Some Laboratory Experiments on Resistance of the Pomace Fly to DDT Poisoning

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Introduction

During 1951 the author carried out two experiments on resistance to DDT poisoning in the pomace fly, *Drosophila melanogaster* Meigen. One experiment was on the development of several strains of flies under p,p'-DDT treatments applied in several generations; and the principal objective was the production of a more-than-normally resistant strain and a more-than-normally susceptible strain. In the second experiment certain possible effects produced within one generation by p,p'-DDT treatments were investigated.

Both experiments were originally regarded as preliminary or exploratory in nature; but, as the author now plans to go no further with this line of work, the present report is made in the hope that others may find the methods or results suggestive.

The Basic Stock of Flies

Eight laboratory strains of pomace flies representing different geographical origins were combined in three series of mass matings (five of each sex of each strain per mating) to produce the basic strain which was designated the X strain.

Culture Methods

The flies were reared in four ounce bottles stoppered with cotton plugs. The medium contained the following ingredients, in the amounts indicated, per liter, made up to volume with water: Molasses, 135 ml.; cornmeal, 100 to 120 gms.; agar, 15 to 20 gms.; and a mold inhibitor (methyl parahydroxybenzoate), 1.5 to 2.0 gms. The prepared culture bottles were autoclaved. Yeast was added before the cultures were used; and, in addition, fresh yeast was added from time to time to provide at all times an obvious excess of food. The intention was to eliminate competition for food.

All fly strains were maintained in duplicate culture.

Temperature and Relative Humidity

The cultures were stored during their developmental periods in an incubator at 26±2°C.; but all the handling and testing of the adult flies had to be done at room temperature and room humidity, both of which varied widely. Statistically significant results were obtained in many cases in spite of large inter-replication differences which are believed to have been due largely to variations in temperature and relative humidity. The author is convinced, however, that control of both temperature and humidity are necessary if closely reproducible results are to be obtained with pomace flies in work of this sort. This conviction agrees with statements made by Weiner and Crow in 1951 (2).

Apparata Devised for Handling Flies

Three improvised apparata greatly facilitated the rapid handling of rather large numbers of flies.

One item was a "fly pipette" which was essentially a small inspirator bottle made from half of a volumetric pipette cut off in the center of the bulb. The end was worked to a tapered tip with a two millimeter opening, the stem was bent about 45°, and the bulb end was fitted with a mouth tube.

An anaesthesia tray was made by fitting a perforated cardboard false bottom in a small enamel tray. Carbon dioxide was led into the bottom part and fed up through the perforations; and a glass plate was used to cover the tray. Anaesthetized flies were poured into the tray and the fly pipette was used to sort and count out samples. It was possible to count out as many as 15 samples of 50 flies each, by sex, in an hour.

A suction apparatus was used for transferring active flies into cages and from one cage to another. The fan and motor from an old vacuum cleaner were mounted under a plywood platform in which a circular opening had been cut. The cages used were made by fitting quart ice cream cartons with cheesecloth bottoms and transparent tops; and the vacuum intake opening was just large enough to accommodate the end of a cage. When flies were to be introduced, the cage was set on the suction apparatus and the flies were poured into the open top. When flies were to be transferred from one cage to another, the occupied cage was set on the suction apparatus and, when the lid was eased off, the flies were blown to the bottom. Then a second cage was fitted on top by means of a tight-fitting collar. When the two cages were inverted, the flies were blown into the second cage. Introductions or transfers could be made at the rate of ten in four minutes.

The Experiment on the Development of Strains

Adult flies of the third generation of the X strain were used to start four experimental strains. The X strain was continued and served as the standard of comparison.

Two of the experimental strains were developed by rearing flies for five successive generations on media containing DDT. One strain, designated the L2 strain, was reared on medium prepared to contain two ppm. of DDT; and the other, designated the L5 strain, was reared on medium containing five ppm. of DDT. The media were prepared by adding appropriate amounts of acetone solution of DDT to freshly prepared standard medium before autoclaving. The other two experimental strains were developed by a selection process.

It was hypothesized that it might be possible in exposures of adults to DDT residues to use rapidity of development of toxic symptoms as the basis for selecting susceptible and resistant strains. The procedure was first, exposure of a group of adults to a DDT residue until about 10 per cent showed incoordination; second, rescue of this early-affected fraction; third, continuation of the exposure until all but about 10 per cent of the flies were in the knock-down state; and, fourth, rescue of this last-affected fraction. The two sexes were exposed and selected separately; and the rescued flies were transferred to clean culture bottles.

