NEW SELF-MARKING DEVICE FOR DISPERSAL STUDIES OF BLACK FLIES (DIPTERA: SIMULIIDAE)

L. M. DOSDALL,[1] M. M. GALLOWAY[2] AND R. M. GADAWSKI[3]

ABSTRACT. An effective and inexpensive device is described for marking newly emerged black flies with fluorescent dust. Survival of marked adults of *Simulium venustum s.l.* and *Stegopterna mutata s.l.* did not differ significantly from unmarked individuals over a 5-day period. Marked individuals were attracted to human hosts in landing biting tests, and the portions of the self-marking trap lying just above the water surface did not appear to significantly hamper black fly emergence success.

Control programs for black flies are reliant upon some knowledge of the range of movement of adults because the application of larvicides in black fly breeding sites is designed to reduce their populations within the boundaries of a specific area. A number of marking techniques for studying black fly dispersal have been developed, including radioactive tags (Fredeen et al. 1953, Bennett 1963, Baldwin et al. 1966), and various dusts and dyes (Dalmat 1950; Thompson 1976a, 1976b). Here, an effective and inexpensive device is described for marking large numbers of newly emerged black flies with fluorescent dust. The device is open-ended, uses little manpower, and facilitates self-marking of adults as they move through the trap. Because the effectiveness of marking systems that employ external pigments may be limited by causing premature mortality of marked individuals, or by interfering with natural behaviors like host-seeking, these factors were evaluated for the self-marking device described here.

The study was conducted in 2 streams in southeastern Ontario, Canada: 1) the Chalk River at Highway 17 (approximately 5 km southeast of the village of Chalk River, 45°57′N, 77°24′W), and 2) Cartier Creek in Canadian Forces Base Petawawa (45°56′N, 77°26′W). The streams were selected because they produce very abundant populations of black flies. Both flow through Precambrian shield, are surrounded by mature coniferous timber stands, and have substrate composed primarily of conglomerate cobble and gravel.

The self-marking device consisted of 4 main components: 1) a styrofoam base for floatation, 2) a metal grid with openings covered with styrofoam chips impregnated with powdered fluorescent pigment (Aurora Pink, AR-Moneith Ltd., Vancouver, BC), 3) a rain shield, and 4) an anchoring device (Fig. 1). The particles of styrofoam were spherical, and ranged from 3 to 5 cm in diameter. The styrofoam was impregnated with pigment by placing both in a large plastic bag and then vigorously shaking the bag and its contents. The self-marking trap was positioned so that the corner with the anchoring device pointed upstream into the current. Black flies emerging from beneath the self-marking device were forced to crawl through the metal grid and then through the layer of styrofoam impregnated with fluorescent pigment. They escaped from the device by flying upward through openings beneath the rain shield. The fluorescent pigment was transferred to the black flies upon contact, thereby marking adults before their dispersal.

Field trials were conducted to assess the effectiveness of the device for marking black fly adults and to determine whether the grid and pigment-impregnated styrofoam chips adversely affected numbers of black fly adults that emerged successfully. Six pyramidal emergence traps of the design described by Westwood and Brust (1981) were positioned along 2 transects of the Chalk River. Each emergence trap sampled an area of 1 m^2. Three traps were selected randomly and modified for self-marking by inserting a metal grid and styrofoam chips impregnated with fluorescent pigment above the styrofoam floats comprising the base. The other 3 emergence traps were not modified. Samples of newly emerged black fly adults were collected hourly by mechanically aspirating specimens from each trap for three, 24-h periods (from 0830 h EST May 17 to 0830 h May 20, 1987). Black fly specimens were cooled to 0°C and then placed in petri dishes and preserved at −15°C. Mean numbers of black fly adults collected from unmodified and self-marking emergence traps were compared using analysis of variance (ANOVA) and Tukey's studentized range test (SAS Institute Inc. 1985), after performing $\log_{10}(x + 1)$ transformations on black fly counts of each sample, to determine whether significant differences occurred in emergence for the 2 trap types.

[1] Alberta Environmental Centre, Bag 4000, Vegreville, Alberta, Canada, T9C 1T4.

