SUBLETHAL EFFECTS OF DDT RESIDUES ON THE CONFUSED FLOUR BEETLE, TRIBOLIUM CONFUSUM, DUV.

A Thesis

Presented to

the Faculty of Science

The University of Manitoba

In Partial Fulfillment

of the Requirements for the degree

Master of Science

---

by

Samuel Joseph Loshiavo

May 1949

TABLE OF CONTENTS

TABLE PAGE

LIST OF FIGURES

INTRODUCTION

Since the object of insecticide research is to kill insects, the usual criterion of insecticidal effectiveness is the mortality suffered by a group of test insects. However, mortality represents the gross physiological response of the insects to the insecticide and is merely the resultant end effect of a complex series of physiological changes. This preoccupation with mortality has tended to divert attention from the possibility of physiological effects other than death. The present study is an experimental investigation of the sublethal effects of insecticides. More specifically the study is concerned with whether or not D.D.T. applied as a residual poison can exert any physiological effect which is normally overlooked because of the preoccupation with induced mortality.

Residual insecticides are being used increasingly in insect control practices. In practice, many difficulties are encountered in attempts to apply uniform deposits of insecticidal sprays over an area to be treated. For example, part of the area to be sprayed may be inaccessible or the treatment may be spotty and haphazard. It is quite probable then, that many insects moving over such a treated surface may not receive a lethal dose of the insecticide. Or again, it is possible that some insects may not remain on a heavily treated area for a sufficient length of time to receive a lethal dose. In any case such insects must be regarded as having received sublethal dosages of the insecticide. The control of insects in the chemical treatments mentioned is usually assessed in terms of mortality, that is; total number killed as a direct result of exposure

to a lethal dosage. However, mortality data does not take into account those insects which have received only sublethal dosages. The question arises as to whether there are any effects less than lethal which can show that control by the use of insecticides is extended beyond that indicated by actual mortality. For example, if it can be shown that adults of the confused flour beetle exposed to sublethal dosages of D.D.T. lay significantly fewer eggs than normal adults, then the control of this insect is better than indicated by the mortality data from lots of test insects. If a D.D.T. residue kills 50 per cent of the insects exposed to it and the sublethal effect is such that the number of eggs deposited by the survivors was reduced by 50 per cent, then the total effect is equivalent to a 75 percent reduction in population.

On the other hand, if a test group of insects, chosen at random, is exposed to D.D.T. residue so that the more susceptible ones are killed off, then the survivors are, by this selective killing, a more resistant group. This more resistant group likely consists of hardy, vigorous, and physiologically sound insects. Furthermore, if the female survivors of this group lay eggs at their normal rate of oviposition, it is likely that the fecundity for such a select group would necessarily be higher than that for a control group containing both weak and strong females. In a control group of this kind, the high rate of oviposition of the hardy, healthy females would be masked by the lower fecundity of the weaker females. A finding such as the one described, where a residual insecticide selectively kills off the weak members of an insect population would not speak well for the use of residual insecticides as a means of insect control.

It is clear that a demonstration of the presence of physiological effects other than death is important in the field of insecticide research. Such effects may be manifested in a disturbance of the physiological function of the genitalia and consequently be reflected in the fecundity and fertility of treated insects. The possibility of two opposite effects as a result of exposing insects to sublethal dosages of D.D.T. has been suggested. The results of these exposures would be significant in either case, that is; where the rate of oviposition is either increased or decreased as a result of exposure. On the assumption that insects treated with sublethal dosages of D.D.T. do exhibit certain physiological effects which are deviations from the norm, the writer will propose to investigate these effects and discuss their significance.

Review of the Literature.

Although much is known about the effects of D.D.T. on various insects, there are relatively few references in the literature on the subject of the effects of sublethal dosages of D.D.T. Most work in the field of insecticides is chiefly concerned with the effectiveness of the insecticide as an insect killer. This effectiveness would be reflected in mortality data. Very few suggest the possibility of sublethal effect.

Recent reports have appeared in the literature which indicate that insecticidal treatment in sublethal dosages lowers the rate of oviposition of the survivors (11) (14). In 1946 Plumner and Baker (11) carried on a laboratory study of the effects of sublethal dosages of

tartar emetic on the Mexican fruit fly. Flies that ingested diets containing tartar emetic laid significantly fewer eggs than flies not given that materia. The percentage hatch of eggs was lower but the percentage of collapsed eggs was higher when tartar emetic was included in the diet. In 1941, Tenhet (14) tested the effect of sublethal dosages of pyrethrum on the oviposition of the cigarette beetle. Test insects in tobacco warehouses that were exposed to spray did not lay as many eggs as did unsprayed beetles. Adults were found to be resistant to pyrethrum. Knockdown was rapid but many recovered. Beetles which survived exposure to pyrethrum-oil spray consistently deposited fewer eggs than untreated beetles, the difference ranging from 25 to 68 per cent. On an average beetles not exposed to spray deposited more than twice as many eggs as surviving beetles that had been exposed. How sublethal dosages reduced oviposition is not known. It is known that pyrethrum tends to paralyze an insect's appendages. A partial paralysis often persists indefinitely, even though the insect may recover sufficiently to fly and crawl. Tenhet suggests that the failure to deposit eggs may have been due to paralysis or partial paralysis of the genitalia.

Kennedy in 1946 (14), working on the excitant and repellent effects on mosquitoes of sublethal contacts with D.D.T. made some interesting findings. As far as mosquitoes were concerned, once visible symptoms develop, death follows: recovery from an early stage of poisoning does not occur. He observed that mosquitoes exposed to sublethal dosages of D.D.T. showed all the symptoms up to, and including knockdown following

contact. Yet these affected mosquitoes recovered within 24 hours and lived in apparent health for a further 48 hours at least. Thus, in view of Kennedy's observations, the appearance of all the characteristic D.D.T. symptoms short of death is no sure indication that the affected mosquitoes are going to die.

Kennedy mentions some work done by Cragg (1945) who showed that sheep blow flies alighting on a D.D.T. treated fleece became so excited they could not oviposit. If they remain on long enough they obtain a lethal dose. The object of applying D.D.T. is to prevent oviposition and this object is achieved through the excitatory action of sublethal doses of D.D.T. Smallman (12) in 1948 found that cotton sacks treated with D.D.T. offered a high degree of protection for the enclosed flour against spider beetles. D.D.T. and certain related compounds act slowly to kill insects, and female spider beetles could have ample time to lay eggs before becoming affected through contact with the D.D.T. How, then, does the D.D.T. function in protecting the sacks? Smallman attributes the protection offered by the treated sacks to an excitatory repellent action of D.D.T. on the spider beetles. Smallman mentions the work of Thomson (1947) in which the author suggests that surfaces treated with D.D.T. have an irritant effect on anopheline mosquitoes preventing them from resting on treated surfaces long enough to absorb a lethal dose.

There are cases where insects have developed a resistance to D.D.T. due to selective breeding of survivors of treated insects.

Lindquist and Wilson (8) in 1948 carried out a series of tests resulting in the development of a strain of houseflies resistant to D.D.T. They used 14th generation flies and the results showed average mortality in several tests was 69 per cent for regular stock and 34 per cent for special stock. This special stock had been reared from the survivors of exposed flies 14 times. The data of these two workers showed that selective breeding produced a strain that was more resistant to D.D.T. spray than were the flies from the regular stock. The findings of Lindquist and his co-workers led other workers to carry out similar tests(1). Questions were raised as to whether or not some detrimental factor may have been instrumental in obtaining the results observed by Lindquist. Such a factor as deteriorated D.D.T. would lead to the false conclusion that the insects were developing a resistance. Therefore, the studies carried out after Lindquist's work were designed to confirm the results obtained by Lindquist. Laboratory and field studies showed that the primary cause for failure of D.D.T. residual sprays to accomplish satisfactory fly control was the development of fly resistance to D.D.T. and related materials. This resistance to D.D.T. has developed to such a magnitude in some of the fly strains collected that it was virtually impossible to obtain 100 per cent knockdown and kill of the flies by means of residual D.D.T. spray treatments.

In summary it may be stated that biological effects other than death have been observed by some investigators. Those studies which show that the sublethal effect is a reduction in the rate of oviposition

suggest that the insects were weakened by exposure to the insecticide. However, those studies which show that a resistance factor is built up, suggest a selection of stronger individuals and a demonstration of an "all or none" effect by the insecticide.

Although most of the literature on the subject deals with sublethal effects of certain insecticides, none of the investigators have designed their experiments to take into account the difference in sublethal effect between the males and females. The present study, therefore, was undertaken not only to demonstrate the effects of sublethal dosages of D.D.T. residues to adults of Tribolium confusum but also to disclose any difference between the sexes when test lots of this species were exposed to sublethal dosages. To demonstrate this difference, if any, an experimental design was employed which used all possible combinations of treatments and sexes.

EXPERIMENTAL PROCEDURE

Design of the Experiment.

