SIMULATED FIELD TESTS WITH OVICIDES AGAINST *AEDES AEGYPTI* EGGS IN TIRES AND CANS[1]

W. L. JAKOB

INTRODUCTION

In spite of the great volume of work that has been done to develop selective insecticides, relatively little effort has been directed to development of ovicides *per se*. Smith and Salkeld (1966) list these prerequisites for effective use of ovicides: (a) the egg must be in an exposed location where lethal concentrations of toxicant can be directed to it, (b) the egg must be susceptible to the toxic effect of the chemical, and (c) a sufficient proportion of the population must be exposed in the egg stage to justify treatment. The *Aedes aegypti* mosquito appears vulnerable to ovicidal attack since its eggs are deposited above the water line and, over much of its range in the U.S., a large proportion of the population is in the egg stage during the cooler months.

Wilton *et al.* (1968) reported preliminary results for relatively nontoxic materials which were effective in preventing hatch of *Ae. aegypti* eggs. The ovicidal activity of long chain aliphatic amines against *Culex pipiens quinquefasciatus* and *Anopheles albimanus* was demonstrated (Mulla and Chaudhury, 1968). Apolar lipophilic amines were found to be effective ovicides against *Ae. aegypti* only when used in the presence of ethanolamine by Cline *et al.* (in press). Such treatments were characterized by collapse and dehydration of the egg. The activity of amine mixtures under a number of temperature and humidity conditions and the functions of the separate components have been investigated (Wilton and Fay, 1969).

In simulated field applications formulations combining ethanolamine with either of two long chain lipophilic amines were applied to *Ae. aegypti* eggs and the results are presented in this paper. Results of evaluations for repellency to ovipositing females, residual larvicide activity, and the effect of temperature on the efficacy of ovicidal applications are also given.

MATERIALS AND METHODS

Eggs of *Ae. aegypti* (Trinidad strain) were obtained by exposing containers to a colony in a large chamber (8 ft. x 12 ft. x 8 ft.) maintained at 80±5° F. and 70±10 percent relative humidity. At weekly intervals additional mosquitoes were introduced to replace those lost due to natural mortality and activities involved in servicing the colony and/or containers. Escapees were, however, confined to a "safety corridor" and destroyed by capturing with a household vacuum cleaner. The colony was provided a rabbit as a source of blood for a period of 4 hours each work day of the week.

Containers (each holding 2,000 ml. tap water) were dated and allowed to remain in the colony chamber for 48 hours. The large food cans (of approximately 1 gallon capacity and with one end removed) had been held outdoors for varying periods to initiate rust formation on the inner surfaces prior to exposure, in an upright position, to egg laying. Tires, obtained from local recapping outlets, were placed in an almost upright position and marked to show that portion of the tire in which eggs were laid. After exposure to oviposition all containers were held in an adjacent room for an additional 48 hours to allow for completion of embryonic development. The water was then removed and the containers stored for subsequent testing.

---

[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.

With the exception of certain tests (*i.e.*, effectiveness at lower temperatures) all formulations contained 0.1 percent Duomeen L–11 [2] or Diamine No. 2 [2] and 1.0 percent ethanolamine in water. All treatments were made with a 1-gallon compression sprayer operating at 40 lb./sq. in. pressure and delivering a hollow cone spray from a TeeJet 5500 adjustable nozzle with an X-18 disc. Preliminary tests had indicated that this nozzle gave better results than did flat fan nozzles of lower delivery capacity. All cans were treated in an upright position by simply rotating the wrist so that the spray covered the upper inside surfaces. Sufficient spray was thus delivered (35–40 ml.) to provide thorough coverage of all inside surfaces. During treatment of tires, except where otherwise indicated, that portion containing the eggs was rotated upwards ¼ turn so that the eggs were in the 3 o'clock position. Spray was then directed around the inner surface of both sides of the tire. The rotation precluded over-treatment due to *all* runoff flowing over the eggs. Approximately 350–400 ml. of ovicide were used in treating each tire.

OVICIDE EVALUATION. The spray remained in the containers for an overnight period and was then removed with a plastic bulb syringe. Final removal of liquid remaining in tires was accomplished by blotting with cellucotton. The eggs were submerged 24 hours later by flooding the containers with hatching medium which had been prepared by adding a small amount of ground lab chow and brewer's yeast to tap water and allowing it to age overnight.

[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.

Domeen L–11 provided through the courtesy of Armour Industrial Chemicals, McCook, Illinois.

Diamine No. 2 provided through the courtesy of Ashland Chemical Co., Division of Ashland Oil and Refining Co., Minneapolis, Minnesota. The Company recently advised that this product is now marketed as Adogen 583.

