TECHNIQUES AND RESULTS OF APPLYING THIOUREA TO SYNTHETIC AND GRASS SILAGE MEDIA FOR THE CONTROL OF *STOMOXYS CALCITRANS* LINNAEUS

by

HARRY B. WEINBURGH

AN ABSTRACT

Submitted to Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

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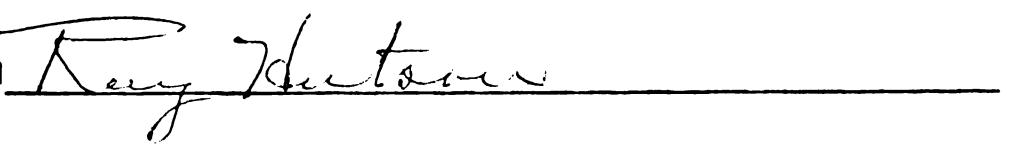
Approved 

HARRY B. WEINBURGH ABSTRACT

This research was initiated because of the need for a compound with low mammalian toxicity, yet exhibiting insecticidal properties, to be used for control of stable fly populations in silage. Since previous workers had shown that thiourea was toxic to house fly and flesh fly larvae, its effectiveness as a larvicide to the stable fly was investigated under laboratory conditions.

Standard procedures were modified and refined for a method of continuous rearing of the stable fly exposed to a varying laboratory environment. The size of the adult rearing cages proved to be not necessarily a limiting factor: large cages (3 x 3 x 3 feet) or small cages (15 x 15 x 24 inches) were equally satisfactory. The adult food, beef blood, was preserved with freshly prepared sodium citrate solution, but could not be retained for more than ten days when stored at 50° Fahrenheit.

Techniques were developed for the evaluation of thiourea as a stable fly larvicide. The test media were composed of CSMA media, oat hulls and distilled water. Counts of emerged adults were facilitated by use of funnels coated with "tanglefoot" and inverted in the test jars. The maintenance of constant temperature and humidity proved to be critical for obtaining reliable results.

The effect of thiourea on the development of the stable fly was determined when mixed with or when sprinkled on the

synthetic media, and when mixed with grass silage. The median lethal dosages were determined to be 63, 13 and 12 parts per million, respectively.

Thiourea sprinkled on the synthetic media was apparently more effective as a toxicant than when mixed with the synthetic media. The data also suggested that thiourea, when mixed with grass silage, produced sufficient mortality to stable flies to warrant future investigations of its toxic properties under field conditions.

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INTRODUCTION

The stable fly, Stomoxys calcitrans Linnaeus, has been a pest of domestic animals and man, causing irritation, discomfort and serious economic loss on occasions.

The stable fly lays its eggs in moist, rotting vegetation. Under laboratory conditions the eggs hatch in 48 hours. The larvae develop by feeding on the decaying matter and usually pupation starts on the ninth day. The adults start emerging on the fourteenth day, mate after four days, and begin egg-laying on the twenty-first day. Both sexes subsist primarily on mammalian blood. The optimum rearing temperatures range between 80° and 85° Fahrenheit.

The recent increase in the use of silage on farms has provided this insect with an excellent growth medium for the immature forms. Since silage is used as food for beef and dairy cattle, the use of chlorinated hydrocarbon and organic phosphate insecticides, which have produced excellent control of stable flies, is prohibitive because of their toxic properties. Because of potential toxicity problems the United States Food and Drug Administration has banned all chemicals in milk.

Consequently, entomologists and farmers have expressed the desire to have made available a compound which would be non-injurious to warm-blooded animals and still destructive to insect life. Previous research by other workers had

produced evidence that at least one compound, known chemically as thiourea, exhibits these desirable qualities. This material has been shown to be toxic to the larvae of the house fly, Musca domestica Linnaeus, and the flesh fly, Lucilia sericata Meigen, is practically non-toxic to mammals, and is partially soluble in water. Since earlier investigators indicated the possible values of thiourea as a larvicide, experiments were conducted to determine the effect of thiourea on the immature forms of Stomoxys calcitrans.

OBJECTIVES

The major objectives in this study were as follows:

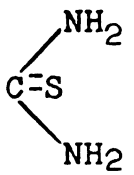
(1): To design a method of continuous rearing of the stable fly, Stomoxys calcitrans, under variable laboratory conditions; and to further design techniques necessary for evaluating the effect of thiourea on stable fly populations.

(2): To determine the effect of various concentrations of thiourea on the immature forms of the stable fly, Stomoxys calcitrans, when (a): mixed with synthetic media, (b): mixed with grass silage, and (c): sprinkled on synthetic media.

MATERIALS USED

1. Thiourea (Thiocarbamide)

Thiourea is a white crystalline solid, stable at normal room temperatures, with a chemical structure as follows:



Thiourea, on heating, isomerizes to ammonia thiocyanate, with which it forms an eutectic mixture. The technical compound has a melting point between 170° and 180° Centigrade. Decomposition takes place at 200° Centigrade; H_2S, CS_2 and NH_3 are evolved and guanidine thiocyanate remains. The solubility in water ranges from three grams per 100 grams of solution at minus five degrees Centigrade to 100 grams per 100 grams of solution at 100° Centigrade. The apparent density of the chemical is fifty pounds per cubic foot.

The thiourea was obtained from the Agricultural Chemicals Division, American Cyanamid Company, 30 Rockefeller Plaza, New York 20, New York.

2. CSMA Standard Fly Larval Medium

The CSMA (Chemical Specialties Manufacturing Association) standard fly larval medium is composed of alfalfa meal, dried brewers grains and soft wheat bran. The media were procured from the Ralston-Purina Company of St. Louis, Missouri.

3. Oat Hulls

The oat hulls, which were mixed with the CSMA media, are known commercially as "Full O'Pep" Poultry Litter. They were purchased from the New Century Company, 3939 South Union Avenue, Chicago 9, Illinois.

4. Beef Blood

The beef blood was obtained from Van Alstine Custom Meats, 5416 North Okemos Road, Okemos, Michigan.

5. "Tanglefoot"

The "tanglefoot" was prepared by mixing two pounds of rosin with one quart of hot castor oil.

6. Battery Jars

The battery jars were the standard type used in many kinds of biological experimentation, having dimensions of six inches in diameter and ten inches in height.

7. Sodium citrate

The crystalline sodium citrate, used to preserve the beef blood, has a molecular weight of 294 and the following formula: $Na_2C_6H_5O_7 \cdot 2H_2O$.

REVIEW OF LITERATURE

Woodbury (1943) stated that the stable fly, Stomoxys calcitrans, can be reared by methods similar to those employed for the house fly, Musca domestica, except that the adults are fed mammalian blood. Campau, Baker and Morrison (1953) reared stable flies by using house fly media for the larvae.

McGregor and Driess (1955) modified earlier procedures to make rearing simpler. The larval medium was prepared by mixing one part by volume of CSMA media with five parts of wood shavings and slightly moistening with water.

Various techniques have been employed to obtain oviposition. Champlain, Fisk and Dowdy (1954) used a water-saturated sponge about one inch thick, fitted horizontally in the bottom of a rectangular box of Plexiglass. The container was then placed away from the source of light within the cage. After sufficient oviposition, the eggs were easily recovered by flushing from the sponge with water. McGregor and Driess (1955) used a one-inch ball of damp cotton batten wrapped in a black cloth which, after moistening with a few drops of five per cent ammonia, was placed in a saucer or Petri-dish in the oviposition cage. Female flies were attracted by the ammonia and therefore eggs were laid on the dark surface, which facilitated the collecting of eggs.

Mold has been a very serious problem in larval media. Champlain, Fisk and Dowdy (1954) added sand to the surface of

the medium to prevent mold formation. Other workers have suggested maintaining a moist media to inhibit mold development.