The experiment was designed to determine whether exposure to D.D.T. had a sublethal effect on either or both sexes of Tribolium confusum which would be reflected in the fecundity and fertility of this insect. Reproduction is a sensitive indicator of any unfavorable factor in the environment of insects. The sublethal effect of insecticides on the female may be determined, therefore, by assessing the fecundity and fertility of treated females of Tribolium. Fecundity is a measure of

egg production or rate of oviposition and fertility a measure of the percentage of eggs, laid by treated females, which hatch into first instar larvae. The fecundity and fertility of treated females can be compared to the fecundity and fertility of untreated females thus affording a comparative method of measuring sublethal effects. However, the reproductive ability of the male may also be affected, and this effect can only be determined by pairing treated males and untreated females as well as untreated males and treated females. The experiment was designed to provide all combinations of sexes with treatments. Since differences due to treatment of adult beetles with uniform deposits of D.D.T. residue were expected to be small, adequate replication and a design suitable for statistical analysis was necessary in order to test the significance of observed differences.

The design of the experiment is indicated in Table I.

Four separate tests were made designated preliminary, A, B, and C. Certain variations and modifications in experimental procedure were introduced in each test for reasons which shall be discussed. Tests B and C are duplicates and therefore, their results are directly comparable. Each test is replicated four times for each combination of treatments and sexes.

Technique employed in the Preliminary Test to Attain 50 percent Mortality.

A preliminary test was carried out to determine the amount of D.D.T. deposit and the length of the exposure period necessary to kill approximately 50 per cent of the test insects. This percentage was arbi-

TABLE I

DESIGN OF ENTIRE INVESTIGATION

Combinations	Tests*			
	Preliminary	A	B	C
Treated males and treated females	♂ 30 30 / 30 30 (1 2 / 3 4) ♀ 30 30 / 30 30 (5 6 / 7 8)	♂ 30 30 / 30 30 ♀ 30 30 / 30 30	♂ 25 25 / 25 25 ♀ 25 25 / 25 25	♂ 25 25 / 25 25 ♀ 25 25 / 25 25
Treated males and untreated females	♂ 30 30 / 30 30 ♀ 30 30 / 30 30	♂ 30 30 / 30 30 ♀ 30 30 / 30 30	♂ 25 25 / 25 25 ♀ 25 25 / 25 25	♂ 25 25 / 25 25 ♀ 25 25 / 25 25
Untreated males and treated females	♂ 30 30 / 30 30 ♀ 30 30 / 30 30	♂ 30 30 / 30 30 ♀ 30 30 / 30 30	♂ 25 25 / 25 25 ♀ 25 25 / 25 25	♂ 25 25 / 25 25 ♀ 25 25 / 25 25
Untreated males and Untreated females	♂ 30 30 / 30 30 ♀ 30 30 / 30 30	♂ 30 30 / 30 30 ♀ 30 30 / 30 30	♂ 25 25 / 25 25 ♀ 25 25 / 25 25	♂ 25 25 / 25 25 ♀ 25 25 / 25 25

* Preliminary - 1% DDT-acetone vol. 4ml/4 plates, exp. 4 hrs. both sexes.
A - 1% DDT-acetone vol. ♂-4ml/4plates, ♀-5ml/4 plates, exp. ♂ 4 hrs, ♀-3hrs.
B - 2.5% DDT-oil, vol. 3ml/plate, exp. ♂ 3 hrs., ♀ 1 hr.
C - Same as for B.

TREATED PLATES - Shaded. UNTREATED PLATES - Clear.

trarily chosen as a convenient measure whereby the survivors could be examined for sublethal effect.

Prior to the preliminary test, random lots of test insects taken from the cultures of the Stored Product Insect Laboratory, Winnipeg, were exposed to residual deposits of D.D.T. on glass plates. Mortality was controlled by varying either the time of exposure or the concentration of D.D.T. Various concentrations and exposure periods were used in the tests to achieve the desired mortality of 50 per cent. After considerable experimentation, reasonable control over mortality was obtained. However, when sexed beetles were exposed to D.D.T. residues separately, as was done in the preliminary test, it was observed that the females suffered higher mortality than the males. This observation is shown in Table II. The sex of the dead beetles was determined by appying gentle pressure on the abdomen with a spatula causing extrusion of the genitalia. The presence of an ovipositor indicated a female. In this manner it was shown that most of the dead beetles were females.

The main object of the preliminary test was to secure 50 per cent mortality of both male and females of Tribolium when exposed to D.D.T. residues. In this test a 1 per cent solution of D.D.T. in acetone was used as the spray liquid. Acetone was adopted as the D.D.T. carrier on account of its high volatility and excellent solvent properties for D.D.T. Four milliliters of this solution was used to spray a set of four glass plates. Both sexes were exposed to a D.D.T. deposit on the plates for the same length of time, namely, 4 hours. The reason for exposing test lots of both sexes to

equal dosages of D.D.T. residue for equal lengths of time was to keep the conditions of treatment equal. This procedure led to the results indicated in Table II.

The higher mortality of the females and the difficulty of inducing higher mortality of males made it necessary to introduce modifications and variations in the experimental procedure. Concentrations of D.D.T. that killed 50 per cent of the females would not kill 50 per cent of the males. Concentrations which did kill 50 per cent of the males killed 95 per cent or more of the females. Hence, changes in technique was necessary to bring the mortality of both sexes closer to 50 per cent. The variations and modifications in experimental technique included the following changes:

1. formulation of D.D.T.
2. concentration of D.D.T.
3. volume of D.D.T.
4. length of the exposure period for males and females.

These changes were introduced in Experiments A, B, and C as indicated in Table I.

Techniques Employed in Experiments A, B and C to Attain 50 Per Cent Mortality.

In Experiment A the period of exposure was 3 hours for females and 4 hours for males. The exposure time was decreased in an effort to reduce the per cent mortality of females from 80 per cent to approximately 50 per cent. The volume of D.D.T. solution sprayed on the targets on which males were to be exposed was increased from 4 ml. to 5 ml. These modifications brought about some improvement as evidenced by the lower mortality of females in Experiment A. However, the mortality of males was still consider-

TABLE III

LEVEL OF INDUCED MORTALITY, AND MORTALITY DURING TEST PERIOD. EXPERIMENT A.

Treatment	Induced Mortality, %		Number of original pairs	Mortality during test, %		
	Males	Females		Males	Females	Total
Both Sexes	10.0	23.3	23	0	0	0
	6.6	36.6	19	0	3.3	3.3
	10.0	50.0	15	0	0	0
	6.6	53.3	14	0	0	0
Mean	8.3	40.8	17.7	0		
Males Only	20.0	0	24	3.3	0	3.3
	23.3	0	23	0	6.6	6.6
	16.6	0	25	0	0	0
	16.6	3.3	25	0	3.3	3.3
Mean	19.1	0.8	24.2			
Females Only	3.3	30.0	21	0	0	0
	0	50.0	15	3.3	0	3.3
	0	63.3	11	0	0	0
	0	46.6	16	0	0	0
Mean	0.8	47.5	15.7			
Neither Sex	3.3	3.3	29	0	3.3	3.3
	0	3.3	29	3.3	0	3.3
	0	3.3	29	0	0	0
	0	0	30	0	3.3	3.3
Mean	0.8	2.5	29.3			

TABLE IV

LEVEL OF INDUCED MORTALITY, AND MORTALITY DURING TEST PERIOD. EXPERIMENT B.

Treatment	Induced Mortality, %		Number of original pairs	Mortality during Test, %.		
	Males	Females		Males	Females	Total
Both Sexes	56	36	11	0	0	0
	52	44	12	3	2	5
	60	36	10	0	2	2
	40	52	12	1	0	1
Mean	52	42				
Males Only	44	0	14	1	0	1
	40	0	15	0	1	1
	64	0	9	1	0	1
	36	0	16	0	0	0
Mean	46	0				
Females Only	0	48	13	1	0	1
	0	52	12	2	0	2
	0	44	14	0	0	0
	0	44	14	0	1	1
Mean	0	47				
Neither Sex	0	0	13*	0	0	0
	0	0	13*	1	2	3
	0	0	13*	0	0	0
	0	0	13*	1	0	1
Mean	0	0				

* half the numbers of pairs of controls used

TABLE V

LEVEL OF INDUCED MORTALITY, AND MORTALITY DURING TEST PERIOD. EXPERIMENT C.

Treatment	Induced Mortality, %		Number of original pairs	Mortality during test, %.		
	Males	Females		Males	Females	Total
Both Sexes	48	52	12	2	0	2
	40	56	11	1	1	2
	36	60	10	0	0	0
	32	52	12	0	1	1
Mean	39	55				
Males Only	44	0	14	1	0	1
	36	0	16	0	0	0
	60	4	10	1	0	1
	36	0	16	0	0	0
Mean	44	1				
Females only	0	48	13	1	0	1
	0	68	8	0	0	0
	0	52	12	0	0	0
	0	52	12	1	1	2
Mean	0	55				
Neither Sex	0	0	13*	0	0	0
	0	0	13*	1	2	3
	0	0	13*	3	3	6
	0	0	13*	1	0	1
Mean	0	0				

* half the number of controls used

ably less than the required 50 per cent. The mortality data for Experiment A is presented in Table III.

