FIELD EVALUATION OF COMPONENTS FOR AN *AEDES AEGYPTI* (L.) OVIPOSITION TRAP

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Laboratory studies of the ovipositional preferences of *Aedes aegypti* led to the development of an oviposition sampling station (oviposition trap or ovitrap) for *Ae. aegypti* (Fay and Perry, 1965). Field evaluations comparing ovitrap sampling to larval inspections suggested that the trap was potentially a sensitive and efficient *Ae. aegypti* surveillance technique (Fay and Eliason, 1966). The prototype unit used in the early field tests consisted of a 1-pint, tapered glass jar (the outside surface painted with black gloss enamel), a strip of brown blotter paper mounted on a wooden tongue depressor (as an oviposition surface or sampling paddle), approximately 100 ml. of water, and a 10-ml. vial containing a chemical attractant (ethyl acetate, A.C.S. grade). The paddle was attached vertically to the inside wall of the jar so that it was partially submerged in the water with the blotter paper facing the center of the container. The vial was suspended in the jar approximately 1 inch below the rim by a wire hook.

Practical or economical considerations indicated that modifications to the basic unit might be incorporated. Efforts were made to simplify the components without reducing the effectiveness of the ovitrap. Data are given on the evaluations of the various trap components based on frequency and amount of oviposition obtained during 1966 and 1967 in Dade County, Florida.

MATERIALS AND METHODS

Ovitraps were maintained in several areas sustaining natural populations of *Ae. aegypti*. Trap locations were selected on the basis of previous experience which indicated that increased effectiveness occurred where shelter, shade, and adult resting surfaces were present. The ovitraps were serviced (new paddles inserted, jars examined for presence of larvae, and water and attractant replenished) at 3- to 4-day intervals. An effort was made to keep the inside of the jars clean, thereby reducing possible oviposition on surfaces other than the paddles. Upon removal, the exposed paddles were promptly placed in a rack to prevent accidental transfer of eggs during transport to the laboratory. Paddles were then inspected microscopically for the presence of eggs and species identification.

Containers. Three types of black, 1-pint, wide-mouth containers were evaluated for effectiveness, durability, and stability by being used in the same test areas. They were: enamel-coated glass jars, ceramic-coated glass jars, and plastic containers.

Sampling Paddles. Preparing the blotter paper-tongue depressor paddles was time consuming and inspecting them for eggs was difficult because the paddles deteriorated. Another material that would be equally effective, easier to prepare, and more durable was needed. Screenback hardboard was considered since it is similar to the brown blotter paper in color and texture. Preliminary tests showed that *Ae. aegypti* would oviposit on this material. The hardboard was easily cut from 1/8-inch-thick sheets into the de-

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sired size (3/4 in. x 5 in.), and did not deteriorate from continuous flooding.

Comparisons were made with the blotter paper paddles and two types of hardboard strips (exterior and interior) in separate areas having high oviposition indices. Tests were made with the competitive materials in the same jar and in adjacent jars at each site. The materials were reversed in location at each servicing.

Fay and Perry (1965) demonstrated that in the laboratory there was a preference for oviposition on paddles clipped in a vertical position. Field studies were conducted in five areas with paddles positioned vertically and with paddles placed in the jars so that they rested against the sides at an approximate 30-degree angle. Comparisons were made between the two paddle positions in adjacent traps at the same location. The traps were reversed in location at each servicing to reduce bias.

To determine if the amount of paddle surface area which had to be examined could be reduced, paddles of 3/8-inch width were compared to the standard (3/4-inch width) paddles. The work was conducted in four separate areas in the same manner as the studies on paddle position, with the exception of testing in adjacent traps.

OVITRAPS WITH AND WITHOUT ETHYL ACETATE. The hazards in the handling, cost, and short residual life of this material indicated a need for studies to determine whether ovitraps without this attractant would be comparable. To obtain data from a variety of environments, a dump and residential areas of differing socioeconomic levels were chosen. Tests were conducted from the period of high oviposition in the summer through the winter, when oviposition was low in all areas. The competing traps were placed at least 20 feet apart in similar locations at each site, and their positions were reversed at each servicing to prevent bias.

