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LABORATORY STUDIES OF OVIPOSITIONAL PREFERENCES OF *AEDES AEGYPTI*[1]

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In programs such as the *Aedes aegypti* eradication campaign in continental U.S.A., Puerto Rico, and the Virgin Islands (Schliessmann, 1964), a surveillance method for detecting the pertinent species at low population levels is a necessity. The data obtained would assist in determining the extent of area to be treated and the point in time for termination of the field applications.

One method for surveillance is the detection of adult female mosquitoes through their egg-laying activities. Although this approach samples the females only at intervals when oviposition occurs, the specificity of the egg-laying sites, *i.e.*, artificial containers or tree holes, limits the environment to be sampled and provides sensitivity in the method. Evidence of egg laying coupled with the limited flight range of the females enables quite accurate delineation of the field area requiring detailed examination. Moreover, confusion of *A. aegypti* eggs with those of other species is limited probably to those of *Aedes triseriatus*.

The present paper describes laboratory studies conducted to secure an attractive egg-laying site based on female *A. aegypti*

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preferences for texture, color, and shape of the container, as well as the odor and taste characteristics of the contents.

The *A. aegypti* test strain (PR), collected and colonized in Puerto Rico by Dr. I. Fox, has been reared at Savannah, Georgia, for 5 years. Each test colony was prepared as follows: (a) on both Monday and Tuesday of the first week pupae were sorted mechanically (Fay and Morlan, 1959), and 54 of each sex were placed in separate beakers in a standard colony cage (Morlan *et al.*, 1963); (b) 10-percent honey water and stewed raisins were supplied three times weekly; (c) the pupae were removed on Friday and the numbers of emerged adults recorded by sex; and (d) a rabbit blood-host was offered Friday and Saturday of week 1; Monday, Thursday and Friday of week 2; and Monday of week 3. Oviposition commenced the Sunday night of week 2. Tests were made Monday through Thursday nights of weeks 2 and 3, and the colony was then discarded. Each colony provided 200 to 3,000 eggs per night during the test periods.

In the first tests four competitive egg-laying containers were placed in a square design in the right corner of a colony cage approximately 1 inch from the rear wall and 1 inch apart. In later tests one container was placed in each corner of the cage. Four colonies were used in each series, and the experimental arrangement was rotated to minimize any bias of test position. Evaluations were made for two nights, and results were expressed as the percent of the total eggs per colony per night in each type of test container.

To determine the effect of lining material on oviposition, four 600-ml. beakers were lined either over the entire inside surface with the test material or by dividing this surface half and half with two competing materials. Each beaker contained 200 ml. of distilled water and remained in the colony from 4:00 p.m. one day until 9:00 a.m. the following morning. Eggs laid on the test material were tabulated, and the mean percent of the total eggs per colony per night on the various surfaces was calculated (Table 1).

TABLE 1.—Percent of the total eggs laid per night per colony found in beakers lined with various test surfaces.

Test Surface	No. of Replicates	Average Percent of total eggs/ colony/night
Brown blotter	32	56
White toweling	32	32
Green blotter	48	30
Brown bag paper	56	29
White blotter	104	27
Brown manila paper	40	20
Green toweling	16	16
White bond paper	40	15
White filter paper	32	14
Aluminum foil	16	1
Clear plastic foil	24	0

Those surfaces having values of 25 percent or higher were considered as attractive. Three factors appear significant (a) the water absorptive properties, (b) the roughness, and (c) to a lesser degree the color of the test materials. With the aluminum foil liners and the clear plastic liners essentially no eggs were found in the water; it appears that these surfaces discourage oviposition almost completely.

The tests of lining materials were followed by examination of the feasibility of using a covered wooden tongue depressor as an egg-collecting device. In each test beaker a tongue depressor was mounted vertically at one side, in one case uncovered, in the others covered with white blotter, brown blotter, or brown bag paper. Results from 16 replicates showed the following mean percent of eggs on the respective test surfaces: uncovered wood—3 percent; brown bag paper—5 percent; white blotter—13 percent; and brown blotter—80 percent.

Since the angle of contact between the test surface and the water might influence oviposition, tongue depressors covered with brown blotter were mounted in the

test beakers in two ways (a) at right angles to the water surface and (b) about 30° from the horizontal. Approximately 59 percent of the eggs were laid on the brown blotter that was placed in the vertical position. Eggs deposited on the horizontally mounted blotter were essentially all on the upper surface.

With 200 ml., 100 ml., 50 ml. and 25 ml. of distilled water per beaker, no significant differences in the number of eggs deposited were observed. All further testing was then done using 100 ml. of solution per beaker.

Comparisons were made of beakers, crystallizing dishes, quart and pint cardboard containers as oviposition sites, in each case using brown-blotter covered tongue depressors as the egg-collecting device. With the cardboard containers eggs were frequently deposited just above the water line on the containers themselves, thus precluding reuse. With the crystallizing dishes a higher percentage of the eggs appeared in the water, whereas with the beakers less than 3 percent of the eggs were found either in the water or on the container. Further tests were based almost entirely on the 600 ml. beaker as a standard container.

