A SELF-MARKING DEVICE FOR EMERGENT ADULT MOSQUITOES[1, 2, 3]

M. L. NIEBYLSKI AND C. L. MEEK

Department of Entomology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

ABSTRACT. A portable, tent-like device with powdered fluorescent pigment for marking emergent adult mosquitoes was evaluated in the field using *Culex quinquefasciatus* adults. The top of the device was equipped with cheesecloth partitions impregnated with the pigment. Eighty-six percent of the emergent adults passed through the partitions, and all were marked.

INTRODUCTION

Numerous methods have been used to label mosquitoes in mark-release-recapture studies to determine adult dispersal (Service 1976). Adult mosquitoes are typically marked with fluorescent dusts and powders by using an insulphator to puff small quantities of pigments onto the external surface of the body or by creating a dust cloud in an enclosed structure (Dunn and Mechelas 1963, Trpis 1971). Singh and Yasuno (1972) developed a self-marking device for emergent adult mosquitoes by artificially crowding immatures within the device. However, crowding of mosquito larvae tends to affect eventual adult dispersal (Nayar and Sauerman 1969).

The effectiveness of these marking systems is often limited by at least 2 factors: 1) possible interference with the natural behavior (e.g., flight) and 2) premature mortality due to inundative covering of the pigment on the insect body (Shapiro et al. 1944, Chang 1946, Sheppard et al. 1969). To avoid these limitations, a self-marking device was constructed and evaluated under field conditions using emergent *Culex quinquefasciatus* Say adults. The marking technique was based on the procedure described by Sheppard et al. (1973).

MATERIALS AND METHODS

The self-marking device consisted of 4 main components: 1) a polyvinylchloride (PVC) tubular framework, 2) an external heavy-duty screen covering, 3) an exit grid equipped with cheesecloth partitions impregnated with powdered fluorescent pigments (Fire Orange and Signal Green, A Series, DAY-GLO Color Corp., Cleveland, OH) and 4) a rain shield. The device in situ is pictured in Fig. 1. Mosquitoes emerging beneath the device were contained by the PVC tubular framework and external heavy-duty screen covering. They escaped from the device by flying upward and through an exit grid. Adults passing through the grid flew into and alit on cheesecloth partitions impregnated with pigment. This pigment was transferred to the mosquitoes upon contact, thereby marking all adults prior to dispersal.

The PVC tubular framework was constructed of 0.5-in thin-walled PVC pipe connected by PVC joints and PVC adhesive. These materials formed a primary support section 2 × 2 × 2 ft (Fig. 2G). Two opposing, lateral extension sections, each 2 × 4 ft, (Fig. 2C) were attached to the upper portion of the support section. Machine bolts (0.38 in diam × 4 in long with 16 threads/in) (Fig. 2F) inserted through 0.5-in PVC pipe caps (Fig. 2E) on the upper horizontal pipes of the support section were connected to the corners of the lateral extension sections with appropriate nuts and washers. These bolts secured the 3 sections into a single unit and on disassembly allow for the collapsibility of the device and subsequent transport to other sites. The most efficient prototype of the device measured 9 ft long × 2 ft high × 2 ft wide. The elongated form of the device was designed primarily for use over sewage ditches which are highly productive larval habitats; however, it is adaptable to other sites. Eight 9-in anchoring stakes (Fig. 2A) attached to each free end of the lateral extension sections by stainless steel wire (Fig. 2B) were used to secure the device to the ground.

The entire framework was covered with black, solar fiberglass screening (New York Wire, Fred Zimmerman Co., Dallas, TX). The 16 × 18 mesh screen (Fig. 3) was loosely stitched by hand around the PVC pipes of the lateral extension

---

[1] Approved by the Director of the Louisiana Agricultural Experiment Station, LSU Agricultural Center as manuscript number 88-17-2507.