[2] Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2.

[3] Department of Parks and Recreation, City of Winnipeg Insect Control Branch, 2799 Roblin Blvd., Winnipeg, Manitoba, Canada, R3R 0B8.

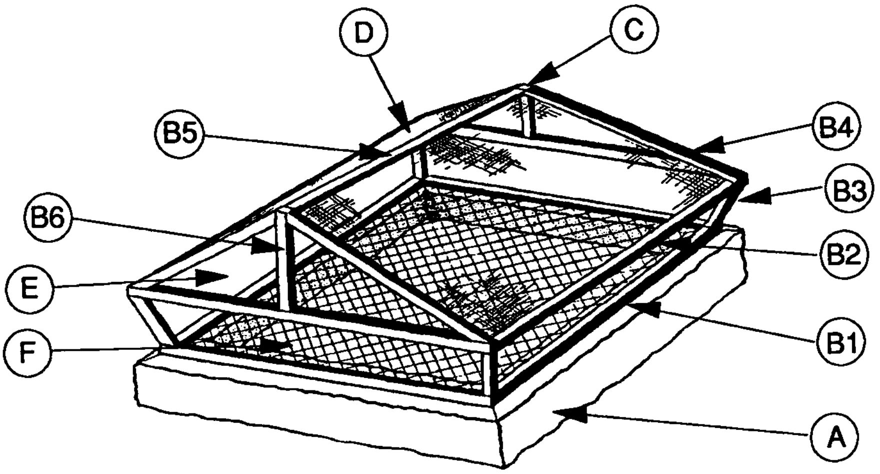

Fig. 1. Diagram of the self-marking trap. (A) Styrofoam blocks (1.20 m × 0.15 m × 0.15 m; inner area = 1 m^2) for floatation, reinforced along their upper surfaces with plywood (thickness = 1.6 cm) glued to the styrofoam, and joined together with bolts (length = 0.20 m); (B) wooden strips comprising the framework: B1 = 1.00 m × 0.05 m, B2 = 1.10 m × 0.05 m, B3 = 0.20 m × 0.05 m, B4 = 0.60 m × 0.05 m, B5 = 1.10 m × 0.05 m, B6 = 0.30 m × 0.05 m; (C) location of an eyebolt for attaching an anchor rope; (D) clear plastic sheets (6 mil) for the rain shield; (E) openings for escape of marked adults (the lower opening can be sealed with plastic to prevent wind damage to the styrofoam chips impregnated with fluorescent dust); (F) metal grid with diamond-shaped openings (2 cm × 1 cm) above the water surface for support of styrofoam chips impregnated with fluorescent dust.

To determine whether the fluorescent pigment affected survival of marked adults, specimens of *Simulium venustum s.l.* Say and *Stegopterna mutata s.l.* (Malloch) were aspirated from closed emergence and self-marking traps and then placed in screened cages. Adults from both trap types were newly emerged because all traps were emptied and samples for the survival study were collected 2 h later. Flies were maintained at 18 ± 4°C, 90% RH, and a photoperiod of 16 h L: 8 h D for 5 days and fed from a cotton wick attached to a bottle containing a saturated solution of sucrose. Dead individuals were removed at 24-h intervals for the duration of the study. The survival study was repeated twice for each black fly species. The LIFETEST procedure (SAS Institute Inc. 1985) was used to calculate survival distribution functions for each species and day of the survival study. The data include censored observations because the exact survival time of some individuals was unknown, and it was known only that their lifetimes exceeded the given value. Such observations were from individuals still alive at the end of the study. Censored observations are not ignored in lifetime analyses because longer-lived individuals are generally more likely to be censored (Lee 1980). The LIFETEST procedure utilized the Kaplan and Meier product-limit method of estimating survivorship function because it applied to the moderate and large sample sizes used here. The Wilcoxon rank test, calculated by the LIFETEST procedure, was used to determine significance of differences ($P = 0.05$) in survival between the treatment (marked) and control (unmarked) samples of each species.

