HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 21

FEBRUARY, 1953

NUMBER 17

LABORATORY EXPERIMENTS ON THE CONTROL OF THREE SPECIES OF FRUIT FLIES (TEPHRITIDAE) WALTER EBELING

FIELD EXPERIMENTS ON THE CONTROL OF THE MELON FLY, DACUS CUCURBITAE

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Laboratory experiments . . .

... on control of the melon fly and the Mediterranean and oriental fruit flies included tests on a total of 26 insecticides. Equipment designed on the venturi principle facilitated application of aerosols, dusts, and sprays, for both space and residue treatments.

Factors that might influence the results of treatments were found to be species, adult age, and sex of the flies and, in residue treatments, period of contact with residues, anesthetization, and number of flies per cage. The last two and the species seem to operate through effects on fly activity.

Of the insecticides tested as wettable powders in residue treatment, the thirteen most effective were parathion, dieldrin, EPN, heptachlor, lindane, aldrin, dilan, chlordane, toxaphene, methoxychlor, DFDT, DDT, and DDD. In other formulations and in space treatment the order was somewhat different.

Dosages of DDT and parathion residues that gave less than 100 per cent kill had little effect on the subsequent mortality and egg laying of melon flies that survived the treatment.

Of 17 wettable-powder residues tested, dieldrin retained effectiveness longest—about 2 weeks—on exposure to sun and rain. When sheltered from sun and rain, the residues from dieldrin, EPN, and parathion sprays resulted in 100 per cent kills of melon flies and oriental fruit flies more than 4 months after treatment.

Field experiments on control of the melon fly in Hawaii . . .

... showed that, owing to the habits of the insect, treating the crop itself is futile in preventing crop damage, even with insecticides that were highly effective in laboratory tests.

In contrast, treating a one- or two-row border of corn planted around the crop field with high concentrations of insecticide once a week (twice a week if it rained) was effective in protecting cucumbers, watermelons, and tomatoes. Treating near-by wild vegetation increased the effectiveness. The insecticides successfully used were DDT, methoxychlor, aldrin, dieldrin, EPN, and parathion wettable powders. The field tests furnished no basis for a comparison of their relative effectiveness.

A number of insecticides were tested by laboratory cage tests for effectiveness of residues after weathering. Among those tested, parathion and chlordane lost effectiveness most rapidly, especially if there were rains; dilan (not used in field tests), dieldrin, EPN, and DDT retained high effectiveness for 10 or 11 days after spraying when there were no heavy rains. The tests indicated wide differences among insecticides in the toxicant concentration required for good control.

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LABORATORY EXPERIMENTS ON THE CONTROL OF THREE SPECIES OF FRUIT FLIES (TEPHRITIDAE)¹ WALTER EBELING^{2, 3}

INTRODUCTION

THE FAMILY TEPHRITIDAE contains many economically important pests of fruits and vegetables that are known as "fruit flies." The three species reported upon in this paper are the melon fly, *Dacus cucurbitae* (Coquillett); the oriental fruit fly, *D. dorsalis* Hendel; and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). All three are serious pests in the Hawaiian Islands and are threats to the fruit and vegetable industries on the mainland—particularly in California—where quarantines have thus far prevented them from becoming established.

The melon fly was apparently first noticed in the Territory of Hawaii on the island of Oahu in the summer of 1897 (Van Dine, 1908). In about two years the species had become so abundant that the growing of melons, once a profitable crop, was abandoned for about a decade. Melons were then again grown in a limited way by Japanese growers who covered them with paper, soil, or straw, immediately after the setting of the fruit, in order to prevent the flies from ovipositing. Chemical control has up to now been ineffective.

The principal food plants of the melon fly in Hawaii are all the cucurbits (watermelons, muskmelons, cucumbers, squashes, pumpkins, and so on, including two wild cucurbits, *Sycos* sp. and *Momordica balsamina* L.), as well as tomatoes and beans. The biology of the melon fly was investigated by Severin, Severin, and Hartung (1914) and Back and Pemberton (1918b).

The Mediterranean fruit fly, since its discovery in the Hawaiian Islands in 1910, has caused as serious a check on horticultural pursuits as the melon fly caused in the growing of cucurbits and tomatoes. A thorough investigation of the biology of this species was made by Back and Pemberton (1918a).