In Experiments B and C further changes in technique were employed. These changes included the use of a different D.D.T. formulation, a greatly increased concentration of D.D.T. and further variation in the length of the exposure period for the males and females. The new formulation of D.D.T. used was a 2.5 per cent solution of D.D.T. in number 9 Imperial refined oil (deodorized kerosene). The solvent was changed from acetone to refined oil for the following reasons:

1. Rapid volatilization of the acetone as it left the spray nozzle may have resulted in the suspension of D.D.T. particles in the air, and prevented maximum deposition on the target.
2. An oil solvent is commonly used for practical control work.

An increased concentration of D.D.T. was necessary to induce higher mortality of males. Further, increasing the concentration made it possible to decrease the period of exposure. Decreasing the period of exposure was considered a desirable feature. During too lengthy an exposure some insects may move about more actively than others and consequently absorb more D.D.T. than the less active ones. The 2.5 per cent solution of D.D.T. in oil was satisfactory in inducing mortality approximately close to 50 per cent for both sexes. In Experiments B and C each target to be treated was sprayed with 3 ml. of the D.D.T. - oil solution. The females were exposed for one hour and the males for three hours. These two duplicate experiments B and C are considered to provide the best measure of

sublethal effects since the induced mortality was more uniform between sexes and within test groups of the same sex. The mortality data for Experiments B and C are presented in Tables IV and V respectively.

In all four experiments the test lots of untreated insects were exposed to targets sprayed with the solvent only.

Preparation of para para D.D.T.

Technical D.D.T. is composed of a group of isomers of which the para para isomer shows the greatest insecticidal activity. Pure para para D.D.T. was obtained by the purification of technical grade D.D.T. after the method of Cristol, Hayes and Haller (3) by refluxing with 75 per cent ethanol. The hot solution thus obtained was filtered through a Buchner funnel which retained all mechanical impurities. Upon cooling, the filtrate recrystallized into para para D.D.T. while the other isomers remained in solution in the alcohol. After five separate refluxings and five recrystallizations the pure para para isomer with a melting point of 105 - 107 degrees C. was obtained.

The amount of D.D.T. prepared proved insufficient for all phases of the work so a sample of pure para para D.D.T., melting point, 105 - 107 degrees C. was procured through the courtesy of a commercial chemical concern.*

Handling the Insect Material before Exposure

Tribolium confusum adults were reared from pupae under carefully

* Naugatuck Chemicals Inc.

controlled conditions of temperature and humidity. The sex of the beetles was determined by the method described by Park (10). The only reliable external sexual characteristic for any stage is found in the pupal stage. The pupae were placed, ventral surface up, on the stage of a binocular microscope and examined under low power. On the ventral surface of the posterior terminal segment, the female has a pair of small appendages appearing very much like miniature mammary glands. These appendages are reduced to indistinct elevations in the male. The insects used in this study were separated into males and females on the basis of this characteristic anatomical difference. The pupae of each sex were reared separately in large pyrex baking dishes containing a standard food medium made up of 95% whole wheat flour sifted twice through a number 60 wire mesh screen and 5 per cent finely powdered brewer's yeast by weight (9). The yeast was thoroughly mixed through the flour so that all parts of the mixture were the same from the standpoint of nutritional value. Only the sifted flour was used in making up the food medium. The purpose in separating the bran from the flour and using only the sifted portion was to facilitate the separation of eggs and first instar larvae from the flour. It is obvious that the presence of bran would make the task of separating the eggs or tiny larvae virtually impossible.

Experimental conditions of temperature, humidity and age of insects were controlled as closely as possible. The temperature and relative humidity were maintained at 28.5 degrees C. and 70 - 75 per cent respectively (9) (10). Temperature has a marked effect upon the number of eggs

laid, whereas, relative humidity has very little effect on oviposition.(4) Increasing the temperature causes increased egg production. Temperatures higher than 34 degrees C. cause a marked decrease in egg production and at about 38 degrees C. mortality of the adult beetles is high. Those pupae which emerged over a 4-day period were retained and used in the study. This procedure ensured that adults of as nearly constant age as possible were used in the study. Females of Tribolium confusum begin to lay eggs at a reasonably uniform rate about 3 weeks after emergence and continue at this level of egg production for about two months. Therefore, about three weeks after emergence, the young adults were prepared for exposure to D.D.T.

Two other factors that could conceivably improve the uniformity of response to the D.D.T. residue are - prior starvation and exposure at constant temperature. Following the finding of Tattersfield (13) that more uniform response is obtained after a period of starvation, the test insects for Experiments B and C were starved for 24 hours before exposure. In the preliminary test and experiment A the insects were exposed to D.D.T. at room temperature but in Experiments B and C, the exposure was carried out at 28.5 degrees C in the constant temperature cabinet. Under these conditions and that of increased concentration the response of the test insects to the D.D.T. deposit was more uniform as indicated in Tables IV and V.

Handling the Insect Material After Exposure.

After the various test lots of insects were exposed to the residual deposits of D.D.T. they were carefully introduced into wide-mouthed

glass jars containing equal volumes of the standard food material. Each test lot was placed in a numbered jar. The treated insects were kept in the jars for a certain period of time during which mortality of some of the exposed insects was induced. This period of time was 8 days for Experiment A and 7 days for Experiments B and C. It was previously noted that after the sixth day any further mortality was negligible; this being the case, it was felt that 7 days was sufficient for insects to succumb to lethal doses of D.D.T. The sex of beetles which died during the course of the experiment was determined by gently squeezing the abdomen of the dead insect and examining the extruded genitalia. Knowledge of the exact number of males and females dead at any given time was essential for the calculation of rate of oviposition.

After the exposed insects had been allowed to remain in the glass jars for the required 7 or 8 days, the male and female survivors were paired according to the design indicated in Table I. For example, the survivors of replicate lot 1 (males) were paired with an equal number of survivors from replicate lot 5 (females) or vice versa, according to which sex suffered the greatest mortality. The paired lots were placed in the wide-mouthed glass jars each containing an equal volume of the standard food medium. It is obvious that the number of pairs is governed by the replicate lot showing the higher mortality. The replicate groups combined as follows: 1 and 5, 2 and 6, 3 and 7, 4 and 8, 9 and 13, etc. This uniform method of pairing was adopted in each of the four experiments. Since each combination of sexes and treatments

was replicated four times (Table I) and there are four such combinations, a total of 16 jars was required for each individual experiment. The four experiments of the whole study, therefore, required a total of 64 jars. Proper ventilation for the insects confined in the oviposition jars was ensured by means of holes 1½ inches in diameter drilled out of the metal tops of the jars. These holes were securely covered with 60 mesh wire screening to prevent the escape of any of the insects during their confinement in the jars. The oviposition jars were kept in a constant temperature cabinet at 28.5 degrees C. and 75% relative humidity. At periodic intervals of 5 days the contents of each jar was sieved through a 20 mesh wire screen which retained the adults, and a 48 mesh wire screen which retained the eggs. The eggs retained by this screen were gently removed on to a black surface. The white eggs contrasted against the dark background facilitated counting. The eggs were counted visually with the aid of a hand tally. The eggs were recorded as number of eggs per female per day. The determinations for numbers of eggs were made for 2 - day periods.

In some experiments (10), no attempts are made to eliminate cannibalism: the rates, therefore, are not, strictly speaking, absolute fecundity rates but measure the eggs remaining in the population after some have been eaten. In the present study the reason for taking 2-day counts rather than weekly counts was to reduce this factor of cannibalism as much as possible without disturbing the adults too much. Thus, after each interval of 5 days, the contents of each jar was sieved as described and the eggs laid during the 5 day period were discarded. The adults were

gently brushed out of the screen. Then the sieved food, free of eggs, was replaced in the respective jars and the adults added. The addition of adults to flour rather than flour to adults was an insurance against injuring the adult beetles. After the adults were added to the jars they were allowed to remain undisturbed in the temperature cabinet for two days; at the end of this time the eggs in each jar were separated from the food medium and counted. These were recorded. This procedure whereby the determinations were made at five-day intervals ensured minimum disturbance of the adults.

In all sifting and transferring operations care was taken to treat the insects gently so that they were not damaged. It was essential to treat the insects gently during handling so that their physiology was disturbed as little as possible. Injury to the insects might be reflected in the rate of oviposition and lead to erroneous conclusions. It has been shown that shaking adversely affects oviposition (10). In this study, however, this disturbance factor is of no consequence because the insects are left undisturbed during the two-day period in which the recorded eggs are laid.

The eggs obtained in each determination were retained for further observations on viability. These eggs were placed in new jars designated with numbers corresponding to those on the oviposition jars whence came the eggs.