Many inorganic and organic insecticides have been used to kill house fly larvae. Hadjinicolaou and Hansens (1953) determined that extremely small amounts of aldrin, dieldrin and chlordane controlled house flies when added to the larval media. Field tests showed that these materials alone could not be expected to result in complete fly control because of the difficulty in finding all breeding places. Tests of these materials on turkey manure and a large manure pit produced excellent control with the first application, but each subsequent application indicated decreasing effectiveness.

Cunningham and Eden (1955) determined the LD_{50} concentrations for endrin, aldrin, dieldrin, chlordane and DDT in the larval media to third instar house fly larvae. Endrin was the most toxic.

Standifer (1955) investigated 25 formulations of fifteen chlorinated hydrocarbon and organic phosphate insecticides to determine their respective toxicities to third instar house fly larvae. Aldrin emulsion was the most toxic of the chemicals tested; EPN-300 wettable powder and diazinon emulsion were nearly as effective; while BHC[a] emulsion and BHC wettable powder were the least effective. McCauley, Grainger, Lindquist and Fay (1955) tested seven chlorinated hydrocarbon insecticides, DDT, methoxychlor, toxaphene, BHC (95 per cent gamma

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[a]Benzene hexachloride

isomer), chlordane, dieldrin and aldrin as larvicides in spray applications on the surface of breeding media containing non-resistant house fly larvae. Chlordane produced superior control of all instars, whereas BHC, aldrin, toxaphene and dieldrin were most effective in media containing early instar larvae. Very low concentrations of endrin, heptachlor, lindane and parathion appeared to be highly effective as house fly larvicides, based on field tests by Sampson (1956). Greater concentrations of other insecticides were required to produce equivalent toxicity.

In an attempt to find a material non-injurious to mammalian life yet toxic to fly larvae, various research workers have experimented with thiourea as a fly larvicide. Hoskins, Bloxham and Van Ess (1940) tested thiourea as a larvicide for the flesh fly, Lucilia sericata. A concentration of four-tenths per cent by weight when added to the synthetic diet resulted in complete control, while a concentration of two-tenths per cent produced 85 per cent control. Green (1946) applied a coarse spray of technical thiourea at the rate of 33 grams per gallon of water per 100 square feet of manure. This type of application resulted in complete control of fly larvae for two weeks. McGovran and Piquett (1945) determined the LD_{50}[a] of thiourea to third instar house fly larvae to be 81 parts per million. Contrary to the findings of Sampson (1956), these

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[a] LD_{50} is the dosage required to kill 50 per cent of the population of test organisms.

workers found DDT and thiourea to be about equally toxic to fly larvae. Konecky and Mitlin (1955) found that a concentration of 5,000 parts per million of thiourea resulted in complete inhibition of house fly larvae.

The mammalian toxicity of thiourea has been investigated, using rabbits, rats, dogs, guinea pigs and humans as test organisms. Flinn and Geary (1940) fed thiourea in small amounts to rabbits, dogs and rats and observed no ill effects. Groups of rabbits given nine grams of thiourea per kilogram of body weight by means of a stomach pump, behaved in the same manner as animals in the control group. In a continuation of this experiment, 50 per cent of the rabbits died after receiving ten grams of thiourea per kilogram of body weight. Mortality increased to 100 per cent when 11 grams of thiourea per kilogram of body weight were administered. Symptoms of toxicity were observed during periods not exceeding 36 hours in these tests. The deaths were probably due to drastic changes of ionic concentrations in the blood stream, rather than direct toxic action by thiourea. Rabbits were fed an aqueous solution containing 25 milligrams of thiourea per kilogram of body weight each day except Sunday for 12 weeks. Increase in weights of test animals did not differ significantly from that of the controls. In subsequent tests, rats were fed thiourea in drinking water at the rate of 60 milligrams per kilogram of body weight for nearly 12 weeks. The weight gain was more gradual in the exposed group when compared with the control group, but the

percentage gain at the end of the test was the same for the two groups. Autopsies on the rats and rabbits receiving low doses of thiourea revealed no lesions in any of the tissues examined.

Hartzell (1940) administered thiourea to guinea pigs at the rate of 45 milligrams per kilogram of body weight each day during a period of six weeks. No evidence of toxic action was observed. Rats that ingested concentrations up to 49 milligrams of thiourea per kilogram of body weight for periods of 22 to 28 days exhibited no symptoms of poisoning and made gains comparable to the controls.

Kennedy (1942) found that 200 milligrams of thiourea in the diet daily for ten days resulted in abnormal thyroid glands in rats. MacKenzie and MacKenzie (1943) also determined that relatively high dosages of thiourea caused enlargement of the thyroid gland.

Purves and Griesbach (1946, 1947) observed tumors of the thyroid gland in rats given 0.25 per cent thiourea in their drinking water for 12 months or more. There was a tendency for such tumors to become malignant after 20 months. Since no abnormal growth changes were found in tissues other than the thyroid gland after long periods of feeding, it was concluded that thiourea had no direct carcinogenic action.

Flinn and Geary (1940) fed dachshund puppies a diet containing 25 milligrams of thiourea per kilogram of body weight for ten weeks. The dogs evidenced no injurious effects; they were lively and gained weight in a normal manner. Hartzell

(1940) fed thiourea to three young males of a litter of five dogs, while the other male and the female served as controls. Thiourea was administered in a ten per cent solution by stomach pump at a dosage equivalent to one gram of thiourea per kilogram of body weight. Every animal increased in weight almost two-fold during the period of the test and no toxic manifestations were evidenced at any time.

Hartzell (1940) also reported that in human feeding trials, 6.8 grams of thiourea were consumed during a period of two days with no discomfort experienced by the subject.

Studies on the metabolism of thiourea, in man by Medes (1937) and in rabbits by Blood and Lewis (1943), indicated that the compound was excreted in urine within 48 hours, probably in unchanged form. Positive tests for thiourea were obtained in urine collected one hour after ingestion. Schulman and Keating (1956) injected thiourea labeled with radioisotopic sulfur in rats and recovered 98 per cent of the radioactivity in the urine within 48 hours. The major portion of the thiourea was excreted unchanged; only 12 per cent of the radioactivity was associated with secondary sulfur products. There was a 55-fold concentration of radioactivity in the thyroid as compared with other tissues of the animals.

Thiourea structurally resembles urea, therefore it is probable that the degradation scheme may be similar. The end products, although not definitely known, are theoretically presumed to be ammonia and sulfur-containing acids. An anonymous

report (1956) indicated that high temperatures were required to initiate hydrolysis. Therefore, since no data to the contrary has been produced, the rate of degradation is assumed to be slow in manure and silage.

PROCEDURE

Wild stable flies were collected at the Michigan State University experimental dairy barn in an attempt to establish a laboratory strain; but, due to some inexplainable reason, the flies did not survive. Consequently, Stomoxys calcitrans pupae were obtained from the Department of Entomology, Rutgers University, New Brunswick, New Jersey. After some refinement in rearing technique, a stock colony was adequately established.

The rearing procedure to initiate and maintain a stock colony, briefly, was as follows:

All experimentation during the winter and spring was conducted under constant environmental conditions (85° Fahrenheit temperature and ten per cent relative humidity). However, the research during the summer and fall was conducted under extremely variable environmental conditions (60° to 85° Fahrenheit temperatures and 20 to 95 per cent relative humidities). The adults were provided fresh beef blood as a daily diet. To prevent coagulation, 64 milliliters of a 0.17 molar sodium citrate solution were added to each liter of blood. Blood was obtained at weekly intervals from a local slaughter house and stored at 50° ± 5° Fahrenheit. Waxed paper sundae cups were used as feeding receptacles within each cage to eliminate extra glass washing. The blood was poured into the cup, after which absorbent cotton was pressed into the liquid with a

stirring rod. Cellucotton was preferred over absorbent cotton since the former rapidly absorbed the blood. Used cups were discarded daily and replaced with freshly prepared feeding containers.

