HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY
THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

Volume 39, Number 10 • July, 1968

Chemosterilants for Control of Reproduction in the Eye Gnat (*Hippelates collusor*) and the Mosquito (*Culex quinquefasciatus*)

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A number of chemical sterilants as well as some antibiotic compounds were evaluated in the laboratory and field for reproduction control in the eye gnat *Hippelates collusor* (Townsend) and the mosquito *Culex quinquefasciatus* Say. Reproduction control here denotes the suppression of fecundity. It is achieved either through the reduction in the number of ova produced or a decrease in their viability, or both. The materials were made available to the adult eye gnat as sugar baits, water solutions, deposits on glass surfaces, and also as pupal dips. The chemosterilants were offered as sugar solutions on cotton pads, to the adult mosquitoes. Against larval and pupal stages the chemosterilants were tested as water solutions or suspensions.

Metepa applied as a contact chemosterilant for 1 hour at 0.8 $\mu\text{g}/\text{cm}^2$ sterilized both male and female eye gnats. Apholate did not appreciably suppress reproduction when tested by the contact technique. Apholate and metepa also produced high levels of reproduction control when offered in water solutions to gnats of both sexes. Apholate was highly effective at 0.1 per cent in sugar bait, as was Upjohn 14743 (porfiromycin) at safe rates. Olin 53356 at 0.1 to 0.5 per cent in sugar bait yielded complete control of reproduction, while Olin 53264 at 0.01 and 0.05 per cent in sugar bait produced high levels of control. No appreciable control resulted from metepa used for pupal dips. In laboratory screening against adult eye gnats, U-15800 (pactamycin), U-14743 (porfiromycin) and U-4527 gave good control, but this was not true of U-7726 and U-10071 (tubercidin). Olin 53356, Olin 53061, and

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Chemosterilants for Control of Reproduction in the Eye Gnat (*Hippelates collusor*) and the Mosquito (*Culex quinquefasciatus*)¹

INTRODUCTION

SEXUAL STERILIZATION has been suggested as a means of insect control or eradication and has been shown to produce more rapid decline of pest populations than would conventional pesticidal applications (Knipling, 1955, 1959, 1960). Much experimental work on the use of chemical sterilants for control of reproduction in insects has already been done, and the concept is being increasingly emphasized. The chemical nature of several of the types of compounds employed in such research programs has been reported by various workers (Alexander, 1960; Bořkovec, 1964; Fye, Gouck, and La Brecque, 1965; Kenaga, 1965; Ratcliffe and Ristich, 1965; Ristich, Ratcliffe, and Perlman, 1965). Smith, La Brecque, and Bořkovec (1964) presented a list of promising chemosterilants, and indicated their biological activity against a number of insects.

A comprehensive review of the alkylating agents, including chemical, clinical, biological, and genetic aspects, comprising 57 articles, was published in *Annals of the New York Academy of Sciences* (1958). Articles relevant to the work reported here are cited under their respective authors.

The alkylating agents as a group are highly reactive compounds, capable of selective actions against hematopoietic

and other proliferating tissues. They are quite diverse in nature, encompassing nitrogen mustards, sulfur mustards, epoxides, ethyleneimines, methane sulfonates, β -lactones, and other compounds (Ross, 1962). These agents have been shown to induce mutagenic and teratogenic changes in insects, and are suspected of being carcinogenic in mammals. These effects are produced only at certain dosage levels.

The alkylating compounds are included among a large number of mutagenic agents (Zamenhof, 1963). The relative efficiency of chemical functional groups in inducing mutagenesis has been discussed by Fahmy and Fahmy (1959) and Auerbach (1958). Some compounds were shown to produce unusually high proportions of visible mutations (Fahmy and Fahmy, 1956).

The carcinogenic potency of alkylating agents is not well established. No highly active carcinogen, even for animals, has been found among these compounds (Hays, 1964). McDonald (1961) has suggested that nitrogen mustards may be co-carcinogenic and not actual carcinogens. The alkylating agents are believed to have a definite place in the treatment of neoplasms, and their therapeutic potential has been amply reviewed (Sykes, Philips, and

¹ Submitted for publication August 24, 1966.

Karnofsky, 1956; Hall, 1962). Teratogenic effects with some chemosterilants have been induced in insects, mammals, birds (Murphy, Delmoro, and Lacon, 1958), and plant tissues (Koller, 1958).

Hays (1964) has discussed the toxicology of alkylating agents to mammals, antimetabolites, and a few other chemosterilants in detail. Although the alkylating agents at times produce acute manifestations, they are primarily noticed for their chronic effects. The delayed effects are accompanied by selective action against hematopoietic cells of bone marrow and lymphoid tissues, the intestinal mucosa, germ cells and embryos, and other proliferating tissues. Ristich, Rateliffe, and Perlman (1965) correlated toxicity in mammals to the percentage of aziridine in the ring of apholate and its analogs, and to the types of substituents. Sterilant activity against insects was also found to be related to the aziridine content, as well as to the water solubility of the compound. Water-soluble analogs were shown to be more effective sterilants than those having low water solubility.

Ross (1962) discussed several effects of alkylating agents: (1) cytotoxic, in which the cell dies; (2) mutagenic, in which cell division proceeds, but the new cells have been altered; (3) cytostatic, in which cell mitosis is delayed or entirely prevented. One or more of these effects which interfere with the fecundity of an organism may be induced with chemosterilants.

Because mosquitoes are important in terms of public health, and can be readily colonized, they have been subjected to experimentation with chemical sterilants by several workers (Bertram,

1963; Mulla, 1964; Murray and Bickley, 1964; Weidhaas, 1962; Weidhaas and Schmidt, 1963; Weidhaas, Schmidt, and Seabrook, 1962). A slight degree of resistance to apholate was demonstrated in *Aedes aegypti* by Hazard *et al.* (1964), suggesting that chemosterilants, like other control agents, can lead to development of resistance through natural selection.

Except for limited studies by Schwartz (1965), no other work has been reported on the potential use of chemical sterilants against *Hippelates* species. Flint (1965) used gamma radiation to suppress or inhibit reproduction in *Hippelates pusio* Loew, and Schwartz (1965) induced complete sterility in this species by feeding tepa, metepa, and apholate in sugar solution.

The data reported here are from studies of a variety of chemical sterilants used against the southern house mosquito, *Culex pipiens quinquefasciatus* Say, and the eye gnat, *Hippelates collusor* (Townsend) (Diptera: Chloropidae). Most of the studies were conducted in the laboratory; a few experiments on the eye gnats were performed on field-collected females.

These studies explored the biological activity of several sterilizing chemicals against two insect species in an attempt to learn more about the techniques and procedures by which chemical sterilants may be rapidly assessed and evaluated. Refined studies were not made on some of the compounds. In general, the data obtained were considered adequate for a preliminary estimate of the effectiveness of the compounds. Sterilants were tested both for sex specificity and reproduction control.

MATERIALS AND METHODS

Eye Gnats (*Hippelates collusor*)

Chemosterilants were evaluated for effectiveness against adult *H. collusor* gnats that were less than eight hours

old. The adults were virgin prior to exposure, except those in isolation and mating experiments. Ten female and 10

male gnats were usually used per treatment. The gnats fed on chemosterilant were contained in small, plastic units provided with water.

The chemosterilants, combined with confectioner's sugar, were exposed in small aluminum dishes. Acetone was added to the mixture to produce a paste or slurry. The acetone evaporated, leaving a dry sugar-chemosterilant residue which was then pulverized and moistened with water. The chemosterilant was thus mixed with the sugar. Adult gnats were allowed to feed on the mixture for predetermined periods (shown in tables) during which no other food was provided.

After the predetermined feeding period, a number of the units were placed in a screened, plastic cage (fig. 1), and standard food was furnished. This consisted of a mixture of mashed prune-honey paste, dabbed on a small piece of plastic screen. The paste was sprinkled with dried-blood powder to make a dryish-moist food readily acceptable to the gnats.

All experiments were conducted at a room temperature maintained at 80° to 85°F, with a photoperiod of 12 hours. The light intensity from one 4-foot fluorescent tube at the surface containing the experimental units was 40 foot-candles.

Eggs were removed, counted and tested for viability twice weekly. Egg viability was determined by placing the eggs on moist blotting papers fitted into small plastic dishes which were then placed in a desiccator at 100 per cent relative humidity, 80°F temperature. After four days, the number of unhatched eggs (which are easily counted) was determined in each treatment, and the balance of hatched eggs was readily obtained.

Reproduction control was determined by the following formula:

$$\frac{H_1 - H_2}{H_1} \times 100$$

where:

H_1 = number of eggs viable in the check;

H_2 = number of eggs viable in the chemosterilant treatments.

