FIELD MATING AND MOVEMENT OF *AEDES AEGYPTI*

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In 1967 insectary-reared *Aedes aegypti* males, homozygous for the incomplete dominant mutant Silver Mesonotum, were released at a single urban site in Meridian, Mississippi, and the extent of mating with field females was studied. Evaluations were based on the recapture of the marked males and on genetically marked adults reared from eggs taken in ovitraps (Fay and Eliason, 1966) in the study area. Positive mating was shown, but the males from the release site were not uniformly dispersed (Fay and Craig, 1969).

The present paper involves studies conducted during June to August, 1968, in two other urban areas of Meridian. Results of a second mating experiment with Silver Mesonotum males released generally over the study areas are presented, and the dispersion pattern of insectary-reared, mated females carrying other genetic markers when released from a single site in each study area is also discussed.

Males for release were obtained from the MISS MARK strain which is homozygous for the mutants: spot abdomen (*s*) and Silver Mesonotum (*Si*) (Craig and Hickey, 1967). Fay and Craig (1969) describe the method for development of MISS MARK; a laboratory stock with *Si* and *s* was crossed to a freshly-collected field strain from Meridian and the F_1 was then backcrossed to the field strain for four generations. Then, the homozygous *Si, s* phenotype was again selected. The MISS MARK strain thus constituted had the markers in a strain with a background largely from Meridian. The *Si* marker is especially useful because it can readily be detected in the heterozygote. Thus, when released males mate with field females, the progeny of these matings show the heterozygous expression of *Si*.

The MISS MARK strain was reared by mass production methods (Morlan, 1966) and the males were selected as pupae. Gallon cardboard containers, each holding approximately 500 adult males, were placed outside overnight before releases which were made from 8:00 to 9:00 a.m. each day. Releases were made each day from June 28 to July 20, 1968, with the exception of July 4, for a total of 22 releases. In releasing the males, an operator walked along each east-west street and allowed the adults to escape slowly through a small aperture in each carton.

To estimate the extent of female dispersal, females of two strains with the genetic markers of either black tarsi (*blt*) or black palps (*blp*) were released from a single site near the center of the same study area. The BLACK TARSI strain was homozygous for the recessive mutant black tarsi; the white scaling on all tarsal segments was much reduced or absent. The BLACK PALP strain was homozygous for two recessive mutants, black palp (*blp*) and compressed antennae (*co*). The palps of the female are entirely black and the white scales of the male palps are much reduced; and both sexes have the two apical antennal segments fused. All of these markers are autosomal, on linkage group 3, and all seem to have no effect on vigor, fecundity or viability. Both strains were constructed by crossing males of the marker strain to females of a strain freshly collected from Meridian. The mutant phenotypes were selected in the F_2, and this time females were crossed to fresh males from Meridian. The F_2 from

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this latter cross was selected and used as a foundation stock to produce the females for release. These females had a significant amount of their genetic material derived from Meridian (theoretically well over 55 percent).

Since the markers in both strains were recessive, males and females were held together in gallon cartons for 4 days to insure mating. *Ae. aegypti* is monogamous (Craig, 1967) and hence the offspring from eggs laid by the released females would show the markers. Females of the BLACK TARSI strain were offered blood meals in the laboratory on the 2nd, 3rd and 4th days after emergence. Females of BLACK PALPS received no food except sugar water. On the 4th day after emergence, July 2, the males were destroyed, and 220 females of each strain were released.

Study area No. 1 was essentially square, measuring about 1,200 feet on each side and comprising 20 blocks in an older section of the city inhabited by families of medium income status. Preliminary inspections showed *Ae. aegypti* larvae in numerous containers of various materials and sizes. Ovitrap surveillance during the week just prior to the release period indicated the occurrence of oviposition.

A 125-foot grid of 103 ovitraps was used with daily inspections the first week and then twice weekly for an additional 6 weeks. All ovitrap paddles showing *Ae. aegypti* eggs were conditioned for 3 days and then stored for further rearing studies. Adults subsequently reared from these eggs were examined for the genetic markers.