The two groups of flies obtained by applying the selection procedure to adults (200 females, and 200 males) of the third generation of the X strain were used to establish two strains. The strain started with the early-affected fraction was designated strain S; and the strain started with the last-affected fraction was designated strain R. Each strain was selected in six successive subsequent generations, the S strain being selected only for the early-affected fraction, and the R strain being selected for the last-affected fraction.

Ice cream carton cages served as selection exposure chambers. Each carton had the curved wall (about 75 per cent of the total inside surface) lined with DDT impregnated paper (1.0 mg. per sq. in.). The bottom of the cage was closed with cheesecloth; and the lid had a transparent insert through which a twelve inch slender glass tube extended. The progress of the exposure was observed through the transparent top; and the glass tube was used as a pipette for rescuing selected flies.

The goals of selection, the first and last 10 per cent fractions, were seldom achieved. Usually about a tenth of the rescued flies failed to recover. In the case of the S strain, this meant that there was in fact a mild selection, presumably for resistance. In the case of the R strain the selection was merely more severe than intended.

TABLE I. Mean mortalities of five strains of pomace flies in residue exposuse tests on adults of the ninth and tenth generations; and differences required for significance.

Strain		Ninth Generation[1]	Tenth Generation[2]
R		36.08	16.83
S		39.50	20.83
X		44.75	28.78
L2		30.83	18.39
L5		39.83	32.44
Differences required for significance	At 19:1	6.77	8.39
	At 99:1	9.04	11.14

[1] Half hour exposures to deposits of 1 mg. per sq. in.; six replications.

[2] One hour exposures to deposits of ⅛ mg. per sq. in.; nine replications.

All the strains were carried through ten generations. In the ninth and tenth generations replicated mortality test comparisons were made to determine the relative resistance of the five strains. The sample groups were 50 flies for each sex and for each strain, making ten tests per replication. The sample groups were exposed to DDT residues in ice cream carton cages for short periods and then were removed to clean cages and held for 24 hours. The 24 hour mortality readings were taken and analyzed statistically.

For both generations the analyses showed first, that there were significant differences (at odds of 19 to 1) between replications; second,

that female flies were significantly more resistant (at odds of 99 to 1) than males; third, that there was no significant interaction of sex and strain; and, fourth, that there were significant differences between strains. The mean mortalities of the five strains, the differences required for significance, the number of replications, and the dosages used in these tests are shown in Table I.

These data show that strains R and L2 were rather highly resistant and that strain S was slightly resistant, as compared with the standard X strain. The L5 strain appears somewhat variable but not very different from the standard.

Less extensive data on adults of the third and fourth generations support this relative ranking of strains R, S, and X; but in those earlier generations the L2 and L5 strains were not shown to be significantly different from the standard.

It seems clear that the high resistance of the R strain resulted from a rather severe selective effect, and that the slight resistance of the S strain resulted from a mild selective effect. The author has, however, no adequate explanation of the results on the L2 and L5 strains. If one explains the high resistance of the L2 strain as the result of a selective effect, he is faced with the question of why the L5 strain, which was treated at a higher rate, was not even more highly selected.

It must be noted that there was an interval of four generations between the last treatments of the L2 and L5 strains and the time of testing for resistance. It appears that, perhaps, during this interval some uncontrolled factor may have acted differentially on these two strains. The author is inclined to suspect there may have been some nutritional factor involved or, possibly, some effect related to an observed mold infestation of the cultures.

It may be stated, by way of conclusion, that the selection method based on rapidity of development of symptoms was successful in producing a resistant strain but failed to produce a susceptible one.

The Experiment on Effects Produced within a Single Generation

The experiment was initiated with eggs obtained from adults of the third generation of the X strain. The eggs were allowed to hatch and develop in culture media containing DDT.

The eggs were obtained by caging several hundred flies for twelve hours (overnight) in an ice cream carton cage in the lid of which a thin layer of agar had been poured to serve as an oviposition site. Batches of 50 eggs were counted and transferred to culture bottles. (A small proportion of newly hatched larvae was included in the "egg" counts, it having been found impractical to attempt to exclude them.)

Four media containing DDT were used. These were prepared to contain one, two, three, or four ppm. of DDT by adding appropriate amounts of acetone solution of DDT to freshly prepared standard medium. Two controls were used. One was the standard (untreated) medium; and the other was acetone-treated medium. The prepared culture bottles were autoclaved to eliminate the acetone; and the cultures were held at $26\pm2°C$. There were fourteen replications.