A rapid method for obtaining a known amount of Stomoxys calcitrans eggs was necessary to expedite the testing procedure. The female flies would deposit eggs in fermenting larval medium, but it was difficult to separate the eggs from the medium for the tests. The method of McGregor and Driess (1955) was tried but did not work under the conditions in the laboratory. The ammonia-moistened cotton quickly dried due to the low humidity and probably was no longer attractive to the females.

During the course of colony maintenance, it was observed that the female flies would deposit many eggs in the feeding containers, particularly when the blood level decreased. Although washing the eggs from the blood-saturated cotton was very time-consuming, it proved to be the most satisfactory manner in which to secure an abundant supply of eggs for testing procedures.

Contrarily, eggs for colony regeneration were easily obtained. Containers of fermenting larval substrata were placed in the breeding cages, and subsequently the females deposited eggs in the medium. The cups were removed each day and the contents added to battery jars containing additional media.

The larval medium was prepared by mixing 180 grams of CSMA media, 20 grams of wood shavings and 500 milliliters of distilled water. The wood shavings varied in weight and water absorption capacity, depending upon the kind of wood. While this variation did not greatly affect rearing of the stock colony, serious inconsistencies occurred in the testing procedures. Consequently, oat hulls were substituted for wood shavings. However, 50 grams of hulls were necessary to manufacture a medium consistent with the other method.

The vessels stocked with egg-seeded media were covered with muslin and dated. If mold formed in the media, it was inhibited by stirring, and additional water was added if necessary. The eggs hatched in one to two days and the larvae began feeding actively until the eighth day. Pupation began on approximately the ninth day. The adults began emerging five days later in the jars and were released in wire screen cages (15 x 15 x 24 inches), closed at one end with muslin and with a muslin sleeve fitted to the opposite end (Figure 1).

When the colony had increased in size to the point that the females were producing a total of several thousand eggs each day, tests with thiourea were started.

The test medium was prepared by mixing thoroughly 90 grams of CSMA media and 25 grams of oat hulls. Known amounts of thiourea were dissolved in 300 milliliters of distilled water, added to the dry ingredients and again mixed.

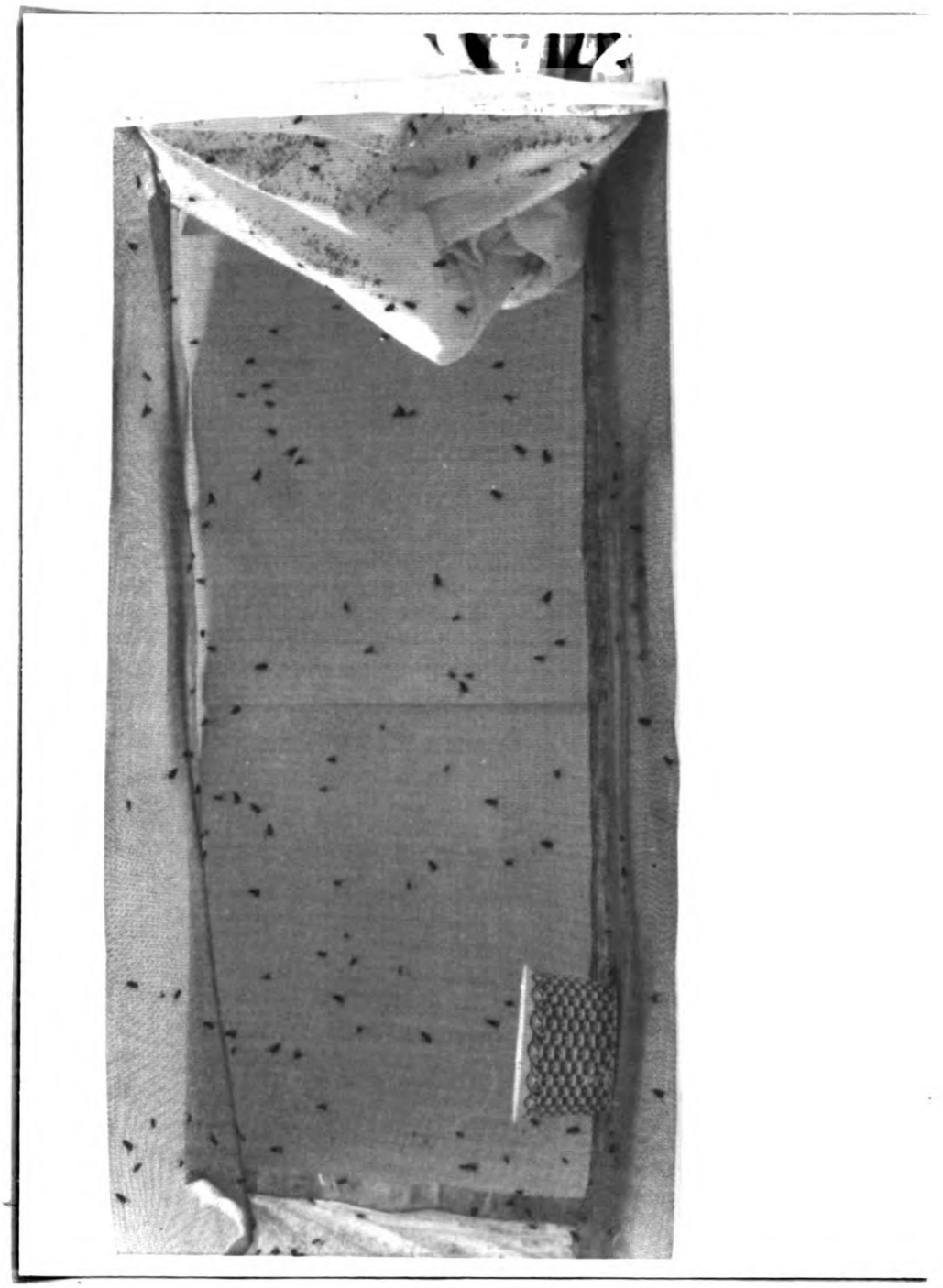

Figure 1. Adult rearing cage.

The test eggs were washed from the blood-soaked cotton, placed in a Petri-dish and gathered in a medicine dropper. One hundred eggs were counted by use of a stereoscopic microscope and deposited on filter paper. The eggs were transferred from the filter paper to the surface of the test media at the rate of 100 eggs per vessel.

The experimental technique consisted of four replications for each concentration of thiourea and equivalent controls. All containers were covered with muslin, labeled for concentration and date, and retained until ample time for emergence elapsed. Mold usually developed in the media within the first three days, but agitation generally inhibited the mold. If stirring was not successful, 50 millliliters of distilled water were added to aid in prevention of mold growth.

Subsequent to pupation and before adult emergence, a short-stemmed six-inch funnel (Figure 2), smeared with "tangle-foot" on the outside, was inverted in each test jar to trap the adults (Figure 3). Daily emergence counts were simplified by this technique.

A second series of experiments were designed to test the effect of various concentrations of thiourea in a grass silage medium. The concentrations of thiourea were dissolved in 50 millliliters of distilled water, thoroughly mixed with the silage, and 100 eggs were introduced. Counts of adult flies were made similarly by the method previously described.

