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CHEMOSTERILIZATION OF *AEDES AEGYPTI* (L.) BY LARVAL TREATMENTS

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The possibility of controlling mosquitoes with chemosterilants has been explored by Weidhaas *et al.* (1961) and Weidhaas (1962), who sterilized both adult and immature stages of mosquitoes in the laboratory. LaBrecque *et al.* (1962) demonstrated that adult house flies (*Musca domestica* L.) can be controlled in the field with chemosterilant bait formulations. Although mosquitoes can also be sterilized by eating chemosterilant-treated food, no satisfactory baits currently available have adequate mosquito attractancy. Thus, although it may be feasible to treat adult mosquito populations in the field, treatment of immature stages in breeding waters might provide the most practical approach. The investigations reported herein are part of a series of studies designed to define and examine the possibilities of chemosterilant treatment of larval breeding waters.

METHODS. Laboratory evaluations were conducted with *Aedes aegypti* (L.) adults exposed as larvae in water containing various concentrations of tepa or apholate.[1] Approximately 300 to 500 third instar larvae were placed in a liter of water in enamel pudding pans, measuring 11 inches in diameter, in a room maintained at 80° ± 4° F. and 70 percent to 80 percent relative humidity. No food was provided for 24 hours, but on the second day of treatment a mixture of ground dog checkers and yeast was added to the water medium. Larvae were maintained in the treated water until they pupated. Pupae were transferred to untreated water and placed in emergence cages. Less than 16 hours after eclosion, adults were immobilized in a cold room at 34° ± 2° F. and the sexes were separated before mating could occur.

Cross matings of untreated and treated adults were performed to evaluate the effects of the treatments. The adults were held in organdy-covered mating cages (9 in. high, 8 in. wide, and 11 in. long) for 3 to 5 days, after which the males were removed. During the mating period a water-soaked cotton pad and a pad saturated with a 20 percent honey solution were available to the mosquitoes. At the end of the mating period the females were allowed to feed on guinea pigs to obtain the blood meal necessary for oviposition, and 2 days later their eggs were collected.

When evaluation of individual egg batches was desired, each female was placed in a 10-dram glass vial lined with filter paper and about one-half inch of tapwater added to the vial. When individual egg batches were not required, a 100-ml. beaker, lined with filter paper and containing about an inch of tapwater, was placed in the cage for oviposition. The eggs were allowed to embryonate and mature on the moist filter paper for 4 days before being flooded for hatching. The total number of eggs and resulting larvae produced per female were separately determined in the tests with individual females; but in the mass evaluations, the total number of eggs was estimated and a sample of 100 to 200 eggs was flooded to determine hatchability. Because each

[1] tepa=tris(1-aziridinyl) phosphine oxide.
apholate=2,2,4,4,6,6,-hexahydro-2,2,4,4,6,6,-hexakis(1-aziridinyl)-1,3,5,2,4,6,-triazatriphosphorine.

female generally oviposited at random over the filter paper and not in one spot, the egg sample represented a cross-section of the total deposition.

In the preliminary tests, apholate and tepa were prepared as 0.1 percent methanol solutions and added to the rearing water to give concentrations ranging from 5 to 25 parts per million (p.p.m.). In addition to the treatments with the sterilant solutions added to the rearing water, treatments with the sterilant apholate incorporated on or in granular or dust diluents were evaluated.

For these treatments, 10-percent formulations were prepared by coating No. 20 corncob grits, vermiculite buffered with ethylene glycol, and pyrophyllite with methanol solutions of apholate. These formulations, prepared in small lots, were stirred until dry and sprinkled directly on the surface of the water in test pans containing the larvae at the rates of 150, 100, and 50 mg. to obtain 15, 10, and 5 p.p.m. of apholate. The formulations were tested when fresh and after aging for 1 week at room temperature. In order to determine the effectiveness of these formulations under less than optimum conditions, 1 inch of soil from a local mosquito breeding area was placed in some rearing pans. The soil was saturated with tapwater and a liter of rearing water added. Procedures as described above for mass egg evaluations were used to obtain the percentage hatch.

