USE OF WORLD HEALTH ORGANIZATION MOSQUITO TEST KITS WITH DDT RESISTANT DROSOPHILA

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Introduction

Widespread use of commercial insecticides in the last two decades has enhanced the development of resistance to chlorinated hydrocarbon insecticides by some important disease vectors and by a number of arthropods which are of importance to public health (Brown, 1958). In connection with this problem, the World Health Organization (W.H.O.) has undertaken the task of coordinating international research in the study of resistance to insecticides. This organization also provides a kit for investigating the resistance of mosquitoes. It also provides all the materials and instructions necessary to carry out a testing program.

The initial problem of insecticide-resistance is the determination of its existence. Probably the best method is by use of a standardized testing procedure with standardized equipment and conditions. One of the goals of the W.H.O. is to develop such a standardized test for comparison of susceptibility and resistance levels of arthropods used for genetic study. Thus, one part of this study dealt with the investigation of the value of the W.H.O. test kit as a standardized testing device for DDT resistant *Drosophila*.

Materials and Methods

Eight strains of *D. melanogaster* were used for these tests. HL 1 - Q and HL 2 - Q were representatives of sibling-selected lines with high DDT-resistance cultured by Bennett (1959). LL 1 - Q and LL 2 - P were low DDT-resistant lines selected during that same period. Cage 1, duplicate, used in this study, was descended from the original unselected wild-type progenitors of both the high and low resistant lines (Bennett, 1959). SySM 1 and ORS 1001 were DDT-resistant strains selected by King (1957) at the Cold Spring Harbor Biological Laboratory. Brown-Eye-R was a strain of DDT-resistant flies which were selected by Crow (1954) at the University of Wisconsin. It should be pointed out that a varying amount of time had elapsed between the time that these flies were originally selected for DDT-resistance and the time that the tests for this study were conducted.

The first resistance testing in this study was done in January and February, 1959, using the W.H.O. dieldrin sets. The flies were etherized and sorted to isolate females for testing purposes. One test for one stock used flies from several cultures. The use of female *D. melanogaster* tends to give more reproduceable results than those based on males (Crow, 1954). Range in age was from 24 hours to one week in most cases. This method gave results which were based on the average aged fly and probably added a greater degree of variation than would results based on flies of a specific age.

Testing methods for either the W.H.O. dieldrin or DDT sets followed this general pattern:

1. Flies of a particular stock were collected from food vials and/or bottles 12 to 24 hours prior to testing, etherized, sorted, and 25 to 30 females transferred to holding vials (this follows the recommendations for adult mosquitoes per the W.H.O. instruction booklet (W.H.O., n.d.). It was later found that this number could be increased without distorting the results.
2. Flies were incubated for a 12- to 24-hour recovery period to reduce any effects caused by etherization, which might have subsequently influenced their reaction to DDT or dieldrin.
3. The following day live, healthy flies were transferred to tubes containing insecticide for the test. During actual exposure the flies were kept in the incubator, to maintain constant conditions as closely as possible, for 1, 8, or 18 hours depending on the time required for the individual test.
4. Upon completion of exposure to insecticide, flies were returned to holding vials marked to indicate the concentration of insecticide to which the flies had been subjected. Flies were placed in and removed from the testing tubes in a serial order so that the first in were the first out. This procedure insured equal test periods.
5. Flies were returned to the incubator for a 24-hour "recovery" period. The live and dead flies were counted (flies unable to walk being considered dead), and the results recorded.

The methods used with Bennett's (1959) test sets (an alternate testing method) which were made up in June, 1958, follow closely the overall pattern used for the W.H.O. 18-hour testing program. Bennett's test sets were originally made by impregnating filter paper with acetone as the vehicle for DDT. The acetone evaporated, leaving pure DDT crystals on the paper.

