1957

A Geographical Study of Housefly Resistance

Vernon H. Lee

A GEOGRAPHICAL STUDY OF HOUSEFLY RESISTANCE

By

Vernon H. Lee

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science at South Dakota
State College of Agriculture
and Mechanic Arts

June, 1957

A GEOGRAPHICAL STUDY OF HOUSEFLY RESISTANCE

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Head of the Major Department

ACKNOWLEDGEMENTS

The writer wishes to express his gratitude to Dr. W. M. Rogoff, Professor of Entomology, for his suggestions, guidance and criticisms during this study. Appreciation is also expressed to those persons who have made suggestions and given assistance with the statistical analysis of the data. The writer is also grateful to other members of the Entomology and Zoology staff for their assistance and suggestions.

VHL

TABLE OF CONTENTS

INTRODUCTION

That insects have the ability to develop an abnormal tolerance, or resistance, to insecticides has been known for many years. In 1914, resistance by scale insects to lime-sulfur sprays was reported by Melander (10). Poisons even as toxic as hydrogen cyanide gas, were found to have lost their effectiveness against certain insects. Since that time, many species of insects have been reported as having an unusual tolerance to a variety of insecticides.

More recently and more significant to entomologists and geneticists was the resistance shown by houseflies, Musca domestica L., to DDT. The widespread use of this insecticide immediately following the second world war, and the subsequent wide-spread resistance to its effect by several species of insects has stimulated much research involving the chemical and genetic roles in resistance.

Shortly following the initial use of DDT, resistance by a housefly strain was reported. Missiroli (11), in 1947, reported on resistance of a strain of housefly found near Rome and Naples, Italy. Also, in 1947, Wiesmann (15) reported on resistance by houseflies from Sweden. In 1948, reports were received at various localities in the United States of poor control of houseflies by DDT. Since that time, nearly all localities of the United States have reported resistance by houseflies, as well as several other insects to DDT.

In 1950 and 1951, attention was called to the poor control of houseflies achieved by DDT at Brookings, South Dakota. At that time, Rogoff (14) made tests to determine the level of resistance of flies

in that community. The results indicated a very high level of resistance.

In 1954, further testing for resistance was done. Having a knowledge of the level of resistance in the city of Brookings, particularly of the fly populations at South Dakota State College, located at Brookings, several questions arose.

> Are fly populations in areas rural to Brookings also resistant to DDT?
>
> If so, what is the level of resistance as compared to the Brookings strain?
>
> If not, where and how quickly does the resistance decline?
>
> Are there small areas of high resistance in a large area of general normal susceptibility, or are there small areas of susceptibility in large areas of general high resistance? If neither condition is the case, then are there enclaves of resistant and susceptible populations in an area of resistance or a level somewhere intermediate to high resistance and susceptibility?

In an attempt to answer these questions, the author, in the early summer of 1954, undertook this project dealing with the geographical distribution of insecticide resistance by houseflies. It was carried on in conjunction with Experiment Station Project #186, which allowed funds for fly control research.

A review of the literature has failed to uncover any other work of this same nature. Decker and Bruce (6), in 1952, reported on survey work done for several years preceeding this date. Their work, however, consisted of a random selection of collection points throughout the state. Any publications of work concerned with resistance over smaller localized areas have not been located, nor have been called to the author's attention.

PROCEDURE

The area investigated surrounded Brookings and extended as far as time would permit. Points of collection were selected at approximately five mile intervals, from the city in all directions. There was no prejudice in the selection of these points--whether it was a farm, hatchery, or whatever type of establishment. The collections of flies at each point were made wherever they were fairly abundant, so that there was no particular difficulty in obtaining sufficient numbers of flies. Also, there was no attempt at segregation of flies as to age, habitat, or sex.

At each point of collection, an attempt to get as much of the history of spraying as possible, was made. Most of the time, the history was confused and incomplete, and was given no further consideration.

The actual collection of flies was made with a sweep net of light weight so that little damage would be done to the flies. Flies collected were then placed in half-gallon cardboard cartons for transfer back to the laboratory at the college. The flies were, whenever possible, kept cool, so that their activity was reduced, thereby tending to reduce any damage caused by the flies injuring themselves by batting themselves against the sides of the container.

In the laboratory, the flies collected were examined for damage and for identification. The flies were anesthetized with carbon dioxide which was kept available in a liquid carbon dioxide cylinder. As soon as they were anesthetized, they were picked individually from the con-

tainer and were identified for species and for physical damage with aid of a stereomicroscope. Each fly that passed for "physical fitness" and was identified as M. domestica was then placed into another container and allowed to recover from anesthesia. This procedure was repeated, until enough flies were obtained to set up the experiment. Many times a repeat of anesthesia was required of the flies in a single container, since dilution of CO_2 occured whenever the lid of the container was lifted to remove a fly and the flies would recover fairly rapidly. However, repeated anesthesia did not seem to injure, or in any way reduce the viability of the fly. Only M. domestica in apparently good condition were used.

When enough flies had been sorted out to complete a setup, all for that series of the experiment were pooled in a single container and held in the refrigerator until other procedures were completed. Preliminary tests indicated that flies could be held in a refrigerator for a substantial length of time without apparently altering their viability.

The insecticides used in the experiments were DDT, lindane, and Diazinon. DDT and lindane are related compounds, both being chlorinated hydrocarbons, with somewhat similar modes of action and effects. Lindane was included because of this relationship, and also because a resistance may be developed to it as resistance to DDT is developed. Diazinon, being unrelated--an organic phosphate, not having been released for commercial use at that time, and highly toxic--served as an extreme check. Flies subjected to this insecticide were all killed fairly quickly.

Chemically, the insecticides were as follows:

DDT- - - --1,1,1-trichloro-2,2-bis (p-chlorophenyl ethane). (The particular sample used was a Merck product with a melting point of 107° C. Purified DDT has a melting point of 109° C.).

Lindane- - an isomer of 1,2,3,4,5,6-Hexachlorocyclohexane. (A 99% pure gamma isomer. The particular sample was an AAEE reference standard).

Diazinon - 0,0-diethyl-0-[2-isopropyl-4-methylpyrimidyl (6)] thiophosphate. (The particular sample used was of a technical grade, 95% pure, brown liquid).