Each of these new jars contained 50 ml. of the standard food material. These jars were placed in the temperature cabinet for a period

of ten days during which the eggs were allowed to hatch into larvae. At the end of the ten-day period the contents of each jar was sieved through a 60 mesh screen to separate the larvae from the food. The larvae were brushed out of the sieve on to a large sheet of white paper. This sheet was gently tapped so that all debris and unhatched eggs fell readily into a receptacle leaving the larvae adhering to the paper. The white background caused the yellowish larvae to stand out clearly and in this manner were easily counted. The debris collected in the receptacle was re-examined several times to ensure that all the larvae were accounted for. The number of larvae obtained from each jar was recorded as per cent viability.

The D.D.T. Deposit

The study was designed so that adults of Tribolium confusum would receive a sublethal dosage of D.D.T. when exposed to a dry D.D.T. deposit. The purified para para D.D.T. was sprayed on a surface so that a dry D.D.T. residue remained after the solvent evaporated. Mention has already been made of the two types of D.D.T. solutions used in this study, namely, a 1 per cent solution of D.D.T. in acetone and a 2.5 per cent solution of D.D.T. in number 9 Imperial refined oil (deodorized kerosene).

After obtaining a suitable solution for spraying, it was necessary next to find a suitable surface which could be sprayed with the solution and which would not absorb any of the solution. The reason for using a non-absorptive surface like glass is that the effectiveness

of residual sprays decreases with the increase in the absorptiveness of the surface sprayed (2). Glass was selected because it possessed this non-absorptive property. Moreover a surface was required to provide suitable footing for the insects, it was important that the insects remain on their feet throughout the period of exposure so that they could be exposed equally on the treated surfaces. Fine sand-blasted glass provided an excellent surface upon which the insects walked without losing their footing. Several sand-blasted glass plates, each four inches square, were made up and used as surfaces on which the D.D.T. spray was deposited and the test insects were exposed.

The spray tower and atomizing nozzle (designed after Hewlett (6)), Fig. 1(a) used at the Stored Product Insect Laboratory, Winnipeg, Manitoba, was used to spray the glass plates with the D.D.T. solution. Several preliminary trials with the spray tower were made to test the replicability of deposit and uniformity of deposit. In these trials four plates were sprayed in one operation. A D.D.T.-dye-acetone solution was used when the ratio of D.D.T. to dye was 10 : 1. The plates were washed off with a fixed volume of acetone and samples of these washings were measured in a colorimeter. The results of the trials as represented by colorimeter readings are indicated in Table XII. The nozzle was adjusted to deliver a fine spray. Air pressure equivalent to 150 mm. Hg. was used in all spraying operations. In the preliminary test and in Experiment A, four of the 4" by 4" glass plates were sprayed at one operation.

Certain experiments are now underway at the Stored Product

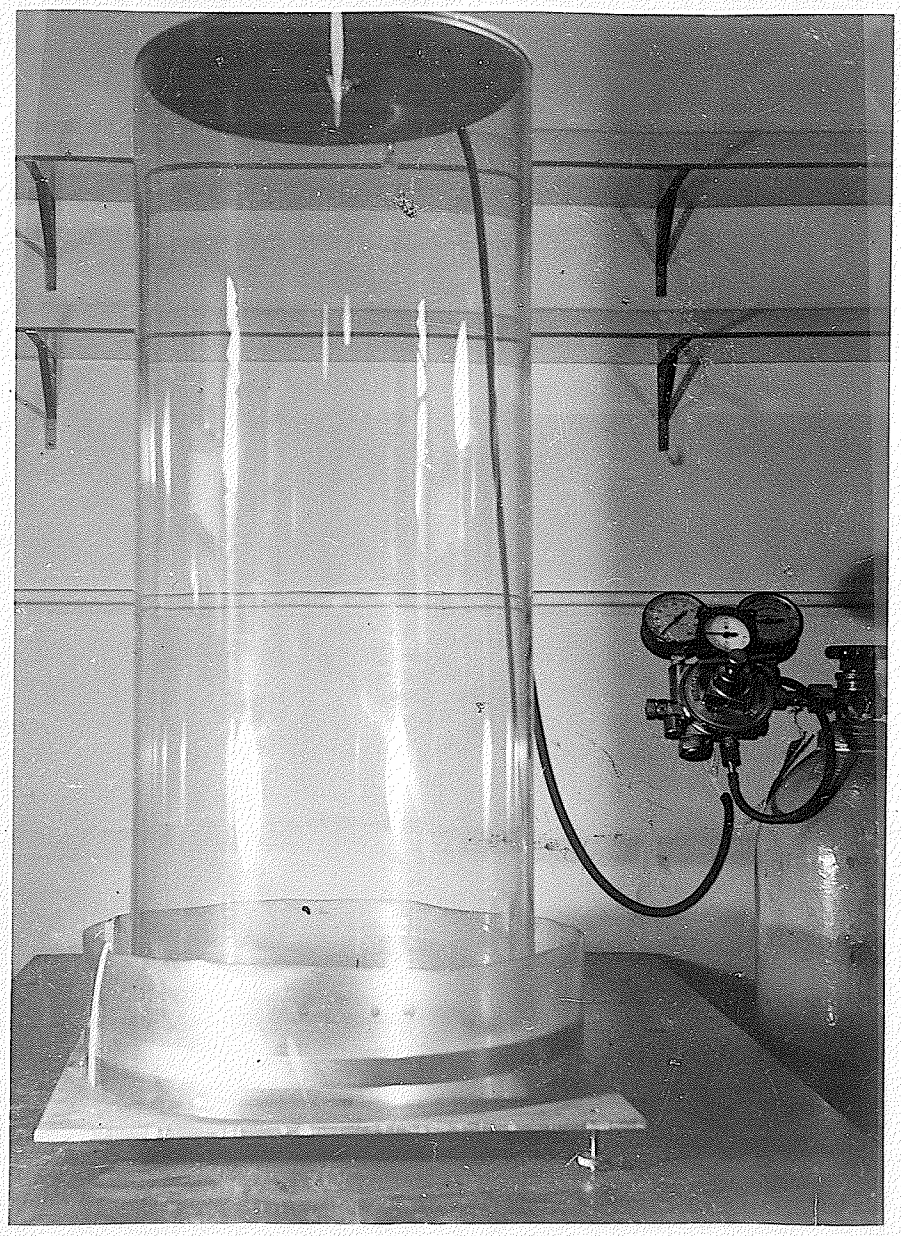

Fig. I(a) - The spray tower assembly.

Insect Laboratory to test the replicability and uniformity of deposit laid down by the spray tower apparatus. Preliminary results seem to indicate that a slightly more uniform deposit is laid down closer to the center of the floor of the spray tower. On the strength of the results of these preliminary tests a variation in spraying technique was introduced in Experiment B. Instead of spraying four plates at one operation, each plate was sprayed separately but was placed in the exact center of the floor of the spray tower prior to being sprayed. Three ml. of solution were sprayed upon each plate. At the end of each spraying operation the plates were left in the tower for an additional five minutes so that any suspended particles of D.D.T. could settle on the plates.

The same plates, without respraying, were used in Experiment C. It was considered unnecessary to respray them since the amount of D.D.T. deposit removed by the insects in Experiment B was extremely small. Cotton (2) has found that D.D.T. residual sprays in all formulations remain effective on glass surfaces for more than a year. Since the sprayed plates used in Experiment B were used again for Experiment C within 5 days, it is probable that no loss in effectiveness occurred in this interval. The insects used in Experiments B and C, therefore, were exposed to dosages of equal concentration and effectiveness.

It should be mentioned that the efficiency of a residual spray deposit is reduced when it becomes coated with dust (2). For this reason precautions were taken to prevent the sprayed glass surfaces from becoming

coated with flour dust prior to and during the exposure period.

Measurement of the D.D.T. Deposit.

It was considered important to know the amount of D.D.T. deposited on the plates and the uniformity of deposit. The amount laid down was measured by colorimetric means according to the following method. A dye of the anthroquinone type was incorporated with the D.D.T. acetone solution used in the preliminary test. The 100 ml. of acetone in the stock solution dissolved completely 0.1 gm. of the dye so that the ratio of D.D.T. to dye was 10 : 1. Equal volumes of the dye solution were sprayed on several replicated sets of plates. The dry D.D.T.-dye residue which remained upon the plates after the acetone had evaporated was removed by washing each plate with 20 ml. of acetone. A sample of this solution was measured in a colorimeter. The galvanometer readings taken directly from an arbitrary scale are indicated in Table XII. The closeness of the results within replicates and between replicates as shown by two separate tests indicate that almost identical amounts of D.D.T. are laid down on each plate during any spraying operation under identical conditions.