[2] This research was conducted as part of a cooperative effort between the State Agricultural Experiment Stations of Arkansas, California, Louisiana, Mississippi, and Texas and the Agricultural Research Service, USDA, as part of the USDA/CSRS Southern Regional Project S-122 on the Biology, Ecology and Management of Riceland Mosquitoes in the Southern Region.

[3] Mention of commercial products does not constitute a recommendation for use or endorsement for sale by Louisiana State University Agricultural Center.

Fig. 1. Photograph of a complete self-marking device positioned over a sewage ditch in East Baton Rouge Parish, LA.

sections and along the upper horizontal pipes of the central support section with heavy-duty thread. The tent-like covering was actually composed of 4 individual panels: 2 rectangular, top panels (Fig. 3A) and 2 trapezoid-like lateral panels (Fig. 3G). Connection flaps along the margins of all 4 panels (Fig. 3D, E) were used to overlap and secure the panels to the PVC pipes with the heavy-duty thread. Extension skirts (Fig. 3F) on all panels, to include maximizing coverage of the larval habitat, assisted in preventing escape of adult mosquitoes along the bottom of the device. Metal grommets (0.25-in diam) were placed at selected points (Fig. 3C) on all 4 panels and secured the device to the central support section and to the ground with anchoring stakes. Inner liners of double-layered cheesecloth (Fig. 3B) were hand-sewn to the upper portions of all 4 panels.

The exit grid (Fig. 2H–L) was composed of 2, 30-in long, 2 × 2 in (milled dimensions) wood pieces and 2 threaded rods (0.38 in diam × 30 in long with 16 threads/in). Half-inch diameter holes were centrally drilled 1.5 in from each end of the wooden pieces for insertion of the threaded rods and secured by appropriate washers and nuts. Ten blind holes, each 0.25 in diam × 1 in deep, were drilled along the inner surface of each wooden piece. Ten stainless steel tubes (24 in long and 0.25 in diam) were inserted into the blind holes. Ten double-layered cheesecloth sheets, each 30 × 12 in, were machine sewn along their respective centerfolds with polyester fabric thread to form 4-in casings. In the field, each sheet was inserted over the individual tubes in the exit grid to form partitions which were impregnated with powdered fluorescent pigment. The complete exit grid consisting of the wood pieces, threaded rods, stainless steel tubes and cheesecloth partitions was assembled and subsequently attached to the top of the primary support section with 0.25-in eyebolts and C-hooks (Fig. 2I). The C-hooks were connected with eyebolts (Fig. 2D) on the crosspiece of the lateral extension section which protruded through grommets in the lateral extension panel.

A rain shield was positioned over the exit grid to prevent pigment loss. The shield consisted of a cast acrylic sheet (30 × 30 × 0.19 in) (Fig. 2N) supported by 4 stainless steel threaded rods (0.75 in diam × 24 in long with 16 threads/in) (Fig. 2M) and associated nuts and washers. The lower ends of the 2 rods were inserted into 4 blind holes (0.75 in diam. × 1 in deep) within the wooden frame and the upper ends connected to