Test solutions were made up at the following concentrations:

DDT- - - - 5.0% (weight-volume)
Lindane- - 0.1% (weight-volume)
Diazinon - 0.1% (weight-volume)

The surface to which the test flies in each replicate were exposed was an 11 cm. filter paper saturated with 1 cc. of test solution. The filter paper was then allowed to dry. When dry, the filter paper was placed over the bottom portion of a Petri dish, approximately 9½ cm. in diameter, and the top portion placed over the bottom portion, thereby folding down the periphery of the filter paper and securing it firmly in place. In this way, when the unit was turned over, a bottom surface of treated filter paper was provided. Also marked on the filter paper was the identification of the test solution.

The effective surface residue for each insecticide was calculated to be as follows:

DDT- - - - 500 mg./sq. ft.
Lindane- - 10 mg./sq. ft.
Diazinon - 10 mg./sq. ft.

In contrast, the recommended surface residues, at that time were (4):

DDT - - - - 200 mg./sq. ft. (approx.)
Lindane - - 25-50 mg./sq. ft.
Diazinon - 100 mg./sq. ft.

To provide some moisture, as well as a nutrient source, a small wedge of apple was placed into each dish.

When the actual setup was begun, the container of flies was again anesthetized. With the test dishes open and ready for receipt of the flies, approximately ten flies (uniform numbers were difficult to maintain during this hasty operation) were gently dumped into the dishes and the covers placed on with the treated filter paper between the two portions of the dish. The time at which the flies were first exposed to the treated surface was noted. The dishes were then turned over so that the filter paper covered the bottom surface. They were then left undisturbed. The flies soon recovered from anesthesia, with much activity of feeding and buzzing around, so that they often made contact with the treated surface.

At regular intervals, first of ten minutes, counts were made of the numbers of flies knocked down. After the first half hour had elapsed, counts were made at fifteen minute intervals. After four or five hours, the intervals between counts was extended to several hours. Counts were continued until every fly in every dish was dead and every count recorded.

The physical setup, as described above, is similar to that used by March and Metcalf (9) and others, and is illustrated by Figure 1 (p. 7).

The first several tests set up were used to test technique and establish procedure. In these tests, concentrations of solutions were de-

Figure 1. Photograph of a typical setup, showing one series of tests, shortly after completion of the procedure. Photo by Rogoff (14).

cided upon. The concentration of lindane was the same as that used by March and Metcalf (9) and Rogoff (14). The concentration for DDT was arbitrarily selected as 5%.

For each chemical tested, there were four replicate units, except in one or two cases where the number of flies would not permit. For each experiment, there were also four replicated dishes for untreated checks. The checks were handled in the same manner as were the test dishes, except that the filter papers were saturated with 1 cc. of acetone only.

A total of forty-nine tests were run, the first seven of which were devoted to establishment of procedure, and covering several points of collection in Brookings, including the city dump, riding stable, sales pavilion, and three points on the campus of South Dakota State College. Collections were made, usually at about five mile intervals, but sometimes the distance was varied for convenience or for lack of some type of establishment at those distances. Toward the end of the experiment the distances were increased in one direction in order to cover a larger area before the season change terminated the work.

Figure 2 shows the general section of the United States in which this investigation was undertaken. The shaded area indicates more specifically the parts of South Dakota and Minnesota involved. Plate I shows in detail the area investigated.

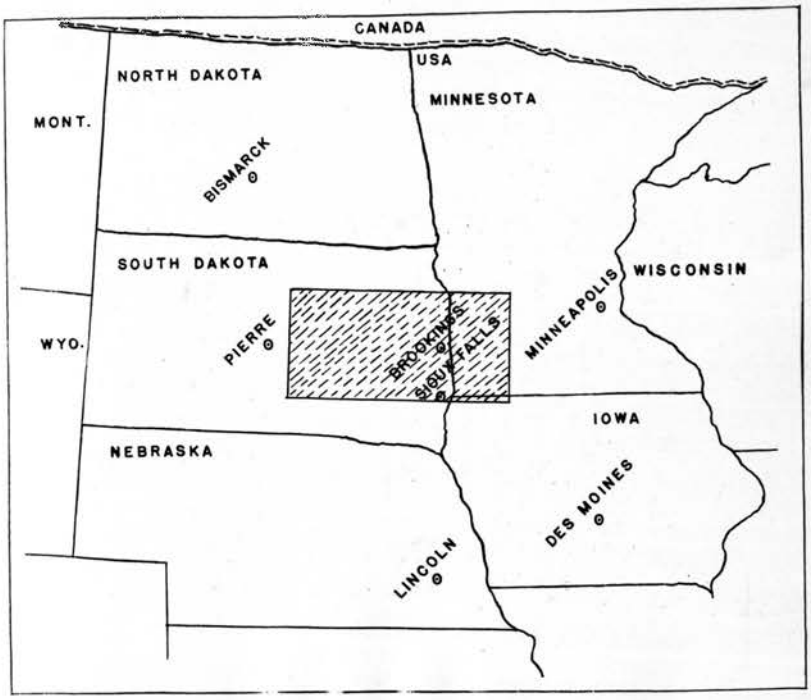

Figure 2. General area of investigation. Shaded area is shown in detail on Plate I.

Plate I (overleaf). The solid black dots are the approximate points of collection. The distance of northern most point is 46.0 miles; of eastern most point, 43.0 miles; of southern most point, 41.0 miles; and of the western most point of collection, 115.0 miles from Brookings.