The actual weight of dye in the acetone washings from each plate was calculated from a standard curve (Fig. 1). This curve was determined as follows: A range of 6 acetone-dye solutions of known concentrations were made up and measured in the colorimeter used at the Stored Product Insect Laboratory. The galvanometer readings observed on the arbitrary scale were converted to figures based on 100. By means of a

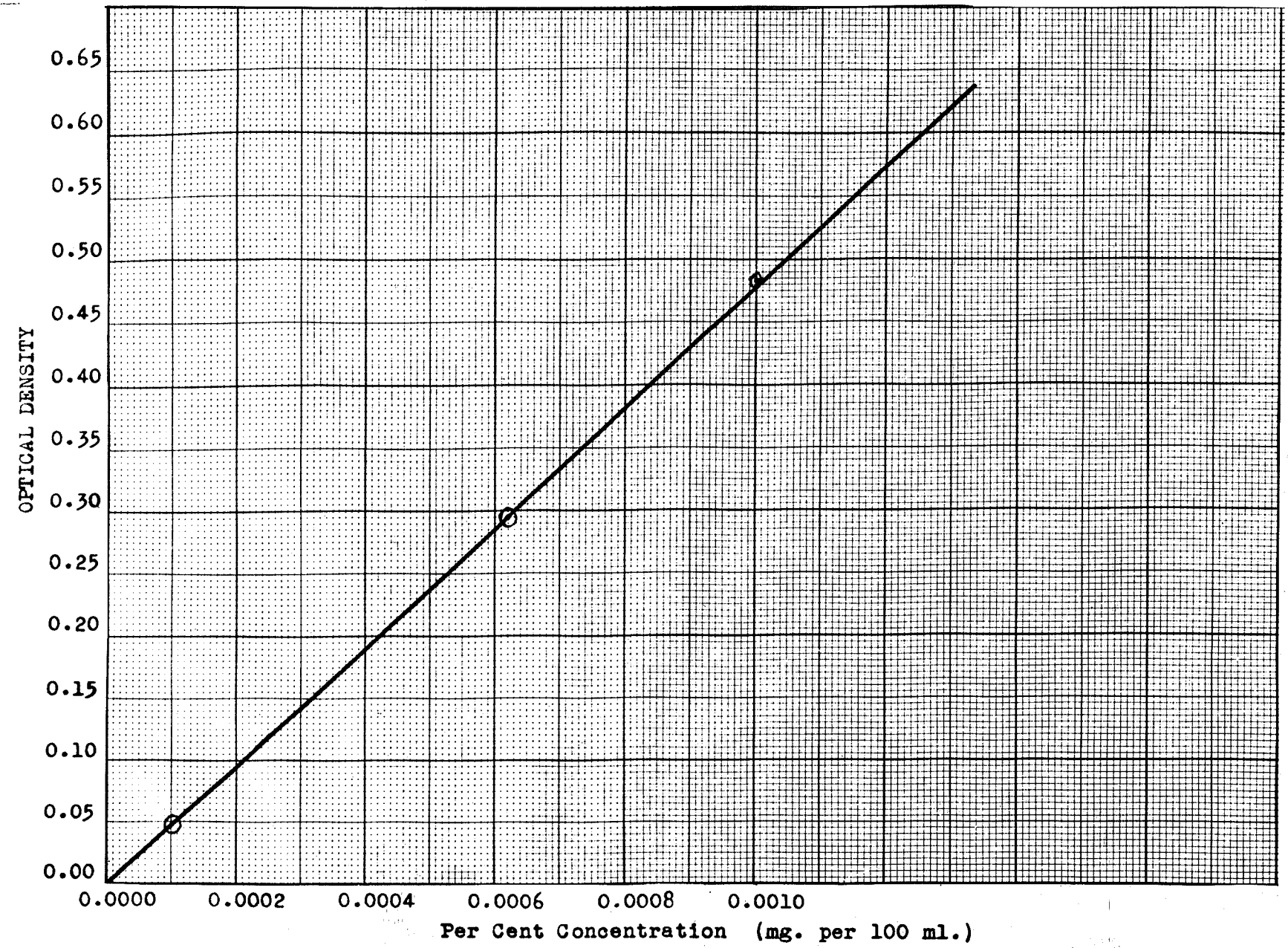
OPTICAL DENSITY
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.0000
0.0002
0.0004
0.0006
0.0008
0.0010
Per Cent Concentration (mg. per 100 ml.)

TABLE XII

UNIFORMITY AND REPLICABILITY OF DDT DEPOSIT ON GLASS PLATES

Numbers of plates in sets of four replicates	SCALE READINGS	
	Trial I	Trial II
1	24.2	24.6
2	23.0	23.8
3	23.5	24.3
4	23.5	25.5
Mean	23.5	24.5
5	22.9	22.9
6	23.1	22.8
7	23.1	22.9
8	22.8	22.8
Mean	23.0	22.8
9	24.6	22.7
10	24.4	22.8
11	24.4	22.3
12	24.4	21.8
Mean	24.4	22.4
21	23.6	22.5
22	23.6	22.1
23	24.1	21.9
24	23.7	22.4
Mean	23.7	22.2
Total Mean for plates	23.6	23.0

table showing density (L = 2-log G) against the galvanometer reading G the figures based on 100 were converted to figures for optical density. The calculations for the standard curve are presented in Table XIII. Optical density was plotted against concentration and a straight-line relationship was established. Using this curve it was possible to measure the concentrations of dye in the solutions made up of acetone and the residues washed from each treated plate. Since the D.D.T. and dye were present in the ratio of 10 : 1, the amount of D.D.T. deposit could easily be calculated.

Method of Exposing the Insects to the D.D.T. Deposit.

Technique I was used in the preliminary experiment and in Experiment A. When glass plates were chosen as areas of exposure the problem arose as to how to confine the insects to the plates for the desired length of time. Two devices were used in combination for this purpose, namely, plastic rings and strong light.

The rings were 4 inches in diameter and approximately $\frac{1}{2}$ inch in height. One was placed on each of the treated glass plates creating in this way a suitable enclosure for the insects. To prevent the insects from climbing the inner wall of the rings and falling on their backs, the lower inner edge of each ring was cut away at an angle, the cut beginning about one sixteenth inch from the lower inner edge and deflecting downwards towards the outer edge.

The second device used to keep the insects on the treated surfaces was strong light since Tribolium confusum is negatively phototropic.

TABLE XIII

CALCULATIONS FOR STANDARD CURVE

Concentration	Scale Reading (Converted)	Density (L = 2-log G) against Galvanometer reading G
0.00250	4.6	-
0.00100	32.9	0.482
0.00062	50.6	0.297
0.00010	98.7	0.0480
0.00000	100.0	0.0000

The procedure was as follows: The treated plates were placed on a large translucent glass surface provided in the laboratory. Two photo-flood lamps were placed below this surface but near the floor. A circle of black paper cut about $\frac{1}{4}$ inch smaller than the plastic rings was placed under each sprayed plate and arranged so that the centre of the plate was exactly over the centre of the paper. When the plastic ring was placed over the plate a circular margin $\frac{1}{4}$ inch in width was evident between the ring and the edge of the paper. When the two lamps were switched on this paper effectively blocked the light rays thus providing a dark island surrounded by a sea of light. The corona of light around the rim of each black circle of paper was so intense that insects venturing to the edge were immediately repelled and forced to turn back. This device seemed to be satisfactory.

The heat generated by the lamps during the period of exposure was dissipated by a fan. This precaution was necessary to prevent the temperature from rising above 35 degrees C. above which mortality becomes pronounced. After exposure the insects were handled as described in the section "Handling the insect material after Exposure."

Technique II.

This technique was used in Experiments B and C. The advisability of using strong light as in Technique I was questionable because photodecomposition of the D.D.T. residue may have occurred. Also, according to Cotton (2) light affects the duration of effectiveness of residual spray deposits. These possibilities precluded the use of strong light in the exposure technique for Experiments B and C. Hitherto, the insects were

exposed at room temperature but in this technique they were exposed at a constant temperature of 28.5 degrees C. Following exposure the insects were handled according to the method described under the heading "Handling the insect material after exposure".

In both techniques no attempt was made to investigate the necessity of movement of insects over the treated surface during exposure. Is the activity of insects important in governing the amount of D.D.T. absorbed by the insects? Hamman (5) placed mobile and non-mobile insects on identical D.D.T.-treated surfaces for definite periods of time. The rate of paralysis for mobile and immobile roaches was practically identical and Hamman concludes that roaches pick up some of the D.D.T. while lying on it. In view of his results, the activity of the insects used in this study was not restricted during exposure.