In order to determine the stability of tepa under field conditions, a pothole was dug at the edge of an existing mosquito breeding area. After the water had percolated into the pothole, it was treated with 0.1 percent methanol solution to give a tepa concentration of 10 p.p.m. Third-instar larvae were introduced and held for 72 hours and then a second group of third instar larvae was introduced and held for another 72 hours. A similar test was run concurrently in fresh tapwater in the laboratory.

The competitiveness of adult males exposed to apholate solutions as larvae was determined by combining equal numbers of untreated and treated males and f males in mating cages. In the tests 10 p.p.m., 50 adults from 0 to 16 hou old from each treatment category we placed in a 17- x 30- x 31-inch screen cag in the tests at 25 p.p.m., 25 one-day-o males of each category were placed in standard mating cage and allowed orient themselves before the 25 virgin f males were introduced. Both treated an untreated females were dusted with co lored powders to facilitate identificatio when combined in the same cage.

The effect of multiple matings on th viability of eggs from the untreated fe males was evaluated. Individual female were introduced into mating cages con taining 5 or 6 males exposed to apholat solutions as larvae and observed unti copulation had occurred. The female wa then removed from the cage and after short interval was placed in a cage con taining 5 or 6 untreated males, and ob served until mating had again occurred The reciprocal sequence was run with un treated males being the first mating part ner and the treated males the second. The eggs from these females were evaluated for hatchability. Since the reliability of these results was dependent upon the observers' ability to differentiate between attempted and completed matings, pretest trials were conducted to evaluate the accuracy of the observations. The presence of sperm in the spermathecae of all females judged to have completed mating confirmed the accuracy of the mating observations.

RESULTS. The effects of exposure to 10 p.p.m. of apholate or tepa in the larval medium are shown in table 1, series I. Both compounds proved to be effective male sterilants. The hatchability of egg from treated females was reduced from the normal by 60 percent and 75 percent with apholate and tepa, respectively When the treated females were mated t treated males, the hatch was 1 percent o less. Although tepa consistently produced greater sterility than apholate, at 10 p.p.m. it reduced adult emergence somewhat. In other tests with *Anopheles quadrimacu-*

TABLE 1.—Effectiveness of apholate and tepa applied in the larval medium as sterilants of *Aedes aegypti* (2 replications).

Concentration (p.p.m.)			
apholate	tepa	Sex treated	Egg hatch (%)
		Series I	
10	..	Male	4
10	..	Female	31
10	..	Both	1
..	..	Neither	>78
..	10	Male	0
..	10	Female	22
..	10	Both	0
..	..	Neither	>87
		Series II	
10	..	Both	5
7	3	Both	3
5	5	Both	5
..	10	Both	2
..	..	Neither	>85

lutus Say, 10 p.p.m. of tepa was highly toxic to larvae and reduced adult emergence severely; but apholate caused little mortality. Combinations of apholate and tepa at total concentrations of 10 p.p.m. of sterilant proved to be as effective as 10 p.p.m. of apholate or tepa alone (table 1, series II).

In the pothole treatments conducted in the field with 10 p.p.m. of tepa, crosses of males and females exposed for the first 72 hours resulted in an egg hatch of only 20 percent while those from the subsequent 72-hour exposure produced eggs with a normal hatch of greater than 85 percent. The concurrent laboratory treatment produced 7 percent and 0 percent hatch for the first and second 72-hour exposures, respectively. Thus, under field conditions the sterilant appeared to break down or become unavailable quite rapidly.

Preliminary investigations with apholate on corncob grits demonstrated that 15 p.p.m. of apholate was needed to obtain the same degree of sterility produced by 10 p.p.m. in solution. Subsequent testing with pyrophyllite, vermiculite, and corncob grits as carriers was conducted with 15 p.p.m. of actual apholate. The results are given in table 2. All the freshly prepared formulations were effective when used in clear water, but after aging for 1 week only the pyrophyllite formulations were effective when applied to water containing 1 inch of soil.

