Chemosterilants for the House Fly

Richard L. Fye[1] and James E. Oliver*[2]

When 33 candidate chemosterilants of miscellaneous structure were administered in the diet of *Musca domestica* L., only 3 sterilized the males but all caused complete sterility at one concentration when tested against mixed sexes. Several chemicals caused both high mortality and sterility. This effect would be undesirable in the production of sterile insects for release programs, but could be advantageous in attractive bait applications.

Previous publications from the Beltsville, Md. laboratory have described the testing of classes of compounds for activity as insect sterilants, for example, alkylating agents (Borkovec, 1969), phosphoramides (Terry and Borkovec, 1967,) *s*-triazines (Borkovec and DeMilo, 1967), and, more recently, dithiobiurets (Oliver *et al.*, 1971) and dithiazolium salts (Oliver *et al.*, 1972). Further work on several of these compounds is in progress; in the meantime, we have continued to screen numerous other unrelated compounds. The present paper describes the results of testing 33 miscellaneous candidate chemosterilants for activity against the house fly, *Musca domestica* L.

MATERIALS AND METHODS

In the present tests, adult flies of the susceptible (Orlando regular) strain were given the candidate chemosterilants in a diet of granulated sugar and/or regular fly food that consisted of 6 parts sugar, 6 parts powdered nonfat dry milk, and 1 part powdered egg yolk. A 6-ml sample of 1% of the chemosterilant in a solution or suspension of acetone, water, or other volatile solvent was first incorporated in 10 g of fly food or sugar; then after evaporation of the solvent, the dried food was repulverized and placed in cages containing 100 newly emerged flies. Water was also provided. Cages containing untreated fly food or sugar were used as checks. After 3 days, the flies were examined to determine mortality caused by the chemical; also, untreated fly food was given to flies that had been fed sugar diets to provide protein for egg development. When the flies were 5–7 days old, 0.25-in. moist Chemical Specialties Manufacturers Association standard fly larva rearing medium in a small waxed paper cup (3 in. in diameter and 2 in. deep) was placed in the cage for oviposition. When the cup was removed 4–6 hr later, it was filled with water, and the medium was stirred. Then a random sample of 100 eggs was collected and placed on a small piece of wet black cloth, which was laid on top of moist larval medium in a rearing container. (If no oviposition occurred, the medium was offered again every 1–2 days until it had been offered 5 times or the flies had oviposited.) Two to three days later, the percentage hatch was determined. Also, when the eggs hatched, the larvae crawled from the cloth into the rearing medium; therefore, about a week after oviposition, the pupae were counted to determine the number of larvae that had reached the pupal stage.

The chemicals that caused death or sterility at the 1% concentration were retested at lower concentrations (0.5–0.0025%) to establish the minimum effective dose. All tests, including those at 1% and the lower concentrations, were duplicate tests of 100 flies per test cage.

Since both sexes were given the chemosterilants, the results did not demonstrate whether sterility, if it occurred, had been induced in the male, the female, or both. Further experiments with the treated male provided part of this information. As soon as we determined that sterile eggs had been laid, 10 males were removed from the test cage and combined with 10 untreated virgin females. At the time of transfer, the males had been fed on the treated diet for 8–10 days; when they were placed in the cage with the untreated females, they received only untreated food. Oviposition medium was made available after 3 days, and sterility was assessed as before. If the eggs from the second mating were sterile, the compound was obviously a male chemosterilant though it might also be a female chemosterilant. If the eggs from the second mating were fertile, the chemosterilant had presumably affected only the females or had only a transient effect on the males. Some compounds caused such high mortality that a second mating could not be tested.

Sixteen of the compounds were purchased or otherwise obtained from outside sources including compounds **28** and **32** which were submitted by Professor Bruce King, University of Georgia. We previously described the preparation of compounds **2** and **3** (Oliver and Stokes, 1970) and of **29** (Oliver *et al.*, 1974b). The remaining ten compounds had previously appeared in the literature and were synthesized according to the published procedure. The syntheses of previously unreported compounds were as follows.