RESULTS

The results of the experiments are presented in tabular form. The data for induced mortality in the preliminary test, and Experiments A, B and C are shown in Tables II, III, IV and V respectively. Table II shows that the mortality of the females is considerably higher than that of the males when both sexes are exposed to equal amounts of D.D.T. residues for equal lengths of time. In Experiment A the period of exposure for the females was shortened and the amount of D.D.T. for the males was increased as indicated in Table I. However, results similar to those of the preliminary test were obtained, namely; that females were killed much more readily than males as a result of exposure to residual deposits of

TABLE VI

OVIPOSITION OF TRIBOLIUM AFTER EXPOSURE TO SUBLETHAL DOSAGES OF DDT. TEST A.
(number of eggs /female/ day over 2-day periods)

Period after exposure, days	Both Sexes treated	Males treated	Females treated	Neither Sex treated
9	8.1 5.7 6.9 8.3	5.2 6.1 7.2 6.6	7.3 6.7 7.0 8.2	6.1 7.0 7.0 7.7
Mean for period	7.2	6.3	7.3	6.9
15	9.0 6.4 7.9 7.9	6.9 5.8 7.3 5.3	6.0 6.0 7.8 6.0	6.3 6.4 5.1 7.3
Mean for period	7.8	6.3	6.4	6.2
21	5.8 3.9 6.9 6.9	3.4 6.6 4.9 5.3	5.8 5.1 5.4 6.0	4.6 5.5 5.4 4.6
Mean for period	5.9	5.0	5.6	5.0
27	5.0 3.0 5.8 6.0	2.8 4.0 5.0 4.7	5.6 4.0 6.6 5.4	3.5 4.7 3.0 4.6
Mean for period	4.9	4.1	5.4	3.9
33	1.1 1.2 2.3 1.5	0.3 0.3 0.8 0.5	1.3 1.4 2.5 2.0	0.1 0.8 0.3 0.5
Mean for period	1.5	0.5	1.8	0.4
39	5.1 3.8 5.1 3.9	3.4 3.3 2.8 2.0	4.3 5.1 5.4 4.6	3.3 3.7 2.2 2.5
Mean for period	4.5	2.9	4.8	2.9
Mean for total period	5.3	4.2	5.2	4.2

Females treated vs. Males treated $P < 0.05$

Females treated vs. Neither Sex treated $P < 0.05$

TABLE VII

OVIPOSITION OF TRIBOLIUM AFTER EXPOSURE TO SUBLETHAL DOSAGES OF DDT. TEST B.
(Number of eggs /female/ day over 2-day periods)

Period after exposure, days	Both Sexes treated	Males treated	Females treated	Neither Sec treated
8	2.5	5.0	3.7	6.1
	3.8	5.8	4.1	5.2
	3.7	4.7	4.4	5.9
	2.6	4.9	6.7	5.2
Mean for period	3.2	5.1	4.8	5.6
14	7.3	6.4	5.9	6.2
	5.9	4.1	6.4	5.3
	6.3	4.7	5.7	4.9
	5.6	6.1	5.7	5.3
Mean for period	6.3	5.3	5.9	5.4
20	6.9	7.7	7.9	6.4
	6.4	3.7	6.8	3.3
	6.0	3.8	6.2	6.4
	5.1	6.5	4.8	7.1
Mean for period	6.1	5.4	6.4	5.8
26 (Temp. 22°C.)	3.6	5.5	5.5	4.8
	4.0	2.3	4.5	2.7
	3.0	3.6	3.1	4.1
	4.2	3.8	2.7	5.5
Mean for period	3.7	3.8	3.9	4.3
32	5.4	6.4	4.5	6.5
	5.9	4.8	5.0	4.8
	7.5	4.4	4.5	6.3
	5.0	3.6	4.1	4.8
Mean for period	5.9	4.8	4.5	5.6
38	8.0	4.1	4.0	8.6
	4.8	5.0	5.4	4.0
	6.7	4.0	4.2	6.4
	6.5	3.7	5.3	6.5
Mean for period	6.5	4.2	4.7	6.4
Mean for total period	5.3	4.8	5.0	5.5

TABLE VIII

OVIPOSITION OF TRIBOLIUM AFTER EXPOSURE TO SUBLETHAL DOSAGES OF DDT. TEST C.
(Number of eggs /female/ day over 2-day periods)

Period after exposure, days	Both Sexes treated	Males treated	Females treated	Neither Sex treated
8	5.8 2.2 4.1 3.8	5.9 5.2 3.6 4.7	2.9 5.2 5.3 5.8	5.7 6.1 4.3 5.7
Mean for period	4.0	4.8	4.8	5.4
14	6.0 5.3 6.2 5.2	6.3 4.4 5.7 6.3	4.4 7.1 7.6 7.3	5.8 4.9 5.9 6.3
Mean for period	5.7	5.7	6.6	5.7
20	5.4 6.5 5.2 5.6	7.2 6.1 5.7 7.1	5.0 7.4 7.5 6.5	6.8 4.3 4.5 6.5
Mean for period	5.7	6.5	6.6	5.5
26	6.7 6.0 8.6 4.6	6.5 4.3 7.6 7.6	6.8 7.9 7.2 4.9	6.7 4.3 4.8 6.0
Mean for period	6.5	6.5	6.7	5.4
32	8.0 6.9 6.1 4.7	9.0 6.0 4.7 6.0	7.9 6.2 7.1 5.3	9.8 4.9 4.2 7.8
Mean for period	6.4	6.4	6.6	6.7
38	5.2 4.9 7.0 5.9	6.5 5.7 6.2 5.5	6.3 8.1 7.6 5.1	6.9 5.5 5.3 6.3
Mean for period	5.7	6.0	6.8	6.0
Mean for total period	5.7	6.0	6.3	5.8

D.D.T. These results are presented in Table III. Tables IV and V show that mortality of the males and females were approximately equal. This was accomplished only by varying the dosage of D.D.T. and period of exposure so that the males received much larger amounts of D.D.T. than the females. The data on fecundity for Experiments A, B and C are shown in Tables VI, VII and VIII respectively. The data on fertility for the same three experiments are indicated in Tables IX, X and XI respectively. The data on fertility and fecundity was subjected to statistical analysis in order to compare any variations existing among the four combinations of treatments with sexes. Wherever significant differences are present they are recorded as P values in footnotes below the appropriate table. Significant differences were observed in the data for Experiment A as indicated in Table VI. These differences were established between the combination: females treated versus males treated and between the combination: females treated versus neither sex treated. The results show that when females are exposed to residual deposits of D.D.T., the average number of eggs laid by the survivors is higher than that for the females of the control group. Furthermore, it seems that treatment of males has no effect on the fecundity of the females. The rate of oviposition of groups of treated females and of untreated females is neither reduced or increased when either of these groups is combined with a group of treated males. The rate of oviposition of the females in the control group, namely; the group where neither sex is treated is considered as a normal rate. This rate is used as a basis of comparison for the rates of oviposition of treated groups. No significant

TABLE X

PER CENT VIABILITY OF EGGS PRODUCED BY TRIBOLIUM AFTER EXPOSURE TO SUBLETHAL DOSAGES OF D.D.T. TEST B.

Period after exposure	Both Sexes treated	Males treated	Females treated	Neither Sex treated
8	71.4	76.4	91.7	70.0
	69.9	64.4	67.0	68.9
	61.3	84.9	75.2	74.7
	53.1	71.5	82.0	80.0
Mean for period	63.9	74.3	79.0	73.4
14	74.5	84.4	81.8	79.6
	74.6	72.3	85.7	89.2
	86.5	87.2	90.0	82.2
	85.8	86.7	85.2	83.3
Mean for period	80.3	82.6	85.7	83.6
20	81.6	92.6	87.4	95.8
	83.1	65.4	90.1	83.5
	83.3	94.1	85.5	87.4
	91.8	87.5	92.8	89.7
Mean for period	84.9	84.9	88.9	89.1
26	72.1	79.1	81.2	80.0
	87.5	59.4	89.8	85.9
	93.4	63.1	87.5	92.4
	76.2	87.8	84.5	91.7
Mean for period	82.3	72.3	85.7	87.5
32	84.2	85.0	90.5	87.6
	83.9	90.3	85.8	82.6
	94.8	88.7	90.4	89.6
	85.0	88.7	93.4	78.2
Mean for period	87.0	88.2	90.0	84.5
38	84.1	78.1	94.2	88.4
	88.6	85.8	81.5	88.8
	79.4	79.4	76.5	86.1
	89.2	89.0	75.4	70.4
Mean for period	85.3	83.1	81.9	83.4
Mean for total period	80.6	80.9	85.2	83.6

TABLE XI

PER CENT VIABILITY OF EGGS PRODUCED BY TRIBOLIUM AFTER EXPOSURE TO SUBLETHAL DOSAGES OF D.D.T. TEST C.

Period after exposure	Both Sexes treated	Males treated	Females treated	Neither Sex treated
8	65.2	69.7	78.7	70.0
	76.0	88.1	63.1	91.2
	54.2	83.3	67.4	73.4
	52.7	78.9	70.2	90.5
Mean for period	62.0	80.0	69.8	81.2
14	77.2	85.3	81.7	81.6
	76.1	84.3	77.2	81.9
	69.3	78.9	76.4	83.7
	88.7	70.8	76.6	93.9
Mean for period	77.8	79.8	78.0	85.3
20	77.9	84.1	81.7	84.2
	79.7	77.3	84.7	75.0
	78.1	95.6	57.2	90.7
	88.9	81.2	81.4	89.9
Mean for period	81.1	84.5	76.2	84.9
26	84.4	91.7	85.8	81.0
	56.7	92.0	77.8	83.1
	73.2	87.6	80.8	88.5
	85.1	73.5	84.1	92.9
Mean for period	74.8	86.2	82.1	86.4
32	71.0	88.5	82.0	75.2
	82.0	82.3	91.0	86.9
	86.2	92.5	75.9	85.9
	87.4	86.9	69.2	86.1
Mean for period	81.6	87.2	79.5	83.5
38	67.2	81.9	78.0	67.2
	59.2	84.1	70.0	79.3
	72.3	85.6	78.7	67.3
	76.0	69.5	76.1	68.3
Mean for period	68.9	80.3	75.7	70.5
Mean for total period	74.3	83.0	76.9	82.0

TABLE XIV

EFFECT ON OVIPOSITION OF DIFFERENT EXPERIMENTAL METHODS FOR EXPOSURE OF TRIBOLIUM TO SUBLETHAL DOSAGES OF DDT.