Information on the chemistry of the sterilizing compounds (table 1) was ob-

TABLE 1
DESCRIPTION OF CHEMOSTERILANTS
USED IN EXPERIMENTS WITH
HIPPELATES COLLUSOR AND
CULEX QUINQUEFASCIATUS

Compound	Chemical description
Eastman Inhibitor HPT tepa	hexamethylphosphoric triamide tris (1-aziridinyl)phosphine oxide
metepa	tris (2-methyl-1-aziridinyl) phosphine oxide
apholate	2,2,4,4,6,6-hexakis(1-aziridinyl)- 2,2,4,4,6,6-hexahydro-1,3,5, 2,4,6-triazatriphosphorine
Olin-53247	N,N'-p-phenylene-bis-1- aziridine carboxamide
Olin-53264	N,N'-(4-methyl-m-phenylene)- bis-1-aziridine carboxamide
Olin-53356	N,N'-pentamethylene bis-1- aziridinyl carboxamide
Olin-53139	N,N'-hexamethylene bis-1- aziridinyl carboxamide
Olin-53362	N,N'-hexamethylene bis (2- methyl-1-aziridine) carboxa- mide
Olin-53061	bis (1-aziridinyl) 3,4-dichloro- phenylureido phosphine oxide
Acti-dione (U-4527)	glutarimide, 3-[2-(3,5-dimethyl- 2-oxocyclohexyl)-2-hydroxy- ethyl]-
Acti-dione oxime (U-7413)	glutarimide, 3-[2-(3,5-dimethyl- 2-oxocyclohexyl)-2-hydroxy- ethyl]-, oxime
U-7726	3-ethoxy-2-oxobutylaldehyde bis-thiosemicarbazone
Tubercidin (U-10071)	4-amino-7-D-ribofuranosyl-7 H-pyrrolo[2,3-d]pyrimidine
Porfirromycin (U-11743)	Azirino [2',3'-3,4]pyrrolo[1,2- a]indole, 6-amino-1,1a,2,8,8a, 8b-hexahydro-8-(hydroxy- methyl)-8a-methoxy-1,5- dimethyl-, carbamate
Pactamycin (U-15800)	C ₂₈ H ₃₈ N ₆ O ₈ - exact structure not known
GC-6936	triphenyl tin acetate
GC-8993	triphenyl tin chloride
Dowco 186	triphenyl tin hydroxide

tained from a recent list by Kenaga (1966), or furnished by the suppliers.

For easier reference, some informa-

tion that properly would appear in this section is presented later, under the discussion of individual tests.

Mosquito (*Culex pipiens quinquefasciatus* Say)

The procedure for evaluation of chemosterilant effectiveness against *Culex quinquefasciatus* larvae has been outlined elsewhere (Mulla, 1964). For the present study, the procedure was slightly modified as follows: Between 50 and 150 immature mosquitoes were placed in 100 ml tap water (pH 8 to 8.5) in 6-ounce, wax-paper cups. Each cup was replicated two or three times. Fresh stock solutions of sterilants were made in tap water. In order to obtain the desired concentration, aliquots of the stock solutions were pipetted into the test cups. Materials not soluble in water were dissolved in dimethyl sulfoxide or acetone. Checks or untreated controls were included in each test.

Larvae and pupae. Both larvae and pupae were exposed continuously in the treated cups. Water lost by evaporation was not replenished. Where various exposure periods were to be studied,

the larvae were removed from the treated cups at prescribed intervals and transferred to untreated water. The cups containing larvae and pupae were placed in small screened cages (9" × 9" × 12"), and the emerging adults were used in a variety of mating combinations. Male and female virgin mosquitoes were obtained by chilling the adult mosquitoes and separating the sexes during a 24- to 40-hour period after start of emergence. Both males and females are easily distinguished by the unaided eye. *Culex quinquefasciatus* was found to begin mating after an age of 40 hours or more. Following desired mating crosses, adult mosquitoes were permitted to feed on raisins and on cotton pads soaked in 5 per cent sucrose solution. Blood meals were provided by placing young chicks in the cages for two nights each week.

Egg rafts laid by the females in each

Fig. 1. Units for evaluation of chemical sterilants against the eye gnat *Hippelates collusor*. To prevent contamination by gnats flying in the room, several units are placed in a plexiglass-screen cage.

treatment were tested for hatch. After three or four days the rafts were transferred into 2 ml of 1 per cent KOH solution and left there for 24 hours (Mulla, 1964), during which they disintegrated into individual eggs. Following raft disintegration, the suspension was brought to 10-ml volume by adding 8 ml of tap water. Aliquots of the egg suspension were streaked out on strips of filter paper, and the number of hatched and unhatched eggs was counted (fig. 2) with the aid of a dissecting microscope. The operculum, or cap, in hatched eggs was easily visible under low-power magnification. This procedure proved a convenient and reliable method of determining hatched eggs.

The degree of control of reproduction and the extent of effective sterilization were calculated by the same formula as that used for the eye gnats (p. 299).

Adults. Mosquitoes less than 42 hours old were used—either 25 or 50 of each sex per treatment. They were chilled, segregated according to sex by means of an aspirator, and placed in small screened cages (9" × 9" × 12"). The insects were provided with cotton pads soaked in 100 ml of 5 per cent sugar solution containing the desired concentration of sterilant. Evaporation of water from the cotton pads tended to change the initial concentrations, provided the materials were stable. Water loss was not replenished. Mosquitoes were given the opportunity to feed on the treated cotton pads for a given period and were then fed on raisins and

Fig. 2. Eggs of the mosquito *Culex quinquefasciatus*. Opercula (caps) are open on hatched eggs, closed on unhatched eggs.

on the cotton pads soaked in 5 per cent sugar solution. Small chicks were placed in the cages two nights a week to provide blood meals. Oviposition, egg viability, and decrease in reproduction were evaluated in the same manner as that described for eye gnats and mosquito larval treatments.

RESULTS AND DISCUSSION

Eye Gnats

Age and mating

In order to assess chemosterilant activity, virgin males and females have to be studied first. It is difficult to sex either *Hippelates collusor* (Townsend) or *H. pusio* Loew in the adult stage, and

it is impossible to do so in the pupal or larval stage at the present time. Isolation of single pupae, in vials, to obtain virgin individuals is tedious, and the survival of the emerging gnats is poor. To obtain virgin females for chemosteri-

lant studies, it became necessary to determine the mating age of eye gnats, especially of the females. Two experiments were therefore conducted with *H. collusor* and *H. pusio*, after emergence.

Females were isolated from males at intervals of 4, 24, 48 and 72 hours after emergence. Ten females were placed in each unit and each treatment was replicated twice. Checks (10 of each sex of like age) were also included with each isolation experiment. The eggs obtained over a period of 21 to 22 days were studied for viability.

In *H. collusor* the noninseminated, isolated females laid essentially the same number of eggs as did the checks for each interval (table 2).

No viability was observed in eggs of females isolated 4, 24, and 48 hours after emergence. Viability of eggs laid by females in corresponding checks was within the normal range. In the 72-hour isolated population, some mating occurred as indicated by a low level of egg viability. It should be noted that, unlike the mosquito, absence of males in *H. collusor* had no effect on the number of eggs laid by the isolated females (table 2). It can be concluded that *H. collusor* females, under the test conditions, become sexually mature after the age of 48 hours or more.

In *H. pusio*, a mating period similar to that of *H. collusor* was observed. No mating occurred up to 48 hours after emergence (table 3) as evidenced by the lack of egg viability. Some mating took place between the 48- to 72-hour period after emergence, as indicated by a low level of viable eggs laid by females isolated 72 hours after emergence. Here, as in the case of *H. collusor*, isolation had no marked influence on the number of eggs laid. Again, as with *H. collusor*, this finding is in contrast to that observed in the mosquito, in which oviposition was markedly decreased by lack of mating.

From these studies it was concluded that eye gnat females are virgin up to

the age of 48 hours. On the basis of this conclusion, virgin females for further experimental work were obtained, from mixed populations, before they attained the age of 48 hours. This technique made it much easier to obtain the desired number of virgin females from mixed populations reared in routine colonies.

The average maximum number of eggs laid per female of *H. collusor* during the 21-day period was 79. These eggs were laid by females kept together with males, and the average is based on the initial number of females; it does not take into account those that died before termination of the experiment. The average minimum number of eggs laid was about 50. Assuming the longevity of gnats to be over 40 days, it would be reasonable to conclude that each gnat female could lay up to 100 to 180 eggs. The fecundity of *H. pusio*, however, was not so high in these tests as in the laboratory colonies. *H. pusio* is able to produce a more vigorous colony than *H. collusor*; however, the test conditions and those in the laboratory were not similar. Survival at subsequent life stages might account for the differences.

Sex specificity of sterilants

Numerous experiments were conducted to determine the sex specificity of a number of chemical sterilants.