Larval counts were made about 5 days after flooding, during which time additional amounts of ground lab chow for larval food were added daily to each container. Contents of the containers were then transferred to glass provision jars to facilitate observation of larvae. The number of larvae was usually estimated if more than 200 were judged to be present but no value of more than 1,000 was given. For example, in an accessory test one tally was in excess of 5,600 larvae before counting was stopped even though many larvae remained to be counted. Although this may be an extreme example, the majority of control containers were judged to contain more than 1,000 larvae.

A series of tests was conducted in which tires and cans, usually in triplicate, were treated but both types of containers were not used in all tests.

REPELLENCY TEST. Tires containing 2,000 ml. tap water were treated with ovicidal sprays and stored outdoors, but protected from rainfall, for specified intervals. After the water level was restored to the original volume, the tires were exposed to oviposition in the colony chamber for 48 hours. Females were not given the opportunity to oviposit in competing untreated containers. Visual observations of oviposition were made and assessment of ovicidal effect conducted as described above except that no further treatment was made.

RESIDUAL LARVICIDE EFFECT. Cans, dry or wet (*i.e.*, containing 1,500 ml. tap water), were treated with ovicidal formulations and stored in an indoor ventilated chamber for specified intervals. Prior to introduction of biological specimens 500 ml. of water were added to the dry cans and the water in the wet cans was restored to its original level. Initially, bioassays were made with third instar larvae (Trinidad strain) using 24-hour mortality counts as the criterion of effectiveness. Later evaluations were made by introducing egg strips and counting the number of larvae surviving 5 to 7 days later.

NATURALLY INFESTED TIRES. Limited studies were conducted with presumed egg-infested tires obtained from a local tire outlet. All tires from this location had an accumulation of organic debris (*e.g.*, old leaves, twigs) indicating that they had been stored there for several months or more. The tires were marked to show their resting positions for subsequent reference in flooding. In two tests the water was removed and the tires allowed to dry out overnight before being processed as described above for artificially infested tires. In a single test, only the larvae and pupae were removed from tires found in the field before they were treated and flooded to capacity with hatching medium. Effectiveness was based on the number of larvae obtained in treated versus control tires.

EFFECTIVENESS OF OVICIDES AT LOWER TEMPERATURES. Tires and cans artificially infested with eggs were exposed outdoors, but protected from rainfall, for a period of 3 days prior to treatment at ambient temperatures. The containers were left outdoors until the following day when the spray material was removed. The containers were conditioned indoors to a temperature of 80°±5° F. for 24 hours before being flooded with hatching medium. Effectiveness of the treatment was based on subsequent larval counts. Ambient temperatures during spray applications were 55° F. and 57° F. Surface temperatures in the containers, as well as the solution temperatures, were only slightly higher than the ambient temperatures given. In both tests only the Duomeen L-11 was tested, at concentrations of 0.1 and 0.2 percent in combination with 2 percent ethanolamine.

RESULTS AND DISCUSSION

OVICIDE EVALUATION. The number of eggs in the test containers, as indicated by the number of larvae recovered in controls, appears to be considerably higher than normally occurs in nature. The average number of larvae found at a given time during the breeding season in south Florida was 38 in medium-sized cans and 75 in tires (von Windeguth and Eliason, unpublished manuscript). Similar data in Mississippi showed an average of 8 and 62 larvae in gallon cans and tires, respectively (Bond and Fay, 1969).

The data (Table 1) show that the Duomeen formulation was completely effective when used in cans but failed to prevent hatch in all tires. The Diamine formulation was less effective, particularly in respect to preventing hatch in cans.

TABLE 1.—Effectiveness of ovicidal formulations against *Ae. aegypti* eggs in containers[1].

	Number of cans		Number of tires	
Ovicide	Hatch	No Hatch	Hatch	No Hatch
Duomeen L-11: ethanolamine (0.1:1.0%)	0	16	6	18
Diamine #2: ethanolamine (0.1:1.0%)	5	13	6	13

[1] Includes seven or more tests with each formulation. Both types of containers not used in all tests.

Since counting of eggs in the containers was virtually impossible, the results are given in definitive terms—no hatch or hatch (=failure). It should be emphasized, however, that in many cases "failures" were charged to replicates which yielded less than 10 larvae as compared to the many hundreds or thousands obtained in control containers. The reason for the "failures" was not investigated but may have been due to poor coverage and/or the presence of layers of eggs which prevented adequate contact of the lower layer by the ovicide. In at least two tests efforts were made to reduce the amount of material applied per tire with resultant poor ovicidal effect.