The oriental fruit fly was discovered in the Hawaiian Islands in 1946. The severe damage it has since done to fruit and vegetable crops in the Hawaiian Islands, as well as the potential threat to agriculture on the mainland, led to a comprehensive and coördinated research program participated in by the Bureau of Entomology and Plant Quarantine of the United States Depart-

¹ Received for publication December 6, 1951.

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[•] The writer was assisted throughout the investigation by Mr. Clarence Nihei and for a period of four months by Mr. Newton Morton.

ment of Agriculture, the Hawaii Agricultural Experiment Station, the Board of Commissioners of Agriculture and Forestry of the Territory of Hawaii, the Pineapple Research Institute of Hawaii, the Experiment Station of the Hawaiian Sugar Planters' Association, the California State Department of Agriculture, and the University of California Agricultural Experiment Station. All oriental-fruit-fly investigations were coördinated under the direction of Dr. Walter Carter (see Carter, 1949–1951).

In the research program of the University of California, organized by Professor Harry S. Smith, provision was made for investigations not only on the oriental fruit fly but also on the melon and Mediterranean fruit flies.

In June, 1950, the writer was stationed in Honolulu, Hawaii, and assigned for one year to that portion of the research program of the University of California that dealt with the laboratory and field investigations of the control of the melon fly. The primary purpose of this part of the program was to evaluate the potentialities of the various insecticides for the eradication of the melon fly if it should become established in California despite the existing quarantines, or for the control of the insect if it should become permanently established in California. In the course of the investigations, opportunity presented itself for a comparative laboratory evaluation of insecticides against the oriental fruit fly and the Mediterranean fruit fly. This paper reports the results of the laboratory experiments with all three species, but with particular emphasis on the melon fly. The results of the field experiments, which were confined to the melon fly, are reported in the companion paper (Ebeling, Nishida, and Bess, 1953).

As a guide in choosing the technique for the laboratory insecticide investigations and in interpreting results, experiments for evaluating the effects of such variables as anesthetization and sex and age of the insects were designed. The circumstances of the coöperative program in Hawaii provided such exceptional facilities for this work that more extensive experiments were conducted than would ordinarily be feasible. Since the findings may have some bearing on other laboratory insecticide investigations, perhaps even with different species of insects, they are reported in considerable detail.

EQUIPMENT, TECHNIQUE, AND MATERIALS TESTED

Ample laboratory space was provided through the courtesy of the Department of Entomology of the University of Hawaii. However, all equipment for the application of insecticides in the laboratory was devised and constructed by the writer for the specific purposes of the melon-fly research program. This equipment was constructed with a view to duplicating as closely as possible the types of treatment that were presumed to have potential utility in the field; namely, aerosols, sprays, and dusts. The equipment designed for spraying or for application of aerosols was based on the venturi principle, and, with modifications, could also be used for dusting.

Settling Tower. Figure 1 shows a portion of the equipment constructed in the laboratory for applying aerosols (or mists) and dusts. Air passing through the tube a t draws 5 ml of liquid from the vial (v) in about 6 seconds through the liquid tube (l t), which has an inner diameter of approximately 1 mm except at its extremely small orifice.

Figure 2 shows the settling-tower assembly. Above the turntable is a glass cylinder 12 inches in diameter and 28 inches high. The greater part of the mist will settle down on the turntable in 3 minutes, the period selected for the experiments made in this laboratory. At the expiration of the 3-minute



Fig. 1. Equipment for generating aerosols: a t, air tube; b, bearing; l t, liquid tube; o, orifice of air tube; δ , orifice of liquid tube; p, pulley and belt; s c, spring clamp for holding vial; t, base of turntable; v, vial.

Fig. 2. Settling-tower assembly: f, exhaust fan; op, opening to permit introduction of liquid or dust charge; p, cover for tower; s, toggle switch; t, glass tower.

period, the plate of glass (p) is removed, and the remaining mist is drawn out of the cabinet and blown through an open window by the fan (f).

Anesthetized adult flies may be placed on petri dishes and exposed to the mist for the 3-minute period, or the insecticide may be allowed to settle on empty petri-dish covers and the flies allowed to crawl over the residue. Five petri-dish covers may be placed on the turntable at one time.