Metepa was tested as a contact sterilant, in 25-ml Erlenmeyer flasks, in the same manner as that described for insecticidal deposits (Georghiou and Mulla, 1961) during 1-hour exposure periods. At the $0.08 \mu\text{g}/\text{cm}^2$ rate, it induced partial sterility in males but not in females (table 4). At a higher rate of deposit ($0.8 \mu\text{g}/\text{cm}^2$), it produced complete sterility in males, but not in females. The number of eggs deposited by females treated at the rate of $0.08 \mu\text{g}/\text{cm}^2$ was essentially the same as for females in the check. However, the number of eggs laid by females treated

TABLE 2
EFFECT ON REPRODUCTION IN THE EYE GNAT *HIPPELATES COLLUSOR* WHEN FEMALES
WERE ISOLATED AT VARIOUS INTERVALS AFTER EMERGENCE*

Time (hrs.) isolated after emergence	Days after start of isolation										Total no. eggs collected	Per cent of total hatched
	7		11		14		18		21			
	No. eggs collected	Eggs hatched	No. eggs collected	Eggs hatched	No. eggs collected	Eggs hatched	No. eggs collected	Eggs hatched	No. eggs collected	Eggs hatched		
		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		
4.....	138	0	286	0	164	0	250	0	152	0	990	0
4 check†.....	197	96	470	84	376	80	324	99	209	86	1,576	86
24.....	126	0	245	0	313	0	277	0	145	0	1,106	0
24 check.....	89	48	255	96	333	90	247	94	319	95	1,243	92
48.....	226	0	382	0	376	0	322	0	190	0	1,496	0
48 check.....	162	80	235	92	288	87	211	86	73	92	1,022	86
72.....	253	47	333	44	292	8	312	1	130	1	1,320	20
72 check.....	266	88	400	94	264	96	248	92	83	96	1,361	92

* Ten females per replicate used at each interval. Each isolation replicated twice. Checks were also duplicated units containing 10 males and 10 females each.
† In one of these replicates no female mortality was observed. In the three-week test period an average of 94.7 eggs was laid per female.

TABLE 3
EFFECT ON REPRODUCTION IN THE EYE GNAT *HIPPELATES PUSIO* WHEN FEMALES
WERE ISOLATED AT VARIOUS INTERVALS AFTER EMERGENCE*

Time (hrs.) isolated after emergence	Days after start of isolation										Total no. eggs collected	Per cent of total hatched		
	6		8		12		15		19				22	
	No. eggs collected	Eggs hatched <i>per cent</i>	No. eggs collected	Eggs hatched <i>per cent</i>	No. eggs collected	Eggs hatched <i>per cent</i>	No. eggs collected	Eggs hatched <i>per cent</i>	No. eggs collected	Eggs hatched <i>per cent</i>			No. eggs collected	Eggs hatched <i>per cent</i>
4.....	66	0	198	0	71	0	17	0	83	0	36	0	471	0
4 check.....	26	100	238	98	98	98	138	91	110	73	26	20	636	90
24.....	18	0	58	0	54	0	58	0	79	0	12	0	279	0
24 check.....	24	92	112	77	99	100	107	92	40	47	62	88	444	80
48.....	13	0	220	0	144	0	128	0	44	0	35	0	584	0
48 check.....	129	88	201	76	120	88	128	94	18	94	50	65	646	84
72.....	25	88	133	52	125	12	87	0	13	0	41	0	424	30
72 check.....	22	14	154	71	95	72	115	82	116	65	51	83	553	69

* Ten females per replicate at each interval. Each isolation replicated twice. Checks were also duplicated units containing 10 males and 10 females each.

TABLE 4
EFFECTIVENESS OF METEPA AS A
SEX-SPECIFIC, CONTACT STERILANT
AGAINST ADULTS OF THE EYE GNAT
HIPPELATES COLLUSOR
EXPOSED FOR ONE HOUR

Mating cross*	Dosage†	No. eggs laid	Eggs hatched‡	Reproduction control
	µg/cm ²		per cent	per cent
T♂ N♀...	0.08	847	49	34
N♂ T♀...	0.08	854	75	0
T♂ T♀...	0.08	900	22	69
T♂ N♀...	0.80	756	0	100
N♂ T♀...	0.80	69	44	95
T♂ T♀...	0.80	104	0	100
N♂ N♀...	(check)	750	85	...

* T = treated; N = not treated.
† Mortality of treated gnats averaged about 20 per cent in the 0.8 µg/cm² treatments within the first week. Experiment lasted 24 days.
‡ Based upon two replicates; each started with 10 males and 10 females.

at 0.8 µg/cm² was markedly lower than for the check females. It is obvious that both sexes can be sterilized by contact with metepa, the females requiring higher doses or longer exposure periods than the males. The ease with which the males can be sterilized was also apparent in fruit flies (Keiser, Steiner, and Kamasaki, 1965) and screw-worm flies, *Cochliomyia hominivorax* (Coquerel) (Crystal, 1965).

Reproduction in eye gnats allowed to feed on a 0.01 per cent metepa-water solution for a period of 24 hours was not affected (table 5), regardless of which sex was treated. However, feeding on 0.1 per cent metepa-water solution for the same period of time controlled reproduction drastically when either sex was treated. When both sexes were treated with the sterilant, reproduction control was 100 per cent. From this experiment it also became apparent that the females are not so easily sterilized as are the males. This is true not only of eye gnats, but also of other insect species.

Apholate at 0.1 per cent concentration in sugar bait, with two exposure periods of 24 and 48 hours, induced

complete sterility in both sexes (table 6, experiment A). When females were treated, no eggs were deposited; oviposition was not markedly affected when males, only, were treated.

Apholate at the 0.01 per cent level induced partial sterility in males and females. The response of females was not so great as that of males (table 6, experiment B). The response of both sexes was higher at the 0.1 per cent concentration than at 0.01 per cent. At the higher concentration almost complete sterility was obtained when both sexes were treated. When one or the other sex was treated, the sterility was not quite complete. At the higher concentration there does not seem to be a marked difference in the level of reproduction control achieved by treating either sex alone.

The sex specificity of four other experimental sterilants was investigated.

Porfiromycin (U-14743) induced partial sterility in eye gnats when either sex was fed alone, or both sexes were fed together on 1 per cent of this material in sugar-bait mixture (table 7). When females were treated in any given mating, a drastic decline in oviposition occurred. This is in contrast to the findings of Kohls, Lemin, and O'Connell (1966) who evaluated this compound against house flies and found no suppression of oviposition. However, the experimental conditions of the two tests were quite different. No such decline in oviposition took place when male eye gnats were treated and crossed with untreated females.

Olin 53362 at 0.1 and 1.0 per cent concentrations in sugar bait, offered to gnats for 24 and 48 hours, did not induce appreciable sterility. At the higher concentration the material was toxic to the gnats; at the lower concentration it induced very little, if any, sterility. Oviposition was not markedly influenced in any of the treatments with this material.

Olin 53356 produced marked effects

TABLE 5
EFFECTIVENESS OF METEPA AS A SEX-SPECIFIC STERILANT
AGAINST THE EYE GNAT *HIPPELATES COLLUSOR* WHEN OFFERED IN
WATER FOR 24 HOURS

Mating cross*	Sterilant concentration	No. eggs laid†	No. eggs hatched	Viability	Reproduction control
	per cent			per cent	per cent
T♂ N♀.....	0.01	1,347	1,104	82	0
N♂ T♀.....	0.01	1,170	1,030	88	0
T♂ T♀.....	0.01	1,354	1,205	89	0
T♂ N♀.....	0.1‡	1,185	154	13	84
N♂ T♀.....	0.1‡	659	448	68	53
T♂ T♀.....	0.1‡	378	0	0	100
T♂ N♀.....	1.0	gnats died		..	...
N♂ N♀ (check).....		1,178	954	81	...

* T = treated; N = not treated.

† Based on two replicates; each had 10 males and 10 females at start of the experiment, which lasted 28 days.

‡ Fifty per cent mortality of the treated gnats occurred within 24 hours; remaining 50 per cent were used in experiment.

at 0.1 per cent in sugar bait for 24 and 48 hours (table 7). At 24 hours, this compound induced almost complete sterility when either sex was treated and crossed with the other untreated sex. When both sexes were treated, the induced sterility was 100 per cent. A drastic decline in oviposition occurred when females in a given mating cross

were treated. When males, only, were treated in the mating cross, no decline in oviposition was apparent. At 0.5 per cent concentration this material was toxic to the gnats.

The relationship of chemical structure to activity is obvious. Olin 53362 is a pentamethylene analog of Olin 53356, a hexamethylene analog having

TABLE 6
EFFECTIVENESS OF APHOLATE-SUGAR BAIT AS A SEX-SPECIFIC
STERILANT AGAINST ADULTS OF THE EYE GNAT
HIPPELATES COLLUSOR

Mating cross*	Sterilant concentration	Feeding period	No. eggs laid	Viability	Reproduction control
	per cent	hours		per cent	per cent
Experiment A:					
T♂ N♀.....	0.1	24	644	0	100
N♂ T♀.....	0.1	48	391	0	100
T♂ T♀.....	0.1	24	0	..	100
N♂ N♀ (check).....	0.1	48	0	..	100
T♂ T♀.....	0.1	24	0	..	100
N♂ N♀ (check).....	0.1	48	0	..	100
N♂ N♀ (check).....	...	24	429	88	..
N♂ N♀ (check).....	...	48	713	70	...
Experiment B:					
T♂ N♀.....	0.01	24	1,343	40	50
N♂ T♀.....	0.01	24	877	86	30
T♂ T♀.....	0.01	24	818	23	75
T♂ N♀.....	0.1	24	1,437	8	89
N♂ T♀.....	0.1	24	161	8	99
T♂ T♀.....	0.1	24	179	2	99+
N♂ N♀ (check).....	...	24	1,208	89	...

* T = treated; N = not treated. Each treatment had 10 males and 10 females at the start.

