ACTION OF AMINE OVICIDES ON *AEDES AEGYPTI* MOSQUITOES[1]

DONALD P. WILTON AND RICHARD W. FAY

Recent laboratory tests with eggs of the yellow fever mosquito, *Aedes aegypti,* have established the effectiveness of ovicide sprays combining an aminoalcohol with any of several long-chain aliphatic amines (Cline *et al.,* in press). In particular, spray treatment of the eggs with emulsions containing 1 percent ethanolamine and 0.1 percent decylamine, dodecylamine, di-n-octylamine, Duomeen L–11,[2,3] or Adogen 583[4] resulted at 24 hours in hatches of 3 percent or less. These mixtures were shown to be much more effective than any of their components used alone. In the present paper the performance of this kind of formulation under a number of temperature and humidity conditions, and the functions of the separate components, are considered.

MATERIALS AND METHODS. Mature eggs, 3 to 10 days old, deposited on 20 x 115 mm. roughened aluminum panels, were used in all tests. Before treatment, each panel was inspected under a dissecting microscope so that excess and abnormal appearing eggs could be eliminated. Ovicides were sprayed to the point of runoff by passing the panels in a vertical position at constant speed under an 8001 Teejet nozzle operated at 40 psi. The procedures used for obtaining, conditioning, and spraying the eggs have been described fully by Wilton *et al.* (1968).

The effects on ovicide action of three temperatures and two relative humidities were tested in constant environment cabinets at 90°, 70° and 60° F. each with 80 percent and 50 percent relative humidity. Temperatures varied only ±1° F. and humidities no more than ±1 percent. The eggs were stored overnight in the cabinets and removed only long enough for ovicide application.

Three formulations known to be highly ovicidal when treated eggs were held for 24 hours at approximately 80° F. and 80 percent humidity (Cline *et al.,* in press) were selected for testing in the specified environments. These were aqueous mixtures of 1 percent ethanolamine with 0.1 percent decylamine, 0.05 percent Duomeen L–11, or 0.05 percent Adogen 583. A fourth mixture of 1 percent ethanolamine and 0.2 percent di-n-octylamine formulated with 1 percent of the surfactant Igepon T–77 was tested at 90° and 60° F. only. Each panel used in these tests held 100 eggs. Equal numbers of sprayed eggs and untreated controls were removed from the environmental cabinets at intervals of 2, 4, 12, and 24 hours after treatment. The percent hatch following a 2-hour submersion at room temperature in a day-old culture containing brewer's yeast and pulverized lab chow in tap water was the criterion of effectiveness for all treatments. Each procedure was replicated four times.

The presumably different functions of the lipophilic and water-soluble components of three amine ovicide mixtures were investigated by separating their applications in time. Panels holding an average of 112 eggs and prepared as described were dipped for 1–2 seconds in 1 percent ethanolamine solution and after an interval of ½, 1, 2, or 4 hours in 0.1 percent emulsions of Duomeen L–11, Adogen 583, or decylamine. Duplicate panels were used at each interval with each material.

---

[1] From the Biology Section, Technical Development Laboratories, Laboratory Division, National Communicable Disease Center, Health Services and Mental Health Administration, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia 31402.

[2] 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.

[3] Furnished by Armour Industrial Chemical Co., Chicago, Illinois.

[4] Furnished by Ashland Chemical Co., Minneapolis, Minnesota.

Parallel tests with the same materials and time intervals but with the reverse order of application were made with an equal number of panels. Controls were provided by dipping panels of eggs in (1) the complete ovicide formulation, (2) 1 percent ethanolamine, or (3) 0.1 percent of the lipophilic component. Use of a magnet stir bar insured uniformity of the emulsions during the dip treatments. All eggs were held at approximately 80° F. and 80 percent relative humidity after treatment and were submerged in hatching medium after 24 hours. Larval counts were made 2 hours later.

The relative effectiveness of the components of an amine ovicide mixture was also investigated by spraying eggs with several concentrations of ethanolamine and Duomeen L-11 used alone and with mixtures in which the concentrations of each material were varied independently. Each treatment was applied to four aluminum panels holding an average of 110 eggs each. Storage and 24-hour hatch determination were as previously described.