1-Acetamidohexahydro-1-methyl-1*H*-azepinium Iodide (1). 1-Aminohexahydro-1*H*-azepin (11.4 g, 0.1 mol) was cooled and stirred while acetic anhydride (15 ml) was added dropwise. After the addition was complete, the solution was warmed to 55° for 0.5 hr and then poured onto ice. The resulting mixture was extracted well with chloroform, and the chloroform extracts were washed with aqueous sodium carbonate. The solution was dried and evaporated, and the resulting oil was diluted with ether (25 ml) and treated with methyl iodide (7.5 ml). The mixture was refluxed 16 hr and cooled, and the white solid was collected and washed with hexane to give 16.4 g (55% overall) of 1, mp 127–128°. Recrystallization from ethyl acetate–methanol gave two crops: 11.0 g, mp 127.5–128.5°, and 4.2 g, mp 127.5–129°. *Anal.* Calcd for $C_9H_{19}N_2OI$: C, 36.25; H, 6.42; N, 9.40. Found: C, 36.32; H, 6.42; N, 9.38.

[(Dimethylcarbamoyl)methyl](iodomethyl)dimethylammonium Iodide (14). Chloroacetyl chloride (11.3 g, 0.1 mol) was added dropwise to chilled 40% aqueous dimethylamine (60 ml). After the addition was complete (extremely vigorous reaction), the mixture was allowed to warm to room temperature and then extracted with several portions of chloroform. The combined chloroform extracts were dried and distilled to give 8.4 g (65%) of *N,N*-dimethyl-2-(dimethylamino)acetamide, bp 50° (0.6–0.7 mm).

The acetamide derivative (1.30 g, 0.01 ml) and methyl-

Agricultural Research Service, U. S. Department of Agriculture, Agricultural Environmental Quality Institute, Beltsville, Maryland 20705.

[1]Insects Affecting Man Research Laboratory, Gainesville, Fla. 32604.

[2]Agricultural Environmental Quality Institute, Beltsville, Md. 20705.

Table I. Sterilizing Effects of Compounds to Both Sexes of House Flies When Administered in the Diet

No.	Chemosterilant	Concn, %	% sterility[a] Fly food	% sterility[a] Sugar
1	1-Acetamidohexahydro-1-methyl-1*H*-azepinium iodide	1.0	NO	8
		0.5	52	
2	1-(1-Adamanyl)biurea	1.0	NO	7
		0.5	42	
3	4-(1-Adamantyl)-3-thiosemicarbazide	1.0	NO	16
		0.5	35	
4	5-Amino-3-(methylthio)-1,2,4-thiadiazole	1.0	NO[c]	NO[c]
		0.5	71	78
5	5-Amino-3-phenyl-1,2,4-thiadiazole	1.0	NO	7
		0.5	NO	
		0.25	NO	
		0.1	10	
6	1-[4-Amino-6-phenyl-*s*-triazin-2-yl)-amino]-2,2,2-trichloroethanol mixture with 1,1′-(6-phenyl-*s*-triazine-2,4-diyl)bis[2,2,2-trichloroethanol]	1.0	NO	11
		0.5	84	
7	3-Amino-*s*-triazole mononitrate	1.0	NO[b]	99
		0.5	94	60
		0.25	100	
		0.1	100	
		0.05	58	
8	3-Amino-4-phenyl-Δ^2-1,2,4-triazoline-5-thione	1.0	NO[c]	12[c]
		0.5	NO	
		0.25	NO[c]	
		0.1	63	
9	2-Butyne-1,4-diol	1.0	NO	100[c]
		0.5	100	5
10	4-Chloro-α,α'-bis(dimethylamino)-2,6-xylenol	1.0	NO[c]	10
		0.5	16	
11	1-(*p*-Chlorophenyl)-3-nitroguanidine	1.0	NO[c]	64[c]
		0.5	NO[c]	
		0.1	75	
12	4-Cyclohexylsemicarbazide	1.0	NO	26
		0.5	15	
13	[2-(Dimethylamino)-4-hydroxy-2-oxo-3-cyclobuten-1-ylidene]dimethylammonium hydroxide inner salt	1.0	NO	6
		0.5	17	
14	[(Dimethylcarbamoyl)methyl](iodomethyl)dimethylammonium iodide	1.0	NO	32
15	*N,N*-Dimethyldithiooxamide	1.0	NO[c]	NO
		0.5	NO[c]	55[c]
		0.25	NO[c]	
		0.1	10	
16	Diphenyliodonium bromide	1.0	NO[c]	NO[c]
		0.5	13	10
17	2,2-Diphenyl-1-picrylhydrazyl (free radical)	1.0	*c*	22
		0.5	*c*	
		0.25	97[c]	
		0.1	*c*	
		0.05	100	
		0.025	42	
18	2,2′-Dithiobis[5-amino-1,3,4-thiadiazole]	5.0	100	
		2.5	NO	
		1.0	98	35
19	Ethyl carbazate	1.0	NO	10
		0.5	21	
20	1-(*p*-Fluorophenyl)-3-nitroguanidine	1.0	*c*	*c*
		0.5	*c*	NO[c]
		0.25	*c*	NO[c]
		0.1	83	28
21	4-(2-Furyl)-2-butanone semicarbazone	1.0	100[c]	26[c]
		0.5	*c*	
22	1-(Hydroxymethyl)-2(1*H*)-pyridone	1.0	NO	3
		0.5	53	
23	1-(Hydroxymethyl)-4(1*H*)-pyridone	1.0	NO	13
		0.5	20	
24	Isoquinaldic acid	1.0	71	100
		0.5		10
25	1-(*m*-Methoxyphenyl)-3-nitroguanidine	1.0	NO	24
26	4-Methyl-3-penten-2-onesemicarbazone	1.0	NO[c]	6[c]
		0.5	73	
27	1-Methyl-2(1*H*)-pyridone	1.0	NO[c]	64[b]
		0.5	75	
28	Octacarbonylbis[tris(dimethylamino)phosphine]dimanganese	1.0	100	74
		0.5	60	
		0.25	54	
29	1,1′-(4-Phenyl-1,2,4-dithiazolidine-3,5-diylidene)bis[3,3′-dimethyl-2-thiourea]	1.0	NO	55
		0.5	96	
30	Tetrazolo[5,1-*b*]quinazolin-9-ol	1.0	100	10
		0.5	94	
31	Tributylpropylammonium iodide	1.0	NO	4[b]
32	Tricarbonylcyclopentadienyl(trimethylstannyl)molybdenum	1.0	*c*	*c*
		0.5	*c*	*c*
		0.25	*c*	*c*
		0.1	*c*	*c*
		0.05	*c*	*c*
		0.025	*c*	NO[c]
		0.01	*c*	39[c]
		0.005	NO	
		0.0025	89	
33	4,4′-Vinylenedipyridine	1.0	NO	13