TABLE 7
EFFECTIVENESS OF VARIOUS CHEMICALS, IN SUGAR BAIT, AS SEX-SPECIFIC STERILANTS AGAINST
ADULTS OF THE EYE GNAT *HIPPELATES COLLUSOR*

Material	Concen- tration	Feeding period	Mating crosses*											
			T ♂ N ♀			N ♂ T ♀			T ♂ T ♀			N ♂ N ♀ (check)		
			No. eggs laid†	No. eggs hatched	Repro. control	No. eggs laid	No. eggs hatched	Repro. control	No. eggs laid	No. eggs hatched	Repro. control	No. eggs laid	No. eggs hatched	Hatch
					per cent			per cent			per cent			per cent
U-14743.....	1.0	24	1,279	137	84	386	147	84	201	70	92	961	913	95
	1.0	48	1,351	122	89	347	174	86	350	94	92	1,371	1,206	88
	0.1	24	1,102	882	0	919	836	0	989	791	4	922	848	92
	0.1	48							663	524	34	850	790	93
Olin 53362...	1.0	24	gnats died											
	1.0	24	1,104	22	98	11	3	99	38	0	100	1,029	906	88
	0.1	48							0	0	100	1,181	1,110	94
	0.1	48												
Olin 53264...	0.5	24	gnats died											
	0.01	24	1,208	459	43	1,064	894	0	978	577	25	898	781	87
	0.01	48	1,100	638	47	910	682	33	1,059	625	38	1,207	1,014	84
	0.05	24	864	78	81	381	221	72	527	32	96	898	781	87
	0.05	48	1,122	168	83	558	307	70	296	9	99	1,207	1,014	84

* Each mating started with 10 males and 10 females. Tests lasted 24 to 29 days. T = treated; N = not treated.

† Based on two replicates.

TABLE 8
EFFECTIVENESS OF METEPA AND APHOLATE AS CONTACT STERILANTS
AGAINST ADULTS OF THE EYE GNAT *HIPPELATES COLLUSOR**

Material	Dosage	Exposure time	No. eggs laid†	No. eggs hatched	Egg viability	Reproduction control
	$\mu\text{g}/\text{cm}^2$	hours			per cent	per cent
Metepa.....	8.0	1	gnats died	...	..	..
		4	gnats died	...	..	..
	0.8	1	317	6	2	99+
		4	gnats died	...	..	..
Apholate.....	80.0	1	733	403	55	63
		4	555	272	49	70
	8.0	1	666	566	85	48
		4	1,578	600	38	33
	0.8	1	850	680	80	38
		4	580	522	90	42
Check.....		1	1,244	1,095	88	..
		4	1,021	898	88	..

* Experiment lasted 26 days.

† Based on two replicates, each having 10 treated males and females.

two additional methyl groups. The latter is more effective as a sterilant than is the former.

Olin 53264 was used at 0.01 and 0.05 per cent concentrations, and offered for 24 and 48 hours. The lower concentration induced only partial sterility when either or both sexes were treated. No drastic decline in oviposition was noted in any of the matings in which one or both sexes were treated with the 0.01 per cent concentration (table 7). At the higher concentration (0.05 per cent), no complete sterility was induced in any of the mating combinations. When males were treated, the level of response to this sterilant was higher than when females, only, were treated. The level of reproduction control, however, was the highest (96 and 99 per cent for 24- and 48-hour feeding periods, respectively) in a mating combination in which both sexes were treated. When females were treated in any given mating, there was a marked decline in oviposition. This is in agreement with the results obtained in similar applications of apholate and other sterilants used in the present study.

Contact exposure

Metepa and apholate were evaluated as contact sterilants, in 25-ml Erlen-

meyer flasks, in the same manner as in earlier studies (p. 302).

Metepa at the rate of $8.0 \mu\text{g}/\text{cm}^2$ was toxic at both one and four hours (table 8). At $0.8 \mu\text{g}/\text{cm}^2$, this material was toxic at the four-hour period, but induced almost complete sterility in the gnat population exposed to it for one hour. This result is in accord with results of the first test (table 4).

Apholate was a poor contact sterilant (table 8). The degree of sterility induced at $80.0 \mu\text{g}/\text{cm}^2$ was negligible. At the 8.0 and $0.8 \mu\text{g}/\text{cm}^2$, the level of sterility was rather low.

The contact method of sterilization offers some interesting possibilities. An effective deposit can be obtained in specially designed traps. The gnats can be directed over the deposit, where contact can be made for a short period, resulting in sterilization. They can then be released back into the environment.

Pupal dip

Metepa was used as a dipping solution for pupae. The pupae were dipped for an instant, and placed on blotting paper. Adults emerging from the treated pupae were confined in egg-laying units. Ten male gnats and 10 females were confined in each of two units per treatment.

TABLE 9
REPRODUCTION CONTROL IN THE EYE GNAT, *HIPPELATES COLLUSOR*,
INDUCED WITH APHOLATE-WATER SOLUTION OFFERED AT DIFFERENT
CONCENTRATIONS AND FOR VARIOUS PERIODS

Apholate conc. (per cent)	Feeding period	No. eggs laid	No. eggs hatched	Eggs hatched	Repro. control
	hours			per cent	per cent
Experiment A:*					
0.1.....	24	275	33	12	93
	48	76	14	19	97
	72	5	0	0	100
1.0.....	24	107	0	0	100
	48	0	0	0	100
	72	2	0	0	100
Check.....	..	762	503	66	...
Experiment B:†					
0.01.....	24	325	193	59	0
	48	219	121	55	30
	72	79	33	42	81
0.1.....	24	438	139	32	20
	48	239	107	45	38
	72	156	22	14	87
1.0.....	24	220	7	3	96
	48	75	0	0	100
	72	348	0	0	100
Check.....	..	279	173	62	...

* Test lasted 28 days.
† Each feeding period started with 50 newly emerged male and female gnats. Test lasted eight days.

None of the metepa solutions in which pupae were dipped induced complete sterility in the treated population. Metepa (1 per cent) in water induced a very low level of sterility. Metepa in acetone, and in water plus a surfactant (Atlox G-1256) did not cause any sterility. Further detailed studies on the sterilization of eye gnat pupae are needed.

Drinking water

Two experiments (A, B) were conducted on the effect of apholate offered in drinking water. At the lowest concentration (0.01 per cent), apholate in water caused no appreciable control of reproduction in the gnats except after the 72-hour period (table 9). At the 0.1 per cent concentration, suppression of reproduction at all the exposure periods was somewhat higher in experiment A than in B. Almost complete control of reproduction was achieved at the 1.0 per cent concentration level at the three periods in the two experiments. Results

of the tests are more or less in agreement, with some variation.

Metepa was also used in drinking water. Gnats of either sex, or both, were treated, and mated. At the 0.01 per cent concentration, metepa in water did not suppress reproduction (table 5). At 0.1 per cent concentration, complete sterility resulted when both sexes were treated. Treatment of either sex alone resulted in partial sterility. The material was somewhat toxic at the 0.1 per cent concentration, causing considerable mortality and thus a reduction in effectiveness. Eye gnats exposed to 1.0 per cent concentration died at the start of the experiment. Obviously, apholate used as a water treatment is a safer and more effective material for the gnats than is metepa.

Sterilant-sugar baits

Apholate and some other sterilants in sugar baits have been evaluated in other studies dealing with sex specificity, as discussed earlier.

TABLE 10
EFFECTIVENESS OF VARIOUS CHEMOSTERILANTS IN SUGAR BAIT
AGAINST ADULTS OF THE EYE GNAT *HIPPELATES COLLUSOR**

Material	Conc.	Feeding period	No. eggs laid	No. eggs hatched	Eggs hatched	Reproduction control
	<i>per cent</i>	<i>hours</i>			<i>per cent</i>	<i>per cent</i>
U-15800.....	0.01	24	185	141	76	81
		48	114	73	64	84
Check.....		24	855	761	89	..
		48	557	457	82	..
U-4527.....	0.01	24	1,075	968	90	0
		48	654	621	95	29
Check.....		24	1,086	912	84	..
		48	955	869	91	..
U-7726.....	0.10	24	708	446	63	44
		48	746	507	68	36
U-10071.....	0.10	24	1,045	794	76	0
		48	336	175	52	78
U-14743.....	0.10	24	1,119	560	50	29
		48	992	436	44	45
U-15800.....	0.10	24	Complete mortality within 14 days			
		48	Complete mortality within 48 hours			
Check.....		24	1,017	793	78	..
U-4527.....	0.10	24	400	164	41	82
		48	Complete mortality within 48 hours			
Check.....		24	1,086	912	84	..
		48	955	869	91	..
U-7726.....	1.00	24	280	252	90	76
		48	922	839	91	20
U-10071.....	1.00	24	413	339	82	68
		48	1,180	979	83	6
U-14743.....	1.00	24	299	60	20	94
		48	632	25	4	98
Check.....		48	1,260	1,046	83	..
U-4527.....	1.00	24	104	101	97	89
		48	Complete mortality within 48 hours			
Check.....		24	1,086	912	84	..
		48	955	869	91	..
Olin 53264.....	0.01	24	710	547	77	28
		48	528	444	84	3
Check.....		24	855	761	89	..
		48	557	457	82	..
Olin 53362.....	0.10	24	711	483	68	12
		48	585	433	74	39
Olin 53356.....	0.10	24	714	186	26	66
		48	377	45	12	94
Check.....		24	686	549	80	..
		48	859	713	83	..
Olin 53061.....	0.10	24	553	227	41	57
		48	809	348	43	36
Olin 53264.....	0.10	24†	0	0	0	100
		48	Complete mortality within 48 hours			
Olin 53247.....	0.10	24	474	441	93	17
		48	662	576	87	0
Olin 53139.....	0.10	24	2	0	0	100
		48	0	0	0	100
Check.....		24	635	533	84	..
		48	685	548	80	..
Olin 53061.....	1.00	24	561	95	17	68
		48	479	34	7	90
Olin 53247.....	1.00	24	550	478	87	0
		48	546	338	62	5