Dust Apparatus. One half gram of insecticide dust is placed in a glass container, constructed from a 1-inch soft-glass tube and flanged above for

added strength. This is stoppered with a rubber cork through which is inserted a glass tube with an inner diameter of 5 mm. The air is blown through the tube into the dust container and thence into the air tube shown in Figure 1. The dust is quickly blown from the container and is allowed to settle in the settling tower (Fig. 2, t) for 2 minutes.

Spray Apparatus. The venturi apparatus shown in Figure 1 was duplicated in essential detail in the construction of the spray equipment except that the



Fig. 3. Container for spray equipment: a b, agitator blade; a t, air tube; b, bearing; j, jar; l, screw-type lid of jar; l t, liquid tube; p, pulley; s, agitator shaft. ($\times 0.5$.)

discharge orifice was left much larger. Consequently a greater volume of liquid is discharged and the spray is of a much greater droplet size, simulating the spray droplets from field spray equipment. In addition, since a much larger quantity of liquid is required, and a longer period is required to empty the container, solids cannot be uniformly suspended for the required period merely by shaking the suspension before using. Consequently an agitator (Fig. 3, a b) is provided.

Figure 4 shows the complete sprayer assembly. The hood (h) is an inverted 5-gallon tin can with one side cut out and the exposed edges turned back as a runway for a sliding door (s d). The sliding door needs to be only part way down to avoid splattering of liquid on the operator. Mists and vapors are

blown away by means of an electric fan. Inside this hood may be seen, in Figure 4, a rectangular board holding in place a circular card (c) upon which the spray stream is directed. This is a waxed paper-cup lid 3^{11}_{16} inches in diameter and with an area of 10.3 square inches (66.5 sq. cm.). (It is ordinarily used as a cover for paper cups containing ice cream, potato salad, and



Fig. 4. Laboratory spray equipment: a b, agitator blade; a t, air tube; b, beaker; c, waxed card (paper-cup lid) upon which spray is directed; h, hood; j, jar; l, jar lid; m, electric motor; p, pulley; s, agitator shaft; s d, sliding door; t s, toggle switch. ($\times 0.125$.)

other types of food.) This card is wet by a spray in a manner similar to that of many types of clean leaf surfaces. Four thumb tacks are placed in the board supporting the card in such a way that the card can easily be slipped into place and is held by the heads of the tacks. It is equally easily removed by means of a tab such as is generally present on paper-cup lids. Flies are later allowed to crawl over the spray residues on the cards.

As shown in Figure 4, the card is held at a 45-degree angle so that the spray droplets roll over it as they do over leaf surfaces. The spray deposit is similar in quantity and distribution to that on the average leaf surface. The spray stream is directed against the card for 5 seconds, then the stream is

cut off by stopping the air compressor by means of the toggle switch (Fig. 4, t s). Twenty-nine cards can be sprayed with one filling of the liquid container. Usually only 3 or 5 cards are sprayed with one material or concentration, but 15 additional cards are sprayed when residues are to be analyzed.



Fig. 5. Card-filing cabinet with two trays out to show how petri-dish covers with insecticide residues from the settling tower were stacked away for drying.

Equipment for Drying Insecticide Residues. As stated previously, sometimes the flies were not treated directly, but were allowed to crawl over insecticide residues deposited on petri-dish covers in the settling tower or on circular waxed cards in the spray chamber. To evaporate the water or solvent from the insecticide residue, the dishes or cards were placed in the trays of a type of card-filing cabinet (Fig. 5) in which there were 15 trays, each capable of holding 15 dishes. Holes were bored into the back end of this cabinet to facilitate the circulation of air. The treated petri-dish covers, or the residue cards inserted into petri-dish covers, then formed the tops of the laboratory test cages, in which flies were confined with residues for 24 hours.