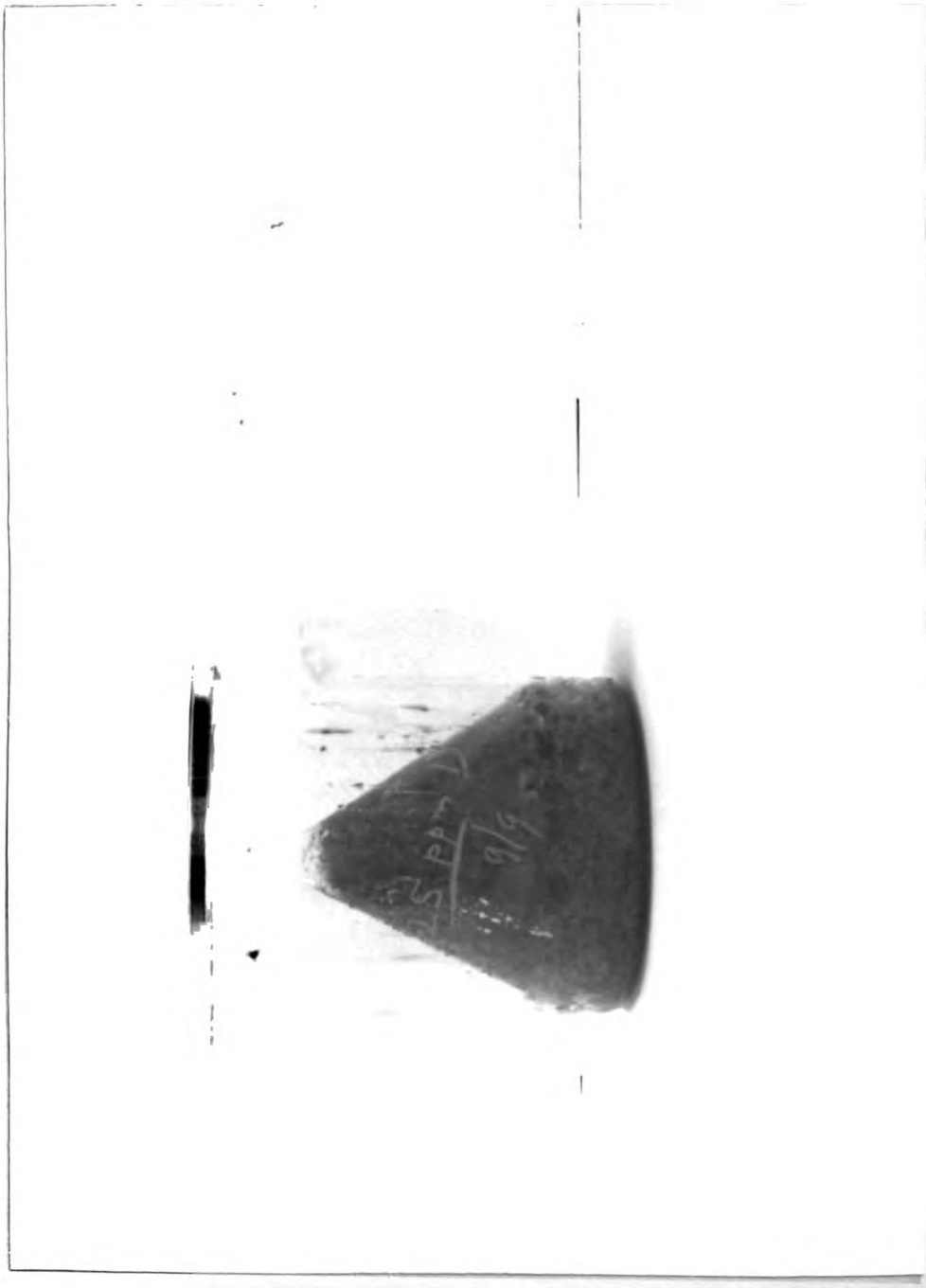

Figure 2. Inverted funnel coated with "tanglefoot."

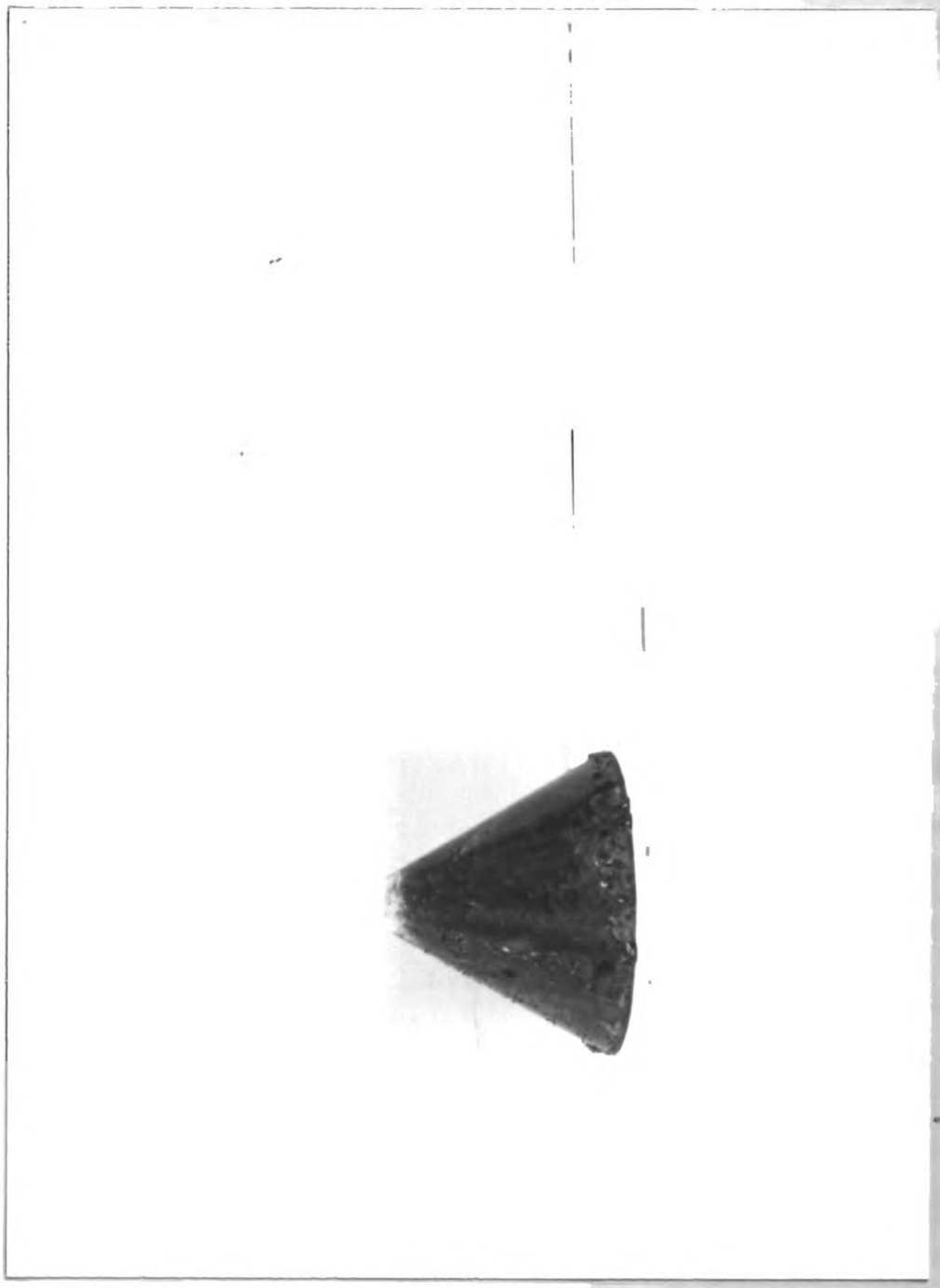

Figure 3. Covered test jar containing inverted funnel.