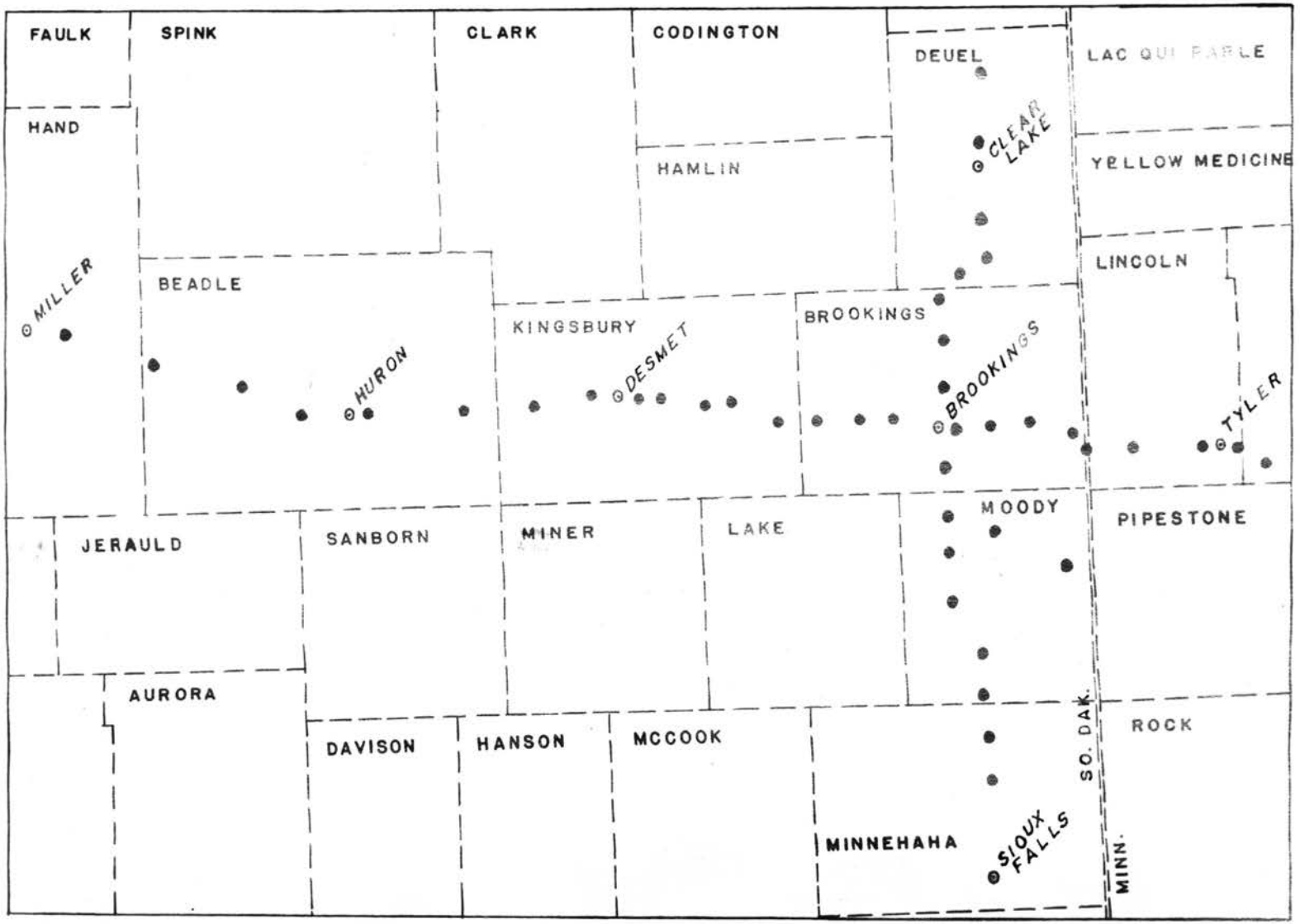
FAULK
SPINK
CLARK
CODINGTON
DEUEL
LAC QUI PARLE
HAND
HAMLIN
CLEAR LAKE
YELLOW MEDICINE
MILLER
BEADLE
KINGSBURY
BROOKINGS
LINCOLN
HURON
DESMET
BROOKINGS
TYLER
JERAULD
SANBORN
MINER
LAKE
MOODY
PIPESTONE
AURORA
DAVISON
HANSON
MCCOOK
SO. DAK.
ROCK
MINNEHAHA
SIOUX FALLS
MINN.

PLATE I

RESULTS

An analysis of the data indicated that a high level of resistance to DDT by houseflies was very generally distributed in the area studied. There were no points of collection which presented what could be considered a susceptible strain of flies. In many cases, there was no significant difference between mortality rates in the DDT tests and in the control tests. There were several instances in which the flies subjected to DDT treatment would outlive flies in the control dishes. These relationships are indicated on Tables 1, 2, 3, 4 and 5 (pp. 13-17), in which the values for all determinations are entered as the time required for a KD_{50} (knock down of 50% of individuals involved). KD_{50} values were used because the length of time required for a KD_{100} was so long that natural mortality became a significant factor.

Peaks and dips appearing on a graph plotting mortality rate versus point of collection have not been demonstrated statistically to be actual levels of resistance. They cannot, therefore, be considered enclaves of high resistance or low resistance populations. Such peaks and dips, without an apparent pattern of response or reason are well illustrated in Figure 5 (p. 22). The points plotted are KD_{50} values which have been corrected for natural mortality as determined from mortality among control flies. The method of correction employed is given by Abbott's formula[1] (1).

[1]Abbott's formula: $\frac{X - Y}{X} \times 100 = \%$ kill by treatment. (X is the percent survival in the control, and Y is the percent survival in the treatment sample).

Any change in resistance through distance traversed in the experiment could not be demonstrated to a reliable degree. However, there is some evidence that a slight over-all change takes place from an eastern most point of collection to a western most point. This change of resistance has little significance and has no practical value concerning the use of DDT for the control of houseflies. Figures 3 and 4 (pp. 19 and 21) show the slight changes in resistance by use of regression lines. The use of regressions will be discussed in the following section.

There was little indication that curves plotted for lindane and Diazinon were related to those for DDT. The curve for Diazinon was low and fairly uniform while that for lindane was higher and less uniform. However, the anticipated increase in level of resistance of lindane in proportion to that of DDT was not indicated. The variability of lindane resistance was present but did not correspond to the fluctuations of DDT resistance as plotted. See Figures 3 and 4 for the curves representing the levels of resistance for lindane and Diazinon.

A resistance to the effects of lindane was apparent, however. A level of resistance of from two to three times the length of time for a KD_{50}, as compared to a similar KD_{50} for susceptible laboratory strains of flies, was apparent. The susceptible laboratory strain to which the South Dakota strain was compared was that used at Riverside, California (9). Being subjected to 10 mg./sq. ft. concentration of lindane, these results were given (See Table 8, p. 23):

KD_{50} - - - - - 13 minutes
KD_{100}- - - - - 20 minutes

The author's results for a KD_{50} at the same concentration of insecticide

ranged from sixteen minutes to forty-two and one-half minutes, with an average of thirty minutes. The KD_{100} ranged from one to three hours with a few single flies surviving up to ten and twenty hours, with the majority being knocked down between one and three-quarters to two and one-quarter hours.