On the morning of July 2, 220 mated and blood-fed BLACK TARSI females were released at a site essentially in the center of the first study area. On the evening of July 2, a 1.9-inch rain was recorded. Additional rain was recorded as follows: 2.2 inches—July 8 to 10, 1 inch—July 14 to 16, 1.25 inches—July 29 to August 2, 1.6 inches—August 14, 1 inch—August 19 to 20, 1.8 inches—August 25, and 1.5 inches—September 3 to 4. Using a hatch indicator (Keirans and Fay, 1970) to guide the timing, we collected larvae and pupae from field containers throughout the area. The specimens were likewise reared to adults and checked for genetic markers. Comprehensive inspections were made for larvae in field containers on July 8–10, July 15–17, August 22–24 and September 3–5.

The BLACK TARSI adults reared from eggs collected on ovitrap paddles and from larvae recovered in field containers are classified (Table 1) as to the number of positive sites, the distance from the release site and the numbers recovered weekly. Ovitrap collections were made daily during the first week after the release July 2; the first positive collection on July 3 was close to the release site, but the second positive one on July 4 was more than 100 yards from the release point. No positive sites were detected during weeks 3 and 4 indicating a possible break in the oviposi-

TABLE 1.—Number and distance of sites positive for collections of BLACK TARSI eggs or larvae following the release of mated, blood-fed *Ae. aegypti* females at a single site. Values are based on adults reared and identified for the genetic marker.

	Weeks after Release					
	0	1	2	5	6	Total
Positive ovitraps	4[a]	2	0	0	3	9
Positive containers	5	7	7	2	1	22
Total positive sites	9	9	7	2	4	31
Sites <100 yards	2	1	3	1	0	7
Sites >100 yards	7	8	4	1	4	24
Total males	21	10	16	2	2	51
Total females	20	4	11	1	2	38

[a] Daily collections (week 0) and twice-weekly collections (weeks 1–6).

tion activity of the released females. Positive sites found during weeks 5 and 6 may indicate egg laying by adults produced in field containers. The presence of positive sites near the periphery of the study area indicates that the dispersion may have exceeded the limits of the study area.

To provide the same environmental conditions as for the BLACK TARSI, we released 220 BLACK PALPS females on July 2. Since these females had been mated but had not obtained a blood meal, it was assumed that oviposition activities would be delayed at least 3 days longer than that of the BLACK TARSI females. A greater loss in the numbers of ovipositing BLACK PALPS females might be expected because of their exposure to various environmental factors for the additional 3 days.

Collections of BLACK PALPS reared adults are classified (Table 2) on the same basis as the BLACK TARSI adults. The first BLACK PALPS eggs were taken at a field site on July 8, whereas BLACK TARSI eggs were first taken on July 3; this difference in time supports the assumed delay in oviposition resulting from the necessity of a blood meal. The number of sites positive for BLACK PALPS per week increased through week 2, whereas the number of sites positive for BLACK TARSI was at its highest in weeks 0 and 1. The BLACK PALPS specimens taken in weeks 4, 5 and 9 may possibly represent an F_2 generation. The total numbers of BLACK PALPS adults recovered were essentially one-third that of the numbers of BLACK TARSI specimens recovered; this variation may indicate either a considerable degree of mortality in the former, incurred from environmental factors, or their inability to obtain a blood meal.

The geographic relationships of the release site to the positive recovery sites for BLACK TARSI and BLACK PALPS are shown in Figure 1; the distances of 100 and 200 yards from the release point are indicated by the circles.

The field collections also supplied the basis for estimating the extent of mating of MISS MARK males with wild females. During the period from June 28 to July 20, a total of 63,600 MISS MARK males were released throughout the study area, an average of 2,765 daily.

