TWO FORMULATIONS EFFECTIVE IN THE LABORATORY AS OVICIDES FOR *AEDES AEGYPTI* (L.)[1]

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The Aedes aegypti Eradication Program in the United States has relied heavily on the traditional control methods of source reduction and larviciding plus a limited amount of residual spraying for adults (Schliessmann, 1966). The egg stage of the mosquito's life cycle has not been a target for chemical control operations and, indeed, it is doubtful whether ovicides have ever been employed in the field against any mosquito species. Their use against *Ae. aegypti,* however, appears feasible. As pointed out by Smith and Salkeld (1966) the essential prerequisites for the effective use of ovicides are, in addition to toxicity, that the egg be in a sufficiently exposed location and that a high enough proportion of the population be present in the egg stage to justify treatment. *Ae. aegypti* appears amply to satisfy these requirements. Its eggs are normally deposited not on water but on the exposed surface of containers just above the water line where they are accessible to spray application and where the spray is not subject to dilution. The proportion of the population represented by eggs will be greatly influenced by the pattern of rainfall and by temperature conditions. Since eggs deposited at the water line do not hatch until submerged, it is likely that after a prolonged dry period most surviving individuals will be in the egg stage. Near the northern and southern limits of its range where breed-

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ing is restricted to the warmer months, the winter population will consist largely of eggs. Proper consideration of such weather factors should make the species highly vulnerable to an effective ovicide.

Since *Ae. aegypti* is a domestic mosquito, any material used in its control will be applied for the most part in densely inhabited places. For this reason, chemicals which present the lowest possible hazard to humans and domestic animals should receive prime consideration. This paper reports on the preliminary results of a search for relatively nontoxic materials effective in preventing hatch of *Ae. aegypti* eggs.

MATERIALS AND METHODS. Only fully embryonated eggs on a nonporous surface were used. Panels of roughened 40-gauge aluminum measuring 20 x 115 mm. served as an oviposition substrate. A flat-bottom, cylindrical glass dish was lined with the panels, which were retained and held upright by a length of coil spring encircling the inside of the dish (Fig. 1). Approximately 4 cm. of tap water were added to the dish and it was left overnight in a colony of several thousand adult *Ae. aegypti.* On removal from the cage, the water was poured off and the dish with panels in place was put into a plastic bag to maintain 100 percent humidity and stored for 2 days at 80° ± 2° F. The bag was then removed and the panels kept at 85±5 percent relative humidity and 80°±2° F. for at least 1 additional day. Eggs thus conditioned were used at ages between 3 and 10 days. Prior to treatment, each panel was examined under a dissecting microscope and excess eggs were removed with a fine needle to permit an accurate count of those to be treated. At this time also any obviously nonviable eggs were discarded.

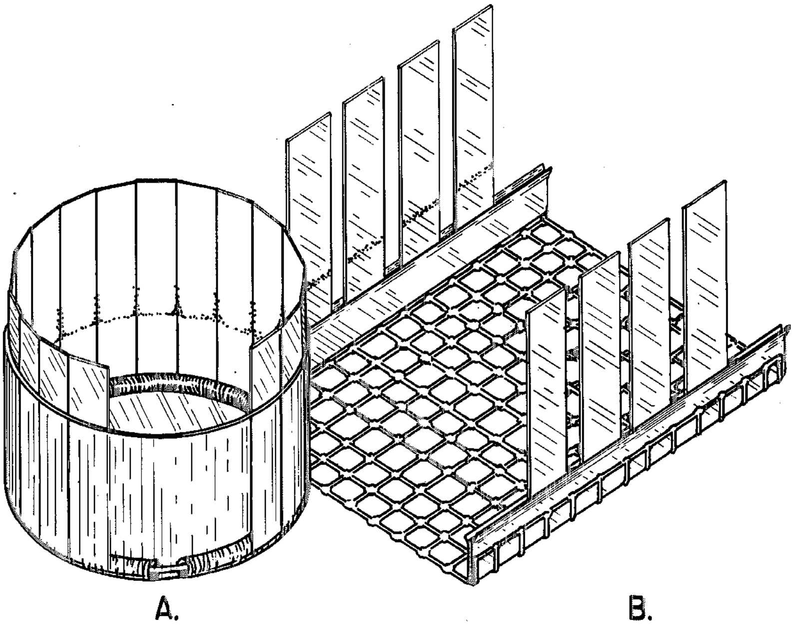

FIG. 1.—A. Oviposition dish with aluminum panels in place. B. Rack holding panels for spray treatment.