RESULTS AND DISCUSSION. Each of the ovicide formulations was most effective at 90° F. (Table 1). At this temperature the highest average hatch at 24 hours was only 1 percent. Two of the formulations, those containing Duomeen L-11 and di-n-octylamine, proved highly effective in only 4 hours. At 90° F. no clear difference in egg hatch attributable to relative humidity was seen. At 70° and 60° F. a marked reduction in the effectiveness of all the test formulations became apparent and the influence of relative humidity, unexpressed at 90° F., became clear-cut. Following treatment with decylamine, Duomeen L-11, or di-n-octylamine preparations and 24-hour storage at 60° F., the mean percent hatch of eggs held at 50 percent humidity was approximately three to four times that of eggs kept at 80 percent humidity. At 70° F. the same humidities caused a similar twofold difference in egg hatch following treatment with both the decylamine and Duomeen L-11 formulations.

The effect of humidity on eggs treated with the Adogen 583 preparation appeared reversed. After 24 hours at 60° F. the effectiveness of this ovicide was four times greater at 50 percent humidity than at 80 percent, and at 70° F. it was twice as effective at the lower humidity.

A great deal of variability in the results

TABLE 1.—Mean percent hatch of 100 *Aedes aegypti* eggs sprayed with four amine ovicides and stored under conditions indicated (four replicates each).

Formulations 1.0% ethanolamine plus	Hours after spray	90° F.		70° F.		60° F.	
		80% RH	50% RH	80% RH	50% RH	80% RH	50% RH
0.1% decylamine	2	58	59	86	95	71	85
	4	11	24	39	68	42	66
	12	5	6	66	64	21	67
	24	1	1	16	32	10	37
0.05% Duomeen L-11	2	28	31	68	88	96	95
	4	0.5	0.3	50	75	68	72
	12	2	1	44	35	39	55
	24	0.5	1	7	15	15	41
0.05% Adogen 583	2	46	34	81	87	93	92
	4	13	0.3	54	82	71	76
	12	11	5	45	69	9	17
	24	1	0	13	6	17	4
0.2% di-n-octylamine	2	2	49			80	91
	4	0.8	2			73	89
1.0% Igepon T 77	12	0.5	3			49	93
	24	0.3	1			24	89
Unsprayed controls	89–100						

is not revealed by the averages shown in Table 1. The Duomeen L–11 and di-n-octylamine formulations gave rather consistent results over the range of test conditions, but the decylamine and Adogen 583 treatments were quite erratic with marked reversals (higher hatches after longer holding time) occurring frequently.

The relatively poor results obtained at 60° and 70° F. with all the formulations appeared to place a severe limitation on their practical use in the field. However, results of additional tests, not included in the tables, indicate that this difficulty can be overcome simply by an increase in the concentration of the spray ingredients. Limited data for eggs held at 60° F. and 50 percent humidity show a 24-hour hatch of less than 1 percent after treatment with mixtures of 2.0 percent ethanolamine and 0.2 percent of either Duomeen L–11 or Adogen 583. Equally good results were obtained at 50° F. and 60 percent humidity when these concentrations were doubled.

When eggs were treated sequentially with the lipophilic and water-soluble components of three highly effective amine ovicide formulations, the results differed greatly, depending upon which component was applied first (Table 2). If the eggs were treated first with the lipophilic amine (Duomeen L–11, Adogen 583, or decylamine), a delay of up to 4 hours before application of the water-soluble ethanolamine had no marked effect on ovicidal action. Hatches remained comparable to those obtained with the complete formulations. If, on the other hand, the ethanolamine were applied first, as short an interval as ½ hour caused substantial reductions in effectiveness compared with the action of both components together.