To determine whether marked black fly adults were attracted to human hosts, 6 open-ended self-marking traps were placed in Cartier Creek, and for 5 days emerging black fly adults (mainly *S. venustum s.l.*) were marked with fluorescent dust. The same color of pigment (Aurora Pink) was used each day. Landing biting tests were conducted over a 4-day period from 1100 to 1200 h EST each day (6 h after sunrise), and at 1 site per day, at distances of 25, 250, 500 and 1,000 m from the self-marking traps. Adults landing on the same 3 human hosts were collected at each site.

Over a 3-day period, 10,813 adult black flies emerged successfully from 3 self-marking traps each of 1 m^2 area on the Chalk River, Ontario, and all were marked with pigment. Over the same period, 13,652 black flies were collected from 3 unmodified emergence traps, also of 1 m^2 area from the same site. Most specimens were *S. venustum s.l.* (62.9%) and *St. mutata s.l.* (36.9%); few were *Simulium decorum s.l.* Walker (0.2%). The body areas marked most frequently were the wings, tibiae, tarsi, scutellum and antennae.

The mean numbers of black flies that emerged from the unmodified emergence traps on each of days 1 and 2 of the trap efficacy study exceeded those emerging from the self-marking traps; on day 3, however, black fly emergence from the self-marking traps exceeded emergence from the unmodified traps (Table 1). On each of the 3 days of the study, no significant differences occurred in the mean numbers of *S. venustum s.l.* or *St. mutata s.l.* adults collected from self-marking traps relative to unmodified emergence traps ($P > 0.05$). These data suggest that the grid and styrofoam barriers above the water surface did not significantly hamper emergence success; however, the data should be considered preliminary because of the low number of replicate traps and the large variability in emergence that occurred between traps of the same type.

Survival distribution functions, or the probabilities (from 0 to 1) that individuals were alive on various days of the study, did not differ

Table 1. Mean numbers of newly emerged black fly adults collected from 3 self-marking and 3 unmodified emergence traps for three, 24-h periods (May 17–20, 1987) from the Chalk River, Ontario.

Day	Species	Mean (±SE) per self-marking trap	Mean (±SE) per unmodified trap	Significance of differences between trap types
1	*S. venustum s.l.*	384.0 ± 146.0	1,068.7 ± 940.7	NS
	St. mutata s.l.	147.0 ± 60.5	310.0 ± 218.2	NS
2	*S. venustum s.l.*	429.0 ± 118.9	977.0 ± 841.9	NS
	St. mutata s.l.	380.7 ± 94.4	366.3 ± 226.8	NS
3	*S. venustum s.l.*	1,261.3 ± 561.9	1,016.0 ± 719.5	NS
	St. mutata s.l.	1,002.3 ± 253.6	812.7 ± 576.3	NS