TABLE 10—Continued

Material	Conc.	Feeding period	No. eggs laid	No. eggs hatched	Eggs hatched	Reproduction control
	<i>per cent</i>	<i>hrs</i>			<i>per cent</i>	<i>per cent</i>
Check.....		24	308	986	96	..
		48	461	355	77	..
Eastman Inhibitor HPT	0.01	24	791	633	80	0
		48	703	534	76	22
	0.10	24	768	92	12	84
		48	535	43	8	94
	1.00	24†	0	0	0	100
		48	Complete mortality within 48 hours			
Check.....		24	735	581	79	..
		48	758	682	90	..
Acti-dione oxime.....	0.01	24	1,424	1,367	96	0
		48	1,147	1,021	89	0
	0.10	24	1,630	1,500	92	0
		48	654	602	92	33
	1.00	24	1,086	1,021	94	0
		48	993	824	83	9
Check.....		24	1,086	912	84	..
		48	955	905	91	..
GC-6936.....	0.05	24	1,391	1,182	85	0
		48	1,126	980	87	10
	0.10	24	1,744	1,639	94	0
		48	941	772	82	29
	1.00	24	1,126	766	68	31
		48	774	534	69	51
Check.....		24	1,284	1,104	86	..
		48	1,144	1,087	95	..
GC-8993.....	0.05	24	1,731	1,229	71	29
		48	1,250	938	75	33
	0.1	24	1,160	789	68	54
		48	1,841	1,105	60	21
	1.0	24	1,895	1,497	79	16
		48	1,282	769	60	45
Check.....		24	2,102	1,724	82	..
		48	1,758	1,406	80	..
Dowco 186.....	0.05	24	1,742	1,532	88	0
		48	1,729	1,418	82	21
	0.1	24	1,894	1,572	83	0
		48	2,052	1,765	86	2
	1.0	24	1,955	1,466	75	3
		48	1,786	1,357	76	25
Check.....		24	1,673	1,506	90	..
		48	1,982	1,834	91	..

* Each test started with 10 males and 10 females, and lasted an average of 26 days.
† 70 per cent mortality of treated gnats. Survivors were tested for sterility.
‡ 50 per cent mortality of treated gnats. Survivors were tested for sterility.

Several new compounds were screened in combination with sugar as bait against mixed sexes of *Hippelates col-lusor*.
The Upjohn series (U-series) of com-pounds were screened at three concen-trations: 0.01, 0.1 and 1.0 per cent. Two exposure periods were used, 24 and 48 hours.
U-15800 (Pactamycin) at 0.01 per cent concentration provided a high level of reproduction control (table

10), resulting in marked decline of egg production. This is in agreement with the results obtained with house flies (Kohls, Lemin, and O'Connell, 1966). Viability of the eggs laid by the gnats, however, was high. At 0.1 per cent this material was toxic. Thus the effective concentration range can be assumed to be between 0.02 to 0.04 per cent.
U-4527 (Acti-dione) was not effective at the lower concentration (0.01 per cent), while at 0.1 per cent, for a 24-

hour period, this material produced an 82 per cent reduction in reproduction. The level of reproduction control at 1.0 per cent and 24 hours was 89 per cent. Although the number of eggs laid was drastically reduced, the level of viability was quite high, indicating that U-4527 is less promising as a chemical sterilant. At 0.1 and 1.0 per cent concentrations and 48 hours of exposure, this material was toxic to the gnats.

U-7726, at concentrations of 0.1 and 1.0 per cent, for 24- and 48-hour periods, was ineffective. This compound at 1.0 per cent concentration and continuous exposure completely inhibited reproduction in the housefly (*Musca domestica*) (Kohls, Lemin, and O'Connell, 1966). At the shorter exposure period (at 1.0 per cent concentration), the level of reproduction control was markedly higher than that achieved with the 48-hour period. The negative correlation with the length of exposure period cannot be explained at present. This unexpected variation could have resulted from unknown errors in the experiment. U-10071 (tubercidin) at the 1.0 per cent concentration yielded similar unexpected results for the two feeding periods. Both of these materials are considered to have low levels of effectiveness as sterilants.

U-14743 (Porfromycin), at 0.1 per cent concentration induced a low level of sterility, but at 1.0 per cent this material produced a high level of reproduction control. A drastic decline in oviposition and level of viability was noted following the use of this compound. These results, as well as those shown in table 7, are in contrast with the results from house fly treatments (Kohls, Lemin, and O'Connell, 1966).

From the standpoint of both effectiveness and margin of safety, U-14743 and U-15800 were the most effective chemical sterilants among the U-series of compounds.

The Olin series of compounds were evaluated at three concentrations: 0.01,

0.1, and 1.0 per cent. Olin 53264 at 0.01 per cent, offered for 24- and 48-hour periods, was ineffective. It was effective at 1.0 per cent concentration (table 10) but showed considerable toxicity. Olin 53356 at 0.1 per cent at the 24- and 48-hour periods was also effective. A direct relationship was noted between level of reproduction control and length of exposure period. Olin 53061 and 53362 at 0.1 per cent concentration were ineffective. At the higher concentration (1.0 per cent), the former material produced a high level of reproduction control at the 48-hour period. Here, also, a direct relationship between level of reproduction control and length of feeding period was noted.

Olin 53247 at 0.1 and 1.0 per cent concentration was ineffective at both periods. Olin 53139 at 0.1 per cent, offered for 24 and 48 hours, produced complete control of reproduction without any noticeable toxicity to the gnats.

Among this series of compounds, Olin 53139 is the most outstanding against adult eye gnats, and was also found to be effective against mosquito larvae and adults. Next in effectiveness were Olin 53264 and 53356. These three compounds are carboxamides, with Olin 53139 and 53356 being closely related. As in previous sex-specificity tests (table 7), Olin 53362 showed little effectiveness in reproduction control.

Eastman inhibitor HPT in sugar bait was evaluated at 0.01, 0.1 and 1.0 per cent concentrations at 24- and 48-hour periods. It resulted in a high level of reproduction control at 0.1 per cent concentration (table 10). A direct relationship was observed between level of reproduction control and length of exposure period. At the 1.0 per cent concentration, this material was toxic to the gnats. These studies indicate that the safe level of effective concentration of this compound would be around 0.2 to 0.4 per cent, in sugar bait, and that from the standpoint of acute mammalian toxicity, HPT shows some

Fig. 3. Portable trap for collecting eye gnats in the field.

promise as a sterilizing agent for eye gnat control.

Acti-dione oxime, tested at similar concentrations as HPT, was ineffective. Three other compounds, two of General Chemicals (GC-6936, GC-8993) and one

of Dow Chemical (Dowco 186) did not yield encouraging results (table 10).

Field experiments

Because apholate was highly effective against laboratory-reared populations of *Hippelates collusor*, we decided to test the material against field populations.

Methods. Eye gnats were collected in portable traps made from polyethylene funnels (4" long $\times$ 3½" outside diameter) attached to inverted 16-ounce polystyrene food containers (fig. 3). The bottom of each container was removed by applying a heated Mason jar lid. Two 1⅛-inch holes were poked into opposite sides of the container, 2 inches from the inverted top. The holes were covered with 8-mesh hardware cloth soldered into the container. The hardware cloth excluded larger flies from the trap. Funnel and container were soldered together at the edges. The joint was supported by two strips of ½-inch fine copper wire about 1 inch long, soldered to the inside of the funnel and the outside of the inverted plastic container. The soldering iron was run along the inside edge of the container to smooth the joined surface and seal any openings.

An inverted paper cup with the bottom removed was lined with a 4" $\times$ 2" $\times$ 8" plastic bag into which bait was placed. The open end was then covered with cheesecloth and pushed up into the plastic container attached to the funnel. A 7-dram glass vial was inverted over the narrow funnel opening, for collecting the gnats. The vial was easily removed and corked. Live gnats thus trapped were used in the experiments.

The traps were hung 3 feet above-ground in eye gnat-infested citrus groves and date gardens. The gnats attracted to the egg bait in the field were mostly females; only 5 to 10 per cent were males. The females were of unknown age, and most of them were parous. The average female contains

TABLE 11
EXPERIMENTS ON REPRODUCTION CONTROL IN FIELD-COLLECTED
FEMALES OF THE EYE GNAT, *HIPPELATES COLLUSOR*, EXPOSED
FOR VARIOUS PERIODS TO APHOLATE IN WATER*

Apholate conc. (per cent) and exposure time	No. eggs laid†	No. eggs hatched	Viability	Reproduction control
			per cent	per cent
½ hour:				
0.5.....	202	32	16	97
1.0.....	93	3	3	99+
2.0‡.....	0	0	0	100
Check.....	1,670	1,252	75	...
1 hour:				
0.5.....	1	0	0	100
1.0.....	4	0	0	100
2.0§.....	...	...	...	...
Check.....	1,262	909	72	...
2 hours:				
0.5.....	0	0	0	100
1.0.....	7	0	0	100
2.0 	1	0	0	...
Check.....	1,018	784	77	...