Diazinon was highly toxic throughout the experiment, only slight variations being noted.

To evaluate the data, methods of statistical analysis were involved. In all cases, where an analysis was made, statistical significance was determined by use of the 't' test or 'F' test. Calculated significance was then compared to theoretical 't' or 'F' values, at the 5% level of probability.

In an analysis of the entire group at any particular point of collection, the significance of treatment differences indicated high reliability. But in an analysis involving DDT treatment versus control, the significance indicated low reliability, except for points in the westerly direction.

In this case the significance increased as the distance in that direction increased. To test this evidence for a change, a regression was run on the points of collection using an over-all KD_{50} of the four replicate DDT tests at each point. The results will be discussed in the following section.

Table 1. Location, date and results of tests in city of Brookings.

Site			KD_{50} in hours			
Test No.	Location	Date	Check	DDT (corrected)	Lindane	Diazinon
1	Beef Barn	6/28/54	- - - - - - not determined at this date - - - - - - - -			
2	Beef Barn	6/29/54	28.00	41.75	0.75	0.52
3	Central Farm	7/1/54	24.05	31.00	0.71	0.43
4	Dairy Barn	7/2/54	92.75	48.94	0.46	0.23
5	Sales Pavilion	7/7/54	33.50	24.22	0.52	0.25
6	Riding Stables	7/13/54	30.00	29.12	0.33	0.17
		Average KD_{50}	41.66	34.98	0.56	0.32

Table 2. Location, date and results of tests covering area north of Brookings.

Site			KD_{50} in hours			
Test No.	Location (in mi.)	Date	Check	DDT (corrected)	Lindane	Diazinon
9	5.5	7/16/54	36.55	30.15	0.40	0.25
14	11.0	7/29/54	48.75	24.95	0.48	0.43
19	15.0	8/10/54	33.80	22.17	0.45	0.29
20	20.0	8/10/54	33.88	44.33	0.47	0.26
27	24.0	8/18/54	41.97	34.64	0.61	0.42
28	30.0	8/18/54	21.19	21.12	0.49	0.46
37	40.2	8/27/54	113.25	29.25	0.41	0.38
38	46.0	8/27/54	33.09	33.25	0.27	0.29
		Average KD_{50}	45.31	29.98	0.45	0.35

Table 3. Location, date and results of tests covering area east of Brookings.

Site			KD_{50} in hours			
Test No.	Location (in mi.)	Date	Check	DDT (corrected)	Lindane	Diazinon
7	1.0	7/14/54	29.50	38.75	0.57	0.28
8	5.0	7/15/54	37.25	30.25	0.39	0.25
13	10.5	7/28/54	47.32	22.61	0.34	0.29
16	15.0	8/3/54	35.25	20.35	0.48	0.42
24	20.0	8/13/54	34.16	21.05	0.47	0.40
25	25.0	8/13/54	32.53	24.02	0.58	0.47
34	34.5	8/26/54	36.00	22.36	0.58	0.38
35	38.0	8/26/54	44.00	31.16	0.62	0.34
36	43.0	8/26/54	19.50	19.56	0.49	0.37
		Average KD_{50}	35.06	25.57	0.50	0.36

Table 4. Location, date and results of tests covering area south of Brookings.

Site			KD_{50} in hours			
Test No.	Location (in mi.)	Date	Check	DDT (corrected)	Lindane	Diazinon
11	6.5	7/26/54	27.00	26.50	0.48	0.33
12	11.0	7/27/54	32.75	22.92	0.47	0.41
17	13.3	8/4/54	30.25	21.92	0.38	0.16
18	21.2	8/4/54	47.15	32.75	0.48	0.42
22	15.0	8/12/54	25.80	25.07	0.58	0.46
23	20.5	8/12/54	29.40	31.20	0.58	0.42
29	25.0	8/19/54	28.25	21.47	0.59	0.43
30	30.0	8/19/54	28.91	20.08	0.40	0.31
39	36.0	8/31/54	40.93	25.40	0.39	0.43
40	41.0	8/31/54	32.79	21.36	0.51	0.41
		Average KD_{50}	32.32	24.87	0.49	0.38

Table 5. Location, date and results of tests covering area west of Brookings.

Site			KD_{50} in hours			
Test No.	Location (in mi.)	Date	Check	DDT (corrected)	Lindane	Diazinon
10	7.0	7/21/54	33.64	25.17	0.54	0.42
15	10.0	8/3/54	26.38	20.46	0.54	0.26
26	15.0	8/16/54	47.28	32.35	0.69	0.66
21	19.5	8/11/54	26.76	21.92	0.50	0.40
31	27.0	8/24/54	47.67	17.83	0.59	0.40
32	31.0	8/24/54	42.00	16.50	0.47	0.40
33	36.5	8/24/54	22.00	24.12	0.44	0.46
41	39.0	9/10/54	-	26.42	0.64	0.68
42	45.0	9/10/54	127.75	23.39	0.68	0.60
43	51.0	9/10/54	198.83	70.00	0.62	0.60
44	60.0	9/23/54	29.37	23.61	0.40	0.28
45	72.0	9/23/54	51.00	46.63	0.41	0.28
46	80.0	9/23/54	132.75	26.41	0.41	0.41
47	91.0	10/19/54	40.65	8.05	0.47	0.40
48	103.0	10/19/54	36.25	32.25	0.56	0.44
49	115.0	10/19/54	35.43	8.25	0.48	0.46
		Average KD_{50}	59.85	36.46	0.53	0.45

Table 6. Pooled data of tables 2 and 4, plotted on Figure 3[1].