In a final series of tests, an attempt was made to determine whether any significant change in inhibition of larval development occurred when the thiourea was combined with eggs or when sprinkled on previously-established larvae. Therefore, six-day-old larvae were added to the media prior to application of various concentrations of thiourea on the basis that under natural conditions this situation might normally exist. The medium was prepared in the usual manner for testing except that the thiourea was omitted. The thiourea was first dissolved in 50 millliliters of distilled water and then sprinkled on the surface of the media by means of a clothes-sprinkling device fitted to an Erlenmeyer flask. Counts of adult emergence were again made by use of the short-stemmed funnels smeared with "tanglefoot."

The experimental data were analyzed by following the procedures exactly as outlined by Finney (1947, 1952) in chapters four and six, except that an adjustment was necessary in Table II because of negative values obtained from two of the concentrations. A second cycle for fitness was computed in Table VII.

EXPERIMENTAL RESULTS

The results in this investigation pertain to the following series of experiments:

A. The determination of the effect of various concentrations of thiourea on stable fly larvae when mixed with synthetic media.

B. The determination of the effect of various concentrations of thiourea on stable fly larvae when mixed with grass silage media.

C. The determination of the effect of various concentrations of thiourea on stable fly larvae when sprinkled on synthetic media.

A. Thiourea Mixed With Synthetic Media

Thiourea solutions ranging in concentrations from 250 to 30,000 parts per million (three per cent by wet weight) resulted in complete inhibition of larval development when mixed with synthetic media (Table I). Response to lower concentrations of 12.5 to 100 parts per million indicated a gradient of larval mortality (Table I). Two additional tests for determining the LD_{50} were conducted, but due to extreme changes in temperature and humidity, results were invalidated. Quantity of thiourea to produce an LD_{50} was determined to be approximately 63 parts per million when mixed with the growth substratum. The statistical analysis of the data is shown in Table II and Figure 4.

B. Thiourea Mixed With Grass Silage Media

Solutions of thiourea mixed with grass silage media produced complete inhibition of larval development at concentrations greater than 75 parts per million, while concentrations of less than 50 parts per million resulted in a reduction gradient of larval survival (Table III). The statistical treatment of the data is provided in Table IV and Figure 5.

C. Thiourea Sprinkled On Synthetic Media

When concentrations of thiourea were sprinkled on infested larval substrata, a gradient of mortality resulted (Table V). The probit analysis of the data is given in Tables VI and VII and accompanying Figures 6 and 7. The sprinkling of various concentrations of thiourea on the synthetic media resulted in a much lower median lethal dose (LD_{50}) when compared with that obtained by mixing equivalent concentrations of thiourea with the synthetic media.

KEY TO SYMBOLS USED IN STATISTICAL ANALYSIS

b	Slope of line
Conc. ppm.	Concentration of thiourea expressed in parts per million
C	Natural mortality
E.P.	Empirical probit
n	Number of insects tested
p'	100 r/n
p(C=)	p = (p'-C)/(1-C): "Abbott's formula"
r	Number of insects killed
w	Weighting factor
x	Natural logarithm of concentration
y	Working probit
Y	Expected probit

TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF THIOUREA ON THE DEVELOPMENT OF THE STABLE FLY, STOMOXYS CALCITRANS, WHEN MIXED WITH SYNTHETIC MEDIA[a]

Conc.	Replications	Total adults emerged A	B	C	D	Total	Mean	Difference from control
ppm.								%
30,000	4					0		
20,000	4					0		
10,000	4					0		
5,000	8					0		
2,500	4					0		
1,250	4					0		
625	4					0		
312	4					0		
100	4	4	8	17	13	42	10.5	-77.3
50	4	59	68	36	33	196	49.0	4.2
25	4	30	62	45	27	162	40.5	-12.7
12.5	4	42	22	52	19	135	33.8	-27.2
0	12	143	132	147	135	557	46.4	

[a]Mean length of test: 20 days

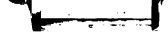

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF THIOUREA ON THE DEVELOPMENT OF THE STABLE FLY, STOMOXYS CALCITRANS, WHEN MIXED WITH SYNTHETIC MEDIA (STATISTICAL ANALYSIS)

Conc. ppm.	x	n	r	p'	(C=64) p	E.P.	Y	nw	y	nwx	nwy
100	4.60	400	358	90	72	5.55	5.58	67.56	5.58	310.776	376.985
50	3.91	400	204	51	-36	4.90	4.85	1.02	3.11	3.988	3.172
25	3.22	400	238	60	-11	4.17	4.05	0.12	2.61	.386	.313
12.5	2.53	400	265	66	6	3.45	3.37	2.85	3.36	7.210	9.576
0	--	400	255	64	--	--	--	71.55	--	322.360	390.046

$\overline{x} = 4.5054$ $\overline{y} = 5.4514$

$Snwx^2$	Snwxy	$Snwy^2$
1,464.647	1,771.766	2,146.434
1,452.355	1,757.306	2,126.288
12.292	14.460	20.146
		17.010

Chi square$_{(3)}$ =3.136

Chi square$_{3,.05}$=9.5

$b = 1.1764$

$Y = \overline{y} + b(x-\overline{x})$

$Y = 5.4514 + 1.1764(x-4.5054)$

$= 0.1512 + 1.1764x$

LD_{50}: $Y = 5$ antilog $x = 63$

$5 = 0.1512 + 1.1764x$ $LD_{50} = 63$ ppm.

$x = 4.8488/1.1764$

$= 4.12172$

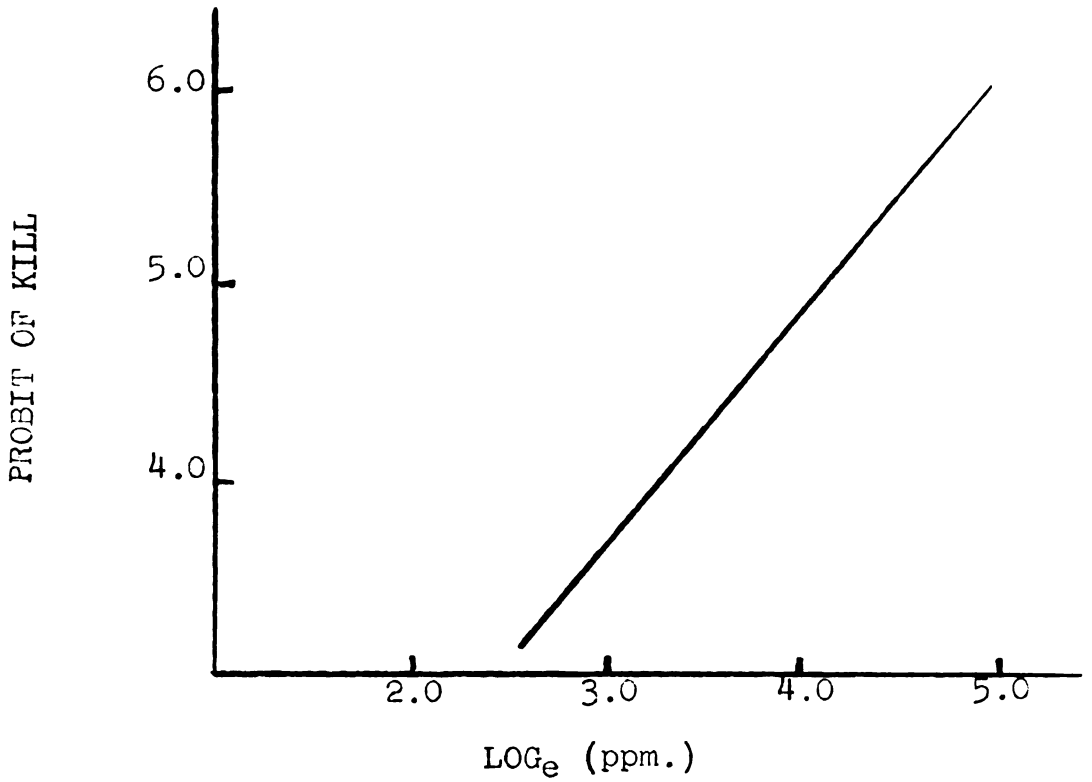

Figure 4. Effect of various concentrations of thiourea on the development of Stomoxys calcitrans when mixed with synthetic media.