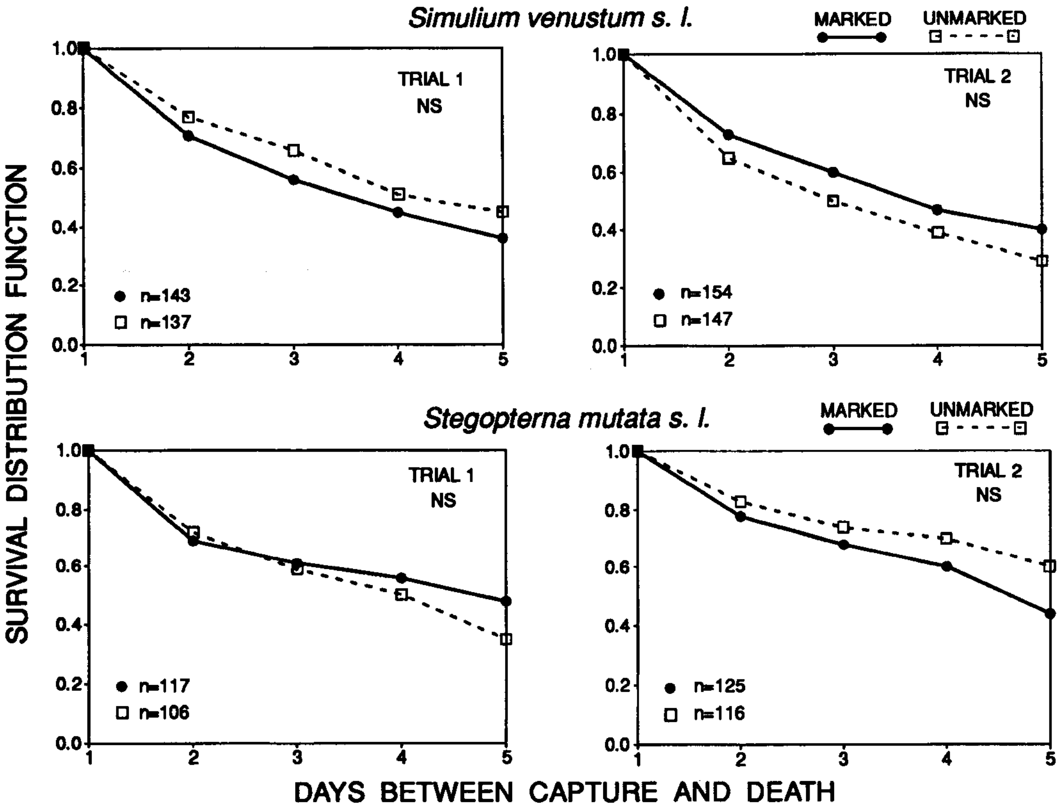

Fig. 2. Survival distribution functions of *Simulium venustum s.l.* and *Stegopterna mutata s.l.* adults collected from self-marking and unmodified emergence traps for each of two 5-day trials. NS = no significant difference between treatment groups using the Wilcoxon rank test.

significantly for marked or unmarked adults of *S. venustum s.l.* ($P > 0.05$) (Fig. 2). In Trial 1, survival of unmarked specimens slightly exceeded that of marked adults, but in Trial 2 this trend was reversed. Survival of marked and unmarked specimens of *St. mutata s.l.* was also similar and not significantly different in each of Trials 1 and 2 ($P > 0.05$). These results support conclusions of Lillie et al. (1981), Service (1976), Schreiber et al. (1988) and Niebylski and Meek (1989) that external marking with fluorescent pigment does not adversely affect insect survival.

Marked adults of *S. venustum s.l.* were attracted to human hosts in landing biting tests, although the frequency of capture of marked vs. unmarked adults declined with distance from the marking site. Recapture frequency was greatest at the site 25 m from the self-marking traps (1.4%, $n = 1{,}247$); at distances of 250, 500 and 1,000 m from the self-marking site the numbers of marked flies captured declined to 0.52% ($n = 968$), 0.18% ($n = 1{,}104$) and 0% ($n = 546$), respectively.

The device was also effective in marking other emerging aquatic insects, including *Hydropsyche* sp. (Trichoptera: Hydropsychidae), *Acroneuria lycorias* (Newman) (Plecoptera: Perlidae) and *Haploperla brevis* (Banks) (Plecoptera: Chloroperlidae). This self-marking trap, therefore, has potential usefulness for dispersal studies of other aquatic insects.

Fluorescent powders have sometimes been mixed with other compounds like gum arabic to increase their adhesive properties (Chang 1946, Sinsko and Craig 1979, Brust 1980). In this study, however, the unmodified fluorescent pigment readily adhered to adult black flies because their bodies were very moist immediately following emergence. The pigment remained effective for at least 5 days, although the quantity of material per adult appeared to decline over time possibly from black fly self-grooming and other behaviors.

Black flies were not inundated with pigment and were marked immediately after leaving the water surface to emerge, resulting in minimal disruption of their behavior. Marked individuals could be recognized by their fluorescence under ultraviolet light without being killed; therefore, further observations or remarkings were possible. The self-marking device was easy to build from readily available materials at a cost of less than $50.00 (U.S.) per m² trap. Modification of the area of coverage could facilitate the usefulness of this trap for dispersal studies of different aquatic insects, or for the same species under different environmental conditions.

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