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# ABSORPTION OF RADIOACTIVE DDT BY RESISTANT AND NONRESISTANT MOSQUITOES

C. M. GJULLIN and ARTHUR W. LINDQUIST

U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine

and

JOSEPH S. BUTTS

Oregon State College [1]

The use of radioactive insecticides appears to be a promising approach to studies of absorption and distribution of the toxicant in insects. Comparing the amount of insecticide absorbed by resistant and nonresistant strains may be of value in explaining causes of resistance. Sternburg, Kearns, and Bruce (1950) and Sternburg and Kearns (1950) determined by chemical analysis the amount of DDT absorbed and some of the metabolites formed. Lindquist, Roth, Yates, Hoffman, and Butts (1951), using radioactive DDT, found that the Orlando strain of resistant house flies absorbed considerably larger amounts of DDT than did susceptible flies.

The objects of the experiments reported in this paper were to determine the amounts of DDT absorbed by resistant and nonresistant *Aedes nigromaculis* (Lud.) larvae and whether any of the DDT absorbed by the resistant larvae was degraded into other less toxic products.

## TESTS WITH *Aedes vexans* AND *A. sticticus*

Tests were first made with nonresistant *Aedes vexans* (Meig.) and *A. sticticus* (Meig.) larvae and pupae to develop satisfactory technique for preparation of the material. Information on the mortality and the amounts of DDT absorbed by larvae of these species at high and low temperatures was also obtained.

Methods. Tests were made in the laboratory at 70° F. unless otherwise stated, with a mixed culture consisting of approximately 55 per cent of *vexans* and 45 per cent of *sticticus* obtained from eggs

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taken in soil near the Columbia River in Oregon. Fourth-instar larvae or pupae were used. The radioactive DDT had been prepared by random substitution of $C^{14}$ in the benzene rings in the DDT molecule.[1]

Radioactivity was determined with a commercial type of windowless gas (pure methane) flow counter attached to a scaler.

Larvae and pupae were treated with radioactive DDT in deodorized kerosene or in acetone suspension to determine the percentage of kill and to provide specimens in which the amount of absorption could be measured with the flow counter. The kerosene solutions were applied on 700 cc. of tap water in 800-cc. beakers and the acetone suspensions on 100 cc. of tap water. From 100 to 300 pupae were used per test, and 300 larvae in all tests except in comparative series at 70° and 90° F., in which 75 larvae were used.

The kerosene preparations were applied to the surface of the water with wire loops. A small loop, delivering 0.505 cu. mm., was used in the tests with larvae and a larger one, delivering 0.742 cu. mm., in those with pupae. The loops were calibrated by comparing the radioactivity obtained from three loopfuls of the solution with that from three 1-cu. mm. drops of the material delivered by a 0.25-cc. syringe operated with a micrometer attachment. The residue clinging to the loops after application to the water surface was measured with the flow counter. There was little loss in the light applications used for larvae, but in the heavier dosages used against pupae larger amounts were withdrawn with the loop.

The mortalities and radioactivity counts from these treatments were determined after 24 hours. The larvae or pupae in these samples were either all alive or all dead. Each sample was washed for 10 to 20 seconds to remove any exterior DDT, allowed to dry, and then ground and extracted with acetone. The radioactivity was determined after the material was thoroughly dry. The results are summarized in Table 1.

Absorption by larvae. Measurable radioactivity was found in larvae treated with both the suspension and the solution of radioactive DDT after 24 hours. With both preparations the radioactivity of larvae increased as the dosage was increased.

The figures given are for the amount of radioactive DDT absorbed up to 24 hours and are not the amounts required to kill. It was found that larvae killed with chloroform gas and subjected to acetone suspensions of DDT for 24 hours absorbed approximately two-thirds as much insecticide as did living larvae. This indicates that the DDT is taken into mosquito larvae largely by absorption and that little is ingested.

Some idea of the large amount of insecticide required in an oil film as compared with the amount actually used is shown by these tests. A 54 per cent kill was obtained among 300 larvae in an 800-cc. beaker that were exposed to a DDT-oil film containing 5 μgm. of DDT on 11.78 square inches of surface. Fifty larvae, which would be a large population for such an area under normal conditions, would use only 0.16 μgm. of DDT, or about 4 per cent of the DDT present in the film.

In tests to determine the mortality of larvae and the amount of DDT absorbed at different temperatures (70° and 90° F.), it was found that in a suspension containing 0.0167 μgm the larvae held at 70° F. averaged 97 per cent mortality with an absorption of 0.0031 μgm of DDT per larva, whereas those held at 90° averaged 71 per cent mortality and the absorption was 0.0056 μgm of DDT per larva. The ability of these larvae to absorb nearly twice as much DDT at 90° as at 70° with less mortality is comparable with results obtained on house flies (unpublished data).