TABLE 2.—Number and distance of sites positive for collections of BLACK PALPS eggs or larvae following the release of mated but not blood-fed *Ae. aegypti* females at a single site. Values are based on adults reared and identified for the genetic marker.

	Weeks after Release						
	0	1	2	4	5	9	Total
Positive ovitraps	0[a]	0	1	1	1	0	3
Positive containers	2	4	4	0	1	1	12
Total positive sites	2	4	5	1	2	1	15
Sites <100 yards	1	0	2	0	0	0	3
Sites >100 yards	1	4	3	1	2	1	12
Total males	2	2	12	2	2	3	23
Total females	0	2	2	0	0	0	4

[a] Daily collections (week 0) and twice-weekly collections (weeks 1–9).

Data summarized on a weekly basis (Table 3) include the number of sites positive for wild and genetically marked specimens, the numbers of wild and genetically marked adults reared, and the percent represented in each category by the genetically marked adults. For a period of 5 weeks (weeks 2–6), essentially one-fourth of all the site collections were positive for genetically marked specimens, and since the distribution of these sites (Figure 2) was quite uniform throughout the study area, the method employed in releasing the MISS MARK males was con-

sidered satisfactory. On week 4, approximately one-eighth of the eggs or larvae collected carried the genetic marker; this percentage provided an estimate of the effectiveness of the MISS MARK males in mating with wild females, probably during week 3.

The second study area selected was about the same size as the first area. It was composed of only 12 city blocks and was located in an area with families of lower income status. Preliminary inspections showed somewhat higher infestations of *Ae. aegypti* and active oviposition.