[a] Percentage based on number of progeny reaching the pupal stage from 100 eggs; NO = no oviposition. [b] Mortality 20–60%. [c] Mortality 61–100%.

ene iodide (2.68 g, 0.01 ml) were combined in acetonitrile (25 ml), and the resulting solution was allowed to stand at room temperature for 2.5 days. A clear solution was obtained that crystallized upon slight agitation. The white solid (1.85 g, 47%) was collected, mp 140–141° dec. An analytical sample was recrystallized from acetonitrile, mp 139–141°. *Anal.* Calcd for $C_7H_{16}I_2N_2O$: C, 21.12; H, 4.10; N, 7.04. Found: C, 21.10; H, 4.06; N, 6.79.

1-(Hydroxymethyl)-4(1*H*)-pyridone (23). The procedure was essentially that described (Cilag, Ltd., 1946) for the preparation of 1-(hydroxymethyl)-2(1*H*)-pyridone (**22**). A solution of 4-pyridone (20 g), 40% formaldehyde (20 ml), and potassium hydroxide (0.3 g) was stirred at room temperature 1 hr. Carbon dioxide was then bubbled through the solution until the pH was approximately 7. The water was evaporated *in vacuo*, the residue was boiled in ethyl acetate, and ethanol was added slowly until most of the material dissolved. The solution was chilled several days, and **23** was collected as a light tan solid (7 g, mp 67–73°). Recrystallization from acetonitrile–dimethylformamide gave a sample melting at 70–76°; however, the odor of formaldehyde was noticeable when solutions of **23** were

heated, and an analytical sample was not prepared. The assigned structure was confirmed by comparison of the nmr spectrum (δ (Me_2SO-d_6) 5.20, CH_2) to that of **22**.

4-Cyclohexylsemicarbazide (12). A solution of cyclohexyl isocyanate (11.5 g) in ether (100 ml) was added dropwise to a rapidly stirred solution of 97% hydrazine (5 g) in ether (150 ml) at 0°. After the addition was complete, the mixture was stirred briefly at room temperature, then the white solid **12** was collected by filtration and recrystallized from a 3:1 mixture of benzene and hexane. The yield was 12.2 g (85%), mp 126.5–127.5°. *Anal.* Calcd for $C_7H_{15}N_3O$: C, 53.48; H, 9.62; N, 26.73. Found: C, 53.44; H, 9.56; N, 26.69.

