SPEED OF ACTION OF AN *AEDES AEGYPTI* OVICIDE [1]

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The yellow fever mosquito, *Aedes aegypti* (Linn.), lays its eggs just above the water level in artificial containers and occasionally in naturally occurring sites, *e.g.*, bromeliads, tree holes, etc.

At oviposition, the egg shell is a two-layered structure consisting of exochorion and endochorion. A third layer, the serosal cuticle, is formed below the endochorion some 16 to 17 hours later (Harwood and Horsfall, 1959). At this time the egg becomes impermeable to water and is very resistant to penetration by many chemicals.

Several formulations tested at Technical Development Laboratories (TDL) have proved to be ovicidal when sprayed on fully embryonated eggs of *Ae. aegypti*. An aqueous mixture of 0.2 percent decanol, 0.2 percent benzylpyridine, and 1.0 percent diethanolamine caused a marked degree of egg rupture and collapse and resulted in only 1 percent hatch after 24 hours (Wilton *et al.*, 1968). In this paper, the minimum time for effective ovicidal action by this formulation on fully embryonated eggs and limited observations on its action on immature eggs will be discussed.

METHODS AND MATERIALS. The eggs, obtained from a stock colony on 20 x 115 mm aluminum panels, were sprayed to run-off by passing the panels vertically under a Teejet 8001 [3] nozzle at 40 feet per minute. The procedures for obtaining, conditioning and spraying the eggs have been detailed by Wilton *et al.* (1968).

To determine the speed of action of the formulation, mature eggs, 3 to 10 days old, were treated and stored at 85 ± 5 percent relative humidity (R.H.) and at 80° ± 2° F. After an accurate count of the eggs, panels were placed in hatching medium at ½, 1, 1½, 2, 3, 4, 5 or 6 hours after spray treatment. Batches of hatching medium (a culture made from brewer's yeast and ground lab chow) were prepared at the same intervals on the preceding day so that all eggs were submerged in hatching medium exactly 24 hours old. Larval counts were made after a 2-hour hatching period.

The effect of the formulation on immature eggs was determined by treatment when the eggs were between 24 and 36 hours old. Immediately following treatment, the eggs were returned to the saturated humidity—80° F. condition in which they had been stored. At intervals of 1, 1½, 2, 3 or 5 hours after treatment, they were washed by dipping the panels several times in 0.1 percent Igepon detergent and then rinsing them three times in water; they were returned to a saturated humidity at 80° F. Twenty-four hours later the eggs were divided into two lots. One was maintained at 100 percent relative humidity for the entire 72- to 84-hour prehatch period. The other lot was transferred to 85 percent humidity for the final 24 hours of the prehatch period in accordance with the standard procedure followed at this laboratory for the conditioning of *Ae. aegypti* eggs. They were then submerged in hatching medium and larval counts were made after 2 hours.

Two kinds of controls were used in all tests: unsprayed eggs to provide a check on viability (dry controls) and eggs sprayed with tap water to assess the hatch-

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[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.

ing stimulant effect of wetting (wet controls).

RESULTS AND DISCUSSION. Mature eggs. Although the eggs showed partial collapse and rupture at 1½ hours after treatment, no effect from this was apparent since the hatches to this time averaged 84 to 86 percent compared with a mean of 85 percent for wet controls (Figure 1). After 2 hours a decrease in hatch to 52 percent was noted. By 4 hours nearly complete rupture or collapse of the eggs had occurred, and hatch reached its minimum of 1 percent at 5 hours. Satisfactory viability of the eggs used in these tests was indicated by an average 90 percent hatch of the dry controls.

These results suggested that effectiveness was due, at least in part, to desiccation of the larvae following shell rupture. The absence of appreciable mortality at 1½ hours, though shell collapse had begun, could be due to the eggs being placed in hatching medium before the effect of the treatment was expressed. The subsequent ½ hour was critical since the average hatch dropped to 52 percent at 2 hours.

Immature eggs. Shell rupture, observed soon after spray application, suggested that the formulation might be acting as a hatching stimulus. If so, it could not be effective on immature eggs. At 30 hours the muscles of the embryo are still incompletely formed, and the hatching spine is not visible until 48 hours (Christophers, 1960). If the formulation action was not a hatching stimulus but caused mortality in some other way, *e.g.*, by being toxic to the embryo and/or causing desiccation, then it should be effective when applied at any stage.