The number of adults of each sex emerging in each 24 hour period was recorded; and the emerged flies were set aside in clean culture bottles. The data provided information on the total number of flies emerging from each batch of eggs, and permitted the calculation of weighted average development times for each sex in each culture.

The analysis of the data on survival to emergence gave only a slight indication that DDT at the higher rates reduced survival. The means for each treatment are given in Table II.

TABLE II. Effects produced within a single generation by DDT treatments of the larval media.

Treatment	Survival to emergence. (Mean no. of adults from 50 eggs)	Length of developmental period. (Mean no. days egg to adult)	Survival from emergence to testing. (Per cent)	Resistance of adults. (Mean mortality values)
Untreated	41.9	9.50	89.8	59.96
Acetone	39.7	9.56	84.2	61.27
1 ppm. DDT	41.9	9.55	92.2	63.24
2 ppm. DDT	37.5	9.70	89.0	65.74
3 ppm. DDT	38.1	9.61	88.6	60.94
4 ppm. DDT	37.6	9.85	89.7	49.11[1]

[1] significantly different from other treatments at odds 19 :1.

The analysis of the data on development time showed first, that the males took significantly longer (about 1/3 day, odds of 99 to one) than females under all treatments; second, that there was no significant interaction of sex with treatment; and, third, that there was a rather strong indication that the DDT treatments increased the length of the developmental period for both sexes. The means for each treatment are shown in Table II.

On the sixteenth day after egg collection the adult flies that had been produced were subjected to DDT residue exposure tests to discover whether the treatments had affected their resistance. Six replications of these tests were run. As the sample groups were rather small and of varied size, the mortality data were expressed as percentages and transformed to arc sine values for analysis.

The analysis showed first, that the males were significantly less resistant (at odds of 99 to 1) than the females under all treatments; second, that there was no significant interaction of sex and treatment; and, third, that there was significantly greater resistance (at odds of 19 to 1) in the flies that had been reared on medium containing four ppm. of DDT. The mean values for the residue exposure tests are shown in Table II.

The records were examined to discover whether there had been any differential in survival of adults of the six treatment-groups in the interval

between emergence and testing. These survival rates are shown in Table II. It is apparent that there were no appreciable differences.

Correlations between sets of means were run to discover whether the results of the residue exposure tests were related to any of the other measured effects of treatment. There was a rather strong indication that increased resistance was related to increased length of the developmental period; and there was a very slight indication that increased resistance was related to decreased survival from egg to adult. There was no indication of any relationship between resistance and survival in the interval between emergence and testing.

In regard to the indicated relationship between length of developmental period and resistance it should be noted that, when the developmental period was longer, the flies, at the time of testing, were necessarily younger in terms of age-as-adults. And it may well be that the apparently increased resistance of the four ppm. flies was merely a reflection of an age difference, perhaps acting in conjunction with a selective effect.

The indication of a lengthened developmental period in flies reared on media containing DDT agrees with observations reported in 1950 by Kalina (1) who reared pomace flies on medium to which five ppm. of DDT had been added in carbon tetrachloride solution.

Kalina reported also that in his experiment all flies died apparently of DDT poisoning at about the time of emergence. In the author's experiment which differed as to rate of treatment, method of treatment, and method of rearing, nothing more than mild toxic symptoms was ever observed; and there was no consistent relationship of these mild symptoms to rate of treatment.

An Experiment on a Temperature Effect

One small experiment was run to discover whether, as suspected, the temperature at which flies were held after exposure to DDT residues affected the 24 hour mortality readings.

A double series of sample groups of flies of the fourth generation of the L5 strain and the corresponding generation of the X strain was exposed to DDT residues. One series was held for 24 hours at 26±2°C. while the other series was held for 24 hours at about 29°C. There were only three replications.

The analysis gave a strong indication that mortality was less for both sexes of both strains at the higher holding temperature.

The author believes that small temperature variations were in part responsible for inter-replications differences in the experiments reported above.

Literature Cited

1. KALINA, BERNARD FRAM. 1950. Development and viability of *Drosophila melanogaster* on a medium containing DDT. Sci. **111**:39-40.

2. WEINER, RICHARD, and JAMES F. CROW. 1951. The resistance of DDT-resistant Drosophila to other insecticides. Sci. **113**:403-404.