Absorption by Pupae. Much larger dosages of DDT were required to kill pupae than larvae. To obtain a 42 per cent kill of pupae eleven times as much

[1] Sample obtained through the courtesy of S. B. Hendricks, Bureau of Plant Industry, Soils and Agricultural Engineering.

TABLE 1.—Absorption of DDT by larvae and pupae of *Aedes vexans* and *sticticus*. Exposure 24 hours.

| Dosage of DDT | Mortality | Living Insects: Samples tested | Living Insects: Radioactivity per sample | Living Insects: DDT absorbed per insect | Dead Insects: Samples tested | Dead Insects: Radioactivity per sample | Dead Insects: DDT absorbed per insect |
|---|---|---|---|---|---|---|---|
| | *Per cent* | *Number* | *C.p.m.* | *Micrograms* | *Number* | *C.p.m.* | *Micrograms* |
| | | | | *Tests with Larvae* | | | |
| In kerosene solution (micrograms per square inch) | | | | | | | |
| 0.2145 | 21 | 9 | 19 | 0.00168 | 4 | 35 | 0.00310 |
| .4290 | 54 | 3 | 32 | 0.00283 | 9 | 42 | 0.00371 |
| In acetone suspension (p.p.m.) | | | | | | | |
| 0.0125 | 23 | 8 | 18 | 0.00194 | 3 | 16 | 0.00173 |
| .0167 | 88 | 2 | 25 | 0.00270 | 11 | 20 | 0.00216 |
| .025 | 97 | – | – | – | 7 | 30 | 0.00324 |
| | | | | *Tests with Pupae* | | | |
| In kerosene solution (micrograms per square inch) | | | | | | | |
| 4.935 | 42 | 9 | 38 | 0.0056 | 4 | 74 | 0.0109 |
| 6.580 | 66 | 4 | 73 | 0.0108 | 8 | 176 | 0.0259 |
| In acetone suspension (p.p.m.) | | | | | | | |
| 0.2 | 14 | 3 | 85 | 0.0153 | – | – | – |
| .5 | 34 | 2 | 63 | 0.0113 | 1 | 61 | 0.0110 |

DDT was needed as to kill 54 per cent of the larvae. Many times as much DDT was actually absorbed by pupae as by the larvae. The ability of pupae to withstand such large dosages may be due to their power of metabolizing DDT into other products more readily than the larvae.

The dead and surviving pupae absorbed approximately equal amounts of DDT applied in acetone suspension. In the tests made with DDT in kerosene the dead pupae absorbed a little over twice as much as the living pupae. Owing to the erratic movements of the pupae that are affected by this insecticide, many parts of their bodies may touch the oil-covered surface of the water, while the pupae less affected remain in their normal position with only the breathing tubes exposed.

### *Aedes nigromaculis*

Absorption tests. For the tests with *nigromaculis* 300 larvae were used in 200 cc. of water and all tests were made with acetone suspensions. DDT-resistant *nigromaculis* larvae from the Merced Mosquito Abatement District and nonresistant larvae from the adjoining Fresno County in California were subjected to similar treatments with radioactive DDT. The larvae were fourth instars collected from seven pastures. Gjullin and Peters (1952) reported on the studies, comparing the resistance of mosquito larvae from intensively treated areas and nontreated areas.

The data in Table 2 show that with 0.1 p.p.m. of DDT only 11 per cent of the resistant larvae were killed but that an average of 0.0325 μgm of DDT was absorbed. All the nonresistant larvae were killed, but the amount of DDT absorbed was only 0.0180 μgm per larva. The resistant larvae were therefore able to live with approximately twice as much absorbed DDT or metabolites in their bodies as were the nonresistant larvae.

The resistant larvae that died from the treatment with 0.2 p.p.m. absorbed over six times as much DDT as the nonresistant larvae treated with 0.02 p.p.m.

Bioassay of extracts of treated larvae. To determine whether the absorbed DDT

TABLE 2.—Absorption of radioactive DDT by resistant and nonresistant *Aedes nigromaculis* larvae.

| DDT | Mortality | 50-larva samples | Condition of larvae | Radioactivity per sample | DDT absorbed per larva |
|---|---|---|---|---|---|
| *P.p.m.* | *Per cent* | *Number* | | *C.p.m.* | *Microgram* |
| | | *Resistant Larvae* | | | |
| 0.2 | 74 | 13 | Dead | 346 | 0.0345 |
| 0.1 | 11 | 20 | Alive | 326 | 0.0325 |
| 0.066 | 7 | 20 | Alive | 205 | 0.0205 |
| | | *Nonresistant Larvae* | | | |
| 0.1 | 100 | 12 | Dead | 181 | 0.0180 |
| 0.066 | 100 | 18 | Dead | 123 | 0.01230 |
| 0.02 | 94 | 15 | Dead | 54 | 0.0054 |

had been degraded to a less toxic substance, extracts of *nigromaculis* larvae that had been subjected to 0.066 p.p.m. were bioassayed. Ten samples (50 larvae per sample) of resistant and nonresistant larvae on which radioactivity had been determined were prepared for bioassay using highly susceptible second-instar larvae of *vexans* and *sticticus*.