Site			Mean KD_{50} in hours			
Test Nos.	Mean location from Brookings (in miles)	Plotted graphically (in miles)	Lindane	Diazinon	DDT (corrected)	Check
39, 40	38.5 S.	1.0	0.45	0.42	23.38	36.86
29, 30	27.5 S.	12.0	0.50	0.37	20.77	28.58
18, 22, 23	18.9 S.	20.6	0.55	0.43	29.67	34.11
11, 12, 17	10.2 S.	29.2	0.45	0.30	23.78	30.00
2, 3, 4, 5, 6 (Brookings)	0.0	39.5	0.56	0.32	34.98	41.66
9, 14, 19	10.5 N.	50.0	0.44	0.32	25.75	39.70
20, 27, 28	24.6 N.	64.1	0.52	0.38	33.36	32.34
37, 38	43.1 N.	82.6	0.34	0.34	31.25	73.17

[1]In Figure 3, on following page, sites of test numbers 39 and 40 (Table 4) are treated as the first point at mile one; sites of test numbers 37 and 38 (Table 2) are treated as the last point at mile 82.6. Other points, as plotted in Figure 3, are represented by average values of grouped locations, as indicated above.

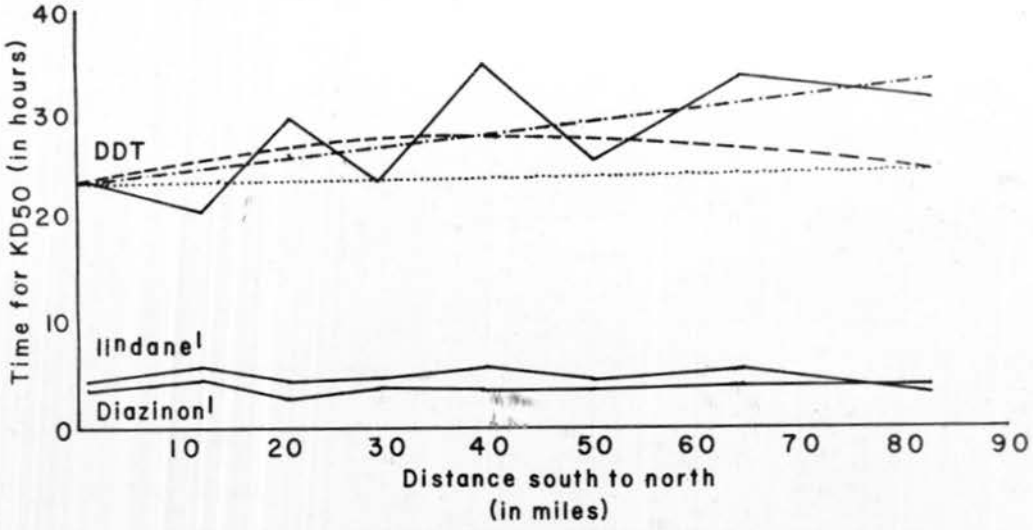

Figure 3. Pooled data on south-north axis (KD_{50} values corrected) for DDT, lindane and Diazinon, with regressions for DDT[1].

[1]Distances are measured from southern most point of collection to northern most point. Lindane and Diazinon plotted at ten times actual value. DDT represented by: actual values plotted ———— average of points -·-·-·-·- actual regression ---------- linear regression ••••••••••

Table 7. Pooled data of tables 3 and 5, plotted on Figure 4[1].

Test Nos.	Site: Mean location from Brookings (in miles)	Site: Plotted graphically (in miles)	Mean KD_{50} in hours: Lindane	Diazinon	DDT (corrected)	Check
34, 35, 36	38.5 E.	1.0	0.56	0.36	24.36	33.16
16, 24, 25	20.0 E.	19.5	0.51	0.43	21.80	33.98
7, 8, 13	5.5 E.	34.0	0.43	0.27	30.53	38.02
2, 3, 4, 5, 6 (Brookings)	0.0	39.5	0.56	0.32	34.98	41.66
10, 15, 26	10.6 W.	50.2	0.59	0.45	25.96	35.76
21, 31, 32	25.8 W.	65.3	0.52	0.40	18.75	38.81
33, 41, 42	40.2 W.	79.7	0.59	0.58	24.64	74.87
43, 44, 45	61.0 W.	100.5	0.48	0.39	46.74	93.06
46, 47	85.5 W.	125.0	0.44	0.41	17.23	86.70
48, 49	109.0 W.	148.5	0.52	0.45	20.25	35.84

[1]In Figure 4, on following page, sites of test numbers 34, 35 and 36 (Table 3) are treated as first point at mile one; sites of test numbers 48 and 49 (Table 5) are treated as the last point at mile 148.5. Other points as plotted in Figure 4 are represented by average values of grouped locations, as indicated above.

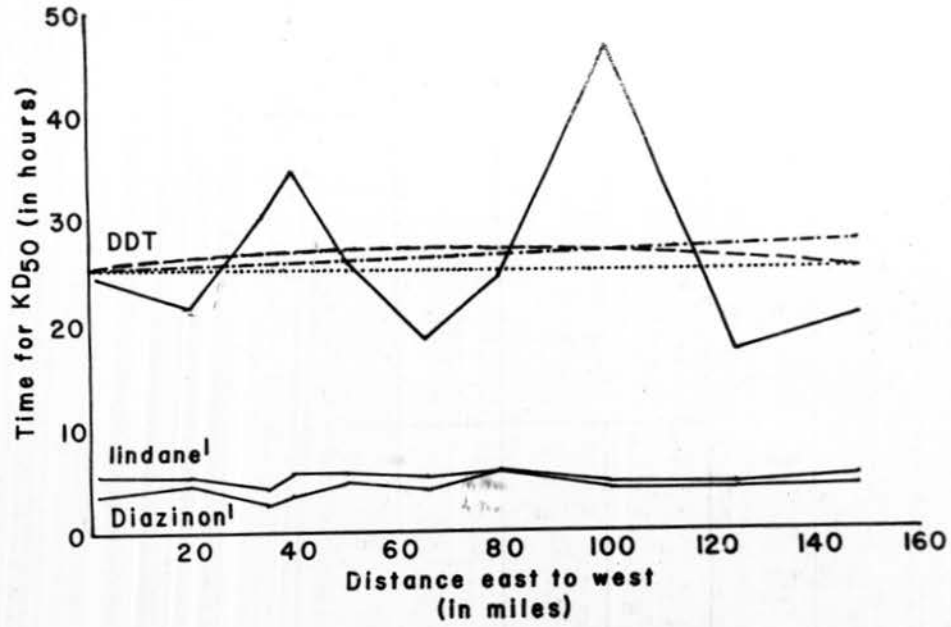

Figure 4. Pooled data on east-west axis (KD_{50} values corrected) for DDT, lindane and Diazinon, with regressions for DDT[1].

