Scientific Note

An automated, field-compatible device for excito-repellency assays in mosquitoes

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The behavioral responses to insecticides by mosquitoes are important components of a chemical's overall effectiveness in reducing human-vector contact and should be carefully evaluated to understand the primary mechanisms involved in controlling vector activity and disease transmission. Excito-repellency (ER) responses of mosquitoes, divided into contact irritancy and noncontact repellency to chemicals (Roberts et al. 1997), have been evaluated in a number of ways. One of the first methods was developed by the World Health Organization using plywood to construct an ER test box that measured the irritant effect of insecticides on mosquitoes, followed by studies using various modifications of the WHO test design (Bondareva et al. 1986, Ree and Loong 1989, Pell et al. 1989, Quinones and Suarez 1989). Rachou et al. (1966) developed a plywood experimental box for testing the escape responses of *Anopheles albimanus* to DDT, and a similar test system was also used to observe the behavioral responses of *Anopheles darlingi* to DDT exposure (Charlwood and Paraluppi 1978). Roberts et al. (1984) developed a collapsible excito-repellency test box for field testing of *An. darlingi* against DDT. Years later, a light proof test chamber was developed to study the behavioral responses of *Anopheles gambiae* to several test compounds (Evans 1993).

Because of the inherent complexities of accurately measuring excito-repellency in mosquitoes, no one test method had been widely accepted as a standard for conducting assays, data gathering, analysis, and interpretation (Brown 1964, Roberts et al. 1984). Improvement came when an experimental escape chamber system was developed that could distinguish irritancy and repellency (Roberts et al. 1997). This test system was first used to study the avoidance behavior of *An. albimanus* to DDT and synthetic pyrethroids in Central America (Chareonviriyaphap et al. 1997). However, it proved to be somewhat cumbersome and required extended time to set up and attach test papers (treated and untreated) on the inside walls of the chambers. Soon afterwards, another version of the excito-repellency test chamber was devised to help alleviate some the burdens associated with the previous test design (Chareonviriyaphap and Aum-Aung 2000, Chareonviriyaphap et al. 2002) and proved valuable in the evaluation of behavioral responses by several laboratory and field populations of mosquitoes in Thailand and Indonesia (Chareonviriyaphap et al. 2001, 2004, Sungvornyothin et al. 2001, Kongmee et al. 2004, Potikasikorn et al. 2005, Chareonviriyaphap et al. 2006). Unlike previous "fixed" construction designs, the new chamber system was a collapsible device for easier transport to the field, and it also greatly reduced the time required to attach the test papers between test trials. However, this system was still cumbersome and required a minimum of two investigators to observe and record data during the 30-min testing period. The test design also required a relatively high number of mosquitoes (25 per test chamber), at times an impractical demand under field conditions. Recently, an assay for evaluating excito-repellency and toxicity in adult mosquitoes was developed (Grieco et al. 2006); but it was not designed as a field-adaptable apparatus. To help overcome this frequent problem when conducting field studies, a more compatible design has evolved. For the device described here, two major modifications from previous models were made: a substantial reduction in the size of the test box and the use of an electronic sensor for automated counting of mosquitoes as they escaped from the test chamber through the opening gate into the external holding cage (Figure 1).

The fundamental structural design of the new ER chamber (1) remains similar to the previous version (Chareonviriyaphap et al. 2002). The main supporting structure is fabricated using stainless steel, each side wall measuring 23 x 23 cm^2 in size. The chamber walls have an aluminum side tongue and groove configuration on joining ends that makes it easier and faster to set up and disassemble for transportation and storage. The frame of the inner chamber is constructed of 22.5 x 19 cm stainless steal beams, which include metal holders for securing test papers on either of two sides for the dual purpose of either providing contact or noncontact exposure designs. For noncontact tests, a thin sheet of fine mesh iron screening secured on the opposite side of the test paper allows for a 1.5 cm gap that prevents mosquito tarsal contact with the test paper. A Plexiglas^{TM} panel at the rear of the chamber is equipped with a 11.5 cm diameter hole sealed with overlapping dental dam, allowing test specimens to be either inserted or removed from the inside of the chamber while minimizing accidental escape during handling. There is a forward exit portal (13.5 cm x 2 cm) connected to a funnel projecting from

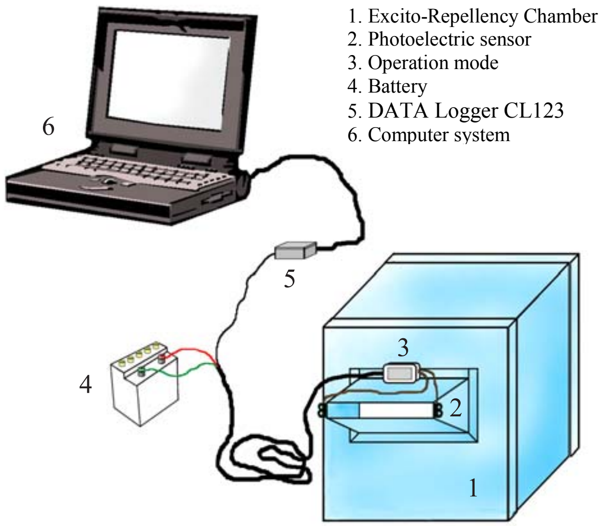

Figure 1. Automated excito-repellency test system.

the box with an electronic sensing device affixed at the point of the exit portal.