Anesthetizing and Counting. The adult flies were brought to the laboratory in 10-inch square screen cages.⁴ They were anesthetized by placing them, in their cage, under a fumatorium (see Sherman, 1950) and subjecting them to CO, gas for about 2 minutes. The flies from several rearing cages, and possibly of different ages, were put into a single rearing cage, after they were anesthetized, and were allowed to revive and fly or crawl about in this cage in order to become thoroughly mixed. The flies were then anesthetized again and placed in a Buchner funnel through which a small volume of CO₂ gas was allowed to pass. The anesthetization was thus continued while the flies were counted. A clump of flies was picked up by means of the fingers or a pair of forceps and placed in a 6-inch test tube. The tube was gently shaken and rolled to scatter the flies throughout its length and a count was made. Then a few more flies were added or taken out, if necessary, so as to leave 25 in the tube. The tube was then corked and set aside until the desired number of lots of 25 flies were counted. When the flies were ready for treatment, more CO, was passed into the test tubes to bring about enough anesthetization to facilitate manipulation in the various types of insecticide treatment.

Some variations from this procedure were necessary for certain experiments. These variations are noted where the experiments are described.

How Flies Were Handled after Treatment. After treatment, the flies were placed in cages made by fitting both ends of a cylinder of fly screen, 4 inches high, into petri-dish covers (Fig. 6). A hole was punched in the screen through which a dental roll of absorbent cotton, $1\frac{1}{2}$ inches long, was inserted. This roll was first soaked in 40 per cent sugar solution. More of the solution can later be added from the outside, if needed, by means of a medicine dropper. Such cages were used by Dr. Martin Sherman at the time the writer arrived in Hawaii.

If the flies were to be exposed to an insecticide residue, the petri-dish cover containing the residue formed the ceiling of the cage. The flies have the habit of resting or crawling on the undersides of horizontal surfaces, so they spent much time on the residues. As explained previously, when the mist residue was deposited in the settling tower, it was allowed to settle on the petri-dish cover itself. The waxed cards treated in the spray chamber were of the right size to be held in place in the petri-dish covers by friction.

The flies were kept in the cages for 24 hours (usually) before mortality counts were made. The number of dead or moribund flies found on the bottom of the test cage was recorded. Moribund flies usually were lying on their backs, but whether they were or not, they were easily identified by their paralyzed condition.

After each test the cages were decontaminated by washing the petri-dish covers with hot water and detergent, then thoroughly rinsing them and drying them by evaporation. The screen part of the cage was plunged in acetone six times, then dipped into a pail of running water three times and dried by evaporation.

⁴ The flies were supplied by the University of California fruit fly and parasite rearing laboratory in Honolulu, where the investigations of G. L. Finney on rearing techniques, K. S. Hagen on nutritional requirements, and Miss Shizuko Maeda on the influence of microorganisms, resulted in a steady production of healthy adult fruit flies in large numbers. (See Carter, 1949–1951.)

The Effect of the Period between Treatment and Counting on Per Cent Mortality. When the flies were directly subjected to a mist or dust (space treatment) and were then removed from further contact with the insecticide, there was very little difference in per cent mortality when counts were made in 24 hours and in 48 hours. In five space-treatment tests with DDT and methoxychlor, in which the average per cent mortality was 44.6 in 24 hours, it was 45.4 in 48 hours, an increase of only 1.8 per cent.



Fig. 6. Insecticide-testing cage.

As is to be expected, when the flies were continuously exposed to insecticide residues, the per cent mortality increased in the period between 24 hours and 48 hours of confinement. Seven lots of male and female melon flies, 3, 5, 7, 9, 11, 12, and 14 days of age, respectively, were exposed to the residue from 1 per cent DDT which settled on petri-dish covers in the settling tower. Among the female flies, 54.3 per cent were dead in 24 hours and 78.3 per cent in 48 hours. Among the males subjected to the same treatment and during the same period, 65.1 per cent were dead in 24 hours and 92.0 per cent in 48 hours. It can be calculated from these data that among the female flies there was 69.3 per cent and among the males 70.8 per cent as high a mortality in 24 hours as in 48 hours. There is no reason to believe, however, that the 48-hour mortality count would offer any advantages over the 24-hour count for the purpose of comparing the insecticidal efficiency of residues. In the present report, a 24-hour period is implied unless otherwise stated. List of Insecticides Used in Tests. Following is a list of the insecticides used in these experiments and their chemical composition.