[1]Distances are measured from eastern most point of collection to western most point. Lindane and Diazinon plotted at ten times actual value. DDT represented by: actual values plotted ———— average of points -•-•-•-•-• actual regression ———— linear regression ••••••••••

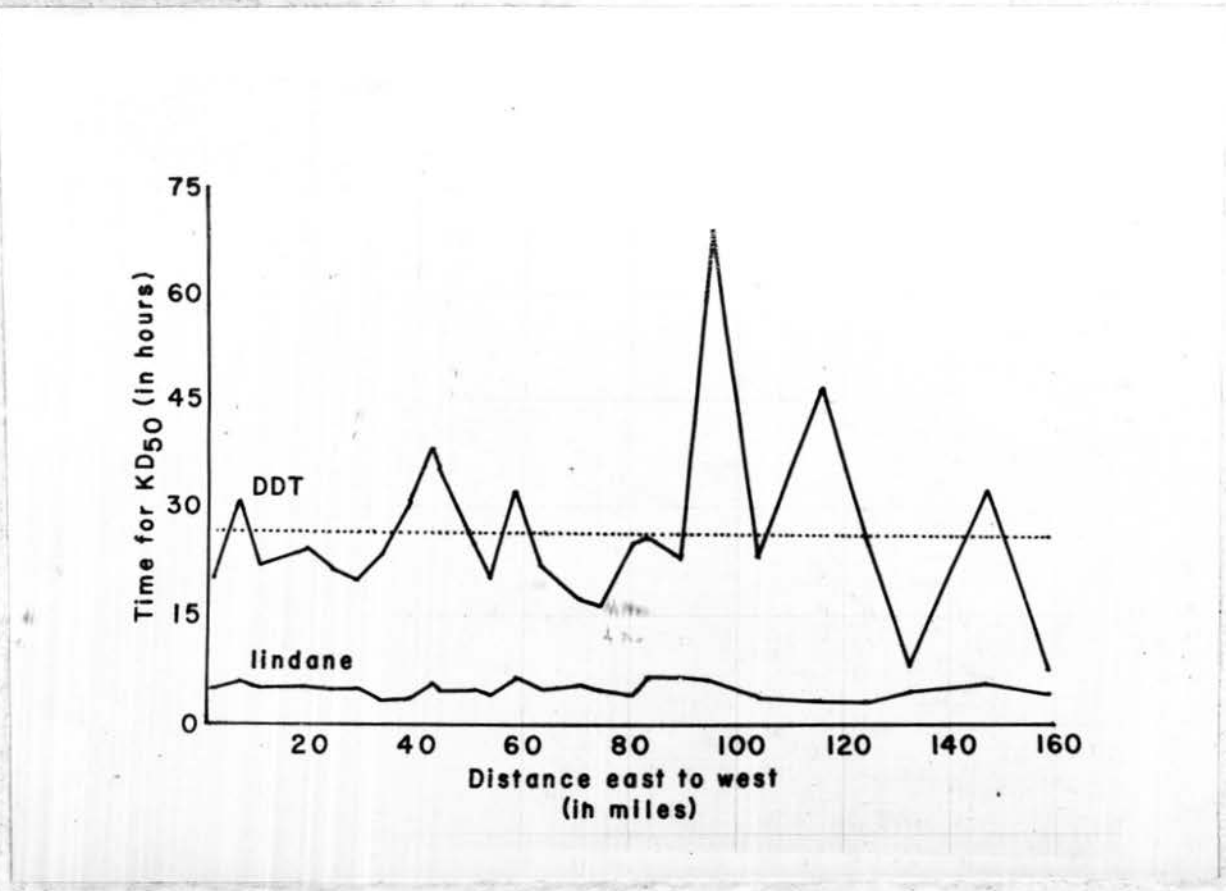

Figure 5. Data, inclusive of all points, on east-west axis (KD_{50} values corrected), for DDT and lindane, with regression for DDT[1].

[1]Distances are measured from eastern most point of collection to western most point. Lindane plotted at ten times actual value. DDT represented by: actual values plotted ———— linear regression ••••••••••

Table 8. Mean levels[1] of resistance of South Dakota strains as compared to published data of a laboratory strain at Riverside, California (9). Values of KD_{50} in minutes.

Treatment	South Dakota data		Riverside data	
	Conc.	KD_{50}	Conc.	KD_{50}
Check	- -	2690	- -	- -
DDT (uncorrected)	500 mg./sq. ft.	1568	100 mg./sq. ft.	91
DDT (corrected)	500 mg./sq. ft.	1665	100 mg./sq. ft.	91
Lindane	10 mg./sq. ft.	30	10 mg./sq. ft.	13
Diazinon	10 mg./sq. ft.	23	- -	- -

[1]Mean levels calculated from values recorded on Tables 1-5.

DISCUSSION

The amount of information revealed by the data was limited by the number of variable factors involved. Since the author was interested in the populations of houseflies as they appear in any locality, it was considered not necessary to rear the populations in the laboratory. Therefore, the conditions under which the tests were run varied from day to day and even within any one day.

Some of the variable factors involved include temperature and humidity to which the flies were subjected during their transportation to the laboratory and during testing. Through lack of facilities there was no attempt to maintain these factors constant. It was not considered necessary at the time to provide such facilities. The results may have been more easily analysed had all the flies been placed into a container, as soon as collected and held until set up for testing, at temperatures and humidities optimum for fly survival.

Other variables related to the previously mentioned factors, are distances that the flies were transported and length of time held in the containers. The length of time that flies were held in the containers varied as the distance from points of collection to Brookings varied, and as the length of time required for work preparatory to the setup varied. All the flies tested were placed into a refrigerator during testing preparations, but for varying lengths of time. Here, too, attempts to hold these factors constant may have affected the nature of the data.

Two other factors which may have had unknown effects on the data

were the amounts of carbon dioxide used in anesthesia and the number of times anesthesia was required for any one container of flies.

Generally, it is believed that any one variable would not seriously affect the data but a combination of all variable factors was responsible for the large variation in the data. The temperatures and humidities in the containers were generally the same as the atmospheric conditions which the flies were experiencing previous to collection. However, when collected, they may have been removed from some essentials to their welfare. It had been previously shown that holding the flies in the refrigerator for varying lengths of time did not seriously affect their viability. Nor did successive anesthesia by carbon dioxide seem to seriously affect their viability.