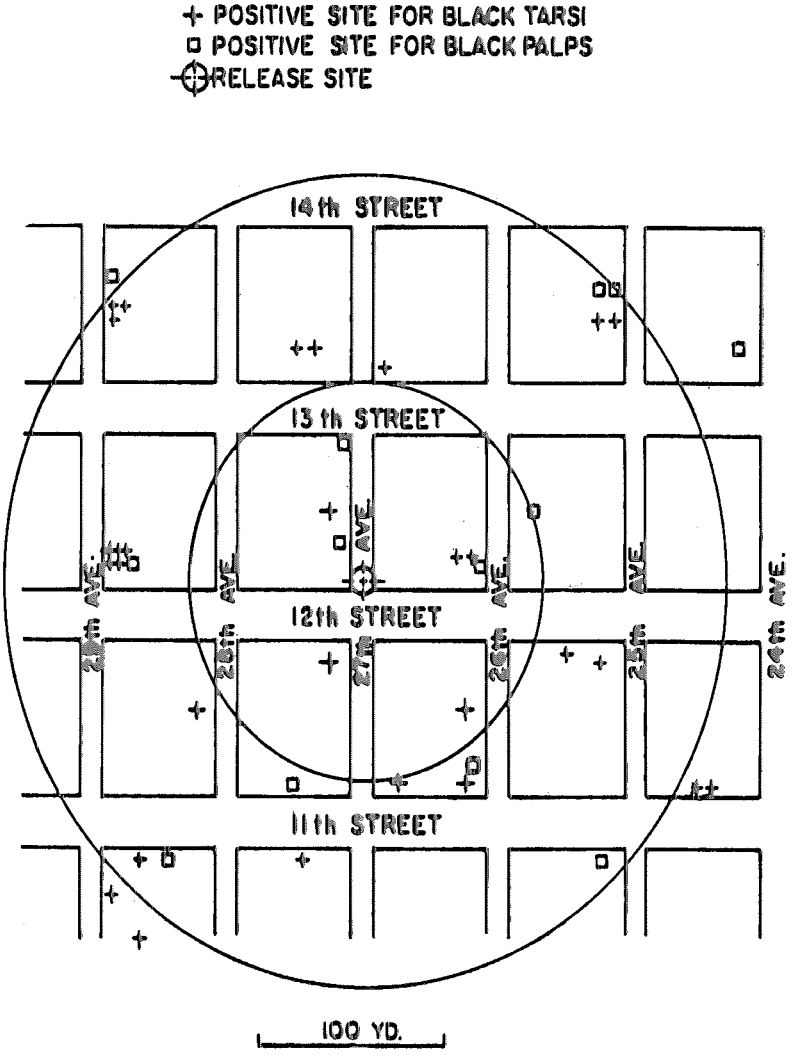

FIG. 1.—Sites positive for BLACK TARSI eggs (crosses) and BLACK PALPS eggs (squares) following release of 220 mated and blood-fed BLACK TARSI females and 220 mated BLACK PALPS females of *Aedes aegypti* at the release site indicated. Area 1, Meridian, Mississippi, 1968.

A total of 78,350 MISS MARK males, or an average of 3,134 per day, were released for a 25-day period from August 21 to September 14, 1968. The males were reared, conditioned and released in the same manner as in study No. 1. Unfortunately, only limited numbers of BLACK TARSI and BLACK PALPS females were available for release. A grid of 131 ovitraps was inspected at 2-day intervals from August 20 to October 14, 1968, and two 7-day collections of larvae and pupae from field containers were made in September and October, respectively. In addition, 12 adult black traps (Fay and Prince, 1970) were placed in the study area and collections were made daily for a 7-week period. Adults taken were examined for genetic markers.

Rainfall during this study period was

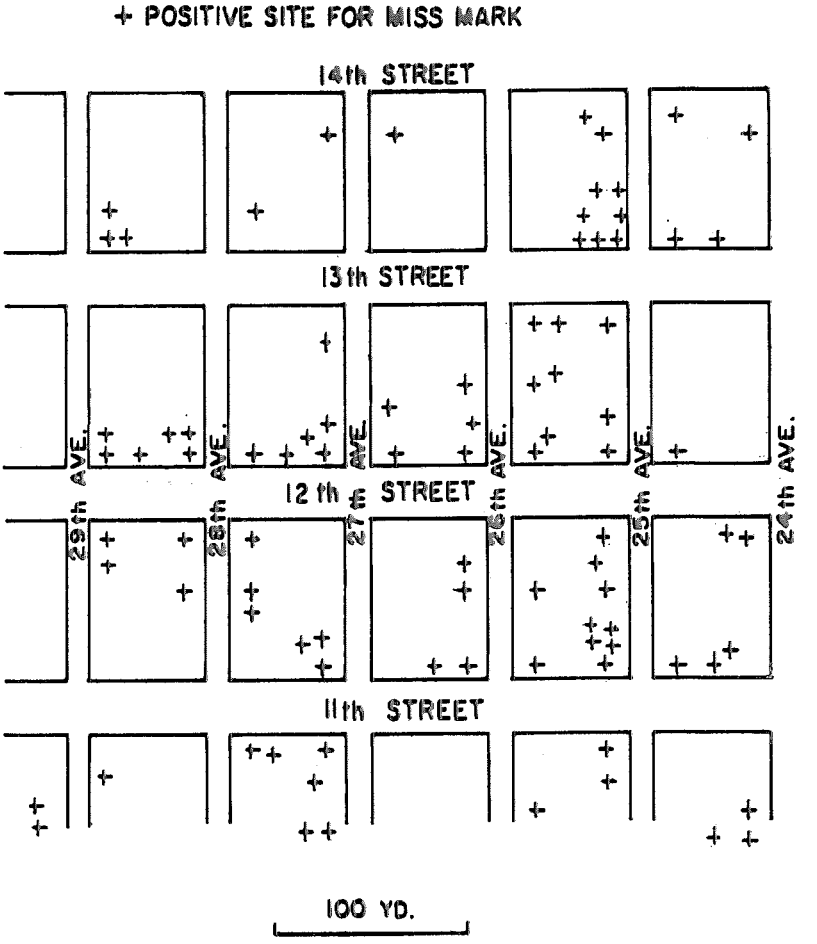

FIG. 2.—Sites positive for eggs of MISS MARK heterozygotes from field females mating with MISS MARK males that were released daily for 4 weeks along 11th, 12th and 13th Streets. Area 1, Meridian, Mississippi, 1968.