Such differential results support the idea that the lipophilic aliphatic amines of these ovicides function by destroying the water impermeability of the egg shell, thus allowing penetration of the water soluble aminoalcohol, which is the actual ovicidal agent. The failure of the aliphatic amines to act as ovicides by themselves, although many are known to be mosquito larvicides (Mulla, 1967), clearly suggests either that they fail to pass completely through the shell or are too slow acting to produce substantial 24-hour mortalities. In this connection, Mulla and Chaudhury (1968) found oleyl amine, a primary aliphatic amine, to be highly effective against eggs of the southern house mosquito, *Culex pipiens quinquefasciatus*. They stated, however, that treatment for less than 24 hours produced no marked effect.

The lipophilic materials, being nonvolatile, apparently remain after application to produce their change in the shell's permeability, and a delay in application of the aminoalcohol has little effect on hatch reduction. The ethanolamine, however, is more volatile and does not persist in sufficient amount to be effective if application of the lipophilic amine is delayed for more than a few minutes.

Results obtained when both the lipophilic and water-soluble parts of an ovicidal

TABLE 2.—Percent hatch of *Ae. aegypti* eggs 24 hours after sequential treatments with 1 percent ethanolamine and 0.1 percent lipophilic amine.[1]

	Lipophilic amine first				Ethanolamine first			
	Hours between application of components							
Lipophilic amine	½	1	2	4	½	1	2	4
Duomeen L–11	0	<1	<1	2	4	20	78	88
Adogen 583	<1	1	<1	2	15	65	56	80
Decylamine	2	6	5	5	81	87	95	65

[1] Percent hatches of controls were: Ethanolamine alone—95; Lipophilic amines alone—98–100; Complete formulations—0–1.

spray were independently varied provide another indication that the lipophilic material is involved with permeability changes in the egg shell. Table 3 shows the percent

TABLE 3.—Percent hatch of *Aedes aegypti* eggs 24 hours after spray treatment with ethanolamine, Duomeen L–11, and mixtures of the two.

Percent Duomeen L–11	Percent Ethanolamine				
	0	0.5	1.0	5.0	10.0
0	..	95	65	39	2
0.01	..	30	3	1	<1
0.05	..	9	0	<1	0
0.1	..	8	<1	<1	<1
0.4	95	1	0	<1	<1
1.0	79	..	..	..	..

hatch obtained from eggs sprayed with Duomeen L–11, ethanolamine, and various mixtures of the two. The lowest concentrations tested in combination were 0.01 percent Duomeen L–11 with 0.5 percent ethanolamine. A 30-percent hatch resulted after treatment with this formulation. If the ethanolamine remained at 0.5 percent, a Duomeen concentration of 0.4 percent was required to lower the hatch to 1 percent. At a 0.01 percent Duomeen concentration, a hatch reduction to approximately the same level (3 percent) was obtained with 1 percent ethanolamine. The total active ingredients in each of these mixtures were nearly equal: 0.9 percent and 1.01 percent, respectively, but Duomeen made up 44 percent of the first preparation and scarcely 1 percent of the second. Apparently the Duomeen had a quantitative effect, whereby a relatively low ethanolamine concentration was effective because of a large change in egg shell permeability, whereas a higher ethanolamine concentration was needed when permeability was less altered.

The lower hatches recorded with 80 percent humidity at 60° F. for three of the four test formulations are consistent with the postulate that ethanolamine is the lethal agent, since the action of a water-soluble material should be favored by high humidity levels. The Adogen 583 formulation provided an exception to the hatch-humidity relationship. At 60° F. it consistently gave better 24-hour results at 50 percent humidity than it did at 80 percent. The factors making it more effective in drier air are as yet undetermined, but its different action very likely correlates with its different structure. Of the four lipophilic materials used in the present tests, Adogen 583 is exceptional in incorporating an ether linkage and a branched carbon chain.

SUMMARY. Ovicide spray mixtures of 1 percent ethanolamine plus either 0.1 percent decylamine, 0.05 percent Duomeen L–11, 0.05 percent Adogen 583, or 0.2 percent di-n-octylamine frequently reduced hatches of *Aedes aegypti* eggs below 1 percent within 4 hours at 90° F. These formulations were only moderately effective at 60° F. in 24 hours, but 2 percent ethanolamine with 0.2 percent Duomeen L–11 or Adogen 583 allowed less than 1 percent hatch at 60° F., and the same materials at 4.0 and 0.4 percent, respectively, performed equally well at 50° F. A high humidity-low hatch relationship, not observed at 90° F., was apparent at 60° F. with three of the four ovicides. The Adogen formulation alone gave a reverse low humidity-low hatch result.