The degree to which any one variable affected the tests is unknown. To describe the variables in terms of correctional values is essentially impossible.

The use of acetone as a solvent may be questioned. Acetone is toxic to flies. However, the flies were never subjected to an acetone treatment. Acetone evaporates very rapidly and leaves little, if any, residue. In all tests, the filter papers saturated with acetone solutions were given sufficient time to allow complete evaporation of acetone.

The methods of statistical analysis used in analysing the data involved the standard analysis of variance and regression procedures as given by Goulden (7).

In the analysis of variance, however, the values for 50% mortality for each replicate in each test thus analysed, were transformed to their logarithmic functions. In this manner, each value retained it's individual variability but was lessened in magnitude. Also, to each value was

added one, so that in cases where values of less than one hour were encountered negative log functions would not be involved.

The results showed high reliability for differences between tests but low reliability between replicate differences. The error terms were consistently very low--indicating small test-replicate interaction.

In separating the treatment degrees of freedom into it's individual components, the comparisons which could be made were DDT test versus control, DDT and control versus lindane and Diazinon, and lindane versus Diazinon. The comparison of main interest was DDT versus control. However, when making this comparison, the analysis was set up using an error term derived only from the DDT-control interaction as if this were the complete test.

The results of this comparison gave the following indications: there was no significant difference between DDT test and control in flies taken from points north of Brookings. The same is true for flies taken from points of collection east and also south of Brookings. However, a significant difference appeared between DDT tests and control tests of flies taken from points of collection west of Brookings, starting at about the twenty-five mile point and continuing through to the most westerly point. Also, this difference became increasingly significant as the distance increased.

The appearance of the data indicated lowered resistance the farther west collections were made. To determine if this were true, if there was a substantial decrease in resistance as indicated, a regression procedure was used. This involved starting at the eastern most point of collection and included each point of collection from there to the western

most point of collection. Actual cumulative mileage intervals were used for ordinate values and the KD_{50} for DDT (corrected for natural mortality by Abbott's formula) was used for the abcissa value. Results of this method gave a positive regression coefficient, contrary to expectations based on the nature of the data. The coefficient was very low, however, being 0.000915, which when plotted was negligible in effect (Figure 5, p. 22).

Realizing that the high variability in KD_{50}s, as plotted on a graph and appearing in high peaks and low dips, may have given an erroneous coefficient of regression, another method was used. By this method, values of distance and KD_{50}s for two or three points were pooled, averaged and treated as a single point of collection. The regression coefficient in this case was negative in character and was of considerably higher value. In this case, it was -0.0186, which when plotted indicated a trend, favoring assumptions made from superficial examination of the data. (Figure 4, p. 21).

It is understood, however, that pooling and averaging values of several points may not give correct values, and may have introduced some errors, resulting in a coefficient of regression which is not altogether true.

A statistical significance for the regression coefficient was calculated by use of the 't' test. The calculated significance was very low as compared to theoretical 't' values at the 5% level of probability. However, the author feels that the slope of the line gives a fair indication of the actual trend in resistance, in support of previous observations.

It had been of interest to locate enclaves of susceptibility or resistance, or both, if they existed. Although, some high peaks and low dips, representing high and low KD_{50}s, at particular sites, appear on a plotted graph, to consider them as enclaves is out of the question. Again, the reason is because of the high variability in treated flies and the checks.

If there were enclaves of different levels of resistance, it would be expected they would appear in a large enough area to be represented at perhaps three points of collection, at least. The distance that flies may be expected to travel, either on their own or by means of vehicles, would probably include this many points or more. However, there were no cases which indicated an area which might represent such enclaves.

In plotting the KD_{50} values for lindane and Diazinon and their regressions, there appeared to be no direct relationship to those plotted for DDT. Whereas, the regression coefficient for Diazinon was very near zero, with a near zero correlation, those for lindane were subject to more wide variations. The regression for lindane did not coincide in proportion to that for DDT. This is contrary to expectations since resistance to lindane appears to increase as the resistance to DDT increases, at least to a certain point. In other words, the sign of the regression coefficient would be expected to be the same for DDT and lindane. The reason for this discrepancy is obscure. Perhaps this is further evidence that the levels of DDT resistance as plotted for particular points do not represent statistically valid levels of resistance.

That no areas of DDT susceptible houseflies were discovered in the

area covered by these data conforms with present knowledge of the genetics of resistance. Considering the widespread use of DDT for several years, from about 1947 until 1952, flies in all communities were probably subjected to this insecticide. Whether the fly populations were subjected to high selective pressure through intensive spray programs or by sparse and irregular programs, the same results have been effected. That is, apparently all fly populations have been subjected to a stimulus resulting in a DDT resistant manifestation.

The apparent universal resistance to DDT has probably resulted through several means. The first, and initial, being subjection to the insecticide, and subsequent development of resistance. The second being a subjection of susceptible flies to resistance through introduction of resistant strains into susceptible areas. The latter method, perhaps being much more important than what is generally attributed to it. One must consider that houseflies may travel, on their own, for a distance of at least thirteen miles (2). However, in a wind their distance of travel and dispersion is indefinite. Also, a large number of flies are carried in vehicles and on farm equipment, which also increases their dispersion to unknown distances.

A third means involves the basic nature of resistance. That resistance to DDT is genetical in nature has been demonstrated quite thoroughly. Because of the fact that resistance is due to genetical make-up, this quality is heritable from parents to offspring.

The nature of the genetic role in resistance indicates a highly integrated gene pool. With the high rate of reproduction, and inbreeding of fly populations, the gene pool is quickly integrated and high resistant

strains are rapidly produced. Through laboratory rearing, subjection of parental stock to selective pressures, and inbreeding of offspring, strains of flies with a tolerance for several hundred times the normal dosage for control of susceptible populations have resulted in ten to fifteen generations (13). That the resistant character of flies is not necessarily disadvantageous to their survival in normal environment is indicated by the length of time which that character is retained even after further subjection to the insecticide has been discontinued. Some reports show that only after many generations does the level of resistance decline significantly. Pimentel, Schwardt, and Dewey (13) reported complete reversion to a non-resistant condition after twenty generations of inbreeding without further exposure to DDT. However, Crow (5) found that his strain had not reverted, appreciably, to a susceptible condition after three years of inbreeding, without further exposure to DDT.

