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ATTRACTANT RESTING BOXES FOR RAPID COLLECTION AND SURVEILLANCE OF *AEDES AEGYPTI* (L.) INSIDE HOUSES

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ABSTRACT. Three different resting station designs made of cardboard covered with black cloth were evaluated for their attractiveness to *Aedes aegypti* populations resting inside houses in Thailand. Box designs attracted more females (but not males) than an open-panel design. Thirty to 60% of all adult *Ae. aegypti* resting inside houses were collected by aspirating from 2–4 resting boxes placed inside houses. Tall boxes (90 cm) did not attract more females or males than short boxes (45 cm). Forty-two percent more females (but fewer males) were attracted to boxes when oviposition bowls were placed inside the boxes. Four boxes per house attracted 32% more mosquitoes than two boxes. Aspirating from artificial resting stations is an efficient and rapid method for sampling male and teneral, bloodfed, and gravid female *Ae. aegypti* resting inside houses.

INTRODUCTION

Aedes aegypti (L.) is the primary human vector of yellow fever and dengue fever viruses (Gubler 1989, Monath 1989). It has been the subject of intense study for over 50 years (Christopher 1960). Collection of wild adults for research, surveillance, and virus isolation attempts is hampered because this diurnal domestic species is not attracted to conventional mosquito traps. Daytime human-biting collection has been the main method for sampling females of this species. Biting collections are costly and laborious and put collectors at increased risk of contracting disease. Moreover, they are limited because they undersample males and teneral, gravid, or engorged females. In recent studies in Puerto Rico (Scott et al. 1993a, Van Handel et al. 1994) and Thailand (Edman et al. 1992, Scott et al. 1993b), these limitations led to the employment of hand-held aspirators to collect resting adults. Although these vacuum devices also are used to collect mosquitoes outdoors, the great majority of *Ae. aegypti* rest inside human dwellings (Scott et al. 1993a, 1993b). Depending on the size and number of rooms, one collector can thoroughly vacuum all potential resting sites in a typical Thai or Puerto Rican house in about 10–15 min.

While vacuum-aspirating *Ae. aegypti* inside houses during previous studies, we commonly observed that adults prefer to rest in darker locations and on dark, nonreflective surfaces such as clothing (see Fay 1968, Fay and Prince 1970, Pant and Yasuno 1970). These field observations are entirely consistent with laboratory experiments characterizing the resting preferences of this species (Muir et al. 1992). Mark-release-recapture studies on *Ae. aegypti* survival and dispersal in rural Thailand required daily collection from a large number of houses (Day et al. 1994). This requirement fostered the idea of accelerating the sampling by aspirating adults from attractant resting stations placed within each house rather than from the entire house.

Initial observations on the potential attraction of adult *Ae. aegypti* to artificial resting stations consisted of tacking 1-m^2 pieces of black muslin cloth near the bottom of the exposed wall joists in dark back corners of 2 different houses. Resting mosquitoes were first aspirated from these surfaces and then from the remainder of the house. Since it appeared that many mosquitoes were resting on the cloth squares, formal experiments were conducted to quantify this attraction.

This paper describes experiments carried out inside houses in Village 6 of Hua Sam Rong District in Chachoengsao Province in eastern Thailand. Our ultimate goal was to be able to artificially concentrate *Ae. aegypti* populations resting inside houses by taking advantage of the distinct resting preferences of this species.

MATERIALS AND METHODS

Initially, two types of resting station were evaluated. One was a box design and the other consisted of two flat panels cut halfway in and positioned in an egg-crate or X design (Fig. 1). Both were 90 cm high and made of medium-weight brown cardboard. All panel surfaces were covered with black muslin cloth, but only the interior surface of the box was covered with cloth. After the initial experiments comparing these two designs, the exterior surfaces of the boxes were covered with cloth as well. Cloth was attached with a staple gun. Later, a shorter version of the all-black box design was made by reducing the box from 90 to 45 cm tall (Fig. 1). For experiments in which an oviposition site was added to the box, a 25-cm-diameter plastic bowl lined with a piece of black cloth was half filled with water from local storage jars and placed in the bottom of the resting box.