In our tests, concentrations of insecticides were used which were regular multiples of the lowest concentration. This was done to make it possible to use "Karber's Method" (Cornfield and Mantel, 1950) to estimate the dose of insecticides which would kill one half of the flies represented in the population of each series of three or more concentrations (LD50).

The LD50 values for individual sets of concentrations were treated as units of data in the statistical analysis.

Results and Discussion

Dieldrin Tests.—The resistance of the strains of *D. melanogaster* to dieldrin (one hour W.H.O. test) was similar with only minor variations to the relative degree of resistance these same stocks displayed for DDT at 18 hours' exposure in Bennett's 1955-1957 study (Bennett, 1959). The stocks resistant to DDT in his study appeared resistant to dieldrin in this study, and those stocks susceptible to DDT also showed susceptibility to dieldrin. The control, Cage 1, retained a mean that was located between those of the resistant and susceptible stocks. Although these data could indicate cross-resistance of these flies to DDT and dieldrin, this phenomenon of a species being resistant to the two insecticides is not necessarily an expected result (Brown, 1958). Analysis of the results using the F test (Pearson and Bennett, 1942) indicated very little significant difference between the means of HL 1-Q and LL 1-Q flies or flies in Cage 1. By this same method of analysis, ORS-1001 showed a significant difference in its reaction to dieldrin when compared to flies in either LL 1 or Cage 1. This indicated that ORS was definitely more resistant to dieldrin

TABLE 1.—Comparison of 18-hour 0.25% DDT resistance and control testing with World Health Organization's test kits (based on percent flies killed).

Test concentration	HL 1-Q	HL 2-Q	ORS-1001	Brown-eye-R	All stocks combined
Control (oil)	100.0	100.0	27.8	100.0	.....
0.25% DDT	88.0	70.8	18.7	90.0	.....
Control (oil)	34.4	92.6	60.6	97.5	.....
0.25% DDT	33.8	50.0	57.4	89.7	.....
Control (oil)	90.5	51.7	90.0	97.7	.....
0.25% DDT	32.4	35.6	65.6	95.3	.....
Control (oil)	63.7	90.8	91.7	.....	.....
0.25% DDT	41.7	86.3	77.3	.....	.....
Average of control	75.6	85.8	61.9	98.7	81.2
Average of 0.25% DDT	49.4	62.2	57.8	90.9	66.1
Ratio: $\frac{\%\text{ kill control}}{\%\text{ kill 0.25\% DDT}}$	1.532	1.379	1.071	1.086	1.227
Probability observed differences due to chance (x^2 method)	<0.001	<0.001	<0.001	$0.2>P>0.1$	.....

than were either of the susceptible lines or the control line.

DDT Tests.—Preliminary tests of one-hour duration were conducted using the W.H.O. DDT test papers. These tests produced no appreciable kill at any concentration, thus rendering them worthless as a test of DDT resistance. The instruction booklet recommends that an increase in exposure times should be the next step in such an instance. An increase to a 2-, a 4-, and if necessary to an 8-hour exposure was prescribed, but since the flies had been selected with 18-hour DDT exposures, the testing program was increased directly to 8 hours.

Results of the eight-hour W.H.O. DDT tests showed that LL 1 - Q, a susceptible stock, was most resistant. HL 1 - Q, a resistant strain, proved to be the most susceptible of all. The control, Cage 1, showed the same general range as most of the resistant stocks. Analysis (F test) of the results indicated that there were significant differences, at the 1% level, between the means for HL-1 and Cage-1 and also HL-2 and LL-1. However, some flies survived the eight-hour test at the 4.0% DDT level, so a longer exposure seemed desirable.