DATA ANALYSIS. Results of comparative tests were analyzed according to standard statistical methodology (Bancroft, 1960; Spiegel, 1961). Probability of ≤ 0.05 was accepted as the significance level for both the Students' t and Chi-square analysis.

RESULTS AND DISCUSSION

CONTAINERS. Although the three types of containers appeared equally effective in egg collection, ceramic-coated jars proved superior to either enamel-coated jars or plastic containers on the basis of durability and stability. The enamel coating tended to chip after exposure to weather and cleaning, thus altering the uniform dark appearance of the jars and potentially influencing their effectiveness. The plastic containers were easily tipped over, affected by ethyl acetate, and cleaning roughed the inside surface.

SAMPLING PADDLES. The interior-type hardboard was significantly more effective than blotter paper paddles for incidence of positivity, but the exterior hardboard and blotter paper paddles were equally effective (Table 1).

When the interior-type hardboard and blotter paper paddles were tested in the same jar, 27 percent of the positive hardboard paddles received eggs when the adjacent blotter paper paddles did not, whereas in no case was the converse true.

Field observations disclosed that the interior-type hardboard was more absorbent than the exterior-type hardboard, thus offering a more extensive moist surface for oviposition. Subsequent investigations (Jakob, W. L. and Kilpatrick, J. W., 1966, personal communication) revealed that most hardboards did not absorb water readily and that the particular interior-type used in these studies was the hardboard most suitable for use as an ovitrap paddle.

Vertically positioned interior hardboard paddles appeared to be more effective ($P=0.09$) for positivity than the leaning paddles when they were tested in separated jars at the same site. Results show an observed difference of 37.8 percent. In another series of tests where the competitive paddles were in adjacent jars at the same location the vertically positioned and leaning paddles were equally effective.

TABLE 1.—Comparative effectiveness of oviposition trap sampling paddles.

Comparison A vs. B	Total tests	Paired samples per test	Percent positive A	Percent positive B	Comparative effectiveness A vs. B	Eggs per positive ($\bar{x}\pm 2SE$) A	Eggs per positive ($\bar{x}\pm 2SE$) B
IHb vs. Bl [1]	1	85	43.5	27.1	A>B [4]	8.5±2.8	17.6±3.7
EHb vs. Bl [1]	1	85	51.8	60.0	A=B	17.0±2.8	11.5±2.5
IHb vs. Bl [2]	1	20	55.0	25.0	A>B [4]	5.7±4.6	17.8±8.2
IHb(V) vs. IHb(L) [3]	5	568	10.2	7.4	A=B	38.7±6.6	26.6±8.0
IHb(V) vs. IHb(L) [1]	5	20	66.0	64.0	A=B	40.9±12.7	28.5±7.6
IHb(S) vs. IHb(N) [3]	4	686	12.1	10.9	A=B	38.0±8.6	41.7±8.0

[1] Comparisons are between paddles in adjacent jars.
[2] Comparisons are between paddles in the same jar.
[3] Comparisons are between paddles in separated jars at the same site.
[4] $P \leq 0.05$ indicates significant difference between A and B.
Paddle Symbols: EHb, exterior hardboard; IHb, interior hardboard; Bl, blotter paper; V, vertical; L, leaning; S, 3/4-inch width; N, 3/8-inch width.

The leaning paddles received large numbers of eggs on their back surfaces, making inspection for eggs more difficult. This was not so with the vertically positioned paddles because they were clipped against the sides of the jars. In addition, the vertically positioned paddles were not easily dislodged when the ovitraps were moved or tipped over. For these reasons the vertically positioned paddles were considered more desirable.

An observed difference in positivity of 11.0 percent occurred between the standard width paddles and the narrow paddles with the standard width paddles appearing to be more effective with a P value of 0.09. The narrow paddles were difficult to handle and number in the field, and also tended to warp. The standard width paddles presented none of these problems.

It is interesting to note that the mean number of eggs per positive paddle was not consistently higher on the materials greatest in positivity (Table 1). In a surveillance activity the number of eggs received in ovitraps is unimportant, as the presence of a single egg demonstrates positivity. Based on this premise, the type of paddle receiving the highest frequency of positivity was considered the most suitable for use in ovitraps.