For these tests each lot was flooded with 3 cc. of acetone to dissolve the toxicant. The debris was then removed by passing the solution through a filter paper. When the filtrate had evaporated, the toxicant was re-dissolved with 0.075 cc. of acetone and 10 cc. of water was added. Twenty second-instar larvae were then added and mortality counts were made after 24 hours. Based on the radioactivity counts the resistant *nigromaculis* larvae averaged 1.14 μgm of DDT or metabolites per sample and the mortality was 7 per cent. The average for the nonresistant larvae was 0.74 μgm per sample, but the mortality was 100 per cent.

When extracts of the resistant and nonresistant *nigromaculis* larvae were tested against *vexans* and *sticticus* larvae in 10 cc. of water, they gave kills of 12 and 70 per cent, respectively. In studies of known amounts of DDT on second-instar larvae 0.005 and 0.025 μgm in 10 cc. of water gave kills of 18 and 72 per cent, respectively. In the case of the resistant larvae, therefore, the original extracts contained 1.14 μgm of radioactive materials but the bioassay showed only 0.005 μgm of DDT or other toxic substances to be in the larvae samples. This is 99.6 per cent loss of toxicity, presumably due largely to degradation of the DDT by the larvae. The nonresistant larvae showed a loss of 96.6 per cent by the bioassay method. The extracts were not bioassayed until nearly six months after preparation, and it is likely that there had also been natural deterioration of the toxic material during this time.

## SUMMARY

The amounts of radioactive DDT absorbed by mosquito larvae and pupae were determined with a commercial type windowless gas flow counter attached to a scaler.

Fourth-instar larvae of *Aedes vexans* and *A. sticticus* absorbed from 0.00310 to 0.00371 μgm of DDT in 24 hours when subjected to kerosene films and from 0.00173 to 0.00324 μgm when treated with acetone suspensions of radioactive DDT. The mortalities ranged from 21 to 97 per cent. Dead larvae absorbed approximately two-thirds as much DDT as did live larvae. Larvae tested in acetone suspensions of DDT absorbed nearly twice as much DDT at 90° F. as at 70° and the mortality was lower at 90°.

Pupae of these species absorbed from 0.0109 to 0.0259 μgm in 24 hours when subjected to kerosene films and from 0.0113 to 0.0153 μgm in acetone suspensions. The mortalities ranged from 14 to 66 per cent.

Resistant *Aedes nigromaculis* larvae from pastures in Merced County in Cali-

fornia that were treated with 0.2 p.p.m. absorbed over six times as much DDT as nonresistant larvae from Fresno County that were treated with 0.02 p.p.m. Mortality of the resistant larvae was 74 per cent and of nonresistant larvae 94 per cent. At 0.1 p.p.m. resistant larvae absorbed nearly twice as much DDT as the nonresistant ones with only 11 per cent mortality as compared with 100 per cent mortality for the nonresistant group.

Bioassay of the extracts of these larvae with second-instar larvae of *vexans* and *sticticus* indicated that both resistant and nonresistant larvae had degraded a large amount of the DDT to non-toxic substances.

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# OBSERVATIONS ON OVERWINTERING OF *CULEX TARSALIS* COQUILLETT (DIPTERA, CULICIDAE) IN WESTERN NEBRASKA

GEORGE G. KEENER, JR.

Entomologist, Communicable Disease Center, Public Health Service, Federal Security Agency, Mitchell, Nebraska

The literature pertaining to the biology of the family Culicidae contain many statements to the effect that *Culex* mosquitoes overwinter as adult females in the northern latitudes of the United States. There appears to be, however, but little specific information available for individual species of this genus showing that specimens may be collected during the coldest winter months (December-March). *Culex tarsalis* Coquillett, one of the common mosquitoes in the Missouri River Basin States, is presently considered as the most important natural vector of Western equine encephalomyelitis (WEE) in the western half of the United States. As pointed out by Jenkins (1950) the question is still not answered as to whether WEE virus can survive the winter period within hibernating *C. tarsalis*. Hammon *et al.* (1945) collected mosquitoes during the winter months from the Yakima Valley, Washington, but WEE was not detected in the 18 specimens of *C. tarsalis* found and tested. In 1950–51 efforts were made to collect hibernating mosquitoes for virus examinations and to make observations on their overwintering habits in western Nebraska. Although literature references commonly state that *C. tarsalis* overwinters as the fertilized adult female and thus produces the new spring generation, no collection records for this species have been located to show that specimens may be collected regularly throughout the winter months.

Preliminary inspections to locate suitable shelters for mosquito hibernation were made during November. On November 14, 1950 several *C. tarsalis* females were