Care was exercised to touch only those eggs to be discarded and to avoid damage to those retained on the panels.

Two types of formulations are reported on here: combinations of (a) decanol, benzylpyridine, and diethanolamine and (b) Diamine #2[2,3] with diethanolamine or ethylenediamine. These formulations were prepared as emulsions and a magnetic stir bar was used to keep them homogeneous during the spray operation. Spray was delivered from an 8001 Teejet nozzle at 40 p.s.i. The panels, held vertically in a rack (Fig. 1), were placed on a belt moving at 40 feet per minute and passed once through the spray which wet them to the point of run-off. Orientation of the panels and their direction of movement were both at right angles to the spray fan. Unsprayed eggs and eggs sprayed with water only were used as controls.

Following treatment, the panels were held for 24 hours at 80° ± 2° F. and 85 ± 5 percent relative humidity. The percent hatch was then determined by comparison of egg and larval counts after a 2-hour hatch period. The hatching medium was a day-old culture containing brewer's yeast and pulverized lab chow.

TABLE 1.—Percent hatch from *Ae. aegypti* eggs sprayed with aqueous mixtures of decanol, benzylpyridine, and diethanolamine.
A = one component; B = two components; C = three components.
Average hatch from 2,881 eggs sprayed with water only = 83.9 percent.

% Decanol		% Benzylpyridine		% Diethanolamine				% Hatch	No. eggs
0.2	0.4	0.2	0.4	0.5	0.75	1.0	2.0		
..	..	B	..	..	..	..	B	0.0	586
C	..	..	C	..	..	C	..	0.4	1896
C	..	..	C	..	C	..	..	0.5	859
C	..	C	..	..	..	C	..	1.0	2368
C	..	C	..	..	C	..	..	1.1	972
C	..	..	C	C	..	..	..	1.6	1283
C	..	C	..	C	..	..	..	6.0	924
B	..	..	..	..	..	..	B	16.6	650
..	..	B	..	..	..	B	..	27.5	2879
..	B	..	..	..	..	..	B	32.6	328
..	B	..	..	..	..	B	..	36.9	763
..	B	B	..	..	..	..	..	74.4	360
..	..	..	..	..	..	A	..	79.3	719
..	A	..	..	..	..	..	..	79.7	956
B	..	B	..	..	..	..	..	88.4	1796
..	..	..	A	..	..	..	..	90.1	667

RESULTS AND DISCUSSION. Certain of the formulations have shown considerable promise. No larvae hatched from eggs treated with a mixture of 0.2 percent 4-benzylpyridine and 2.0 percent diethanolamine (Table 1). Other two-component formulations tested had only a limited effect. Combinations of decanol and benzylpyridine allowed from 74 to 88 percent hatch and sprays containing decanol plus diethanolamine gave hatches of 16 to 37 percent. Decanol was included in most of the effective test formulations because it resulted in a better emulsion and permitted use of a lower concentration of diethanolamine. Egg hatch was held to approximately 1.0 percent by the addition of 0.2 percent decanol to mixtures of 0.2 percent benzylpyridine and 0.75 to 1.0 percent diethanolamine. Eggs sprayed with 0.4 percent decanol, 0.4 percent benzylpyridine, or 1.0 percent

[2] Furnished through the courtesy of Ashland Oil and Refining Co., Minneapolis, Minnesota.

[3] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health, Education, and Welfare.

diethanolamine used alone yielded hatches of 80, 90, and 79 percent, respectively.