The initial evaluations of chemical additives to the distilled water were made simultaneously with the surface evaluations, and since the value of the brown blotter was unrecognized initially, the test beakers were lined half and half with brown bag paper and white blotter. Comparisons of attractiveness were made between distilled water and three candidate chemical solutions or in some cases between four candidate chemicals. Solutions showing attraction were sodium benzoate at 0.5 percent (48 percent), beta-mercaptoethanol at 0.1 percent (44 percent) and ammonium carbonate at 0.3 percent concentration (36 percent). Other compounds tested, namely thiourea, ammonium chloride, lysine, sodium lactate, lactic acid, glucose, and a synthetic mixture resembling human sweat failed to show appreciable attraction.

A round-robin pair test of 0.5 percent sodium benzoate, 0.1 percent beta-mercaptoethanol and 0.3 percent ammonium carbonate using two beakers of each solution in each test and tongue depressors covered with brown blotter or white toweling showed values of 78 percent for sodium benzoate, 34 percent for beta-mercaptoethanol and 9 percent for ammonium carbonate. Eggs laid in the beakers containing beta-mercaptoethanol failed to darken and did not hatch when flooded.

Although the 0.5 percent sodium benzoate solution appeared promising, when it was checked against distilled water using the brown-blotter-covered tongue depressors, more eggs were laid in the distilled water container. The distilled water extracted a colored material from the blotter causing a shift in pH from 5.6 to 7.5. This did not occur with the sodium benzoate solution. A quantity of brown blotter extract was prepared and the attractiveness of distilled water and of sodium benzoate with and without the extract was tested. The mean percentage of eggs from 16 replicates were as follows: distilled water alone—14; sodium benzoate—8; distilled water with extract—38; and sodium benzoate plus extract—40. In addition, brown blotter and new blotter were compared in distilled water and in 0.5 percent sodium benzoate. New brown blotter in distilled water drew 36 percent of the eggs and in sodium benzoate 25 percent of the eggs; while the extracted brown blotter drew only 22 percent and 17 percent of the eggs in distilled water and sodium benzoate, respectively. Comparable results were obtained with the extract and extracted blotter in parallel tests using quart cardboard containers instead of the glass beakers.

Further comparisons of the extract with a neutral vegetable coloring material indicated that color of the solution rather than chemical composition was the controlling factor. Higher percentages of eggs were consistently obtained as the color of solution was darkened with no

significant differences in a choice of red, green or brown. From these observations, tests were made to compare the attractiveness of beakers placed in carbon-paper sleeves with clear beakers using only distilled water. In a series of 16 replicates, 75 percent of the eggs were deposited in the darkened beakers. Further evaluations were made then with the beakers enclosed in carbon-paper sleeves to compare them with beakers coated on the outside with black enamel. In these tests a slight but consistent preference was shown for the black-enameled beakers with 55 percent of the eggs against 45 percent in the carbon-paper sleeve beakers in a series of 8 replicates.

With the four beakers in one corner of the cage, a position bias existed for the two beakers closest to the rear wall. By rotating the test combinations in each replicate this bias had little influence on the results. However, with one beaker in each corner of the cage, the bias was reduced by one-fourth and comparable results were obtained. By testing only two combinations per replicate and using one rear and one front station for each combination, the mean difference for the paired positions was less than 2 percent. With the paired tests a combined value of more than 50 percent of the eggs indicated attraction.

Methyl butyrate, tested against distilled water, in a series of concentrations showed considerable attraction. Two types of evaluations were made: (a) with the chemical in the exposed water of the beaker to evaluate odor and taste combined and (b) with the chemical solution in a gauze-covered vial surrounded by distilled water to evaluate odor alone. The results (Table 2) indicate 0.05 and 0.1 percent concentrations as more attractive. Although the methyl butyrate solutions in the vials were attractive, those in the beakers were more attractive, indicating that both odor and taste affect selection.

Several other organic esters were tested at 0.1 percent concentration for attraction (Table 3), in each case using paired tests with distilled water. The present data indicate that methyl butyrate was the most attractive and that the methyl moiety contributed more to the overall attractiveness than higher alkyl groups in the series.

In comparing the effectiveness of 0.1 percent methyl butyrate, brown blotter and black-enameled beakers, a series of tests (Table 4) indicate that each factor contributes markedly to the attraction of the egg-laying site.

Several tests were made with single

TABLE 2.—Attractiveness of methyl butyrate concentrations expressed as the percent of total eggs laid in beakers holding (a) an exposed water solution or (b) a protected solution in gauze-covered vial surrounded by water.

