insect will be able to perceive a chemical and whether there is likely to elicit behavioral response. Odorant binding proteins, which may transport odor molecules through the sensilla lymph, have been identified in several insect orders (Honson *et al*., 2005) and olfactory receptors have also been identified (Clyne *et al*., 1999). These could be use as lure or attractants for new generation of traps. Application of the same principle may work for the future control of *Ae. aegypti*.

Assessment of the performance of traps in public spaces and non-residential areas, application of BGS traps in other vectors, push-pull strategy in other disease vector systems maybe future push-pull strategy applications.

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