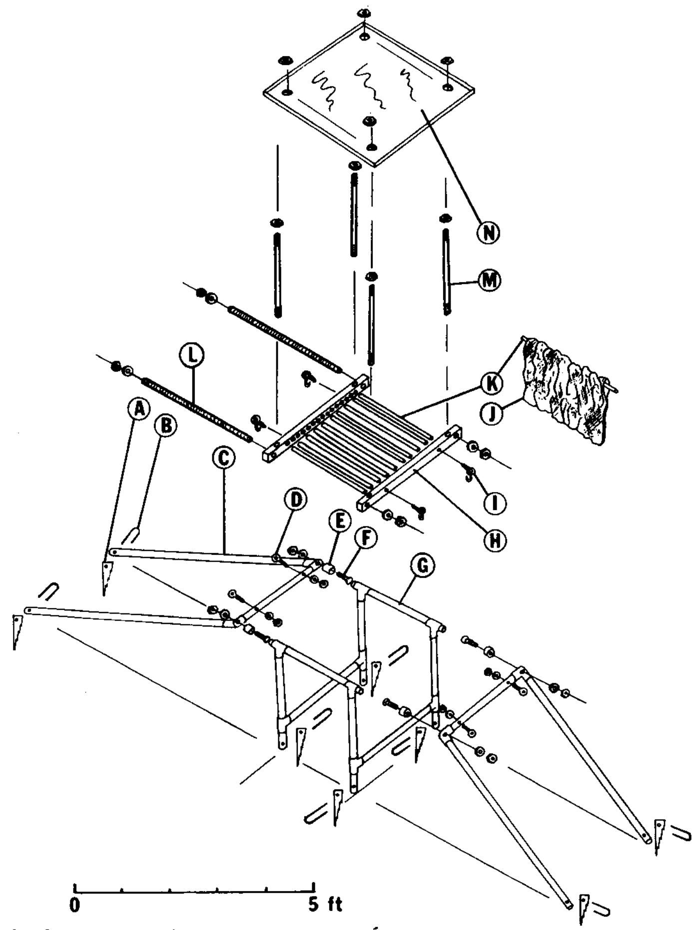

Fig. 2. Diagram of polyvinylchloride (PVC) tubular framework, exit grid and rain shield for a self-marking device.[4] (A) Anchoring stake (9 in), (B) stainless steel wire, (C) lateral extension section (0.5 in PVC pipes and joints), (D) eyebolt, (E) PVC pipe cap (0.5 in diam), (F) machine bolt (0.38 in diam × 4 in long with 16 threads/in and accompanying nuts and washers), (G) primary support section (0.5 in PVC pipes and joints), (H) wood piece (30 in long, 2 × 2 in milled dimensions), (I) eyebolt with C-hook, (J) double-layered cheesecloth partition (30 × 12 in), (K) stainless steel tube (24 in long and 0.25 in diam), (L) threaded stainless steel rod (0.38 in diam × 30 in long with 16 threads/in), (M) threaded stainless steel rod (0.75 in diam × 24 in long with 16 threads/in) and associated nuts and washers and (N) cast acrylic sheet (30 × 30 × 0.19 in).

0.75 in holes drilled in each corner of the cast acrylic sheet.

Preliminary field trials were conducted to assess the efficacy of the device and fluorescent pigment. In each of 6 replicates, 100 *Cx. quinquefasciatus* pupae were allowed to emerge beneath the device and pass through the exit grid into a screened collecting chamber (10 × 7 × 7 ft). Enclosed adults were collected daily for 3.5 days and examined under ultraviolet light for pigment. No added significance was attributed to those individuals with more than one mark.

The capacity of fluorescent pigment to induce premature mortality in adult mosquitoes was tested. In each of 5 replicates, 250 freshly emerged *Cx. quinquefasciatus* adults marked with fluorescent pigment and 250 unmarked freshly emerged *Cx. quinquefasciatus* adults were placed in separate aluminum cages (8 ft^3) along with water and carbohydrate sources (honey and raisins). The pigment on marked mosquitoes was applied by an insulphator and was barely visible with the naked eye, but very evident

[4] The distance scale represents 12 in (equivalent to 30.48 cm), and all numbers represent distances in inches.

