APHOLATE AND GAMMA IRRADIATION COMPARED AS STERILANTS FOR *CULEX PIPIENS QUINQUEFASCIATUS* SAY (DIPTERA:CULICIDAE)

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INTRODUCTION. Research on control of *Culex pipiens quinquefasciatus* Say (= *fatigans* Wiedemann) has recently been increased because of the world-wide importance of these insects as transmitters of disease. New and old methods of chemical control are being studied, and the sterile male method of control is being evaluated. Ramakrishnan *et al.* (1962) reported that male sterilization was obtained with 7700 R of gamma rays. Krishnamurthy *et al.* (1962) reported that in a small scale field trial the release of sterile males into the native population was apparently followed by an increase in the percentage of rafts with embryonated but unhatched eggs. Mulla (1964) used apholate in both larval and adult treatments in sterilization studies on both sexes. Murray and Bickley (1964) and Das (1967) used apholate to sterilize males, and these sterilized males were sexually competitive with normal males. Laven (1967) reported successful eradication of the pest from a small village in Burma after the release of cytoplasmically incompatible males.

The studies reported here were made to compare the sterilizing effects of gamma irradiation and apholate dusts on *C. p. quinquefasciatus* and the mating competitiveness of males exposed to these treatments.

METHODS AND MATERIALS. Mosquitoes were obtained from a colony started from egg rafts collected near Gainesville, Florida. This colony had been reared in the laboratory for three generations when the tests were begun.

In the studies of apholate, the adult males were separated from the females within 20 hours of emergence. Then after 2 to 3 days, the males were immobilized in a cold room at 2° ± 1° C., placed in 10-dram glass vials, and treated with a dust of technical apholate or with dilutions in pyrophyllite. For each treatment, enough dust was added to the vials so each mosquito was thoroughly coated by gentle rotation of the capped container. Also, the excess dust was transferred with the treated mosquitoes to a petri dish that was then placed in a holding cage and maintained at room temperature (26° C.). These treated males were always held 1 day after recovery before they were confined with 3- to 4-day-old virgin females. Fifty treated males and 50 untreated females were used for each mating cross, and at least 3 days were allowed for mating before the females were offered a blood meal.

Initially, the females were offered guinea pigs or mice, but large numbers would not feed on them even though the animals were placed in the cage four successive nights. However, about 90 percent of the females would feed on baby chicks (1 to 14 days old) in one night, and chicks were used in the subsequent studies. A 10 percent sugar-water solution was provided as a supplementary food for the adults in all tests. Three or four days after the insects had their blood meal, paper cups containing water were placed in the test cages to serve as the oviposition medium. Also, eggs were given at least 3 days to hatch before a determination of percent sterility was made by comparing the number of unhatched eggs with the total number of eggs in each raft.

Permanency of sterility in males dusted with technical apholate was studied by

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two methods. In the first, virgin males were aged 7 and 11 days after treatment and then confined with untreated virgin females. In the second, treated males were combined with additional groups of untreated virgin females 1, 5, and 10 days after treatment.

For the tests of sterility induced by irradiation, groups of 1-day-old male pupae were placed in 50 ml of distilled water 1 cm deep and exposed to doses of gamma rays ranging from 6 to 12 kR in a cobalt-60 source similar to that described by Jefferson (1960). The dose rate was about 420 R/min. After irradiation the pupae were rinsed, transferred to paper cups containing distilled water, and placed in cages. After adult emergence, the adult males were crossed with untreated females. The remaining procedures were as described for the chemosterilant tests.

In the mating competitiveness tests, the treated males and untreated males were taken from the same batch of mosquitoes so differences in age and nutritional background could be minimized. All mosquitoes were 2- to 4-day-old virgins when they were introduced into the test cages. For most of the tests made with 1:1:1 ratios of treated males:untreated males:untreated females, 16 x 24 x 25 cm cages were used. For tests made with other ratios and thus greater numbers of mosquitoes, cages as large as 61 x 61 x 61 cm were used. Both treated and untreated males were introduced into the test cages at least 1 hour before the females were added, and from 50 to 100 females were used per test.

TABLE 1.—Effect of apholate dusts on fertility of male *C. p. quinquefasciatus*.[1]

Concentration of apholate (%)	No. of egg rafts examined	Sterility (%)
10	52	11.0
25	61	42.0
50	54	89.0
75	40	97.5
100	54	99.6
Check (untreated males)	41	15.0

[1] Average of three tests with 50 treated males and 50 untreated females per test.

RESULTS AND DISCUSSION. The results of exposing mosquitoes to various concentrations of apholate are shown in Table 1. Apholate alone produced 99.6 percent sterility in treated males; the 75 percent dust was slightly less effective since it caused 97.5 percent sterility. Concentrations of 50, 25, and 10 percent apholate all produced less than 90 percent sterility.