Larval mortality due to residues of the treatment remaining in the container appears negligible in view of the number of containers producing larvae after treatment and the lack of dead specimens in the medium during larval counts.

REPELLENCY TEST. A preliminary series had indicated that cans and tires, treated

1 week previously, were not as attractive to the ovipositing female as were untreated containers. This study further indicated that an ovicidal effect was present against eggs deposited in the treated containers.

Tires treated 4 weeks earlier were repellent to ovipositing females which were not offered the chance to lay in untreated containers (Table 2). One may assume that most eggs were retained, a phenomenon known to occur with this species in the absence of water. Oviposition observations were based upon the number of eggs which would normally be expected from exposure of untreated containers to the oviposition potential of the colony.

Although relatively few eggs were deposited, hatch was obtained in only one tire (treated with Diamine formulation). Thus the ovicides appear to be quite effective against newly deposited eggs which are quite vulnerable while the process of melanization and impermeability is in progress.

RESIDUAL LARVICIDE EFFECT. The ovicides, in cans with or without water at time of treatment, were effective against newly emerging larvae for up to 6 weeks (Table 3). Dry cans treated with the same formulation yielded larvae in only one of three replicates in the 4-week post-treatment test. The Diamine formulation showed markedly less residual larvicide activity than the Duomeen formulation. Both treatments, however, produced 100 percent mortalities (moribund+dead) of the third instar larvae introduced 1 day after treatment.

Hatch and survival of larvae occurred at week 9 in wet cans and in all replicates of dry cans at week 6. However, in each case, surviving larvae were retarded in development in comparison to control larvae. Such biostatic phenomena were observed in evaluations of aliphatic amines against *C. p. quinquefasciatus* (Mulla, 1967).

It is interesting to speculate that these materials, under actual conditions of use, will be detrimental to larvae which might emerge weeks after application of the ovicide.

NATURALLY INFESTED TIRES. In two tests in which tires were treated dry and the treatment removed before flooding, one of seven tires treated with the Duomeen formulation yielded larvae (only five) whereas four of seven treated with the Diamine formulation produced a total of

TABLE 2.—Observations on the number of eggs deposited and the number of larvae obtained in tires treated with ovicides.

Ovicide	Age of treatment	Number replicates	Oviposition observation	Number larvae
Duomeen L-11: ethanolamine (0.1:1.0%)	2 weeks	4	Very few—very light	0
	4 weeks	4	Few, if any	0
Diamine #2: ethanolamine (0.1:1.0%)	2 weeks	4	Very few—<50	0
	4 weeks	3	Few	0
		1	Very Light	>200

TABLE 3.—Number of weeks of residual larvicide activity against *Ae. aegypti* in cans sprayed with test formulations of ovicide.

Treatment	Condition of cans[1]	24-hr. Mortality tests[2]	Egg strips with emerging larvae
Diamine #2: ethanolamine (0.1:1.0%)	Wet	<2	<4
	Dry	<2	<4
Duomeen L-11: ethanolamine (0.1:1.0%)	Wet	2	6
	Dry	2	<4

[1] Wet—cans contained 1,500 ml. tap water at treatment. Dry—no water in cans at treatment.
[2] Based on 90 percent or higher mortality of third instar larvae.

25 larvae (range 1 to 12). The seven control tires yielded a total of 86 larvae (range 3 to 37).

In a single test, tires (three replicates each), treated as they would be during actual field applications, failed to yield larvae when sprayed with either ovicidal formulation. Control tires yielded from 2 to 10 larvae.

Although larval yield in control tires was quite low, the data indicate that the presence of organic debris in tires may not necessarily be detrimental to the action of the ovicides, at least in the case of the Duomeen formulation. In the single test in which applications and evaluations were made under practical field conditions excellent results were obtained with both ovicide formulations.

EFFECTIVENESS OF OVICIDES AT LOWER TEMPERATURES. With the lower concentration of Duomeen (0.1 percent) less than 15 larvae were obtained from three of a total of eight tires treated at temperatures of 55 to 57° F. No larvae were found in cans treated with this formulation at these temperatures.

In neither test were larvae found in either type of container treated with the formulation containing 0.2 percent Duomeen.

The data verify laboratory results (Wilton and Fay, 1969) indicating that when the ovicide is sprayed at temperatures below 60° F. an increase in concentration of both constituents is necessary to achieve the efficacy obtained with treatments applied at temperatures of 70° F. or higher. Thus, the ovicide may be productively applied during periods when larval and adult activity has decreased or is absent. This would effect a longer anti-*Ae. aegypti* control period than was considered advisable during the recent eradication program in the U.S. which primarily employed residual larvicide applications.