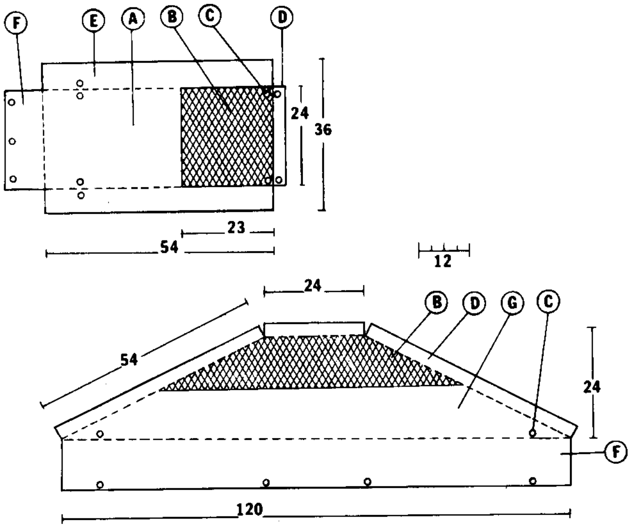

Fig. 3. Diagram of an external heavy-duty screen covering for a self-marking device.[5] (A) Top panel (black, solar fiberglass screen), (B) double-layered cheesecloth, inner liners, (C) metal grommets (0.25 in diam), (D) connection flaps (3 in wide) (black, solar fiberglass screen), (E) connection flap (6 in wide) (black, solar fiberglass screen), (F) extension flap (6 in wide) (black, solar fiberglass screen) and (G) lateral skirt (black, solar fiberglass screen).

under ultraviolet light. Dead adults were collected from each cage and counted 2, 4, 6, 8 and 9 days after placement of adults in the cages.

The incidence of fluorescent pigment transference among adult mosquito mating pairs was tested. One hundred recently emerged male *Cx. quinquefasciatus*, marked with fluorescent pigment, and 100 unmarked, freshly emerged, virgin female *Cx. quinquefasciatus* were placed in an aluminum cage (8 ft^3) containing water, carbohydrate sources (honey and raisins) and an adult quail, *Colinus virginianus* (Linn.). Females were collected 6 days after placement in the cage and examined under ultraviolet light for pigment and evidence of pigment transfer. This experiment had 2 replicates.

The capacity of fluorescent pigment to be transferred among adult mosquitoes during ovitrap collections and aspirator sampling was tested. In each of 5 replicates, an average of 50 freshly emerged *Cx. quinquefasciatus* adults, marked with fluorescent pigment, and an average of 50 unmarked, freshly emerged adults were collected with an ovitrap (Reiter 1983). The ovitrap was operated for 1 day and the mosquitoes were then collected with a battery-powered aspirator (Meek et al. 1985) and examined under an ultraviolet light for pigment and evidence of pigment transfer.

RESULTS

Four hundred and seventy-one out of 550 (86%) enclosed adults successfully exited the device, and all of these were marked with pigment after 6 replicates. A total of 118, 203, 116 and 34 marked *Culex* departed the device after days 1, 2, 3 and 3.5, respectively. The body areas most often marked were the tibiae, tarsi, scutellum and ventral abdomen. There were 5–15 pigment spots per mosquito. The pigment did not cover the entire body of the mosquito and rarely adhered to the mosquitoes' wings or antennae.

Daily mortality rates for marked and unmarked *Culex* adults during days 0–2, 2–4, 4–6, 6–8 and 8–9 were calculated according to Mayfield (1961). Pooled daily mortality rates for marked and unmarked adults during each time period were compared using a *t* test (SAS 1985). No significant differences ($P < 0.1$, $df = 4$ per replicate) were observed between mortality in marked and unmarked *Culex*, which indicates the pigment had no adverse effect on adult survivorship. These results support the conclusions of Lillie et al. (1981), Service (1976) and Schreiber et al. (1988) who also reported no increased mortality among mosquitoes marked with fluorescent pigment.

No pigment transfer occurred between marked and unmarked *Cx. quinquefasciatus*

---

[5] All measurements are given in inches for ease in purchasing required hardware. The distance scale represents 5 ft (equivalent to 1.524 m).

mating pairs. A total of 142 out of 200 (71%) unmarked virgin females became parous after 6 days of confinement with marked males, but none acquired the pigment. Additionally, no pigment transfer occurred between marked and unmarked adults subjected to ovitrap and aspirator collections.

DISCUSSION

The self-marking device effectively marked adult mosquito populations for dispersal studies. Departing *Cx. quinquefasciatus* adults were all marked with a safe and inexpensive fluorescent pigment ($150.00/25 lb).