The photoelectric sensor (FX-301, SUNX Limited, Aichi, Japan) (Figure 1, #2) detects and counts escaping mosquitoes, automatically recording any flying object < 0.1 mm in size without requiring physical contact. The sensor has two operation mode switches (#3), a jog switch, and a MODE key required for operating the system. The MODE key operates the "mode selection" and "mode cancel" functions, while the jog switch selects the desired numerical values available for each mode. To record data during the observation period, the DATA Logger CL123 (#5) is connected to the photoelectric sensor and records values at three signal channels, one analog and two digital. The DATA Logger CL123 is a small, battery-operated device (#4) with software to record and transfer data in tabular and graphic form to the computer system (#6). The entire system can be programmed to record escaping mosquitoes at 30 s-intervals until test completion (30 min). The previous recording interval with human observation was set at 1-min periods.

This improved system provides distinct advantages over the previous version as it can accurately and automatically count and record escaping mosquitoes, thereby eliminating error by human observation alone while also preventing any possible confounding factors or bias produced by human attractant/stimulant cues (e.g., carbon-dioxide, odor, body heat) that could influence test results. The reduction in size of the device also makes it easier to transport to and from the field.

This improved excito-repellency device has been used to measure the behavioral responses of a field population of *Aedes aegypti* from Bangkok to single standard operational field concentrations of 0.02 g/m^2 of deltamethrin. Assay results revealed that test mosquito populations quickly departed chambers, indicating strong irritancy following direct contact with deltamethrin (Figure 2). As in previous studies, a complete test trial consists of four chambers, two treated with insecticides (one for contact, the other for noncontact) and two paired control (without treatment) chambers, respectively. However, we have reduced the number of unfed female mosquitoes required for each chamber from 25 to 15, a 40% reduction per trial, while retaining the statistical accuracy of the analysis (Roberts et al. 1997). This improved test chamber provides a highly reliable and objective record of the precise

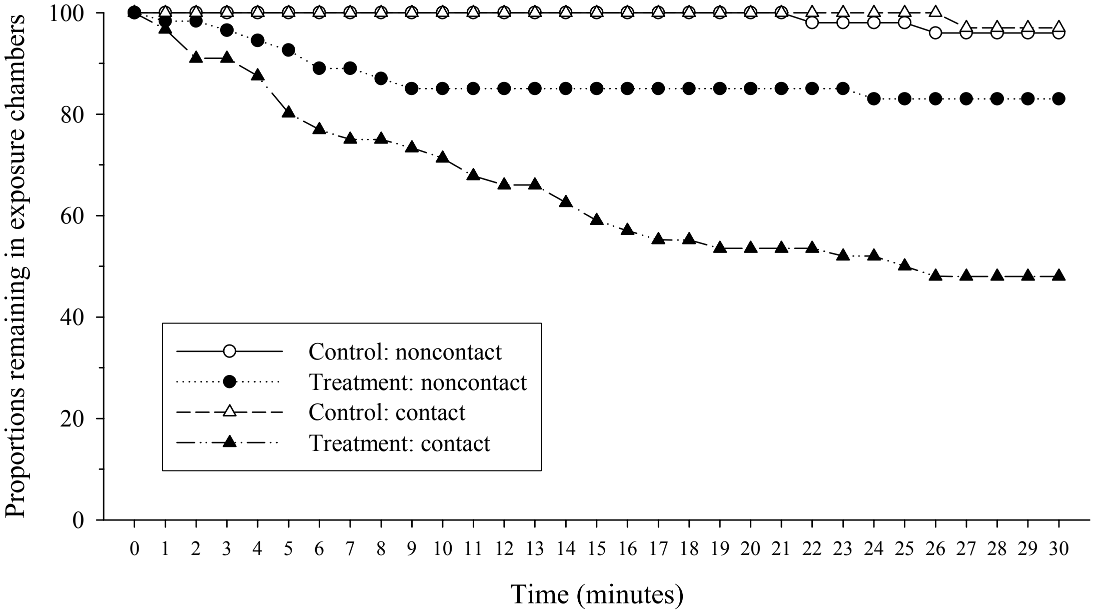

Figure 2. Behavioral responses of *Aedes aegypti* exposed to deltamethrin (0.02 g/m^2) in contact and noncontact exposures.

time interval when mosquitoes exit the test chambers. The design retains the ability to be easily transported to the field and, together with a substantial reduction in the previous number of mosquitoes required per test and the automated counting of exiting mosquitoes using a photoelectric sensor, allows greater flexibility to conduct excito-repellency tests. This automated detection system is easy to operate and eliminates human observer error.

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