* Gnats collected in field with egg-bait attractant; allowed to feed on apholate in the field and then kept in the laboratory for 28 days. Fed honey, prune, and blood-meal wick during that period.

† Based upon two replicates, each with 10 treated females and 10 nontreated males from laboratory colony.

‡ Mortality of treated females was 75 per cent within 24 hours. Results based upon remaining 25 per cent.

§ 100 per cent mortality of treated females within 24 hours of treatment.

|| 90 per cent mortality of treated females within 24 hours. Results based upon remaining 10 per cent.

eggs of stages 5 to 6 (Mulla, unpublished data) if the gonotrophic cycle is divided into 10 stages (Adams and Mulla, 1967a, b). It was of considerable interest to determine whether such parous females could be irreversibly sterilized. Temperature during the exposure period of the sterilants in the field was 90 to 100° F; relative humidity was 5 to 15 per cent.

After exposure, the females were transferred to the plastic units used in laboratory experiments for egg laying, and maintained in the laboratory in a manner described earlier. Male gnats in equal ratio, from a laboratory colony, were added to each unit to assure an adequate supply of males for mating. The females deposited eggs for a period of three to four weeks, and the extent of reproduction decline was determined in the same manner as described for the laboratory studies.

Apholate was offered to the field-collected gnats at 0.5, 1.0, and 2.0 per cent concentrations in tap water, for periods of one half, one and two hours. After this exposure period, the gnats

were kept on untreated water and food for 28 days, and the level of fecundity was determined.

The treatment was highly effective (table 11). At the lowest exposure period (one half hour), the lowest concentration (0.5 per cent) produced a high level of reproduction control. At the 1.0 per cent concentration, reproduction control was almost 100 per cent. With all the apholate treatments, there was a drastic reduction in oviposition.

Apholate mixed in sugar at 0.1, 0.5, 1.0, 2.0 and 5.0 per cent concentrations and offered for two and four hours did not yield a high level of reproduction control (table 12). Even at 5 per cent concentration and an exposure period of four hours, apholate in sugar produced only 60 per cent control of reproduction. An exposure of 0.1 per cent apholate in sugar for 24 hours, however, produced complete sterility in the gnats (not shown in table 12). Generally, a marked reduction in oviposition was noticed in most of the apholate-treated gnats.

TABLE 12
EXPERIMENTS ON REPRODUCTION CONTROL IN FIELD-COLLECTED
FEMALES OF THE EYE GNAT, *HIPPELATES COLLUSOR*, EXPOSED
FOR VARIOUS PERIODS TO APHOLATE IN SUGAR*

Apholate conc. (per cent) and exposure time	No. eggs laid†	No. eggs hatched	Viability	Reproduction control
			per cent	per cent
2 hours:				
0.1.....	676	473	70	0
0.5.....	398	223	56	51
1.0.....	446	227	51	50
2.0.....	306	95	31	79
5.0.....	363	152	42	67
Check.....	557	457	82	..
4 hours:				
0.1.....	581	343	59	36
0.5.....	401	232	58	56
1.0.....	192	92	48	83
2.0.....	201	42	21	92
5.0.....	357	214	60	60
Check.....	691	532	77	..

* Gnats collected in field in egg-bait traps, and fed apholate. Fed honey, prune, and blood meal on wicks after treatments. Experiment lasted 26 days.
† Total based on two replicates, each with 10 males and 10 females.

Sterilization of female eye gnats in the field, with apholate in water, seems promising, and was more effective than in the laboratory. This trend in differential activity is to be expected. The gnats in the laboratory are newly emerged, and are maintained at lower temperature and high humidity (84° F and 60 per cent relative humidity). The demand for water by the laboratory gnats is quite low. On the other hand, field populations maintained at 90° to 100° F and 5 to 15 per cent relative humidity before and during the exposure period, congregated on the water source contaminated with apholate, thus picking up sterilizing dosages in a short period of time. These differences in activity depend, therefore, on the physiological condition of the insect and its immediate environmental parameters. In order to advance field-scale studies, specially designed traps are required, that would permit auto-

matic treatment of the females, and their automatic release back into the environment. Before such a research program can be embarked upon, many biological facts concerning population dynamics of eye gnats must be determined. The influence on population control of parous females sterilized after they have laid a clutch or two of eggs has to be determined. Another matter to be investigated is whether sterilized females released into the environment utilize the mating capacity of normal males. These and similar questions have to be answered before efficacy of chemical sterilants against eye gnats in the field can be critically assessed. One solution to this problem may come through the discovery of attractants for male eye gnats, or release of sterilized males. The latter possibility holds promise if an automatic method for separation of the sexes is developed.

Mosquitoes

Survival

Some chemosterilants caused considerable mortality at effective sterilizing concentrations—an undesirable feature for laboratory experimentation.

TABLE 13
EFFECT ON PUPATION, EMERGENCE, AND ADULT SURVIVAL OF THE
MOSQUITO, *CULEX QUINQUEFASCIATUS*, WHEN LARVAE WERE EXPOSED
FOR VARIOUS PERIODS TO APHOLATE (0.001 PER CENT) IN
TAP WATER AND IN DISTILLED WATER

Apholate combination and exposure period	Larvae* exposed as:					
	4th instar			2nd and 3rd instars		
	Pupating	Emerging (adults)	Surviving (adults)	Pupating	Emerging (adults)	Surviving (adults)
	per cent	per cent	per cent	per cent	per cent	per cent
Tap water:						
24 hrs.....	99	98	88	93	92	90
48 hrs.....	91	91	86	97	96	68
72 hrs.....	97	92	88	99	99	90
Check.....	98	97	96	85	84	81
Distilled water:						
24 hrs.....	97	96	91	89	87	75
48 hrs.....	98	88	87	72	65	65
72 hrs.....	100	98	97	85	79	77
Check.....	99	92	83	100	100	88

* 50 larvae per wax-paper cup in 100 ml of sterilant solutions. After being exposed, larvae were strained out and transferred to clean tap water.

It was determined that apholate in water, at 0.001 per cent concentration, when used against larvae, had no drastic effect on the extent of pupation, adult emergence, and survival (table 13). No marked difference was observed between distilled and tap water.

Olin 53264 and Olin 53139 were found to be toxic at 0.01 per cent con-

centration in tap water (table 14). The former compound was slightly more toxic to the second- and third-stage larvae than to the fourth, at 0.001 and 0.0001 per cent concentrations. Olin 53139 was toxic at 0.01 and 0.001 per cent concentrations. This compound produced some larvae and pupae mortality at 0.0001 per cent concentration.

TABLE 14
EFFECT ON PUPATION, EMERGENCE, AND ADULT SURVIVAL OF
THE MOSQUITO, *CULEX QUINQUEFASCIATUS*, WHEN LARVAE
WERE EXPOSED TO VARIOUS CONCENTRATIONS
OF TWO CHEMOSTERILANTS

Sterilant†	Conc.	Larvae* exposed as:					
		4th instar			2nd and 3rd instars		
		Pupating	Emerging (adults)	Surviving (adults)	Pupating	Emerging (adults)	Surviving (adults)
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Olin 53264.....	0.01	0	..	..	0	..	..
	0.001	68	61	55	34	32	32
	0.0001	61	54	50	36	36	35
Olin 53139.....	0.01	0	..	..	0	..	..
	0.001	0	..	..	0	..	..
	0.0001	60	54	51	65	56	49
Check.....	A	63	60	58	70	68	67
	B	73	67	65	60	59	58

* 50 larvae per wax-paper cup in 100 ml of sterilant solution in tap water. Total of 100 larvae per treatment. Exposure was continuous.

† Olin 53264 dissolves slowly in water; Olin 53139 dissolves instantly.

For experimental studies, the two Olin compounds have to be used at concentrations that do not impair the survival of various stages of mosquitoes. The tests presented in tables 13 and 14 were undertaken to illustrate this point. From these studies as well as from other unpublished work it seems that practically every sterilant has its own characteristic toxic and sterilizing levels. In general, the margin of safety between these two dosages or concentrations is not sufficiently wide.

Age and mating

To obtain virgin females, the sexes must be separated in the pupal stage or individual pupae must be isolated. In the first procedure, complete separation of sexes is not always assured; the second procedure requires a great deal of time and effort. To overcome these difficulties it was necessary to determine the age of adult mosquitoes at the time mating occurs.

Twenty-five females were isolated from the males at intervals after emergence. Checks of 25 females and 25 males of like age were also established. The number of egg rafts and eggs, and the percentage of viability were determined for each interval. Two replicates were used per treatment.