High egg mortalities have also been produced by a combination of 0.2 percent Diamine #2, a lipid-soluble aliphatic amine, with 1.0 percent diethanolamine or ethylenediamine (Table 2). Inclusion of 0.2 percent decanol appeared to have little effect on the hatch. Incomplete data, not included in the table, suggest that the Diamine #2 in this formulation remains effective at a concentration of 0.1 percent. It was ineffective when sprayed alone at 0.4 percent.

TABLE 2.—Percent hatch from *Ae. aegypti* eggs sprayed with 0.2 percent Diamine #2 and 1.0 percent diethanolamine or ethylenediamine. Numbers in parentheses indicate number of eggs tested.

	Diethanolamine	Ethylenediamine
Diamine #2 with 0.2% decanol	3.3(1084)	0.0(646)
Diamine #2 without 0.2% decanol	1.5 (734)	0.4(448)

The average hatch of 2,881 eggs sprayed with water alone was 83.9 percent, compared with 89.0 percent for 11,188 unsprayed eggs. The difference (5.1 percent) represents those eggs stimulated to hatch merely by being wetted.

The data reported here are preliminary, and a great deal remains to be learned about the kinds of chemicals which combine to form effective ovicides for *Ae. aegypti*. It seems significant, however, that each of the formulations discussed above includes both lipid-soluble and water-soluble components. Unless it acts simply by coating the egg and interfering with normal gas exchange (Smith and Pearce, 1948) an ovicide must be able to penetrate the shell.

The marked resistance to desiccation, which characterizes the mature egg of *Ae. aegypti*, is believed to result from a continuous lipid layer secreted into the shell some hours after oviposition (Clements, 1963, p. 29). Such a lipid layer would account for the known failure of such water-soluble toxins as ethanol, phenol, and mercuric chloride to penetrate the egg. A similar barrier to lipid-soluble materials is also suggested. Benzylpyridine has been shown to cause 100 percent mortality of human body louse eggs at a concentration of 0.25 percent in ethanol (Eddy and Carson, 1948) and Mulla (1967) found aliphatic *alpha*-diamines as a group to be highly effective larvicides in tests against the southern house mosquito, *Culex pipiens quinquefasciatus*. LC-50 values of less than 2 ppm for fourth stage larvae were reported for the several compounds of this group tested. Despite their indicated toxicities, however, neither benzylpyridine nor Diamine #2 caused any reduction in egg hatch of *Ae. aegypti* at 0.4 percent concentration in the present experiments.

When examined prior to hatching, panels sprayed with both effective formulations revealed a proportion of collapsed eggs which correlated well with the percent hatch obtained. This suggests that desiccation may have been a cause of mortality and that an ovicide need not be directly toxic but might achieve its effect indirectly through exposure of the embryo to dehydration, either by altering the shell's permeability or by causing it to rupture. Such an indirect action was noted by Judson *et al.* (1962) in fumigant tests of various chemicals on *Ae. aegypti* eggs. Several lachrymatory materials had the effect, in addition to their toxicity, of inducing a hatch-like response in the embryo which resulted in rupture of the shell. Such a condition occurring out of water would be just as lethal to the embryo as direct toxic action.

SUMMARY. Initial results of a search for materials effective as ovicides for *Ae. aegypti* include two types of formulation which caused very high mortalities when sprayed on mature eggs deposited on a roughened aluminum substrate. No eggs

hatched after treatment with a mixture of 0.2 percent 4-benzylpyridine and 2.0 percent diethanolamine in water. Egg hatch was held to approximately 1.0 percent by addition of 0.2 percent decanol when the diethanolamine concentration was reduced to 0.75 percent. Similar mortalities resulted from treatment of eggs with an aqueous mixture of 0.2 percent Diamine #2 and 1.0 percent of either diethanolamine or ethylenediamine. These formulations each include both lipid-soluble and water-soluble components which had only limited effectiveness when used alone. Controls sprayed with water alone averaged 84 percent hatch.

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