Material	Composition
Parathion	O, O-diethyl O-p-nitrophenyl thiophosphate
Paraoxon	Phosphate analog (oxy analog) of parathion
EPN	· O-ethyl O-p-nitrophenyl benzene thiophosphonate
Dimethyl parathion	Dimethyl analog of parathion
Metacide	Mixture of 25 per cent dimethyl parathion and 6 per cent parathion
Diisopropyl paraoxon	Diisopropyl analog of paraoxon
TEPP	Tetraethyl pyrophosphate
OMPA (Pestox III)	Octamethyl pyrophosphoramide
E-838	Diethoxy thiophosphoric acid ester of 7-hydroxymethyl coumarin
Compound 3975	O-(1, 2-dicarbomethoxyethyl) O, O-diethyl dithiophosphate
Compound 4018	Dicarbomethoxyethyl analog of 3975
Compound 4049	Dimethyl analog of 3975
Compound 4124	O-(2-chloro-4-nitrophenyl) O, O-dimethyl thiophosphate
Lindane	Gamma isomer of 1, 2, 3, 4, 5, 6-hexachlorocyclohexane
Chlordane	1, 2, 4, 5, 6, 7, 8, 8-octachloro-2, 3, 3a, 4, 7, 7a-hexahydro-4, 7-methano indene
Heptachlor	1, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 6, 6a-tetrahydro-4, 7- methano indene
Dieldrin	1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a- octahydro-1:4, 5:8-dimethano naphthalene
Aldrin	1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1:4, 5:8-dimethano naphthalene
Toxaphene	Chlorinated camphene $(C_{10}H_{10}Cl_8)$
DDT	1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl)-ethane
DDD (TDE)	1, 1-dichloro-2, 2-bis(p-chlorophenyl)-ethane
DFDT	1, 1, 1-trichloro-2, 2-bis(p-fluorophenyl)-ethane
Ditolyl trichloroethane	1, 1, 1-trichloro-2, 2-bis(p-tolyl)-ethane
Methoxychlor	1, 1, 1-trichloro-2, 2-bis(p-methoxyphenyl)-ethane
DNP	1, 1-bis(p-chlorophenyl)-2-nitropropane
DNB	1, 1-bis(p-chlorophenyl)-2-nitrobutane
Dilan	Mixture of DNP and DNB
DNCHP .	2, 4-dinitro-6-cyclohexylphenol
Neotran	bis(p-chlorophenoxy) methane
Genitol 923	2, 4-dichlorophenyl ester of benzenesulfonic acid
Pyrethrins	Pyrethrins I and II
Allethrin	Allyl homolog of cinerin I
Nicotine	3-(1-methyl-2-pyrrolidyl)-pyridine
Lethane	Beta-butoxy-beta'-thiocyanodiethyl ether
Rotenone	······
Sabadilla	••••••

The formulations used were wettable powders, emulsifiable concentrates, kerosene solutions, and dusts, though not all the insecticides listed were tested in all four formulations. The wettable powders and emulsifiable concentrates were all proprietary formulations. The kerosene solutions were prepared from wettable powders—or, if necessary, from emulsifiable concentrates—except with DDT, for which a technical grade was available. The dusts were made by diluting proprietary dusts or wettable powders to the required concentrations with pyrophyllite.

The suspensions, emulsions, and solutions were all freshly prepared the day they were used.

Throughout the paper, "per cent concentration" is in grams per 100 milliliters of suspension, emulsion, or solution, and in grams per 100 grams of dust.

FACTORS THAT MAY CAUSE VARIATION IN THE RESULTS

Natural Mortality. How a factor causing high natural mortality might affect toxicity evaluations is shown in one experiment in which certain lots of flies were deprived of water. The residues of four insecticides were being tested. The usual 3 lots of 25 flies were used for each concentration and for an untreated check group. One of these 3 lots was not given water for the 24hour period during which the flies were confined to the insecticide residues. In the checks, the mortality among the flies without water was 44 per cent, while among the flies receiving water it averaged only 4 per cent. In the lots exposed to residues, however, when the mortality was above 64 per cent there was no significant difference in the mortalities among the watered and the unwatered flies.