OVITRAPS WITH AND WITHOUT ETHYL ACETATE. Although variations in *Ae. aegypti* oviposition were reflected by the positives found in the different socioeconomic areas, the effectiveness of the ovitraps with ethyl acetate attractant and those without were consistently equal (Table 2).

No extended periods of cold weather occurred in South Florida during these studies. Minimum daily temperatures less than 50° F. rarely persisted throughout a complete service cycle of the ovitraps (U. S. Weather Bureau, 1966–1967). The overall rate of oviposition was reduced during colder periods but there were no significant differences in the effectiveness of ovitraps with or without ethyl acetate.

SUMMARY

Field studies were conducted on individual components of the *Aedes aegypti* oviposition trap in Dade County, Florida.

Under field conditions glossy black, ceramic coated pint glass jars were more durable and suitable than enamel-coated jars or black plastic containers.

Interior-type screenback hardboard strips (5 x 3/4 x 1/8 inches) of a specific manufacture were found to be suitable as ovitrap paddles and were more durable than the original blotter paper-tongue depressor sampling paddles. Paddles clipped vertically inside the traps were not easily dislodged, received very few eggs on the back surfaces, and were equal or more

TABLE 2.—Comparative effectiveness of oviposition traps with and without attractant.

Habitat description	Period of study	Paired samples	Percent positive[1] WEA	Percent positive[1] WOEA	Comparative[2] effectiveness
Dump, high *Ae. aegypti* density	Sep–Oct	300	57.0	57.3	WEA=WOEA
	Nov–Feb	642	17.8	18.8	WEA=WOEA
	Mar–Apr	260	33.5	40.0	WEA=WOEA
Poorly kept, moderate *Ae. aegypti* density	Jul–Sep	895	30.2	31.6	WEA=WOEA
	Jan–Feb	932	6.6	7.4	WEA=WOEA
Well kept, low *Ae. aegypti* density	Nov–Feb	3,358	3.9	4.1	WEA=WOEA

[1] WEA, traps containing ethyl acetate attractant; WOEA, traps without ethyl acetate attractant.
[2] A P value of ≤0.05 indicated a significant difference between WEA and WOEA.

effective in frequency of positivity than paddles resting at an angle. Hardboard paddles of standard width (3/4 inch) were more frequently positive than a narrower unit, but the difference was not statistically significant. The narrower paddles were found to be undesirable because of difficulty in handling and their tendency to warp.

Competitive tests under varying field conditions showed ovitraps with and without ethyl acetate attractant to be equally effective.

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EVALUATION OF TECHNIQUES USED FOR MASS REARING *AEDES NIGROMACULIS* BY INDUCED MATING

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The development of resistance to insecticides by the irrigated pasture mosquito, *Aedes nigromaculis* (Ludlow), in the Central Valley of California, has necessitated a search for long-range population-suppression methods as well as for new insecticides.

Since the report of Gjullin and Peters (1952) of mosquito resistance to insecticides in California, many attempts have been made to develop a self-sustaining colony of this species, but, in all cases, this mosquito has refused to reproduce in captivity.

At this laboratory, *A. nigromaculis* is reared routinely from eggs laid by wild-caught females. The progeny are used in testing new insecticides and for biological studies. However, this is a time-consuming and seasonal operation; furthermore, such mosquitoes are quite heterogeneous. To overcome these problems, a modified version of the induced mating technique of McDaniel and Horsfall (1957) has been used for maintaining laboratory colonies of *A. nigromaculis* at this laboratory (Miura 1967).

This paper describes further technical modifications for improving the production of eggs for use in laboratory investigations.

MATERIALS AND METHODS. Eggs were obtained from females maintained by induced copulation and from females collected in the vicinity of Fresno, California.

Eggs were collected on filter papers and stored in a chamber with a relative humidity of 100 percent. The chamber was kept in the laboratory at 24° C. Four to five hundred eggs at a time were hatched by flooding them with 200 ml of deoxygenated water in a pan (30 x 18 x 5 cm). After about 12 hours, the first instar larvae were transferred to a rearing pan (40 x 25 x 6 cm) containing grass and straw in 1000 ml of tap water; the water

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