Four to 12 adjacent houses were used for trap comparisons and 1–4 boxes were placed within each test house depending on the experiment. Trap loca-

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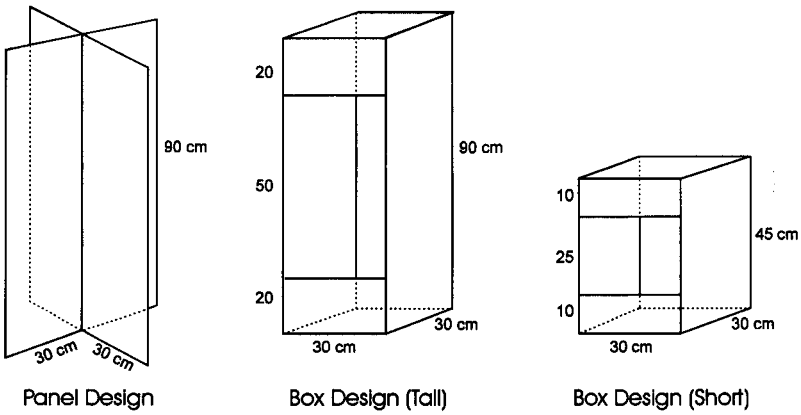

Fig. 1. Diagram of the 3 resting station designs evaluated for collecting *Ae. aegypti* inside houses in Hua Sam Rong, Chachoengsao Province, Thailand.

tions within houses as well as the type of trap assigned to the house were rotated daily so that all houses and sites within houses were equally exposed to all experimental treatments. All collections were made between 9:30 a.m. and 11:30 a.m. using a modified vacuum cleaner aspirator (Scott et al. 1993b). In the initial experiment comparing box and panel designs, all houses were sampled a second time between 1:30 p.m. and 3:30 p.m. Test boxes were aspirated first, for about 30 sec per box; then the remainder of the house was aspirated until no more mosquitoes were observed (8–10 min). Collections were separated by replacing the collection carton on the aspirator and labeling it with removable surgical tape. Collection cartons were immediately placed on ice and brought to a field laboratory for identification, sexing, and numeration of all *Ae. aegypti* with the aid of a dissecting microscope. A large number of *Culex* and *Anopheles* species also were collected in the houses and in the resting stations, but these were not identified or counted.

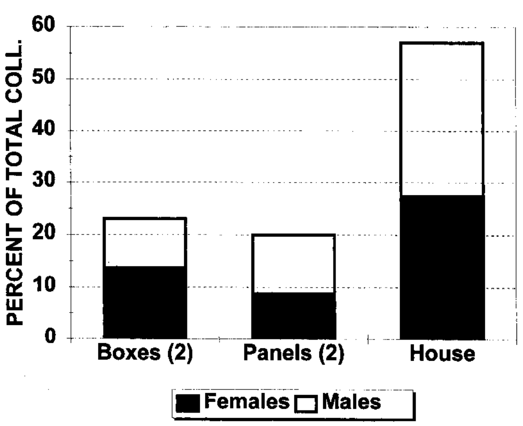

Fig. 2. Comparison of the proportion of male and female *Ae. aegypti* (n = 2,703) collected from resting boxes vs. resting panels vs. the remainder of the house.

EXPERIMENTAL DESIGN AND RESULTS

Comparison of box and panel design: Two box and 2 panel resting stations were placed within each of 12 houses. Stations were placed in the darker sections of the house and in locations where they would not be disrupted by household activities. Locations of the boxes and panels were rotated daily following the afternoon collection. Collections for the 2 boxes and the 2 panels were combined into a single 1-min collection for each design. The third, separate collection, 8–10 min from the entire house, followed the collections from the resting stations. Collections were made for 8 consecutive days (Fig. 2).

The total number of male and female *Ae. aegypti* collected from the 12 houses during the 8 test days was nearly identical (1,358 males vs. 1,345 females). About 30% more mosquitoes were collected in morning collections (1,510) than in afternoon collections (1,193) from the same houses. However, the percentage of the collections taken in the resting stations as opposed to the houses proper did not vary significantly between morning (41%) and afternoon (46%) collections. Therefore, morning and afternoon collections were combined when assessing the efficiency of the resting stations. Forty percent of all males and 46% of all females collected from the 12 houses were collected from the 4 resting stations as opposed to the houses themselves. The boxes were more efficient for collecting resting females (374 vs. 231) but the panels produced more males (297 vs. 252).