This pigment did not transfer from marked male mosquitoes to unmarked females during mating. Lillie et al. (1981) found similar results among mating pairs of *Culicoides variipennis* (Coquillett) using fluorescent pigments manufactured by a commercial company other than Day-Glo Color Corporation. In addition, no pigment was transferred among marked and unmarked individuals during ovitrap and aspirator collections. These data agree with Schreiber et al. (1988) who used a different powdered dye which was manufactured by Radiant Pigment Corporation.

Mosquitoes were not inundated with pigment and were marked as they attempted to disperse, resulting in minimal disruption of behavior. The self-marking device was collapsible for transport to other study sites, easy to build and made from readily available hardware materials. The cost per unit, excluding labor and fluorescent pigment, was less than $60.00.

We have used this self-marking device to study other insect species, in addition to mosquitoes, whose larvae develop in localized areas (Muscidae, Calliphoridae and Psychodidae). The area of coverage, marking grid and cheesecloth partitions may be readily altered to suit environmental conditions. Therefore, the self-marking device has a broad range of applications in marking insects for dispersal evaluations.

ACKNOWLEDGMENTS

Sincere gratitude is expressed to L. M. Niebylski for assisting in architectural design and hardware allocation and Amy Niebylski for aid in construction of materials. The authors also thank, P. Reiter, V. L. Wright, E. A. Heinrichs, and R. C. Lowrie, Jr. for reviewing this manuscript.

REFERENCES CITED

Chang, H. T. 1946. Studies on the use of fluorescent dyes for marking *Anopheles quadrimaculatus*. Mosq. News 6:122–125.

Dunn, P. H. and B. J. Mechalas. 1963. An easily constructed vacuum duster. J. Econ. Entomol. 56:899.

Lillie, T. H., R. H. Jones and W. C. Marquardt. 1981. Micronized fluorescent dusts for marking *Culicoides variipennis* adults. Mosq. News 41:356–358.

Mayfield, H. 1961. Nesting success calculated from exposure. Wilson. Bull. 73:255–261.

Meek, C. L., M. V. Meisch, and T. W. Walker. 1985. Portable, battery-operated aspirators for collecting adult mosquitoes. J. Am. Mosq. Control Assoc. 1:102–105.

Nayar, J. K. and D. M. Sauerman. 1969. Flight behavior and phase polymorphism in the mosquito *Aedes taeniorhynchus*. Entomologia Exp. Appl. 12:363–375.

Reiter, P. 1983. A portable, battery-powered trap for collecting gravid *Culex* mosquitoes. Mosq. News 43:496–498.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5 Edition. SAS Institute Inc., Cary, NC. 956 pp.

Schreiber, E. T., M. S. Mulla, J. D. Chaney and M. S. Dhillon. 1988. Dispersal of *Culex quinquefasciatus* from a dairy in southern California. J. Am. Mosq. Control Assoc. 4:300–304.

Service, M. W. 1976. Mosquito ecology. Field sampling methods. Halsted Press (John Wiley & Sons), New York and Toronto. 583 pp.

Shapiro, J. M., Z. Saliternik, and S. Belferman. 1944. Malaria survey of the Dead Sea area during 1942, including the description of a mosquito flight test and its results. Trans. R. Soc. Trop. Med. Hyg. 38:95-116.

Sheppard, D. C., B. H. Wilson and J. A. Hawkins. 1973. A device for self-marking of Tabanidae. Environ. Entomol. 2:960–961.

Sheppard, P. M., W. W. Macdonald, R. J. Tonn, and B. Grab. 1969. The dynamics of an adult population of *Aedes aegypti* in relation to dengue haemorrhagic fever in Bangkok. J. Anim. Ecol. 38:661–702.

Singh, N. and M. Yasuno. 1972. A device for self-marking of mosquitoes. Bull. W.H.O. 47:677–679.

Trpis, M. 1971. Seasonal variation in the adult populations of *Aedes aegypti* in the Dar es Salaam area, Tanzania. W.H.O./V.B.C. 71.291, 29 pp.