(Mean number of eggs /female/ day over total period for each replicate)

Experimental methods	TREATMENTS				Means for methods
	Both Sexes	Males Only	Females Only	Neither Sex	
Preliminary test	6.27 6.18 4.47 4.84	5.41 6.08 4.90 5.74	6.67 4.30 3.37 6.00	4.42 5.27 4.98 7.00	
Means for treatments	5.6	5.1	5.0	5.0	5.2
A	5.69 4.00 5.81 5.75	3.66 4.35 4.66 4.06	5.05 4.71 5.78 5.36	3.98 4.68 3.83 4.53	
Means for treatments	5.3	4.2	5.2	4.2	4.7
B	5.61 5.13 5.53 4.83	5.85 4.28 4.20 4.76	5.25 5.38 4.68 4.89	6.43 4.21 5.66 5.73	
Means for treatments	5.3	4.8	5.0	5.5	5.1
C	6.18 5.30 6.20 4.96	6.90 5.28 5.58 6.20	5.55 6.98 7.05 5.81	6.95 5.00 4.83 6.43	
Means for treatments	5.7	6.0	6.3	5.8	5.9

TABLE XV

EFFECT ON VIABILITY OF DIFFERENT EXPERIMENTAL METHODS FOR EXPOSURE OF TRIBOLIUM TO SUBLETHAL DOSAGES OF D.D.T.
(Mean per cent viability over total period for each replicate)

Experimental mthods	TREATMENTS				Means for methods
	Both Sexes	Males only	Females only	Neither Sex	
Preliminary	63.1 64.0 68.3 67.8	64.2 60.3 59.0 65.6	67.4 65.3 71.2 62.0	71.8 64.6 74.3 69.4	
Means for treatments	65.8	62.3	66.5	70.1	66.1
A	78.25 73.12 73.28 68.50	70.56 69.14 67.26 76.80	77.62 80.70 72.52 77.76	62.90 68.54 72.86 77.38	
Means for treatments	73.3	70.9	77.1	70.4	72.9
B	78.0 81.3 83.1 80.2	82.6 72.9 82.9 85.2	87.8 83.3 84.2 85.5	83.6 83.1 85.4 82.2	
Means for treatments	80.6	80.9	85.2	83.6	82.6
C	73.8 71.6 72.2 79.8	83.5 84.7 87.2 76.8	81.3 77.3 72.7 76.3	76.5 82.9 81.6 86.9	
Means for treatments	74.3	83.0	76.9	82.0	79.0

differences were observed between the various combinations of treatments and sexes with regard to viability. In other words, the eggs laid by treated females are no more or less viable than those laid by untreated females.

In Experiments B and C no significant differences were observed between combinations of treated and untreated sexes, in either the fecundity or fertility data. In both experiments the rate of oviposition of the female survivors was neither reduced nor increased as a result of exposure to D.D.T. In other words, the female survivors were not affected sublethally at least as far as reproduction is concerned.

In all experiments the rate of oviposition of treated females was lower in the initial counts than in any subsequent counts. This initially low rate of oviposition was more evident in Experiments B and C than in Experiment A. Further, the eggs from these first counts showed a correspondingly low viability.

The results of the effect on oviposition and on viability of different experimental methods for exposure of Tribolium confusum to sublethal dosages of D.D.T. are presented in Tables XIV and XV respectively. The data was subjected to statistical analysis but no significant differences were observed among any of the methods.

Throughout the whole investigation oviposition and viability of eggs were not reduced as a result of exposing the parent females to deposits of D.D.T. residues.

DISCUSSION

An examination of the experimental data of induced mortality, oviposition, and viability reveals that considerable variation exists among the replicates. Actually there is no real discrepancy in the results since experimental conditions and procedure have been different in each case. Different concentrations of D.D.T. and different lengths of exposure were used in an effort to induce 50 per cent mortality of the test insects. It is possible that, as a result of these experimental changes the physiological responses of the insects may have changed accordingly.

It is possible that more uniform results were not obtained because certain factors were not closely controlled, for example, the factor of movement. It will be remembered that the factor of movement over a treated surface during exposure was not considered important in governing the amount of D.D.T. absorbed. This conclusion was based on the results of Hamman's work with cockroaches (5). However, it may well be that with Tribolium the factor of movement is important during exposure.

The observed results on fecundity for all three experiments are different from those obtained by other workers investigating sublethal effects of insecticides (11), (14). This study shows, for the first time, that treatment with D.D.T. does not lower the rate of oviposition of an insect surviving the treatment. In other studies of a similar nature, oviposition has been reduced as a result of treatment. The differences in results are likely due to the differences in methods and to the different species and insecticides used by other investigators.

A few statements concerning the findings of the study are made in the following paragraphs.

The higher mortalities of the females of Tribolium confusum indicate that this sex is more susceptible to D.D.T. residues than the male when both sexes are exposed to equal concentrations of this insecticide for equal lengths of time. In Experiment A where concentration was increased for the males, the females were killed more readily than the males. This result further demonstrates the difference in susceptibility between the two sexes.

The results of Experiment A with regard to fecundity and fertility show that treatment of females is correlated with an increased number of eggs. This result indicates a relationship between dosage, mortality and rate of oviposition of the females. If the dosage and period of exposure is such that weak females are selectively killed, it is logical to assume that the survivors constitute a strong strain of insects. These survivors lay eggs at their normal rate. However, the rate of oviposition of such a select group may be higher than that for a control group containing both weak and strong females. In a control group of this kind the high rate of oviposition of the strong, healthy females is masked by the low rate of oviposition of the weak females. The viability of the eggs laid by the female survivors is higher than that of the eggs laid by the control groups. This result is to be expected for it is obvious that eggs laid by sound females would show a high degree of viability.

In the hypothesis formulated for Experiment A the rate of oviposition of the female survivors is considered to reflect the vigour of these survivors. Therefore, the high numbers of eggs laid by the females which survi-

ved exposure to D.D.T. are high because these females are considered to constitute a select, resistant group. It must be borne in mind, however, that this consideration is theoretical and that more extensive studies are required to verify or disprove it.

In Experiments B and C it was observed that the rate of oviposition of the female survivors was neither significantly increased or decreased as a result of exposure to D.D.T. residue. In other words, no sublethal effect was manifested. Therefore, the reproductive function of the female survivors was not impaired by dosages which killed approximately fifty per cent of the exposed insects. These results seem to indicate an "all-or-none" effect of D.D.T., that is; if the insects are affected to any great extent by exposure to a certain dosage of D.D.T. they will die as a result. Those insects that do not die are probably resistant to that particular dosage. It is logical to conclude that in long term control operations more and more selection occurs, resulting in the development of a highly resistant strain of Tribolium. Lindquist's work with house flies (8) and related work (1) has established that insect resistance to insecticides can be developed.

In Experiments B and C the observed results do not indicate that treated females have a higher fecundity and fertility than untreated ones. The results as indicated in Tables VII and VIII show that the rate of oviposition of the treated females is the same as that of untreated females. The fecundity of females surviving exposure to a dosage of D.D.T. which killed approximately 50 per cent of the exposed insects is not significantly changed.

All three experiments show that oviposition is not reduced as a result of exposure to D.D.T. This is a most important finding in view of the popular conception concerning the function of insecticides.

Generally speaking, the purpose of an insecticide is to eliminate an insect population. Although they may seem to reduce the numbers of adults present, are they really accomplishing their purpose in eliminating the total population? What of the egg population? Insecticides do not achieve their purpose unless the adult survivors are affected so that their fecundity is lowered. As has been observed, under certain experimental conditions the fecundity of adult females which survived exposure to D.D.T. has increased. In defence of insecticides one may say that the viability of the eggs produced by these female survivors is "probably" low. However, as this study has shown, the viability of these eggs is not lowered.

The results of all three experiments suggest that the level of mortality is not important in determining the number of eggs laid by female survivors. In Experiment A, for example, a low level of mortality was observed but the survivors showed a high fecundity. In Experiments B and C, although the level of mortality was higher, the fecundity of the survivors was approximately the same as that for Experiment A.

One reason which may account for the difference in results between Experiment A and Experiments B and C is the different dosage and exposure used in each case. In Experiment A the females were treated with a 1 per

cent solution of D.D.T. but were exposed for three hours. In the other two experiments a 2.5 per cent solution of D.D.T. was used but the females were exposed for only one hour. It is likely that the groups exposed to higher dosages for shorter periods of time received more D.D.T. than the groups exposed to lower dosages for longer periods. This might explain why the mortality of females in Experiments B and C was generally higher than in Experiment A.