Females isolated 24 hours and 42 hours after emergence laid fewer eggs than did the checks, and none of the eggs laid were viable (table 15). Females isolated 48 hours after emergence also laid fewer eggs than did their respective checks, with viability of 9 per cent as compared with 72 per cent in the check. Number of eggs laid, and their viability increased as the length of the pre-isolation interval increased. Females isolated 72 hours after emergence laid almost as many eggs as did their respective checks, but the percentage of viability was lower. The number of eggs laid by females separated 96 hours after treatment was essentially the same as for the females in

TABLE 15
EFFECT ON REPRODUCTION
OF THE MOSQUITO,
CULEX QUINQUEFASCIATUS,
WHEN FEMALES WERE ISOLATED AT
VARIOUS INTERVALS AFTER
EMERGENCE

Time (hrs) females isolated after emergence*	Egg production and viability		
	No. egg rafts	No. eggs	Viability
			per cent
24.....	12	747	0
Check (♀ ♂)....	40	3,530	77
42.....	17		0
Check (♀ ♂)....	47		93
48.....	16	1,682	9
Check (♀ ♂)....	57	7,060	72
72.....	22	2,888	45
Check (♀ ♂)....	38	3,396	86
96.....	60	7,633	80
Check (♀ ♂)....	66	7,322	79

* 25 females were isolated from a mixed population at the indicated intervals after emergence. Checks consisted of 25 females and 25 males.

the check, as was the degree of viability.

Obviously, *Culex quinquefasciatus* females are sexually mature and undergo maximum mating during the 72 to 96 hours after emergence. Before that age, the females are not sexually mature and thus do not become fully inseminated. It should be noted that lack of mating resulted in decreased oviposition, a phenomenon which should be considered in studies on chemical sterilants. This finding is in contrast to that with *Hippelates collusor* and *H. pusio*, in which absence of mating did not influence oviposition (tables 2 and 3). Differential mortality of males caused by a chemosterilant will deprive the females of full mating and insemination; this will lead to fewer eggs, and thus to misleading results. Male, as well as female, mortalities should be assessed after treatment. Results of tests conducted during the course of these studies in which high male or female mortality occurred were discarded.

Exposure periods

Young and fourth-stage larvae were exposed for various periods to a 0.001

TABLE 16
EFFECT OF APHOLATE (0.001 PER CENT), IN TAP WATER AND IN DISTILLED WATER, ON OVIPOSITION AND EGG VIABILITY OF THE MOSQUITO, *CULEX QUINQUEFASCIATUS*, WHEN LARVAE WERE EXPOSED FOR VARIOUS PERIODS

Apholate combination and exposure period (hours)	Larvae exposed as:					
	4th instar			2nd and 3rd instars		
	No. egg rafts	No. eggs	Viability	No. egg rafts	No. eggs	Viability
			per cent			per cent
Tap water:						
24.....	51	4,950	92	39*	3,130	88
48.....	42	3,610	81	18*	1,330	71
72.....	48	6,640	67	19*	1,470	76
Check.....	54	6,480	95	41	5,002	92
Distilled water:						
24.....	35	3,590	89	12	1,220	94
48.....	42	4,700	82	40	4,050	93
72.....	61	5,660	82	42	5,699	79
Check.....	43	7,400	92	60	8,970	97

* Egg rafts were small.

per cent solution of apholate in tap water and in distilled water. At the end of the exposure period, the larvae were transferred to clean water and provided with food; they were then placed in cages for pupation and adult emergence. Eggs laid by the adults were counted and studied for viability.

Apholate in both tap and distilled water caused little reduction in egg viability (table 16). The decrease in viability was slightly more pronounced in the tap water (pH 7.5 to 8.0) than in the distilled water (pH 6.5). None of the exposure periods, however, produced marked degrees of reproduction control; therefore, the larvae in subsequent tests were continuously reared in treated water until pupation occurred. The pupae were transferred to clean water for emergence.

Sex specificity

Second- and third-stage larvae were exposed to apholate and metepa, and adult mosquitoes were allowed to feed on cotton pads soaked with the compounds and 5 per cent sucrose solution.

Larvae continuously exposed to 0.002 per cent solution of apholate and 0.01 per cent solution of metepa in tap water were reared to the adult stage. In preliminary studies, these concentrations had been found to sterilize mixed populations. Proper mating crosses were made by using 25 females and 25 males per treatment. When apholate-sterilized males (T) were crossed with fertile females (N), a large number of eggs were laid, but none hatched; thus, control of reproduction was complete (table 17). A similar mating technique with metepa produced the normal complement of eggs, a small percentage of which hatched. By reversing the mating (fertile males + sterile females), egg production was completely eliminated in the apholate-treated females, and the number of eggs laid by metepa-treated females was markedly lower than in the previous treatment or the check. A mating cross of both sterile males and females also resulted in no oviposition following the apholate treatment, and in very few eggs being laid after the metepa treatment. The highest degree

TABLE 17
APHOLATE AND METEPA AS SEX-SPECIFIC STERILANTS AGAINST SECOND- AND THIRD-STAGE LARVAE*
OF THE MOSQUITO, *CULEX QUINQUEFASCIATUS*

Mating cross†	Apholate (0.002 per cent)						Metepa			
	No. rafts	No. eggs	No. eggs hatched	Egg viability per cent	Reproduction control per cent		No. rafts	No. eggs	No. eggs hatched	Egg viability per cent
T♂ N♀	82	12,355	0	0	100		63	7,870	787	10
N♂ T♀	0	0	0	..	100		17	1,861	912	49
T♂ T♀	0	0	0	..	100		10	678	81	12
— N♀	32	3,651	0	0	100		..	..	..	..
N♂ N♀ (check)	104	20,539	18,074	88	..		76	7,945	6,833	86

* Continuous exposure.
† 25 males and 25 females per mating. T = treated; N = not treated.

of sterilization was obtained when both males and females were treated with metepa. As in previous studies (table 15), females isolated from males at the start of the test produced a much smaller number of eggs as compared with the check.

Sex specificity of apholate (0.01 per cent and metepa (0.1 per cent) against the adult mosquitoes was also studied (table 18). Here various feeding periods were employed. Matings of apholate-sterilized males (fed for 16 and 24 hours) with fertile females resulted in a large number of eggs laid, but only a small fraction of them hatched. A mating of sterilized males and females (fed for 24 hours on apholate) yielded a smaller number of eggs, none of which hatched.

A study was also made of reverse matings in which females had been sterilized with apholate by extending the feeding period. This extension was necessary to effect sterilization, possibly because females do not feed on soaked cotton pads as readily as do males. Or it may be that a higher concentration of sterilants is needed to impair female fertility markedly. The results of three exposure periods tend to confirm these suppositions. Females fed for 24 hours on apholate, and crossed with fertile males, produced some eggs, a few of which hatched. However, extending the feeding period to 48 hours resulted in the deposition of only 26 eggs of which 14 hatched, yielding 99+ per cent control of reproduction. Further extension of the feeding period to 72 and 96 hours prevented egg deposition. It is thus obvious that males are much more readily sterilized than are females. This observation is also true for eye gnats (see tables 4, 5, and 6), fruit flies (Keiser, Steiner, and Kamasaki, 1965) and screw-worm flies (Crystal, 1965).

The sex specificity of metepa with adult mosquitoes was similar to that of

TABLE 18
APHOLATE AND METEPA, IN SUGAR SOLUTION ON COTTON PADS,
AS SEX-SPECIFIC STERILANTS AGAINST ADULTS OF THE
MOSQUITO, *CULEX QUINQUEFASCIATUS*

Sterilant and mating cross*	Feeding period	No. rafts	No. eggs	No. eggs hatched	Egg viability	Reproduction control
	hours				per cent	per cent
Apholate (0.01 per cent):						
T♂ N♀	16	46	4,304	172	4	97
T♂ N♀	24	47	5,439	163	3	97
T♂ N♀	24	15		...	0	100
N♂ T♀	24	8	598	335	56	94
N♂ T♀	48	1	26	14	55	99+
N♂ T♀	72	0	0	0	..	100
N♂ T♀	96	0	0	0	..	100
N♂ N♀ (check)	..	57	6,860	5,762	84	...
Metepa (0.1 per cent):						
T♂ N♀	8	64	6,580	4,409	67	42
T♂ N♀	24	57	5,760	230	4	97
T♂ N♀	48	61	7,550	226	3	97
N♂ T♀	8	9	1,167	630	54	92
N♂ T♀	24	4	235	122	52	98
N♂ T♀	48	0	0	0	..	100
T♂ T♀	8	61	7,140	5,998	84	21
T♂ T♀	24	2	117	5	4	99+
T♂ T♀	48	0	0	0	..	100
— N♀	..	17	2,100	0	0	100
N♂ N♀ (check)	..	68	8,260	7,599	92	...

* 25 males and 25 females per mating. T = treated; N = not treated.

apholate. Matings of males sterilized by metepa with fertile females caused little reduction in egg production, but decreased the percentage of viability as the males' feeding period on metepa increased. It is interesting to note that only a small percentage of eggs hatched, indicating either resistant sperm or resistant individual males in the population.

When the mating was reversed, i.e., sterilized females crossed with fertile males, fewer eggs were produced. The number of eggs deposited decreased as the feeding period increased from 8 hours to 48 hours (table 18). No eggs were laid after the 48-hour feeding period, indicating complete control of reproduction.

Mating between metepa-sterilized males and females produced results similar to those with single-sex sterilized matings. As the feeding period increased, the number of eggs deposited markedly decreased, producing effects similar to those in the tests in which

females alone were sterilized. Females isolated from the males deposited fewer eggs, as usual, than did the checks, and none of the eggs hatched.