TABLE 3.—Field sites positive for eggs or larvae of wild and heterozygous MISS MARK (MM/+) *Aedes aegypti* and the percent of reared adults showing the genetic marker. Adult MISS MARK (MM) males released daily during weeks 1–3, inclusive. Study area 1, Meridian, Mississippi, 1968.

		Positive Sites			Adults Reared		
Week	Dates	Wild	MM/+	% MM**	Wild	MM/+	% MM/+
0*	6/28–7/4	27	4	15	274	10	4
1*	7/5–7/11	62	14	22	904	68	7
2*	7/12–7/18	109	30	26	1405	114	8
3	7/19–7/25	106	26	25	1889	249	12
4	7/26–8/1	116	31	25	3174	301	9
5	8/2–8/8	115	27	23	1598	137	8
6	8/9–8/15	103	20	19	1594	102	6
7	8/16–8/22	92	19	21	900	36	4
8	8/23–8/29	27	1	4	359	3	1
9	8/30–9/5	24	7	29	443	19	4

* Daily releases of MM "MISS MARK" males.
** Certain sites positive for MM+ only.

1.6 inches on August 25, 3.0 inches from September 3–5, 0.1 inch on September 10, 1.1 inches from September 16–19, 0.5 inch on September 26, and 3.7 inches from October 7–10. Two comprehensive inspections of field containers during the periods September 10–18 and October 11–17 were conducted as a consequence of this precipitation. Adults reared from larvae collected from containers and from eggs collected from ovitraps were examined for the genetic marker.

A summarization of these data on a weekly basis (Table 4) includes the percentage of sites positive for MISS MARK and the percentage of reared adults which were MISS MARK. The positive sites are shown in Figure 3. In the first study area, approximately 25 percent of all sites were positive for MISS MARK for 5 consecutive weeks (weeks 2–6), but in this second study area, an average of 17 percent of all positive sites harbored marked mosquitoes for 7 consecutive weeks (weeks 2–8). Again the distribution of the positive sites indicates adequate release methods for marked males. Of all eggs or larvae collected 9 and 10 percent gave rise to marked individuals during weeks 4 and 5 in study area No. 2, compared with 12 percent during week 3 in study area No. 1. Nineteen percent of all reared adults during

TABLE 4.—Field sites positive for eggs or larvae of wild and heterozygous MISS MARK (MM/+) *Aedes aegypti* and the percent of reared adults showing the genetic marker. Study area 2, Meridian, Miss., 1968.

		Positive Sites			Adults Reared		
Week	Dates	Wild	MM/+	% MM	Wild	MM/+	% MM/+
0*	8/21–8/27	49	0	0	359	0	0
1*	8/28–9/3	62	7	10	369	15	4
2*	9/4–9/10	167	27	14	1752	90	5
3*	9/11–9/17	190	45	19	2995	135	4
4	9/18–9/24	111	25	18	1420	133	9
5	9/25–10/1	95	19	17	859	90	10
6	10/2–10/8	42	9	18	438	103	19
7	10/9–10/15	208	36	15	2807	108	4
8**	10/16–10/17	113	22	16	1786	63	3

* Daily releases of "MISS MARK" (MM) males from Aug. 21–Sept. 14.
** 2 days only.