Apparently those flies that succumb to such adverse conditions as lack of water over a limited period are the same ones that succumb to comparatively low dosages of insecticide, and the flies that can resist such adverse conditions are the ones that resist all but the higher dosages of insecticide. The resistance of the latter to insecticides does not appear to be reduced by an environmental condition sufficiently severe to cause a 44 per cent mortality of the general population. Possibly other natural-mortality factors would show the same tendency. It would be difficult to make corrections for natural mortality unless it were certain that natural mortality and insecticide mortality operate independently on the entire test population.

In the experiments reported in this paper, natural mortality of adult fruit flies was never more than 10 per cent, and usually below 5 per cent. Often no flies died in the 3 untreated test cages used as checks. For this reason, along with the considerations discussed above, no attempt was made to correct for natural mortality.

Effects of Anesthetization. In order that the flies might be easily handled, they were inactivated with CO_2 . In the experiments reported in this paper, it was seldom necessary to keep the flies in the gas for as long as 1 hour. Ordinarily the tubes in which the flies were kept before treatment were filled with CO_2 in groups, so that the flies were in the gas less than half an hour. Preliminary experiments indicated that under such conditions the anesthetization did not affect mortality during the usual 24-hour period that the flies were in the cages. Flies left in CO_2 for 1 hour showed 0 per cent mortality and those left in for 2 hours, 3 per cent mortality in 24 hours; mortality in 48 hours was 3 and 8 per cent.

It was found, however, that flies became much less susceptible to insecticide residues if they were reanesthetized or if they were exposed to the gas for prolonged periods or—even for as little as 5 minutes—to high concentrations. The kill from exposure to a 1 per cent DDT aerosol residue for 24 hours was only about 55 per cent as high among 5 lots of twice-anesthetized flies as among 5 lots of once-anesthetized flies; and among flies left in CO_2 for 1 and

2 hours it was 37 and 32 per cent respectively as high as among flies exposed immediately after anesthetization. Differences in kill after 48 hours' exposure to the residue were smaller.

The decrease in susceptibility seems to be due to decreased activity of the flies. Flies that were completely anesthetized with a rapid flow of CO_2 for 3 minutes and kept in the gas for 1 minute longer required less than an hour to reach what seemed to be normal activity; whereas flies first completely anesthetized and then left in test tubes filled with CO_2 for 10 minutes required about 4 hours to appear on the ceilings of their cages in as great numbers as the first group, and even then were much less active.



Fig. 7. Variation in the susceptibility of male and female melon flies of different adult ages to the residue from a DDT suspension containing 1.0 per cent actual toxicant.

On the other hand, a group that were subjected to a feeble flow of CO_2 gas for 2 minutes, so that they were incompletely anesthetized, required about 2 hours—1 hour longer than the first group—to reach what seemed to be normal activity. It appears anomalous that a single complete anesthetization from a brief exposure to CO_2 should leave the flies in a more active state than a less complete anesthetization. This third group eventually became about as active as the first group but in the 24-hour period of observation did not appear on the ceilings of the cages in as great numbers.

Thus the method of anesthetization and preparation of the flies to be placed in the residue cages has an effect on their subsequent behavior that influences not only the percentage of flies in a cage in contact with the insecticide residue at a given moment, but also the number of times the flies crawl over the residue, in both cases influencing the mortality. Both a light anesthetization and prolonged exposure to the anesthetic reduce the percentage of flies in contact with the residue, which is always placed on the petri-dish cover that forms the ceiling of the cage. Prolonged exposure to the anesthetic, in addition, greatly retards the rate of movement of the flies over the residue surface. The reduced kills from exposure to DDT residue among flies subjected to CO_2 for prolonged periods are thus shown to be the result of the retardation of fly activity caused by the gas. Ililgardia

There has been no evidence that CO_2 as used in this project for the purpose of inactivating the flies has any effect on per cent kill from insecticides used as space treatments, in which no residues are involved. Metcalf (1940) likewise concluded that CO_2 did not influence per cent kill from topical applications under the conditions of his experiments.