TABLE III

EFFECT OF VARIOUS CONCENTRATIONS OF THIOUREA ON THE DEVELOPMENT OF THE STABLE FLY, STOMOXYS CALCITRANS, WHEN MIXED WITH GRASS SILAGE MEDIA[a]

Conc.	Replications	Total adults emerged A	B	C	D	Total	Mean	Difference from control
ppm.								%
100	4					0		
75	4					0		
50	4	0	3	2	0	5	1.3	-94.0
25	4	3	13	4	0	20	5.0	-76.4
0	4	13	22	19	31	85	21.3	

[a]Length of test: 28 days

TABLE IV

EFFECT OF VARIOUS CONCENTRATIONS OF THIOUREA
ON THE DEVELOPMENT OF THE STABLE FLY, STOMOXYS CALCITRANS, WHEN MIXED WITH GRASS SILAGE MEDIA (STATISTICAL ANALYSIS)

Conc. ppm.	x	n	r	p'	(C=79) p	E.P.	Y	nw	y	nwx	nwy
100	4.60	400	400	100	100		7.30	23.84	7.369	109.664	175.677
75	4.32	400	400	100	100		6.95	44.82	6.880	193.622	308.362
50	3.91	400	395	99	94	6.55	6.55	86.12	6.553	336.729	564.344
25	3.22	400	380	95	76	5.71	5.71	106.05	5.706	341.481	605.121
0	--	400	315	79	--	--	--	260.83	--	981.496	1,653.504

$\bar{x} = 3.7630$ $\bar{y} = 6.3399$

$Snwx^2$	Snwxy	$Snwy^2$
3,757.081	6,295.309	10,567.061
3,693.342	6,222.036	10,482.162
63.739	73.273	84.899
		84.233

Chi square(3) = 0.666

Chi square$_{3,.05}$ = 9.5

b = 1.1496

Y = 6.3399 + 1.1496(x-3.7630)

= 2.1040 + 1.1496x

LD_{50}:

5 = 2.0140 + 1.1496x

x = 2.9860/1.1496

= 2.59742

antilog x = 13

LD_{50} = 13 ppm.

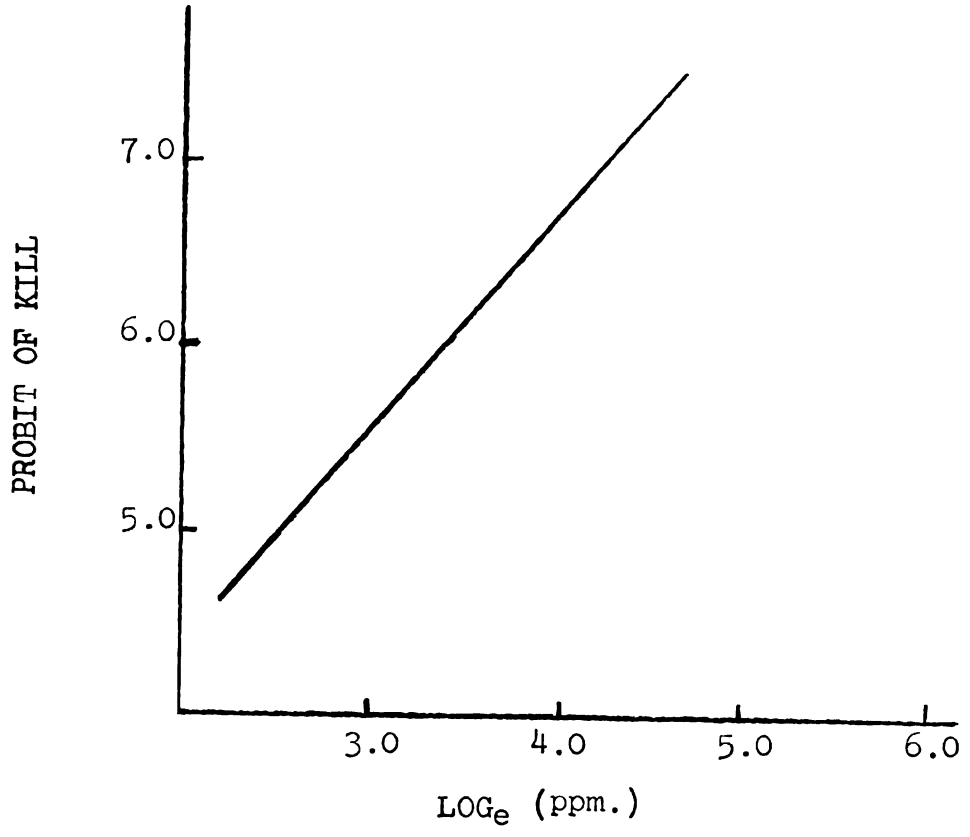

Figure 5. Effect of various concentrations of thiourea on the development of Stomoxys calcitrans when mixed with grass silage.

TABLE V

EFFECT OF VARIOUS CONCENTRATIONS OF THIOUREA ON THE DEVELOPMENT OF THE STABLE FLY, STOMOXYS CALCITRANS, WHEN SPRINKLED ON SYNTHETIC MEDIA (Tests 1 & 2)[a]

Conc.	Replications	Total larvae added	Adults emerged			Total	Mean	Difference from control
			A	B	C			
ppm.	#	#	#	#	#	#	#	%
6,400	6	330				0		
3,200	6	330				0		
1,600	6	330				0		
800	6	330				0		
400	9	480	1	3	3	7	0.8	-97.4
200	9	480	6	6	2	14	1.6	-93.8
100	3	150	2	1	1	4	1.7	-93.4
92	3	150	19	3	2	24	8.0	-68.4
50	3	150	4	3	5	12	4.0	-84.2
42	3	150	21	17	18	56	18.7	-26.2
25	3	150	19	11	22	52	14.0	-24.9
0	9	480	105	110	103	318	25.3	

[a]Mean length of test: 23 days

TABLE VI

EFFECT OF VARIOUS CONCENTRATIONS OF THIOUREA ON THE DEVELOPMENT OF THE STABLE FLY, STOMOXYS CALCITRANS, WHEN SPRINKLED ON SYNTHETIC MEDIA (STATISTICAL ANALYSIS - Test 1)

Conc. ppm.	x	n	r	p'	p (C=40)	E.P.	Y	nw	y	nwx	nwy
800	6.68	150	150	100	100		8.10	2.65	8.12	14.702	21.518
400	5.99	150	149	99	98.9	7.28	7.25	24.15	7.54	144.659	182.091
200	5.30	150	146	97	95.6	6.71	6.95	38.60	6.94	204.580	268.270
100	4.60	150	146	97	95.6	6.71	6.85	39.30	6.59	180.780	269.205
50	3.91	150	138	92	86.7	6.11	5.80	109.03	5.81	426.307	632.374
0	--	150	61	40	--	--	--	213.73	--	971.028	1,373.458

$\bar{x}$ = 4.5325 $\bar{y}$ = 6.4261 b = .4279

$Snwx^2$	Snwxy	$Snwy^2$
4,547.439	6,298.078	8,857.640
4,411.619	6,239.958	8,826.028
135.820	58.120	31.612
		24.871

Chi square(4) = 6.741

Chi square4,.05 = 9.49

Y = 6.4261 + .4279(x-4.5325)
= 4.4866 + .4279x

LD_{50}:5 = 4.4866 + .2479x
x = .5134/.4279
= 1.19981

antilog x = 3
LD_{50} = 3 ppm.