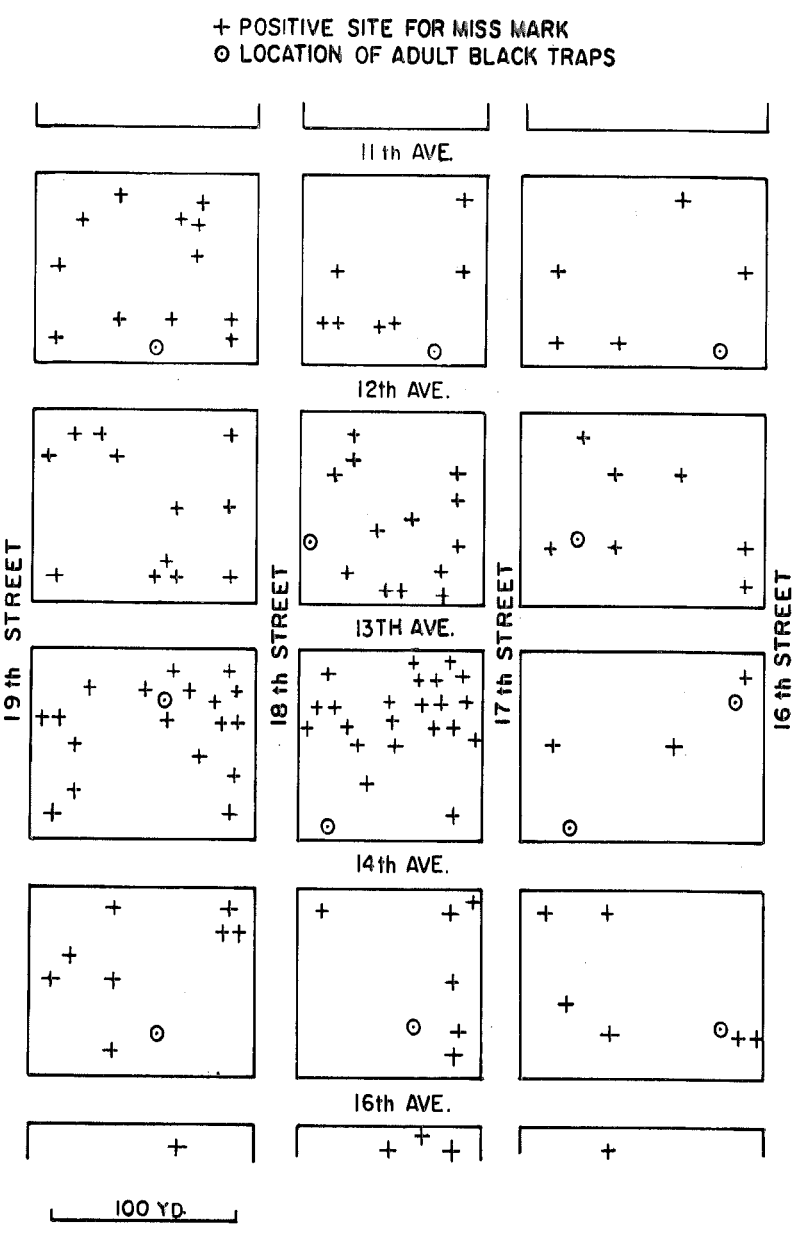

FIG. 3.—Sites positive for eggs of MISS MARK heterozygotes from field females mating with MISS MARK males that were released daily for 4 weeks along 12th, 13th and 14th Avenues. Locations of the adult black traps shown by circles, Area 2, Meridian, Mississippi, 1968.

week 6 were marked; they were derived solely from eggs collected in ovitraps. This increase in marked individuals over those collected in the previous 2 weeks indicates breeding of F_1 individuals, or it may be the result of prolonged oviposition by the wild females originally mated with the MISS MARK males released. In any case, the results indicate considerable mating between released males and wild females.

In this study area, 12 adult black traps (Fay and Prince, 1970) were located so that each trap was 300–400 feet from any other black trap. With this pattern, four traps were 600 feet, four were 440 feet and four were 300 feet from the center of the study area (Figure 3). Traps were operated from 9:00 a.m.—4:00 p.m., 5 days a week over a 7-week period. Data from this operation (Table 5) indicate

TABLE 5.—Adult *Ae. aegypti* mosquitoes taken in black traps each week in study area 2, Meridian, Miss., 1968.