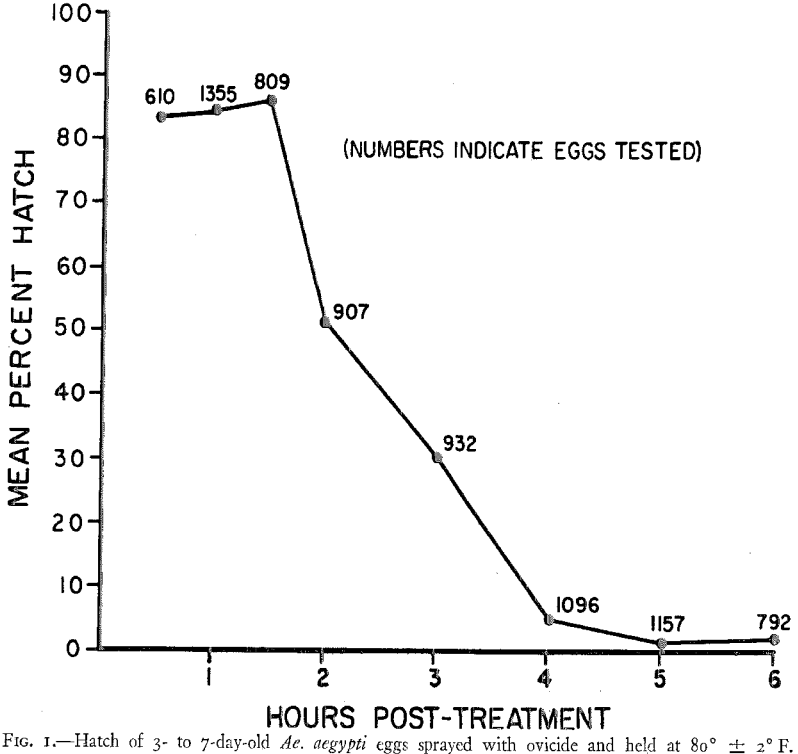

FIG. 1.—Hatch of 3- to 7-day-old *Ae. aegypti* eggs sprayed with ovicide and held at 80° ± 2° F. and 85 ± 5 percent R.H. for various periods.

When 590 eggs between 24 and 36 hours old were sprayed and washed after 1 hour, and when adequate time was allowed for maturation under routine conditions, the hatch obtained was only 1.9 percent. When the interval between spray treatment and washing was 1½ hours the hatch from 803 eggs was reduced to 0.5 percent. Spray-wash intervals of 2, 3 and 5 hours all resulted in zero hatch from a total of 1,645 eggs at the end of the maturation period. Untreated controls averaged 71 percent hatch when mature. The effectiveness of the formulation made it apparent that a hatching stimulus mode of action could be ruled out since at this stage of development the pre-larvae are incapable of such a response.

A test was set up to determine if relative humidity played a part in the effectiveness of the ovicide on immature eggs. Table 1 shows the percent hatch from unsprayed controls and from eggs sprayed at 36 hours of age and washed 4 hours later. One set of each was kept at 100 percent R.H. until 72 hours old. The other set was kept at 100 percent R.H. for 48 hours, then removed to 85 percent R.H. for the remaining 24 hours.

No shell collapse occurred in those treated eggs held continuously at 100 percent R.H., but the eggs taken from 100 percent to 85 percent R.H. showed collapse within 15 minutes. This finding indicates that the formulation evidently altered the permeability of the shell. The hatch obtained from treated eggs removed at 48 hours of age to 85 percent relative humidity differed by less than 1 percent from that of similar eggs held for the entire 72-hour maturation period at 100 percent R.H. Apparently desiccation was not an important mortality factor.

TABLE 1.—Hatch of ovicide-treated immature *Ae. aegypti* eggs conditioned under two humidity schedules.

	Relative Humidity			
	Continuous 100%		100%/85%	
	No. Eggs	% Hatch	No. Eggs	% Hatch
Treatment	408	3.7	262	2.9
Control	316	86.4	324	80.9

SUMMARY. Mature eggs of *Ae. aegypti* showed pronounced rupture and collapse and gave only 1 percent hatch 5 hours after spray treatment with an aqueous mixture of 0.2 percent decanol, 0.2 percent benzylpyridine, and 1.0 percent diethanolamine. Immature eggs similarly treated at 24–36 hours of age yielded zero hatch if the ovicide remained on them for 2 hours or longer. The mode of action, therefore, did not involve a hatch stimulus. In contrast to those held at 100 percent R.H. for the entire posttreatment period, immature eggs transferred to 85 percent R.H. when 48 hours old showed collapse but little difference in mortalities.

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