Confidence limits: probability .05

1.66086 ± 1.03497
or
2.69565
antilog = 15 ppm.
0.62571
antilog = 2 ppm.

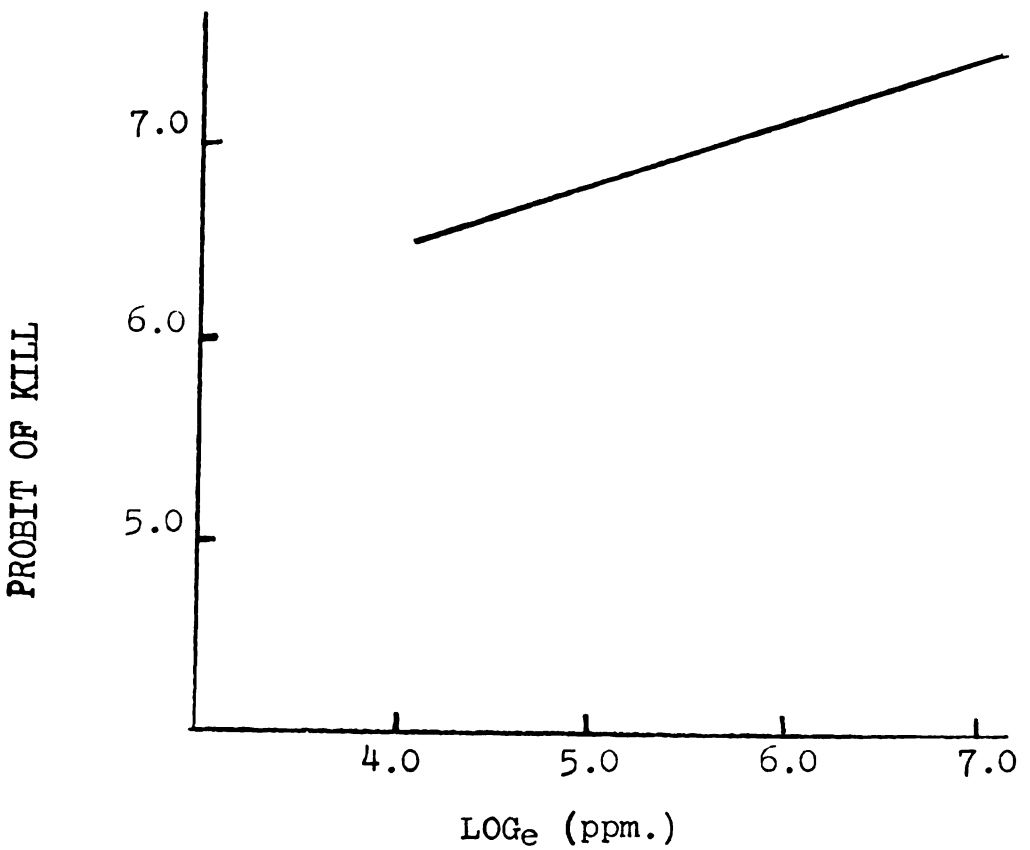

Figure 6. Effect of various concentrations of thiourea on the development of Stomoxys calcitrans when sprinkled on synthetic media (test I).

TABLE VII

EFFECT OF VARIOUS CONCENTRATIONS OF THIOUREA
ON THE DEVELOPMENT OF THE STABLE FLY, STOMOXYS CALCITRANS,
WHEN SPRINKLED ON SYNTHETIC MEDIA (STATISTICAL ANALYSIS - Test 2)

Conc. ppm.	x	n	r	p'	(C=47) p	E.P.	Y	nw	y	nwx	nwy
400	5.99	150	144	96	92	6.44	6.44	42.10	6.40	252.179	269.524
200	5.30	150	140	93	88	6.15	5.95	32.77	6.17	173.469	202.075
92	4.52	150	126	84	70	5.52	5.40	40.37	5.52	182.472	222.883
42	3.74	150	94	63	30	4.48	4.90	24.05	4.50	89.947	108.129
25	3.22	150	98	65	35	4.61	4.52	24.66	4.62	79.405	113.880
0	--	150	70	47	--	--	--	163.95	--	777.472	916.491

$\bar{x}$ = 4.7421 $\bar{y}$ = 5.6058

$Snwx^2$	Snwxy	$Snwy^2$
3,846.797	4,463.969	5,215.687
3,686.872	4,346.118	5,123.243
159.925	117.851	92.444
		8.684

b = .5768

Y = 2.8705 + .5768x

Chi square(4) = 83.760 ***

Chi square$_{4,.05}$ = 9.49

***Highly significant figure; therefore, computed second cycle for fitness.

TABLE VII (Continued)

x	n	Y	nw	y	nwx	nwy
5.99	150	6.33	25.52	6.34	152.841	161.771
5.30	150	5.93	33.97	5.92	180.062	200.956
4.52	150	5.48	37.82	5.47	170.955	206.773
3.74	150	5.03	34.87	5.02	130.399	175.202
3.22	150	4.73	28.23	4.72	90.891	133.260
--	--	--	160.41	--	725.148	877.962

$\bar{x}$ = 4.52073 $\bar{y}$ = 5.47341 b = .33703

$Snwx^2$ Snwxy $Snwy^2$

3,422.924 4,053.041 4,854.221

3,278.200 3,969.034 4,805.444

144.724 84.007 48.777

44.000

Chi square(4) = 4.777

Chi square$_{4,.05}$ = 9.49

$Y = 5.473 + .3370(x-\bar{x})$

$= 3.9498 + .3370x$

LD_{50}: $5 = 3.9498 + .3370x$

$x = 1.0502/.3370$

$= 3.11607$

antilog x = 23

LD_{50} = 23 ppm.

Confidence limits: probability .05

3.86202 ± 1.08838

or

4.94642

antilog = 140 ppm.

2.77762

antilog = 16 ppm.

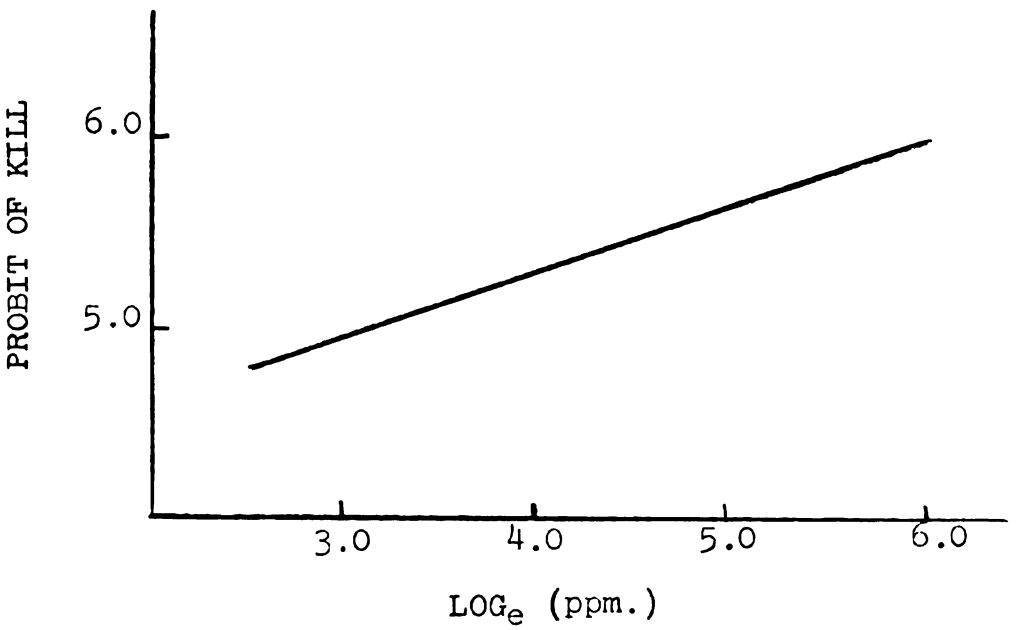

Figure 7. Effect of various concentrations of thiourea on the development of Stomoxys calcitrans when sprinkled on synthetic media (test 2).