Week	Dates	Wild		MISS MARK
		Female	Male	Male
0*	8/18–8/24	48	60	6
1*	8/25–8/31	19	99	93
2*	9/1–9/7	32	49	252
3*	9/8–9/14	13	22	253
4	9/15–9/21	9	18	6
5	9/22–9/28	16	22	0
6	9/29–10/5	7	7	0

* MISS MARK males released Aug. 21–Sept. 14.

a general decline in the wild *Ae. aegypti* population after week 2 and an increase in the marked male populations from weeks 1–3. Recaptures during week 3 and 4 indicate that the population of marked males was sustained through the repeated releases. One percent or less of the released males was recaptured in the black traps.

The ratio of the number of released males captured to the number of wild males captured may be used to estimate the wild male population. The proportions of wild males to MISS MARK males taken in the traps were 10:1, 1:1, 1:5 and 1:12 for weeks 1 to 4, respectively, during the release period. These proportions may provide estimates for the wild male population since the numbers of released MISS MARK males were known. The rapid drop in the numbers of MISS MARK males on week 5 indicates their survival time in the field to be relatively short, however.

If mating of F_1 individuals occurred in the field, some marked individuals could be expected in the traps during weeks 6 and 7. In their absence, the higher percentage of marked adults reared from eggs taken on week 7, namely 19 percent (Table 4), may well reflect a delayed oviposition by females mated on week 4 when the 1:12 ratio existed between wild and MISS MARK males.

On a theoretical basis, the assumed ratio of 1:12 of wild to MISS MARK males should have resulted in more than 19 percent of the eggs having the genetic marker if both types of males were equally competitive. It must be pointed out, however, that the dispersion patterns of the ovipositing BLACK TARSI and BLACK PALPS females indicated movement to the periphery of the study area in a relatively short period of time. This may be interpreted to mean that reverse migration of females from the area outside the study area into the study area likewise took place. These females would not be affected to any degree by the released males and would provide wild type adults from their eggs. The study areas, then, were in all probability too small for obtaining accurate data on the effects of the male ratios. It is therefore important to obtain further data on the size of an experimental area. The results can then be applied to studies with applications in genetic control through the use of chemosterilized or irradiated male releases.

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A CONSTANT-RATE LIQUID DISPENSER FOR USE IN BLACKFLY LARVICIDING[1]

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Treatment of a stream or river with blackfly larvicide requires injection of the chemical at a constant rate. Notwithstanding the need for uniform injection rates during treatments of test plots, uneven rates can also subject non-target organisms to unnecessary pressures.

Various means of achieving constant injection rates have been used, one of the most sophisticated being that described by Hocking (1950). In his larvicide dispenser, liquid pressures on both sides of an adjustable orifice plate were visible in manometers. With the aid of calibration charts any desired discharge rate could be achieved by inserting a suitable orifice plate and then adjusting valves on both sides of the plate until the required pressure differential was achieved. To maintain a steady discharge rate, however, continued valve adjustments were required as the head of concentrate in the supply tank fell.

A dispenser that I have used on numerous occasions utilizes an air inlet tube to maintain a constant head of liquid above the outlet orifice and thus obviates the need for continuing valve adjustments to compensate for a declining head of liquid in the reservoir (Figs. 1 and 2).

In small plot tests of larvicides in the St. Lawrence River (Fredeen, 1969) a 25-litre polyethylene carboy was used as a reservoir for the larvicide (Fig. 1). The top was fitted with an airtight rubber stopper through which a single, copper air-inlet tube was inserted so that the lower end rested on the bottom of the carboy. This copper tube had an inside diameter of approximately 3 mm and was open at both ends. It was curved about 100° at the bottom end so that the inflow of air was not impeded by contact with the bottom. A spigot at the rim of the bottom of the carboy drained through a 15 cm length of rubber tubing into an open funnel connected to a second piece of tubing that was anchored to the river bed.

The rate of discharge from the carboy was adjusted to 500 ml per minute, either

[1] Contribution No. 395 of the Research Station, Saskatoon.