On average, 14.2 female *Ae. aegypti* were collected per house per day (a.m. and p.m. collections combined) and 6.5 of those were collected in the 4 resting stations (*ca.* 2 min) while 7.7 females were taken while aspirating the remainder of the houses (8–10 min). Thus, collecting from resting stations was nearly 4 times more productive than aspirating from the houses themselves.

Results indicated that boxes were significantly better than panels for attracting female *Ae. aegypti* (χ^2 =

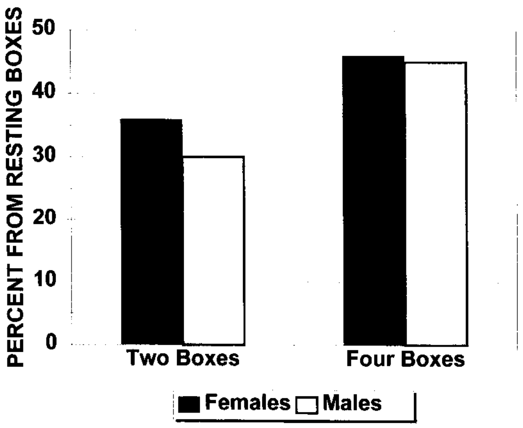

Fig. 3. Comparison of the efficiency of 2 vs. 4 resting boxes per house for collecting male and female *Ae. aegypti* (n = 1,373).

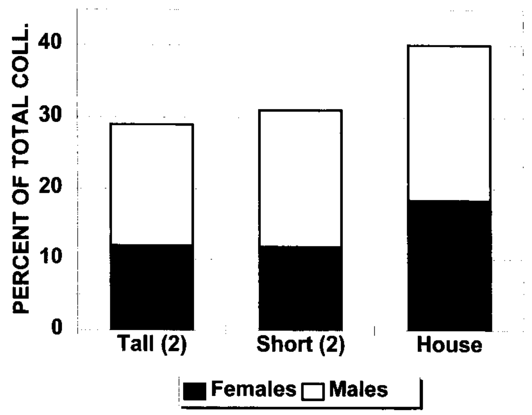

Fig. 4. Comparison of the proportion of male and female *Ae. aegypti* (n = 509) collected from tall resting boxes vs. short resting boxes vs. the remainder of the house.

29.38, $P < 0.00001$, df 1) and led us to cover the outside surfaces of the boxes with black cloth in order to make them equally attractive to males. In a 2-day preliminary test in 6 houses, 2 all-black boxes with added water bowls produced 154 adult *Ae. aegypti* vs. 89 in the 2 standard boxes that were black only on the inside and contained no water bowls.

Number of boxes per house: Two all-black boxes were placed in 4 houses and 4 all-black boxes were placed in 4 neighboring houses. The houses containing 2 or 4 boxes were reversed each day following the morning collection so that each house contained 2 boxes for 10 days and 4 boxes for 10 days (Fig. 3).

Forty-five percent (147 of 324) of adult *Ae. aegypti* collected in houses with 4 boxes were aspirated from the boxes, whereas 32% (116 of 358) were collected from the boxes when only 2 boxes were present in the same house. Halving the number of boxes did not halve the number of mosquitoes in the boxes; the reduction was about 30%.

Size of boxes: All-black boxes 90 cm tall were compared with boxes 45 cm tall to assess the effect of box size on the efficiency of attraction. Box size was evaluated with and without the addition of a bowl containing water to provide an oviposition site and enhanced humidity within the box. Two short and two tall boxes were placed in each of 4 houses. One short and one tall box in each house had water added and the position of short and tall and wet and dry boxes was rotated daily for a total of 20 collection days.

