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Abstract. *Increased resistance to the sterilizing effects of apholate was observed in two colonies of* Aedes aegypti *(L.) exposed in the larval stage of each generation to concentrations of apholate that induced about 90 to 40 percent sterility in the eggs laid by the ensuing adults.*

Since the inception of research on the potential of chemosterilants as insect control agents, many persons have asked whether insects could develop resistance to the sterilizing effects of these compounds. On the other hand, because the action of certain chemosterilants influences the induction of dominant lethal mutations, it has been theorized that continued exposure to substerilizing dosages or to dosages causing only partial sterility might result in accumulation of genetic defects and ultimate death of a colony.

In 1962 efforts were initiated to determine whether the yellow fever mosquito, *Aedes aegypti* (L.), could develop resistance to apholate (*1*). Weidhaas *et al.* (*2*) had reported that sterility could be induced in *A. aegypti* by feeding adult mosquitoes on honey solution containing 0.1 percent of apholate. Weidhaas (*3*) had also demonstrated that exposure of larvae of *A. aegypti*, from the third instar to pupation, in water containing 10 parts of apholate per million produced about 90 percent sterility in the ensuing males and about 50 percent sterility in females. When both sexes were treated, sterility was about 98 percent. In later experiments at this laboratory sterility induced by this larval treatment sometimes reached 100 percent. For our experiments in development of resist-

ance, selection pressure was exerted by treatment in the larval stage.

Two different colonies of *A. aegypti* were subjected to selection. Treatment of the first colony began in December 1962, and a second colony was subjected to selection pressure beginning in July 1963.

The strain of *A. aegypti* used in both experiments was from our regular colony, maintained at this laboratory for more than 20 years without deliberate exposure to insecticides. In each generation several hundred third-instar larvae were placed in shallow exposure pans that contained 1 liter of a solution of apholate (5 ppm) in tapwater. In the first experiment the number of larvae in each pan varied, depending on the quantity available; in the second experiment 300 larvae were always used in each pan. After 24 hours, food consisting of powdered dog biscuit was added, and larvae were allowed to continue development in the treated water until they reached the pupal stage. Pupae were removed to untreated water, and emerging adults were allowed to remain together in a screen cage for about 5 days to permit mating. Females were then given a blood meal on a guinea pig. A day later a container which had been lined with filter paper, and containing water to keep the filter paper damp, was placed in the cage for oviposition. Eggs deposited by all the laying females were used to produce the next generation of the colony. In addition, the percentage hatch of eggs laid in each generation was determined separately. In the first experiment females were individually confined for oviposition, and the percentage hatch was calculated for all eggs laid. In each generation 1000 to 5000 eggs were checked for sterility. In the second experiment a sample of about 200 eggs, taken at random from eggs laid by all females about 1 week after deposition, was used to determine percentage hatch. A complete cycle from third instar to third instar required about 3 weeks.

In the second experiment, beginning with the F_7 generation of the apholate-treated colony, larvae from the regular colony were treated with the same dosage of apholate as those in the experimental colony. This procedure provided a direct comparison between the sterility induced in the selected and in the unselected colonies. The percentage sterility in eggs from untreated mosquitoes from the regular colony was also determined in each generation (Table 1).

Table 1. Sterility of eggs from a colony of *Aedes aegypti* exposed in the larval stage of each generation to selection pressure with apholate and from treated and untreated mosquitoes from the regular colony.

Generation of apholate colony	Concentration apholate (ppm)	Sterility of mosquitoes exposed to apholate		Sterility of eggs from untreated regular colony (%)
		Apholate colony (%)	Regular colony (%)	
		Experiment 1		
Parent	5	96		2
1	5	89		20
2	5	75		48
3	5	59		3
4	5	46		6
5	15	72		2
		Experiment 2		
Parent	5	91		6
1	5	85		1
2	5	90		0
3	5	82		1
4	5	85		2
5	5	77		19
6	5	38		15
7	5	52	84	8
8	5	50	93	17
9	10	81	100	4
10	10	88	100	18
11	10	59	100	4

In the first experiment sterility of eggs from females exposed to 5 ppm of apholate gradually declined from 96 percent in the parent generation to 46 percent in the F_4 generation. Larvae from the F_5 generation were exposed to 15 ppm, a dosage which usually causes complete or almost complete sterility. Resulting sterility of eggs from the females was 72 percent or 24 percent less than that obtained with the initial selection dosage of 5 ppm. The percentage sterility in the control colony ranged from 2 percent to 48 percent (average 13.5 percent); however, in 4 out of the 6 hatchings sterility was 6 percent or less.

In the second experiment, sterility ranged from 77 percent to 91 percent for the parent and first five generations, but decreased in the next three generations (38 percent to 52 percent). In the F_9 generation the selection concentration was increased to 10 ppm. This concentration caused 81 percent and 88 percent sterility in the next two generations, but in the F_{11} generation sterility dropped to 59 percent. At this selection concentration, complete sterility was obtained with treated mosquitoes from the regular colony. In addition, these females produced far fewer eggs than females from the apholate colony. Sterility among untreated control females ranged from 0 percent to 19 percent.

These data indicate that *A. aegypti* can develop resistance to apholate. The degree of resistance encountered to date is not great, probably between four and five times that of the normal strain.

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References and Notes

1. The chemical name of apholate is 2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis-(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine.
2. D. E. Weidhaas, H. R. Ford, J. B. Gahan, C. N. Smith, *New Jersey Mosquito Extermination Assoc. Proc.* **48**, 106 (1961).
3. D. E. Weidhaas, *Nature* **195**, 786 (1962).
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