The results of the tests to determine male competitiveness are shown in table 3. With both the 25- and 10-p.p.m. treatments, the treated males reduced the total egg hatch by approximately 33 percent of the normal. The expected reduction of 50 percent was not achieved, indicating some loss of mating vigor at both levels of treatment. Furthermore, the hatch in individual egg batches was not what would be expected. Since the treated males were completely sterilized at the 25 p.p.m. level, one would expect, theoretically, that 50 percent of the egg masses would be completely sterile in the competitive cross if mating were unaffected by the treatment. Even with the resulting 33 percent sterility, one would expect approximately 30 percent of the egg masses to be completely

TABLE 2.—Effect of 10 percent formulations of apholate in the larval medium of *Aedes aegypti* on the viability of eggs from the ensuing adults (2 replications).

Concentration (p.p.m.)	Carrier	Percent hatch			
		In clear water		In water over soil	
		Fresh formulation	Aged formulation	Fresh formulation	Aged formulation
15	Pyrophyllite	2	3	6[a]	4
15	Buffered vermiculite	5	4	17	22
15	Corncob grits	2	36	21[a]	18
0	.....	>85	..	..	..

[a] 3 replications.

TABLE 3.—Competitiveness of *Aedes aegypti* males sterilized as larvae with apholate as shown by fertility of untreated females caged with treated and untreated males (2 replicates).

Concentration (p.p.m.)	Number of mosquitoes per cage				Percentage of egg batches from untreated females with hatch of				Percentage hatch	
	Treated males	Treated females	Untreated males	Untreated females	0%	1–9%	10–59%	60–100%	Actual	Theoretically expected
25	25	0	25	25	7	7	25	61	63	46
	25	0	0	25	100	0	0	0	0	..
10	50	50	50	50	14	11	9	66	62	45
	50	0	0	50	34	48	18	0	4	..
0[a]	0	0	50	50	0	1	3	96	93	..

[a] 3 replicates.

TABLE 4.—Viability of *Aedes aegypti* eggs from individual females mated by untreated males and/or males treated as larvae with 10 p.p.m. of apholate.

Condition of male		Number of females examined	Percentage of egg batches with hatch of				Total percentage hatch
1st Mating	2nd Mating		0%	1–9%	10–59%	60–100%	
Treated	Untreated	18	0	39	33	28	31
Untreated	Treated	10	10	0	40	50	57
Treated	..	6	100	0	0	0	0
Untreated	..	5	0	0	60	40	50

sterile. In actuality, only 7 percent to 14 percent were completely sterile, strongly indicating that multiple matings had occurred.

The effects of such multiple matings are shown in table 4. In those females mated first with treated males and subsequently with untreated males, no completely sterile egg masses resulted, and the overall egg hatch was only 38 percent less than that of the untreated cross. With the reciprocal cross the overall hatch was not diminished, and the distribution of egg batches in the various hatch categories was affected only to a slight degree. The net effect was that the mixing of treated and untreated sperm in normal females mated first to treated males and then to untreated males severely reduced the effect of the treated mating. The mixture of sperm when normal females were first mated with untreated males and then to treated males did not reduce the effect of the first mating.

DISCUSSION. The use of alkylating agents in the field on a broad-scale basis must necessarily await a more thorough knowledge of their effects on both plant and animal life. The apparent short life of these mutagens in nature is encouraging, but their acute and chronic effects are not adequately understood. Thus, their unrestricted use can only be considered as extremely hazardous at the present time.

Many factors, however, must be weighed in determining the potential effectiveness of the direct application of chemosterilants to mosquito breeding waters. Our data demonstrated that it is feasible to obtain *A. aegypti* males with a high degree of sterility which are capable of competing to some extent with untreated males. Interpretation of data in table 3 shows that if individual *A. aegypti* females have opportunities to mate with both treated and untreated males, the effectiveness of the treated male is severely reduced regardless of the sequence of the mating. Thus, field applications might be effective if a high proportion of the males in a given breeding area were sterilized, and if there were relatively few untreated males migrating into the area. On the other hand, the effectiveness of migrating sterilized males would be seriously impeded in areas where untreated males were present in any number.

It is pertinent to note that laboratory investigations by Weidhaas and Schmidt (1963) showed that the effectiveness of *A. aegypti* males sterilized as adults by feeding on apholate was apparently not reduced either by multiple matings or lack of competitiveness. It might be speculated that the larval treatment reduces the ability of the male to satisfy the sperm complement necessary for the female, whereas males treated as adults are able to satisfy this requirement. If such were the case, one would expect a low proportion of multiple matings following adult treatments but a high proportion with larval treatments.