Neither sex showed greater attraction to larger boxes, so data for both sexes were combined (Fig. 4). Females responded positively to the addition of water (71 collected in wet boxes vs. 50 in dry boxes), as suggested by an earlier preliminary test, but this difference is not significant by chi-square analysis. This increased attraction (42%) was not observed for males. More males were collected in dry boxes (112 in dry boxes vs. 72 in wet boxes); 36% fewer males were taken when water was added to the boxes (Fig. 5). The overall efficiency of the 4 boxes combined, when compared with collections from throughout the house, was 57% for females and 62% for males. This was the highest efficiency rate of any experiment.

DISCUSSION

Artificial resting boxes (usually black or red in color) of various sizes and shapes have been placed in a variety of field situations, mainly to collect species of *Anopheles, Culex,* and *Culiesta* mosquitoes that naturally prefer to rest in shaded and protected locations (Service 1993). The principal advantage of collecting resting rather than active mosquitoes is that the sample is more representative of the entire adult population. Despite the fact that *Ae. aegypti* have been known for many years to have resting preferences, this knowledge has not been

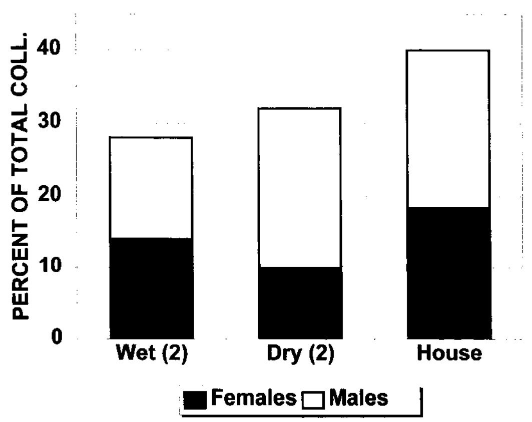

Fig. 5. Comparison of the proportions of male and female *Ae. aegypti* (n = 509) collected from wet vs. dry resting boxes.

exploited to develop indoor resting boxes to expedite the collection of this important vector species.

Our experiments in Thailand clearly demonstrate the utility of such a strategy for quickly sampling the indoor-resting population of this species. Further experiments are needed to better establish the optimum and most practical size, number, and location for resting boxes, but this may depend as much on local conditions and the purpose for which collections are being made. Depending on the size and layout of the house, anywhere from 20 to 70% of all the *Ae. aegypti* in a given house can be enticed to rest in artificial resting boxes placed in the house. The number of boxes per house and whether they contain a water source can be readily manipulated to influence the efficiency of this collection procedure. Resting boxes need not be large, but black, nonreflective surfaces are recommended for maximum attractancy (Muir et al. 1992). Males are as attracted to dark surfaces as are females, but they appeared to remain in more exposed areas. Perhaps this affords them more opportunities for locating and mating with host-seeking females.

Resting boxes also appear to have excellent potential as a surveillance tool. Landing rate counts in our study village and in neighboring villages averaged about 2–4 female *Ae. aegypti* per house per hour (Strickman and Kittayapong, personal communication.). Approximately the same number of females can be aspirated in only 1 min from 2–4 resting boxes placed within a house.

Because up to 50% or more of the indoor population can be readily attracted to artificial resting boxes at any point in time, we speculated that if the black cloth lining the boxes were impregnated with a nonrepellent pyrethriod (e.g., deltamethrin), these resting boxes might be deployed in a community to prevent or suppress transmission of dengue fever during epidemic periods. Adult females must survive 10–14 days to become potential vectors of dengue virus, so vector mortality need not be immediate for the interruption of transmission. In fact, delayed efficiency may be a positive attribute of this strategy if it serves to avoid development of resistance to the pesticide, as has been reported for impregnated bednets used to control *Anopheles* vectors of malaria (Vulule et al. 1994). We are currently exploring this idea and, in some very preliminary results, both the age of females (determined by parity) and the number of adult *Ae. aegypti* were significantly reduced almost immediately after the introduction of 4 boxes with deltamethrin-impregnated cloth lining the inside (4 treatment and 4 control houses). Dramatic population reduction of *Ae. aegypti* has been reported recently in houses containing impregnated bednets for malaria control in Zaire (Karch et al. 1995). This provides further evidence of the potential of this vector control strategy in dengue prevention.

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