When eggs were dipped sequentially in the separate components of three amine ovicides, a 4-hour interval between dips had no marked influence on effectiveness if the liposoluble amine was applied first. If the water-soluble ethanolamine came first, ½ hour between dips caused substantially increased hatches.

Equivalent reduction of the 30 percent hatch obtained from eggs sprayed with 0.5 percent ethanolamine plus 0.01 percent Duomeen L–11 was effected by either 1 percent ethanolamine or 0.4 percent Duomeen L–11 when the concentration of the alternate component was unchanged.

The data suggest that the aliphatic amines in these ovicides produce a quantitative change in the water impermeability of the egg shell, allowing penetration of the ethanolamine.

ACKNOWLEDGMENTS. The authors thank Dr. R. E. Cline, Chemistry Section, for his valuable suggestions and cooperation in the chemical aspects of this investigation and Mrs. Betsy Noonan, Biological Laboratory Technician, Biology Section, for making most of the egg and larval counts.

Literature Cited

CLINE, R. E., WILTON, D. P., and FAY, R. W. Aliphatic amines ovicidal for the mosquito, *Aedes aegypti* (L.). J. Econ. Entomol., in press.

MULLA, M. S. 1967. Biocidal and biostatic activity of aliphatic amines against southern house mosquito larvae and pupae. J. Econ. Entomol. 60(2):515–522.

MULLA, M. S. and CHAUDHURY, M. F. B. 1968. Ovicidal activity of aliphatic amines and petroleum oil against two species of mosquitoes. J. Econ. Entomol. 61(2):510–515.

WILTON, D. P., CLINE, R. E., and FAY, R. W. 1968. Two formulations effective in the laboratory as ovicides for *Aedes aegypti* (L.). Mosq. News 28(4):602–606.

THREE ESTUARINE KILLIFISH AS FRESH WATER MOSQUITO LARVIVORES

J. M. DOLL AND T. F. BAST

Bur. of Epidemiology, New York State Dept. of Health, Albany, N.Y.

Inland fresh waters of New York State lack indigenous fish that are efficient mosquito larvivores. Recently, specimens of three killifish, *Fundulus confluentus,* Goode and Bean, *Fundulus heteroclitus* (L), and *Cyprinodon variegatus* Lacepede partially acclimatized for fresh water, have been obtained for colonization and field observation.

The value of these fish as mosquito larvivores has been studied by several authors. *Fundulus heteroclitus* and *Cyprinodon variegatus* were listed as suitable salt water or brackish water fish by the Bureau of Fisheries as early as 1915 (Radcliff, 1915). About this same time the New Jersey Agricultural Experiment Station studied these two species and named *F. heteroclitus* as by far the most important salt marsh larvivore of those studied, (Childester, 1917). In Florida a study indicated that where abundant, mosquitoes contributed to total food volumes up to 85.5 percent for *F. confluentus,* while in a similar situation mosquitoes made up about 78 percent of the food volumes of *Gambusia,* (Harrington and Harrington 1961).

All three species are euryhaline and native to the Atlantic Coast, *C. variegatus* and *F. confluentus* ranging generally farther south than *F. heteroclitus* (Miller, 1955).

Since these killifish all range into fresh water under certain situations, introduction into most of New York would constitute little more than a slight extension of their natural range and they should, therefore, not be considered as "exotic."

Fundulus confluentus has been observed to lay stranded eggs which are resistant to desiccation for several months, an accomplishment previously only associated with Grunion in California and certain South American and African Cyprinodontidae, (Harrington, 1959).

The more northern ranging *F. heteroclitus* has been reported living in pools where the water temperature dropped to the 40° Fahrenheit range. Burrowing into soft muck on the bottom of the pool enabled them to withstand the low water