Data presented in tables 17 and 18 show that, in larval exposures to apholate, sterilization of both sexes results in reproduction control similar to that obtained by sterilization of either sex. The results with metepa, although not so conclusive, show a similar pattern. This same trend is more or less obvious for adults sterilized with apholate or metepa. Sterilization of either sex produced results similar to those from matings between both sexes, sterilized.

Tests against immature stages

Five Olin compounds, four Upjohn compounds (U-series), and Eastman inhibitor HPT were evaluated against second-, third-, and fourth-stage larvae, and pupae. These studies were preliminary in nature, aimed at finding the relative effectiveness. Exposure was continuous for the larvae until pupation

took place. Pupae were also exposed continuously in the treated water until all the adults emerged. In each case, 25 female and 25 male emerging mosquitoes were segregated in each cage, for assessment of their reproductive capacity in a manner described earlier.

Olin 53139 caused 69 per cent reduction in reproduction when second- and third-stage larvae were exposed at the 0.0001 per cent concentration. At 0.0005 per cent the immature stages did not develop to the adult stage. It is possible that an effective sterilizing dosage lies between these two concentrations, indicating a narrow margin of safety for this compound against the young larvae. The same trend was observed with the fourth-stage larvae, except that at 0.0001 per cent reproduction control was lower than for the second- and third-stage larvae.

Olin 53264 produced results similar to those with Olin 53139, except that it had to be used at higher concentrations.

Olin 53362 reduced reproduction markedly at 0.0001 per cent concentration when the second-, third-, and fourth-stage larvae were exposed. At 0.001 per cent and higher concentrations, this compound was toxic to larvae. Effective concentration possibly lies between 0.0001 and 0.001 per cent, again pointing to the lower margin of safety for this compound. While the margin of safety must be wide in field studies, that noted here would possibly pose no serious problems in the laboratory. Up to 0.01 per cent concentration, Olin 53362 was not an effective sterilant against the pupae.

Olin 53356 at 0.002 per cent resulted in 99 per cent control of reproduction when used against second- and third-stage larvae. Against fourth-stage larvae it gave 79 per cent control. At higher concentrations, there was evidence of interference with larval and pupal development, and against pupae, the compound was ineffective.

Olin 53061 was insoluble in water or

acetone, and had to be solubilized in dimethyl sulfoxide (DMSO). This compound was found to be essentially ineffective against both larvae and pupae.

U-10071 effected good control of reproduction at 0.001 per cent when second-, third-, and fourth-stage larvae were exposed. An effective sterilizing concentration for this compound, to induce complete sterility, lies above this level.

U-15800 at the 0.001 per cent concentration was toxic to the larvae. Its range of activity against larvae is therefore still undetermined.

U-7726 gave good reproduction control.

U-14743 gave complete control of reproduction at 0.001 per cent concentration. It produced certain abnormalities in the tracheal system of the larvae that resulted in convoluted tracheal trunks. Larvae contracted and assumed wavy shapes.

Of the experimental compounds evaluated, Olin 53362 and 53356, and U-7726 and U-14743 are quite promising, based on their initial biological activity, as well as margin of safety.

Eastman inhibitor HPT, a supposedly less toxic chemosterilant, resulted in mediocre reduction in reproduction at 0.005 per cent and 0.01 per cent concentrations. At a slightly higher concentration (0.05 per cent), this material proved toxic to the larvae. Against pupae, it was not effective up to a concentration of 0.1 per cent.

Reproduction control in adults

Four Olin compounds and four Upjohn (U-series) compounds were evaluated against adult mosquitoes, with both sexes receiving treatment. Mosquito adults that were 24 hours old were segregated; 25 females and 25 males were used per treatment. The feeding period on the various concentrations of the sterilants in 5 per cent sucrose solution was either 24 or 48 hours.

TABLE 19
EFFECT OF VARIOUS CHEMOSTERILANTS ON REPRODUCTION IN ADULTS
OF THE MOSQUITO, *CULEX QUINQUEFASCIATUS*

Sterilant	Conc.	Feeding period	No. rafts	No. eggs	No. eggs attached	Egg viability	Reproduction control
	<i>per cent</i>	<i>hours</i>				<i>per cent</i>	<i>per cent</i>
Olin 53139.....	0.001	24	28	4,627	2,869	62	29
	0.01	24	1	34	0	0	100
Olin 53264.....	0.001	24	35	5,023	4,470	89	0
	0.01	24	26	3,877	1,560	41	61
Check.....		24	31	4,292	4,034	94	...
Olin 53362.....	0.01	24	4	220	165	75	96
	0.1	24	0	0	0	0	100
Olin 53356.....	0.01	24	0	0	0	0	100
	0.1	24	Adults died				
Check.....		24	32	4,502	4,142	92	...
U-10071.....	0.01	24	34	3,457	2,109	61	50
	0.01	48	6	635	495	78	91
U-15800.....	0.01	24	34	3,806	3,311	87	22
	0.01	48	5	557	484	87	91
U-7726.....	0.01	24	48	4,899	4,311	88	0
	0.01	48	56	6,654	6,188	93	0
U-14743.....	0.01	24	3	248	35	14	99
	0.01	48	Adults died				
Check.....		24	44	4,964	4,269	86	..
Check.....		48	51	5,846	5,495	94	..

Olin 53139 at 0.01 per cent concentration gave complete reproduction control (table 19). Very few eggs were laid by the females.

Olin 53264 effected only mediocre control at 0.01 per cent concentration. It was not evaluated at a higher concentration, where the complete sterilization potential possibly lies.

Olin 53362 produced total sterility at 0.01 per cent concentration, completely eliminating egg production. Reproduction control was 96 per cent at the 0.01 per cent concentration, suggesting that this compound is equivalent to Olin 53139.

Olin 53356 produced complete sterility at 0.01 per cent concentration, indicating that it is slightly more effective than Olin 53362.

U-10071 caused considerable sterility at 0.01 per cent concentration when fed to mosquitoes for 48 hours (table 19). The concentration producing complete sterility has not been determined and probably lies slightly above that used in this study.

U-15800 showed the same trend, in

which sterilizing concentration lies slightly above the 0.01 per cent concentration level.

U-7726 at 0.01 per cent concentration, when fed to adult mosquitoes for either 24 or 48 hours, produced no sterility.

U-14743, used against larvae, was found to be more effective than the other U-series compounds. At the 0.01 per cent concentration, this compound produced almost complete sterility when fed upon by adults for 24 hours. Extending the feeding period to 48 hours caused complete mortality of the adults.

From the data in table 19, it is apparent that Olin 53139, Olin 53362, Olin 53356, and U-14743 are quite promising sterilizing chemicals when used against adult mosquitoes.

The data presented in this paper clearly demonstrate the efficacy of chemical sterilants as a tool for the control or eradication of insects harmful to humans and animals. Much more work in both the laboratory and field is needed before chemical sterilants can

be employed in operational programs. Biological parameters, behavior, ecological trends, and other fundamental problems in relation to the use of chem-

ical sterilants have to be studied in experimental, area-wide programs before recommendations can be made for practical vector-control programs.

ACKNOWLEDGMENTS

Many persons gave able assistance during the course of these studies, including H. Axelrod, G. Kats, H. A. Darwazeh and M. F. B. Chaudhury of the Citrus Research Center and Agricultural Experiment Station, Riverside, and L. D. Moore of the Coachella Valley Mosquito Abatement District.

Experimental samples of the com-

pounds evaluated were kindly furnished by the Interchemical Company, American Cyanamid Company, Olin Mathieson Chemical Company, Eastman Chemical Products, Inc., and the Upjohn Company. The assistance of these firms in furnishing samples and technical information is greatly appreciated.

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Olin 53139 produced good to complete suppression, while Olin 53362, and Olin 53247 produced low levels of reproduction control. Eastman Inhibitor HPT at 0.1 and 1.0 per cent resulted in good to complete suppression; Acti-dione Oxime, GC-6936, GC-8993, and Dowco 186 yielded low to moderate levels of control.

In the field, female eye gnats (attractant available for females only) were readily sterilized with apholate offered in sugar bait or water solution. Metepa as a contact material also readily sterilized the natural populations of female gnats.

Against mosquito larvae, apholate (0.002 per cent water solution) yielded complete control of reproduction in the resulting adults, while metepa at 0.01 per cent water solution gave a very high level of control. Both materials at 0.01 and 0.1 per cent, respectively, yielded good to complete control. Apholate used in this manner proved over 10 times as effective as metepa. Against larvae, none of the Olin series of compounds (with the exception of Olin 53356) showed any appreciable activity, and none was found effective against pupae. U-7726 against larvae suppressed reproduction markedly, U-10071 produced a moderate reduction, while U-14743 resulted in complete control. Results with U-15800 are inconclusive.

Sugar baits of Olin 53139, Olin 53356, and Olin 53362, used against adult mosquitoes, completely suppressed reproduction. U-10071 and U-15800, offered as sugar bait to the adult mosquito, yielded a high level of control; with U-14743, control was almost complete. U-7726 was not effective at the concentrations used.

In general, the margin of safety between sterilizing concentrations and those causing appreciable mortality was found to be narrow. This would possibly pose some problems in the sterilization of laboratory and field populations of insects. The full potential of chemosterilants for the control or eradication of insects harmful to humans and animals is not well established as yet. Many more fundamental studies will have to be conducted before these potentially useful compounds can be recommended for the control or eradication of insects.

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