% Conc.	% of Total Eggs	
	Tested in solution	Tested in vial
0.025	57	59
0.05	73	60
0.1	72	56
0.2	65	56
0.4	63	60

TABLE 3.—Attractiveness of 0.1 percent solutions of various esters for oviposition of *Aedes aegypti* when compared with distilled water and with methyl butyrate

Ester	Percent of Total Eggs in Ester Solution *	
	Vs. Distilled Water	Vs. Methyl butyrate
Butyl formate	46	..
Methyl propionate	68	45
Ethyl propionate	67	44
Butyl propionate	55	..
Methyl butyrate	72	..
Ethyl butyrate	63	..
Butyl butyrate	33	45
Sodium butyrate (salt)	..	24
Butyl caproate	45	..

* Each value represents 8 replicates.

TABLE 4.—Attractiveness of various combinations of color, surface and chemical solution for egg-laying sites of *Aedes aegypti* in paired tests.

Series *	Beaker Color		Surface		Solution		% of Total eggs
	Clear	Black	White Paper	Brown Blotter	Distilled water	0.1% Methyl butyrate	
1a	..	×	×	..	..	×	48
1b	..	×	..	×	×	..	52
2a	..	×	×	..	..	×	32
2b	..	×	..	×	..	×	68
3a	..	×	..	×	×	..	28
3b	..	×	..	×	..	×	72
4a	×	..	..	×	..	×	24
4b	..	×	..	×	..	×	76
5a	×	..	×	..	×	..	8
5b	..	×	..	×	..	×	92

* Each series represents 8 replicates per test combination.

females to determine the total number of eggs laid per night and the distribution of these eggs when four identical egg-laying sites were available. In an initial series involving 50 replicates, a single female was placed in a colony cage, and four beakers each with a brown-blotter-covered tongue depressor immersed in distilled water were introduced in the cage at 4:00 p.m. one day and removed at 9:00 a.m. the following morning. In a second series a similar procedure was utilized except that the beakers were placed in the cage at 9:00 a.m. one day and removed at 9:00 a.m. the following day. Following egg laying, the test female was discarded and another female introduced.

In Series 2 (Table 5), egg counts made by 4 p.m. showed that considerable egg laying occurred during the daylight hours as well as during the later hours, and this is reflected in the larger numbers of eggs laid. It is also evident that most females deposited their eggs in more than one container (Series 2), a factor that offers promise in the field trials of a surveillance method based on preferred ovipositional sites.

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TABLE 5.—Single female oviposition tests to determine number of eggs per test and distribution in four identical containers.

Total No. eggs per female per test	Percent of Frequency				
	Series 1 * 4 pm–9 am 17 hr.	Series 2 * 9 am–9 am 24 hr.	No. containers with eggs per test	Series 1 4 pm–9 am 17 hr.	Series 2 9 am–9 am 24 hr.
0–20	10	8	1	48	14
20–40	22	18	2	24	36
40–60	26	10	3	14	36
60–80	16	18	4	14	14
80–100	10	22			
100–120	14	18			
120–140	2	6			
140–160	2	0			

* Each series based on tests with 50 *Aedes aegypti* females.

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A STEER-BAITED TRAP FOR SAMPLING INSECTS AFFECTING CATTLE

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In recent years, the methods used for sampling and studying populations of insects have become more specialized as the accumulated knowledge concerning various taxonomic groups has suggested more suitable sampling techniques. With regard to insects affecting livestock, especially cattle, the principal method of study has been based on counts made at a particular time on animals in the field. This method has been effective in estimating populations of host-inhabiting species, but it does not provide an accurate estimate of nocturnally active species. Estimates of the nocturnally active insects have been based on light-trap collections. Although light-trap collections yield usable data, we cannot assume that cattle and light will attract the same species and in the same proportions. Because of the possibility of erroneous interpretation of light-trap data, a study was conducted on the efficacy of a steer-baited insect trap for sampling the populations of Diptera attracted to and attacking cattle.

The trap designed and used in this study was a modification of the stable trap described by Magoon (1935). Two main objectives were sought in the modifications: (1) To allow as much circulation of air through the trap as possible for insect attraction and (2) to enable insects approaching the trap to have ingress at several heights above ground level.

This paper includes the trap design, materials required for its construction, and a check list of the insects collected in the baited trap as compared with those collected in light traps. A report on a comprehensive study on the seasonal collections is planned for a later paper.

TRAP DESIGN AND MATERIALS. The structural plan for the trap is shown in Fig. 1 and a photograph of the completed trap in Fig. 2. The following materials were used in the construction of this trap:

Lumber (number and size of pieces):

7—2″ x 4″ x 14′
7—2″ x 4″ x 12′
1—2″ x 4″ x 8′
3—2″ x 4″ x 10′
2—1″ x 4″ x 12′
6—1″ x 10″ x 14′
2—1″ x 10″ x 12′
250 linear ft of screen molding

Hardware:

1 box corrugated fasteners, ⅜″ x 5 gauge
1 pair strap hinges, 4″
1 safety hasp, 4″

[1] In cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station.