For species not performing multiple matings following larval treatments, this approach might be especially useful regardless of the migratory habits of the untreated males. The treatment of breeding waters, however, would require varying approaches depending on the type of breeding. Because of the apparent rapid breakdown of the sterilant in natural environments, the water of continuous breeders such as anopheline species would require multiple applications to maintain high enough sterilant levels to cause sterility. On the other hand, floodwater mosquitoes, *e.g., Aedes* and *Psorophora* species, might be successfully treated with a single application at a time when the majority of the population reached the third instar.

The sterilant dosages which have proved successful in the laboratory are extremely heavy in terms of field applications. The amount of actual sterilant necessary in floodwater pools might range between 10 and 30 pounds per acre, depending on the depth of the water. One can immediately perceive the impracticality of such heavy applications in the

extended patterns which would be necessary to obtain control or eradication in a given area. Attempts to find larval sterilants which are effective at lower concentrations have been unsuccessful to date.

SUMMARY. Apholate and tepa in solution were effective sexual sterilants of *Aedes aegypti* (L.) larvae in the laboratory at 10 parts per million either alone or in combination. Fifteen parts per million of apholate coated on pyrophyllite gave similar results, even when used over soil. In the field, tepa solutions became ineffective after 3 days. When in competition with untreated males, males treated with apholate reduced the viability of eggs from untreated females by approximately 66 percent of the theoretically expected reduction. Multiple matings severely reduced the effectiveness of individual sterile matings.

References Cited

LABRECQUE, G. C., SMITH, C. N., and MEIFERT, D. W. 1962. A field experiment in the control of house flies with chemosterilant baits. Jour. Econ. Ent. 55(4):449–51.

WEIDHAAS, D. E. 1962. Chemical sterilization of mosquitoes. Nature 195:786–787.

WEIDHAAS, D. E., FORD, H. R., GAHAN, J. B., and SMITH, C. N. 1961. Preliminary observations on chemosterilization of mosquitoes. N. J. Mosq. Exterm. Assoc. Proc. 48:106–9.

WEIDHAAS, D. E., and SCHMIDT, C. H. 1963. Mating ability of male mosquitoes, *Aedes aegypti* (L.), sterilized chemically or by gamma radiation. Mosq. News 23:32–34.

FIELD BEHAVIOR OF SEXUALLY STERILE *ANOPHELES QUADRIMACULATUS* MALES

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Field experiments on the use of sterile males to control *Anopheles quadrimaculatus* Say were first attempted in 1959–60 at Lake Okeechobee and subsequently at Lake Panasoffkee, Florida (Weidhaas *et al.*, 1962). Although trials with radiosterilized males apparently failed to reduce the natural populations, they did stimulate an awareness of the need for increased knowledge of the biology of *quadrimaculatus* in relation to the sterilization approach. Dame and Schmidt (1962) discussed the possible factors influencing the ability of sterile *quadrimaculatus* males to inseminate females in the field. Release of sufficient numbers of males to overwhelm the natural male population, vigor of treated males, male sexual compatibility with females in the natural population, and a knowledge of the behavioral characteristics which guide both sexes to potential mates are all prerequisites to the success of the sterile-male technique.

Since the termination of the 1959–60 sterile-male release programs, intensive studies have been conducted in the Panasoffkee release area to gain insight on the mating behavior, physiological age, and natural fertility of the local *quadrimaculatus* population. In conducting these investigations Woodard *et al.* (1962) de-

[1] The authors express their appreciation to: Dr. Carroll N. Smith, of this Division, for supervision of the experiments; to Lt. Col. R. M. Altman, of the U. S. Army, and Dr. G. A. Mount, and Mr. W. M. Borah, of this Division, for assistance in various segments of the field investigation; to Mr. E. Nottingham and Mr. H. Rizer, of this Division, who were responsible for rearing the colony mosquitoes; and to Mr. O. Marsh, who donated the use of his private property for these investigations.