It is evident that control of Tribolium confusum by the use of residual insecticides is practicable only if they are applied in sufficiently large dosages to kill 95 per cent or more of the insect population present. Although the results of the study with regard to fecundity are inconclusive, the data may be interpreted as indicating that as far as Tribolium is concerned treatment with D.D.T. at the 50 per cent level does not change the rate of oviposition of the females. However, if any change does occur, it is in the direction of increased egg production. This change was indicated by one of three tests, that is, Test A. It is significant to note, however, that the rate of oviposition is not reduced as a result of treatment. This inability of D.D.T. to lower the rate of oviposition of females exposed to it questions the advisability of applying residual insecticides where only a part of the insect population is lethally affected.

The initially low numbers of eggs laid shortly after treatment may possibly be due to an initial paralytic effect of D.D.T. on the reproductive apparatus of the female. Since subsequent counts showed increasingly

higher fecundity rates, this effect, if present, was only temporary. The fertility of the females was also affected as shown in the viability data. The eggs laid shortly after treatment were not only low in numbers but also were of low viability. This observation may seem to contradict the possibility of the "all or none" effect of D.D.T. mentioned earlier. The "all or none" effect likely exists as a general over all effect throughout the experiment. It is still possible, however, to have slight variations in any one period such as the period mentioned where the numbers of eggs produced were low.

Before a final conclusion may be drawn as to the significance of the effects of sublethal dosage of D.D.T. residues on the confused flour beetle, a more extensive experimental study is necessary. Such an experiment should be carried out under closely controlled conditions especially with regard to variable factors. For example, the factor of movement may play a more important part in influencing the amount of insecticide absorbed than at first realized. Therefore, to give more accurate results the test insects should be immobilized with some inert, harmless gas such as carbon dioxide during exposure to a uniform deposit of D.D.T. In this way the amount of D.D.T. absorbed by each insect can be more closely controlled.

The high rate of oviposition of the female survivors in Experiment A led to the belief that selective killing occurred during treatment, leaving a strain of physiologically sound females. This hypothesis may be verified by setting up an experiment whereby groups of test insects

are exposed to different dosages of D.D.T. residues for varying lengths of time. The treated insects can then be separated into groups which suffered different degrees of mortality, for example, 25, 50, 75 and 90 per cent. If selection of strong females does occur then we would expect the highest rate of oviposition in the group which suffered 90 per cent mortality and the lowest rate in the group which suffered 25 per cent mortality. In the former group all weak females are likely killed as a result of long exposure to large amounts of D.D.T. The rate of oviposition of the select survivors is therefore quite high. The latter group likely contains weak females because the low dosage of D.D.T. or short exposure period was not sufficient to kill all the weak females. In this group the over-all rate of oviposition is lower than that of the select survivors of the former group because of the presence of weak females. The lower rate of oviposition of these weak females can lower the rate for the group as a whole. Consequently, the high fecundity of the strong females is not apparent.

Approaching the problem from another direction, an experiment may be set up as follows: Male and female adults of Tribolium confusum of constant age can be paired and reared in separate vials. These can be observed for ovipoisition over a period and arranged into three groups on the basis of rate of oviposition as follows:

Group I showing lowest rate of oviposition.

Group II showing an intermediate rate of oviposition

Group III showing highest rate of oviposition

These three groups can be exposed to identical dosages of D.D.T. residue for the same length of time and observed for the degree of mortality that is induced. If the hypothesis is correct, the lowest mortality will be observed in Group III, because, according to the hypothesis the females of this group are considered to be a select group of physiologically sound insects. By the same line of reasoning we would expect the highest mortality to be induced in Group I.

Lindquist and Wilson have shown that a resistant strain of house flies can be developed as a result of exposure to D.D.T. (8). The possibility of developing strains of D.D.T. resistant flour beetles has been suggested in view of the results of Experiment A. Whether or not resistance to D.D.T. is actually developed by flour beetles and passed on to subsequent generations is unknown. The question can be answered by setting up an experiment designed to test the resistance of flour beetles to D.D.T. Adults of Tribolium can be exposed to D.D.T. residues of known concentration for a known period of time to induce 50 per cent mortality. After a few days the survivors can be exposed until 50 per cent mortality is induced by varying either the concentration of D.D.T. or the period of exposure. If either of these two factors has to be increased in order to kill 50 per cent of the insects, then these insects must be considered as having developed a resistance to D.D.T. Further, the experiment may be extended to test the resistance of the progeny of treated adults. Eggs laid by females which have survived treatment can be allowed to develop into second generation adults. These adults can be exposed to D.D.T. residues. If it is necessary to use higher concentrations or to expose them for longer periods than the parents,

then the progeny must have developed a resistance to D.D.T.

Pending further investigation into the problem of sublethal effects of D.D.T., it would not be desirable to draw too many definite conclusions from the data presented. A few statements concerning the effects of sublethal dosages of D.D.T. on Tribolium have been made but the need for further studies on the subject is clear.

SUMMARY

A laboratory study was carried out to determine the sublethal effects of D.D.T. residues on the confused flour beetle. Test groups of males and females were exposed separately to uniform deposits of D.D.T. residues for a sufficient length of time to induce 50 per cent mortality. The survivors were paired and assessed for sublethal effect by means of two criteria, namely; the rate of oviposition of the females and viability of the eggs produced by these females. The experiment was designed to determine the effect, if any, on the reproductive ability of either or both sexes of Tribolium. The rate of oviposition (fecundity) and the viability of eggs produced (fertility) of the female survivors was observed periodically over an interval of almost 40 days. Records of data were tabulated and subjected to statistical analysis.

The principal findings of the study were as follows:

1. Females of Tribolium confusum are much more susceptible than males to deposits of D.D.T. residue when both sexes are exposed to equal dosages for equal lengths of time.

2. The first counts of oviposition show that the rate of oviposition is considerably lower than that for subsequent counts. This is probably due to an intial paralytic effect of D.D.T. on the reproductive apparatus of the female. The eggs obtained from these counts are of low viability.

3. Treatment of males has no effect on the fecundity and fertility of the females.

4. Oviposition is not significantly reduced as a result of treatment with D.D.T. The fecundity of female survivors may be higher under certain experimental conditions, but it is never lower than the fecundity of unexposed females.

5. Suggestions are made for further studies designed to test the hypothesis that selective killing of individuals occurs when insects are exposed to residual deposits of D.D.T.

BIBLIOGRAPHY

(1) Anon. Controlling flies with BHC. Agricultural Chemicals 4 : 30. April, 1939.

(2) Cotton, R. T., J. C. Frankenfeld and N. M. Dennis. Residual sprays for use against the confused flour beetle. Report of the Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, U.S.D.A., E-766, December, 1948.

(3) Cristol, S. J., R. A. Hayes and H. L. Haller. Determination of p,p' D.D.T. in technical D.D.T. Ind. Eng. Chem., anal. ed. 17 : 470-2. 1945.

(4) Gray, H. E. The biology of the flour beetle. Contribution No. 2577, Division of Entomology, Science Service, Department of Agriculture, Dominion of Canada.

(5) Hamman, R. E. Factors involved in poisoning German roaches by exposing them to surfaces treated with chlorinated hydrocarbons. Journ. Econ. Ent. 4 (3) : 516 - 17. 1948.

(6) Hewlett, P. S. The design and performance of an atomizing nozzle for use with a spraying tower for testing liquid insecticides. Ann. App. Biol. 33 : 303. 1946.

(7) Kennedy, J. S. The excitant and repellent effects on mosquitoes of sublethal contacts with D.D.T. Bull. Ent. Res. 37 (4) : 593 - 607. 1946.

(8) Lindquist, A. W., and H. G. Wilson. Development of a strain of house flies resistant to D.D.T. Science. 107 : 276-7. 1948.

(9) Park, Thomas, and M. B. Frank. The fecundity and development of the flour beetles, Tribolium confusum and Tribolium castaneum at three constant temperatures. Ecology 29 (3) : 368 - 74. July 1948.

(10) Park, T. The biology of the flour beetle. Quarterly Review of Biology. 9 : 36 - 54. 1934.

(11) Plumner, C. C., and Baker. Effect of sublethal dosages of tartar emetic on the Mexican fruit fly. Journ. Econ. Ent. 39 (6) : 775-80. 1946.

(12) Smallman, B. N. Residual insecticides for the control of spider beetles in cereal warehouses. Journ. Econ. Ent. 41 (6) : 869 - 874. Dec. 1948.

(13) Tattersfield, F. and C. Potter. Biological methods of determining the insecticidal values of pyrethrum preparations. Ann. Appl. Biol. 30 (3) : 259 - 79. 1943.

(14) Tenhet, J. N. Effect of sublethal dosages of pyrethrum on the oviposition of the cigarette beetle. Journ. Econ. Ent. 40 : 910 1947.