RESULTS AND DISCUSSION

All the compounds in Table I caused complete sterility with at least one concentration, though high mortality accompanied the sterility in several cases. The occurrence of mortality is indicated by footnotes. In all other cases NO refers to no oviposition by live flies. Some compounds were tested at lower concentrations than those shown in Table I but were ineffective at those levels. Of the 33 compounds effective against mixed sexes, only three, **27** (96% sterility at 1% in fly food), **17** (98% sterility at 0.05% in fly food), and **12** (100% sterility at 1% in fly food) were effective male sterilants. Several of the same compounds (**12, 18, 22–24, 27**) have also shown sterilant activity against the screwworm, *Cochliomyia hominivorax* (Coquerel) (Oliver and Crystal, 1972), and **10** was active against the boll weevil, *Anthonomus grandis* Boheman (Oliver *et al.*, 1974a).

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Received for review June 18, 1973. Accepted January 24, 1974.

Isolation and Identification of Host Compounds Eliciting Attraction and Bite Stimuli in the Fruit Tree Bark Beetle, *Scolytus mediterraneus*

Elie C. Levy,*[1] Isaac Ishaaya,[2] Eliahu Gurevitz,[2] Raymond Cooper,[1] and David Lavie[1]

The host extracts and their chemical components eliciting attraction and bite stimuli in *Scolytus mediterraneus* were investigated. The ether extract exhibited the highest activity as expressed by the number of holes on a Styropor disk impregnated with the extract. Taxifolin (V), pinocembrin (I), and dihydrokaempferol (III) showed high activity, whereas naringenin (II), quercetin (VI), kaempferol (IV), 5,7-dihydroxy-2-methylchromone (VIII), and scopoletin (VII) exhibited low activity. These compounds may function as primary attractants of the beetle in the field.

Scolytus mediterraneus (Egger) is a bark beetle that attacks and inflicts much damage to deciduous fruit trees in various parts of Israel. The heavy economic loss and the lack of a general applicable means of regulating the beetle population in the field induced us to study host factors affecting the behavioral response of these beetles. The initial attack of the beetle, usually occurring on physiologically damaged trees, is followed by a secondary mass attack which causes severe damage to the tree.

Advanced studies of the behavioral response of bark beetles attacking forest trees have been reported and reviewed by various authors (Pesson and Chararas, 1969; McNew, 1970; Renwick, 1970; Silverstein, 1970; Coster, 1970). Sex pheromones have been extracted and identified from various bark beetles (Pitman *et al.*, 1968; Silverstein *et al.*, 1968; Renwick and Vité, 1968; Kinzer *et al.*, 1969). However, field bioassays indicated that, in general, these pheromones had little or no activity on their own, but their combination with host resins usually induced attraction (Vité and Pitman, 1969a,b). The behavioral patterns of bark beetles and the compounds involved in their attraction vary considerably in different bark beetle species. α-Pinene was found to be the most effective host terpene for *Dendroctonus frontalis* and *D. penderosae* while 3-carene, β-pinene, myrcene, or their mixtures were effective for *D. brevicomis* (Renwick and Vité, 1970).

In contrast, very little is known about the behavioral response of bark beetles attacking deciduous trees. Preliminary studies with *Scolytus mediterraneus* (Gurevitz and Ishaaya, 1972; Ascher and Gurevitz, 1972) showed that host extracts in general elicited attraction and bite stimuli in this species, whereas those from an infested host were the most effective. This study was conducted in order to isolate and identify the active host compounds eliciting attraction and bite stimuli in *Scolytus mediterraneus*.

MATERIALS AND METHODS

Rearing Method and Bioassay. The beetles were reared on plum or apricot twigs as previously described (Gurevitz and Ishaaya, 1972). The bioassay tests were carried out by employing the Styropor method; the number of holes made by the beetles was used as a record for attraction and bite stimuli (Gurevitz and Ishaaya, 1972; Ascher and Gurevitz, 1972).

Isolation and Identification of Compounds. Melting points were made on a Fisher-Johns apparatus and are uncorrected. All uv spectra were taken in methanol solution on a Cary 14 spectrophotometer. Nmr spectra were taken in deuterated Me_2SO solution with TMS as an in-

[1] Department of Chemistry, Weizmann Institute of Science, Rehovot, Israel.
[2] Division of Entomology, The Volcani Center, Agricultural Research Organization, Bet Dagan, Israel. Contribution No. 242-E, 1973 Series.