CONCLUSIONS

An aqueous formulation containing Duomeen L-11 and ethanolamine was superior in ovicidal activity against *Ae. aegypti* to a similar formulation with Diamine No. 2 and ethanolamine. Although evaluated primarily as ovicides, both formulations exhibited residual larvicide properties and were repellent to the ovipositing females; Duomeen in each case was again superior to Diamine.

Although the effectiveness of the Duomeen-ethanolamine formulation requires further evaluation under field conditions, the attack on *Ae. aegypti* eggs offers a new approach to control or extermination. Experience in these studies has indicated that thorough coverage of the inner surfaces of each container is essential for maximum effectiveness. Destruction of eggs would preclude reliance on repeated applications of residual insecticides for larval control since the ovicide appears effective in both dry containers and those holding water. Use of ovicides during the breeding season would probably require some means of adult control to eliminate oviposition potential. However, in the "off season" application of ovicides also offers the possibility of increased advantageous utilization of available manpower at a time when residual larvicide treatments are of questionable benefit. Thorough coverage of all potential breeding sites appears essential.

ACKNOWLEDGMENTS

All ovicide formulations were prepared by Dr. Richard E. Cline, Chemistry Section, TDL. The author also acknowledges the technical assistance of Messrs. Bernard O. Smith and John Olson.

These studies were supported in part by funds provided by the Environmental Control Administration.

Literature Cited

BOND, H. A., and FAY, R. W. 1969. Factors influencing *Aedes aegypti* occurrence in containers. Mosq. News 29(1):113–116.

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 oils against two species of mosquitoes. J. Econ. Entomol. 61(2):510–515.

SMITH, E. H., and SALKELD, E. H. 1966. The use and action of ovicides. Annual Rev. Ent. 11: 331–368.

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.

WILTON, D. P., and FAY, R. W. 1969. Action of amine ovicides on *Aedes aegypti* mosquitoes. Mosq. News 29(3):361–365.

SEASONAL DISTRIBUTION OF MOSQUITOES OF HANCOCK COUNTY, MISSISSIPPI, 1964–1968

FREDERICK W. HARDEN AND B. J. POOLSON [1]

There are many references to the mosquitoes of Mississippi, based primarily on work done during World War II by the Malaria Control in War Areas (MCWA): Anon. (1946), Young (1944), Peterson (1945), Michener (1947), Rings (1953), and Richmond (1962). Carpenter *et al.* (1955) and King *et al.* (1960) refer to many of these publications.

Mosquito studies of Hancock County, Mississippi, per se, are quite limited. T. H. D. Griffitts (1928, unpublished) referred to flights of *Aedes sollicitans, Anopheles crucians,* and *Culex salinarius* occurring in annoying numbers. Fehn *et al.* (1963), in a brief observation of the mosquito problem at the National Aeronautics and Space Administration's (NASA) Mississippi Test Facility (MTF) Site in Hancock County, estimated that during June 1963, the massive mosquito invasion consisted of approximately 74 percent *Aedes sollicitans,* 14 percent *Aedes taeniorhynchus,* and 9 percent *Aedes vexans.*

In 1964, following Fehn's evaluation of the infestation, General Electric Company, the support contractor to NASA, employed an entomologist for MTF, who established a control program directed toward abating the mosquito problem. Harden *et al.* (1965, 1967 and 1968) published on the problem and mosquitoes of the area, but an evaluation of the overall mosquito population and distribution had not been done. It is the purpose of this paper to fill that gap.

Surveillance of the mosquitoes of Hancock County was conducted over the period May 1964 through December 1968. This study reflects the seasonal distribution and relative abundance based on collections taken in New Jersey light traps, Communicable Disease Center (CDC) miniature light traps, truck traps, larval samples, biting and resting observations.

Of the 53 species recorded by King *et al.* (1960) from Mississippi, 45 were taken during this period. Heretofore only 20 species had been reported from Hancock County. (Anon., 1946).

The study centered around the Mississippi Test Facility, latitude 30°22′N., longitude 89°35′W. MTF consists of two principal areas: the Fee Area and the Buffer Zone. The Fee Area includes approximately 13,500 acres of pine forest and hardwood swamps. This is the area where the Saturn Rocket is tested and all work activity concentrated. The Buffer Zone is an unpopulated area consisting of approximately 128,500 acres of pine forest, river swamp and transitional salt marsh. The study included the 485 square miles of Hancock County. Bordered to the west by the Pearl River and to the east by the Jordan River, Hancock County has almost 18 miles of salt marsh coastline overlook-

[1] General Electric Company, Mississippi Test Support Department, Bay Saint Louis, Mississippi.