DISCUSSION OF TECHNIQUES AND RESULTS

Great difficulties were encountered in establishing a laboratory stock colony of stable flies. Wild stable flies were collected at the Michigan State University experimental dairy barn and deposited in a jar containing grass silage. The breeding chamber was placed in a constant temperature room maintained at 80° Fahrenheit. The eggs hatched and larval development continued in a normal manner. After emergence, adults were transferred to a codling moth cage that had been modified by replacing the door with a cloth sleeve, and citrated beef blood was provided as food. However, the flies perished without producing eggs. This procedure was repeated with additional wild flies, but these also were unable to reproduce. Reasons for death were not definitely known, but probable factors included contamination of breeding cage and presence of toxic substances in the adult food supply.

Therefore, because of lack of time, arrangements were made with the Department of Entomology, Rutgers University, New Brunswick, New Jersey, through the courtesy of Dr. Phillip Granett, to provide stable fly pupae at intervals so that a stock colony could be initiated and maintained. Considerable difficulty was experienced in rearing consecutive generations. Persistent loss of adult flies may have been due to failure

to eliminate completely pesticide contamination of cages as well as possible toxic substances in the food.

In addition to cage contamination hazards, it was believed that small cages provided inadequate breeding space, and as a consequence, insufficient mating and egg deposition would occur. To avoid the above-mentioned sources of error, a cubical cage three feet in each dimension was constructed of non-pesticide-contaminated materials. However, subsequent modification of rearing procedure indicated conclusively that stable flies could be satisfactorily maintained in either a large cubical cage (3 x 3 x 3 feet) or in a smaller rectangular cage (15 x 15 x 24 inches).

Since mortality of adult flies continued to occur after the contamination problem had been eliminated, attention was directed to the adult food supply. In the original procedure an excess quantity of 0.17 molar solution of sodium citrate prepared with tap water had been stored at 50° Fahrenheit and used until coagulation or spoilage occurred. However, it was believed that under the above system of constituting and maintaining an adult food supply it was quite likely that certain toxic elements evolved to cause prolonged mortality within the stock colony.

To eliminate suspected toxic effects from adult food retained under these conditions, a fresh 0.17 molar solution of sodium citrate prepared with distilled water was added to each new quantity of blood. As a further precaution citrated blood

was never retained more than seven days. With these changes in procedure, mortality numbers decreased and the stock colony progressed sufficiently.

The experimental details were next undertaken. The larval substrata for the initial tests were prepared by mixing CSMA media and wood shavings with distilled water. However, since extreme variations in composition of the wood shavings existed, a relatively non-porous substance with a constant texture was sought as an alternate. Oat hulls have been utilized as a component of stable fly media by other investigators. Therefore, after preliminary experiments indicated that oat hulls would suffice for this project, they were substituted for wood shavings.

The system for making daily counts proved quite efficient. Glass funnels, despite a coating of "tanglefoot," were easily manipulated by using the shortened stems as handles. When flies emerged, the majority would fly out of the funnels and subsequently become affixed in the "tanglefoot." The trapped flies were removed each day with a pair of forceps and individually counted. The few flies that escaped the trap were counted without difficulty. Therefore, since the total tabulation was essentially accurate, the method was considered to be reliable.

The first tests of thiourea mixed with synthetic media indicated that the LD_{50} for stable fly larvae was approximately 63 parts per million. Efforts to reproduce this data failed

because of extreme variability in laboratory conditions. Before subsequent tests could be conducted, and because of space requirements, both stock and test colonies were transferred to a room with inadequate temperature and humidity controls. As a consequence, considerable time was lost in readjusting the colonies to the changeable conditions.

During the course of developing experimental techniques, observations were made of the influence of temperature and humidity on the consistency of test results. It became apparent that both factors were critical in effecting good duplication. As an example, natural mortality of adults increased when room temperatures ranged more that 5° from a mean of 80° Fahrenheit. Humidity appeared less critical than temperature, but radical changes in air moisture were indirectly related to variation in testing procedure.

In addition to temperature and humidity factors, mold formation in the media was an interfering factor. Prevention of mold growth was accomplished either by agitation of media or by replenishment of moisture content. Additional distilled water was necessary only when mold formation rapidly increased. Mold development was more readily observed at low humidity values than at the optimum range. Usually the appearance of mold would occur within the first 100 hours. The reasons for this phenomenon were not definitely known but it was assumed that newly-hatched larvae were unable to maintain sufficient agitation of media to inhibit mold growth during this interval.

However, as larvae matured and feeding activity increased, mold formation was noticeably absent.

In a second series of tests thiourea, when mixed with grass silage, produced an LD_{50} of 12 parts per million with stable fly larvae. However, results were not too conclusive as time permitted only one test, and additional complications occurred with this series. Predacious mites were discovered at the termination of the experiment among the surviving adults, and it was unknown to what extent this condition may have affected the outcome of the test.

Despite difficulties encountered in this series, evidence of a trend of apparent toxic effect by thiourea to stable fly larvae in grass silage is present in the results. If subsequent research substantiates these findings, thiourea may have definite practical application and commercial usage.

Should thiourea prove to be effective and safe for use in grass silage as a toxicant to stable fly populations, the matter of application would be problematical. Therefore, in a final series of investigations, an attempt was made to determine whether thiourea would produce similar toxic reactions if various concentrations were sprinkled on synthetic media.

Results from duplicate tests in this series indicated the possibility that thiourea increased mortality of stable flies when distributed in the above-described manner. The data suggested that thiourea sprinkled on media was more effective as a toxicant to stable flies than when mixed with the media. It

was not definitely determined that such was the case, but it is reasonable to assume that a greater degree of contact action was responsible for the increased mortality. The mode of toxicity by thiourea to stable flies is also unknown; however, it is probable that toxicological symptoms could result from either direct contact or by metabolic processes following ingestion.

Although the results in each series of tests were not definitely conclusive because of insufficient data, statistical interpretations provided evidence that thiourea was toxic to stable fly populations in either grass silage or synthetic media under laboratory conditions. Field testing may yield different results, but it is logical that if application techniques are properly designed, similar responses are likely to occur.

SUMMARY AND CONCLUSIONS

This investigation can be summarized briefly by the following statements:

1. Standard techniques for initiating and maintaining a stock colony of Stomoxys calcitrans were revised and refined to meet certain environmental conditions in the laboratory.

2. Techniques were developed for evaluating thiourea as a larvicide for the stable fly under laboratory conditions.

3. The effects of thiourea on the development of the stable fly were determined when various concentrations were mixed with or sprinkled on synthetic media, and when mixed with grass silage. The LD_{50} values were as follows: mixed with synthetic media, 63 parts per million; mixed with grass silage, 12 parts per million; sprinkled on synthetic media, 13 parts per million (mean value).

4. Counts of emerged flies were facilitated by use of funnels coated with "tanglefoot" and inverted in the test jars.

The following conclusions were drawn from the results of this research:

1. For maximum colony sustenance, the adult food supply (beef blood) should be preserved with freshly prepared sodium citrate solution, and should not be retained more than ten days, if stored at 50° Fahrenheit.

2. Size of the rearing cage for adult flies is not necessarily a limiting factor.

3. Maintaining constant temperature and humidity conditions is necessary for obtaining reliable results in research of this nature.

4. Thiourea sprinkled on media was more effective as a toxicant to stable flies than when mixed with the media.

5. The mode of toxicity of thiourea may have been initiated either by direct contact action or by metabolic processes following ingestion.

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Date Due