The 18-hour W.H.O. tests of DDT resistance produced a shifting of the relative order of the strains as to resistance, with the exception of ORS-1001 which remained fairly constant. There was a lower degree of significance in these 18-hour tests, which might have been due to the number of tests rather than to the number of flies tested. Differences between stocks tested at 8 and 18 hours were all significant

with the exception of HL 1-Q. This was unexpected because HL-1 showed the greatest resistance of all tested stocks at 18 hours and the least at 8 hours. This can be accounted for by the assumption that either there was a large genetic variation within members of this stock since resistance is determined by genetic control (Brown, 1958), or that different exposure times produced different results for the same stock. If the latter is true, and it does seem possible, the W.H.O. test kits, in order to be a standardized test device for *Drosophila*, should specify a certain period of time. This time limit could be established only after a large number of data from other research had been collected and analyzed.

A comparison was made of the 18-hour W.H.O. DDT test, the 18-hour Bennett DDT test, and an 18-hour test made by Bennett during the summer of 1958 at the University of Wisconsin. The Bennett sets used in this study were constructed and used in June, 1958. Therefore, it would be logical to assume that the test sets were more potent then than for this study, due to the volatilization of the DDT in the eight-month interim. The median lethal dose was significantly higher for the same stocks in the earlier testing program (plus some other minor variations), than it was in the tests we conducted. This would indicate a possible change in resistance between the testing dates. It is also possible that change in resistance was correlated with change from banana to cornmeal agar food. It is also possible that the conditions Bennett maintained in 1958 were enough different from the conditions in this study to cause varying results, even though the former conditions were the pattern for our testing program. Both sets of test data obtained with Bennett's test sets were vastly different from the W.H.O. test results for 18 hours, enough so that the question arises as to what stocks should be compared for relative susceptibility and resistance. Again, a greater number of tests should be conducted before this matter can be settled.

One feature is very apparent in the final analysis of the 18-hour W.H.O. DDT tests. Table 1 shows that the control paper which was impregnated with mineral oil alone produced a greater kill of *D. melanogaster* than did any test at a 0.25% DDT concentration, conducted at the same time with the same strain of flies. This difference was very significant in three of the four stocks and was prevalent in all controlled tests. Survival for the 18-hour W.H.O. test was low enough that it did not warrant testing the susceptible lines. The high percentage of kill appeared to be the result both of DDT and of the mineral oil. The oil definitely took the role of a poison or toxic substance rather than that of a neutral agent in the control experiments. We assume that there was also some effect from the oil used with the DDT, this would distort the data on the number killed by DDT alone. We feel this fact renders the W.H.O. test kit ineffective as a standardized device at 18 hours, for *Drosophila*, because it measures two different results at the same time with no discrimination. Although mineral oil did not

appear lethal in our tests at 1 or 8 hours, its effect was outstanding at 18 hours.

It is possible that HL-1 and HL-2 were resistant to DDT by behavior, in that they could recognize DDT and avoid it. Assuming this, it would then seem likely that they were unable to recognize the mineral oil as being potentially lethal, and thus did not avoid it in the control tubes. But in the 0.25% DDT tubes they may have recognized DDT and thus avoided both. This testing equipment would be improved by using a better solvent, such as acetone, which evaporates readily.

Fluctuations in temperature and humidity were occurring during the tests, and undoubtedly affected the results (Brown, 1958), although an attempt was made to regulate them. With the equipment available such errors were expected and must be considered when reviewing the results.

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Summary

1. A number of DDT resistant and susceptible strains of *Drosophila melanogaster* were tested for resistance to DDT and dieldrin, using the W.H.O. mosquito test kits. The same strains were also tested for DDT resistance by a method designed for *Drosophila*.

2. DDT and dieldrin resistance appeared to be correlated, suggesting some kind of cross reaction.

3. Apparent DDT resistance, as evidenced by ranked order of the strains, changed markedly with exposure time.

4. The mineral oil used as a carrier of insecticide in the W.H.O. test kits proved highly toxic in the 18-hour exposure tests with DDT.

5. The mosquito test kits in their present form are not suitable for use with *D. melanogaster*.

6. The most valuable aspect of the kits, for use with *D. melanogaster*, lies in the provision of standardized insecticide impregnated papers for use in equipment available locally.

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