Males dusted with technical apholate and held as virgins for 7 or 11 days before mating with virgin females (Table 2) or mated with three groups of females over 11 days (Table 3) remained completely or almost completely sterile.

The results of inducing sterility by gamma irradiation are shown in Table 4. Over 99 percent sterility of males can be produced by exposure to doses of gamma irradiation of 10 to 12 kR. However, 100 percent sterility was not obtained with any dose tested. Sterility produced by doses of 6 to 9 kR ranged from 46.3 to 97.1 percent.

The mating competitiveness tests (Ta-

TABLE 2.—Permanency of sterility in virgin male *C. p. quinquefasciatus* held for 7 and 11 days after dusting with technical apholate.[1]

Type of male mated with untreated female	Age of males (days)	Days after treatment	No. of egg rafts examined	Sterility (%)
Sterile male	9–10	7	6	100
Untreated male	9–10	..	19	7
Sterile male	13–14	11	11	100
Untreated male	13–14	..	18	9

[1] One test with 50 males and 50 females per cage.

TABLE 3.—Permanancy of sterility in male *C. p. quinquefasciatus* dusted with technical apholate and mated to three groups of untreated virgin females.[1]

Age of males (days)	Days after treatment	No. of egg rafts examined	Sterility (%)
		Sterile males	
3–4	1	77	99.7
7–8	5	69	99.3
12–13	10	71	100.0
		Untreated males	
3–4	..	40	4.0
7–8	..	24	3.0
12–13	..	48	8.0

[1] Three tests with 25 to 50 males and 25 to 50 females per test.

TABLE 4.—Sterility of eggs of untreated female *C. p. quinquefasciatus* mated with irradiated males.

Exposure dose (kR)	No. of egg rafts examined	% Sterility of eggs
6	42	46.3
7	47	92.3
8	35	86.6
8.5	63	94.9
9	84	97.1
10	192	99.6
11	32	99.7
12	33	99.7
Check	125	4.2

ble 5) showed that males treated with apholate were about equally competitive with untreated males at all ratios tested. However, males treated with gamma irradiation were not equally competitive: 16 to 38 percent fewer females mated with irradiated males than the number that would be expected theoretically if all males were equally competitive. These results compare favorably with those reported by other authors on *C. p. quinquefasciatus* and other species. Murray and Bickley (1964) found no adverse effect of apholate

TABLE 5.—Sterility of eggs laid by female *C. p. quinquefasciatus* mated with various ratios of sterile males in competitive mating tests.

Sterilizing treatment	Mating ratio[1]	No. of egg rafts examined	% Sterility of eggs	
			Actual	Theoretical
Apholate[2]	1:1:1	86	50	50
	2:1:1	20	70	67
	4:1:1	32	85	80
	9:1:1	27	89	90
	9:1:3	17	94	90
8500 R	4:1:1	147	64	80
9000 R	1:1:1	60	17	50
	9:1:1	41	58	90
	9:1:2	50	58	90
10000 R	1:1:1	83	13	50
	4:1:1	96	44	80
	9:1:1	56	52	90
	9:1:2	55	69	90

[1] Treated males:untreated males:untreated females.
[2] Technical apholate used as a dust.

on male competitiveness in *C. p. quinquefasciatus*. Das (1967) using the apholate dusting technique found treated males more competitive than untreated males. Weidhaas and Schmidt (1963) found that males of *Aedes aegypti* (L.) exposed to gamma irradiation were not as competitive with normal males as those treated with apholate. Schmidt *et al.* (1964) reported that male *Anopheles quadrimaculatus* Say given 10 kR were about equally competitive with normal males but that males given 12 kR or treated with apholate were about 26 percent less competitive, and Davis *et al.* (1959) found that 11,820 R reduced the mating competitiveness of the same species.

SUMMARY. Tests conducted to compare the relative effectiveness of two methods of sterilizing *Culex pipiens quinquefasciatus* showed that apholate, a chemosterilant, was superior to gamma irradiation because it produced a higher degree of sterility without impairing the competitiveness of the male.

When 2- to 3-day-old males were dusted with technical apholate or dilutions in pyrophyllite, technical apholate caused over 99 percent sterility, a 75 percent dust caused 97.5 percent sterility, and lower concentrations caused less than 90 percent sterility. After treatment, males retained their sterility at least 11 days and through three mating periods with virgin females. In contrast, exposure of pupae to 10 to 12 kR of gamma rays produced over 99 percent sterility in the resulting adult males, and exposure to 6 to 9 kR produced 46.3 to 97.1 percent sterility. Males treated with apholate were about equally competitive with normal males; those exposed to gamma irradiation were 16 to 38 percent less effective.

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