Effect of Adult Age of Fly. To test the effect of age of flies on toxicity results, male and female melon flies of adult ages ranging from 2 to 16 days were subjected simultaneously to DDT residues on petri-dish covers. The



Fig. 8. Variation in the susceptibility of melon flies of mixed sexes and of different ages to DDT suspension as a space spray (0.5 per cent of toxicant) and as a residue (1.0 per cent of toxicant).

residues were from 1 per cent DDT aerosol made from a 50 per cent wettable powder, applied in the settling tower. In each of four tests, 3 lots of males and 3 lots of females were used with each adult age treated. All ages were represented in the course of the experiment, in which 4,200 flies were used. The four tests were made at widely separate dates, ranging over a period of about 2 months (August and September, 1950).

The results of the experiment are graphically depicted in Figure 7. A distinct change in the susceptibility of the flies occurred on the eighth day of their adult life when oviposition began: flies from 8 to 16 days old, inclusive, were uniformly more susceptible to DDT than those from 2 to 7 days old, inclusive. Except for the 2-day-old flies, the males were always more susceptible than the females, but the abrupt change in susceptibility on the eighth day of adult life was equally marked with both sexes.

In January, 1951, the effect of age on the susceptibility of melon flies to DDT was further investigated. Flies of each of 10 ages, ranging from 2 to 17 days, were used in the experiment. Since in the previous experiment the difference in susceptibility at different ages was the same for males and females, the sexes were no longer segregated. As before, the flies were exposed to 1 per cent DDT aerosol residues, but in addition, an equal number of flies

were given a space treatment with a 0.5 per cent actual DDT suspension made with a 50 per cent wettable DDT powder. The suspension was allowed to settle on anesthetized flies in the settling tower.

Figure 8 shows the per cent mortality for the flies of the various age groups sprayed directly (left) and those allowed to crawl over the residues for 24 hours (right). Since the temperatures were considerably lower than for the experiment depicted in Figure 7, the sharp increase in per cent mortality did not occur until the tenth day of adult life instead of on the eighth day;



Fig. 9. Variation in the susceptibility of male and female oriental fruit flies of different adult ages to a DDT emulsion containing 0.05 per cent of actual toxicant and used as a space spray.

but as before, it coincided with the termination of the preoviposition period.

Soon after this experiment the rearing rooms were artificially heated to simulate summer conditions and the period of comparative resistance was again found to be approximately 7 days. Consequently no melon flies over 7 days old were used in insecticide evaluations. As stated before, if flies of more than one age were used, they were thoroughly mixed.

The results of a similar experiment with oriental fruit flies of age groups from 2 to 17 days, inclusive, are shown in Figure 9. Each histogram represents the average per cent mortality from three experiments, two made about 1 month after the first, but under practically identical conditions in the rearing room. A 0.05 per cent DDT aerosol from a 25 per cent emulsifiable concentrate was allowed to settle on anesthetized flies in the settling tower.

The tendency was for oriental fruit flies to become more resistant to the DDT up to the adult age of 13 days. On the fourteenth day the flies became highly susceptible, as compared to flies one to several days younger or older. This tendency was equally marked in all three experiments. In the third experiment, made on February 24, 1951, the flies were taken from the same rearing cages as those of the second experiment, made the previous day. The results were similar. The flies from the same group that had been resistant



Fig. 10. Variation in the susceptibility of male and female Mediterranean fruit flies of different adult ages to a DDT suspension containing 0.25 per cent of actual toxicant and used as a space spray.

to DDT on February 23, at the age of 13 days, were highly susceptible the following day.

In the insecticide evaluation with the oriental fruit fly, insects a week or less in adult age, or mixtures of age groups within that age category, were used.

In Figure 10 is shown the effect of age on the susceptibility of male and female Mediterranean fruit flies subjected to 0.25 per cent DDT aerosol from

TABLE 1 EFFECT OF SEX ON SUSCEPTIBILITY TO DDT AEROSOL SPRAYS IN THREE SPECIES OF FRUIT FLIES*

Species	Date	Sex	Weight, mg	Per cent mortality
Melon fly, Dacus cucurbitae	Sept. 13, 1950	{ Male Female	14.4 19.2	47 36
	Dec. 4, 1950	{ Male Female	15.9 20.7	72 63
Oriental fruit fly, Dacus dorsalis	Jan. 15, 1951	{ Male { Female	5.9 6.2	36 51
	Feb. 23, 1951	{ Male Female	11.8 12.6	46 56
Mediterranean fruit fly, Ceratitis capitata	Jan. 16, 1951	{ Male { Female	4.2 4.4	87 59
	Dec. 23, 1950	{ Male { Female	7.7 8.5	61 38