To consider the genetics of resistance further may be of importance. In laboratory procedures of rearing, testing and analysing (5, 8, 13) it was found that two different lines may respond differently to selection. Lines subjected to low levels of selective pressure responded more rapidly and consistently than lines subjected to higher levels. In any case, about a dozen generations (perhaps the number of generations produced in a year and one-half in the climate of the area in question) were required before resistance could be clearly demonstrated.

In crosses made between a resistant line and it's susceptible parental stock, and between two resistant lines, Pimentel (13) found that resistance was not sex-linked. Nor does it appear that there is simple dominance of either of the characters in question, since the F_1

generation of crosses between resistant and susceptible stock always displayed a level of resistance intermediate between the two.

Furthermore, crosses between two nearly equally resistant lines, the F_1 are always of about the same resistance. But when the F_1 are inbred, the F_2 show a sharp decline in resistance, which is again recovered in the F_3. An explanation of this unusual situation in the F_2, is that the two parental lines, whose resistant factors were brought together in the F_1, did not carry the same factors for resistance. Apparently, in the F_2, a segregation of the dissimilar factors occurred. This lends more weight to the indication of a highly integrated gene pool (5, 8).

It was also found that there are some susceptible strains which cannot develop resistance (12). They are genetically pure. In view of this, one would not expect to find any such susceptible strains since they would have been killed during the period of time that DDT was used to such a large extent.

Considering this knowledge of the genetics of resistance, the information taken from the data is not at all surprising. It is, in fact, quite in accordance with what one would expect. If a resistant population can be produced as quickly by exposure to low selective pressures as to high pressures, then at establishments where spray programs are erratic and not at all conducive to high selective pressures, one may well expect populations as resistant as in places where a well planned control program is carried on. One might expect also, that exposure to DDT only occasionally might maintain the level of resistance for long periods of time since a population will retain it's resistance

for more than a dozen generations without significant decline.

If this characteristic is not sex-linked, nor of simple dominance, then one would not expect to find a build-up of susceptible populations immediately after the removal of the selective agent. Instead, it would remain fairly uniform in level of resistance as well as in geographical distribution.

To more conclusively establish as facts the observations taken from the data, much more work would be required. More points of collection would be necessary, especially at greater distances in any direction. Points of collection, should also be established at intervals between the main directions of collection, i.e. to the north-east, south-east, south-west, and north-west.

With this plan of approach, and with necessary precautions to reduce, as much as possible, the variable factors encountered, data with increased reliability could be obtained.

SUMMARY

In the summer of 1954, forty-nine tests were run involving flies collected at that many points. The points of collection covered an area extending from forty-six miles north of Brookings, South Dakota, to forty-one miles south, and from forty-three miles east to one hundred fifteen miles west of Brookings. These tests were conducted in an attempt to learn more of the geographical distribution of housefly resistance. Included in the tests were three insecticides; DDT, lindane, and Diazinon, and control groups.

The unit of measure used to determine levels of resistance was the length of time required to reach a KD_{50} of the flies being tested.

The analysis of the data indicated a general, high level resistance by houseflies to DDT. A resistance to lindane appeared to be perhaps two to three times the level reported for a susceptible laboratory strain. No resistance to Diazinon was detected.

The data indicated a very slight decrease in resistance as the distance in the westerly direction increased. The reliability of this lowered resistance, however, could not be satisfactorily demonstrated.

No enclaves of highly resistant or DDT susceptible fly populations were detected.

A study of the genetic nature of resistance supplements and substantiates the information taken from the data.

There were many variable factors which could have affected the data, leaving the results highly variable and difficult to interpret.

To more satisfactorily reach a conclusion, many more tests involving

a larger area would be required. Elimination or reduction of the variable factors would allow for more uniform data making possible a more satisfactory analysis.

LITERATURE CITED

1. Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Jour. Econ. Ent. 18:265-7.

2. Bishopp, F. C., and Cornelius B. Philip. 1952. Carriers of human diseases. Insects, The Yearbook of Agriculture, USDA, 1952: 152.

3. Brown, A. W. A. 1951. Insect control by chemicals. New York, John Wiley and Sons, Inc. p. 740.

4. Communicable Disease Center, Public Health Service, U. S. Dept. of Health, Education and Welfare. 1957. Public Health pesticides. Pest Control, March 1957: 20-22.

5. Crow, James F. 1954. Analysis of a DDT-resistant strain of Drosophila. Jour. Econ. Ent. 47(3): 393-98.

6. Decker, G. C., and W. N. Bruce. 1952. Housefly resistance to chemicals. Amer. Jour. Trop. Med. and Hyg. 1(3): 395-403.

7. Goulden, Cyril H. 1956. Methods of statistical analysis. 2nd ed. New York, John Wiley and Sons, Inc. p. 102-107.

8. King, James C. 1954. The genetics of resistance to DDT in D. melanogaster. Jour. Econ. Ent. 47(3): 387-93.

9. March, Ralph B., and R. L. Metcalf. 1949. Laboratory and field studies of DDT resistant houseflies in southern California. Bull. Dept. Agr. Calif. XXXVIII(2): 93-101.

10. Melander, A. L. 1914. Can insects become resistant to sprays? Jour. Econ. Ent. 7(2): 167-72.

11. Missiroli, A. 1947. Resistant houseflies at Naples. Riv. di parasitol., 8:141-169. (from 3).

12. Norton, Robert J. 1953. Inheritance of DDT tolerance in the housefly. Contrib. Boyce Thompson Inst. 17(2): 105-126.

13. Pimentel, David, H. H. Schwardt, and J. E. Dewey. 1954. The inheritance of DDT-resistance in the housefly. Ann. Ent. Soc. Amer. 47(1): 208-13.

14. Rogoff, Wm. M. 1952. Some observations on insecticide tolerance in houseflies. Proc. S. D. Acad. Sci. XXXI:36-40.

15. Wiesmann, R. von. 1947. Resistant houseflies in northern Sweden. Schweiz. Ent. Gesell. Mitterl., 20:484-504. (from 3).