* Various percentages of DDT wettable powder or emulsifiable concentrate in space treatments were used in the course of the experiments so as to result in kills of less than 100 per cent. In all tests males and females were treated with the same dosage.

a 50 per cent wettable powder, allowed to settle on anesthetized flies in the settling tower. With this species there was a marked increase in susceptibility to DDT at the adult ages of 10 and 14 days. This experiment was made during the month of February. In May the experiment was repeated, but without segregation of sexes. As occurred in the earlier experiment also, there was a sharp decrease in susceptibility up to the adult age of 4 days, when the flies were more resistant than at any other age. In the later experiment the two peaks in susceptibility occurred at the adult ages of 8 and 13 days (see Fig. 11). In general, however, the younger flies were the more resist-



Fig. 11. Variation in the susceptibility of mixtures of male and female Mediterranean fruit flies of different adult ages to a DDT suspension containing 0.25 per cent of actual toxicant and used as a space spray.

ant, and in practice, flies from 3 to 8 days of age, inclusive, or mixtures of these ages, were used in the experiments on insecticide evaluation with this species.

Effects of Sex and Crowding. The sexes were segregated in the experiments depicted in Figures 7, 9, and 10. The data on which these figures are based and those of a number of other experiments are summarized in Table 1. It will be noted that with the melon fly and Mediterranean fruit fly, the males were more susceptible to DDT residue than the females, but the opposite was true for the oriental fruit fly.

A further experiment was conducted to determine not only the effect of segregating versus randomizing the sexes, but also the effect of number of flies per lot, on insecticide evaluation. In this experiment the lots of melon flies exposed to 1 per cent DDT aerosol residues were of four categories: (1) 25 females, (2) 25 randomized males and females, (3) 50 females, and (4) 50 randomized males and females. Five replications of 3 lots were made for each category. This experiment was designed to determine (1) the degree of variance in fly mortality in the mixed male and female groups as com-

pared with the groups containing only one sex; (2) the degree of variance in the lots containing 25 flies as compared with those containing 50 flies, and (3) the effect of crowding on per cent kill.

The results are shown in Table 2. There is not enough difference in the coefficients of variation to indicate that there would be an advantage in using one category in preference to another. Practical considerations greatly favor

TABLE 2 EFFECT OF SEX AND CROWDING ON SUSCEPTIBILITY OF MELON FLIES TO RESIDUE FROM 1 PER CENT DDT AEROSOL

I		25 fe pe	emale er lot	es		25 of sex	mix per lo	ed ot		50 fe pe	emale er lot	8		50 of sex	mixe per la	ed ot
Ttem	Lot 1	${f Lot}_2$	Lot	Av.	Lot 1	Lot 2	Lot	Av.	Lot 1	Lot 2	Lot 3	Av.	Lot 1	$_2^{\rm Lot}$	Lot	Av.
No. dead, treated groups: Test 1. Test 2. Test 3. Test 4. Test 5. Average per lot, all tests.	12 14 16 14 13	11 15 14 12 12	15 18 13 10 11 	12.7 15.7 14.3 12.0 12.0 13.4	18 17 16 19 17	21 17 13 19 14	17 21 14 16 13	18.7 18.3 14.3 18.0 14.7 16.8	23 26 21 22 18	22 19 17 14 25	21 21 15 17 19 	22.0 22.0 17.7 17.7 20.7 20.0	23 20 21 19 20 	24 23 18 18 22	24 27 21 27 21 	23.7 23.3 20.3 18.0 21.0 21.2
Untreated	0	0	2	0.7	1	0	1	0.7	0	2	2	1.3	0	0	0	0.0
Per cent mortality				53.4				67.2				40.0				42.4
Standard deviation for number per lot				1.54				2.12				2.18	•			2.3
Coefficient of variation				11.4				12.6				10.9				11.0

L.S.D. of average number dead per lot (all tests) at P = 0.05, 1.97.

the use of the lower number of flies and the use of a random mixture instead of one sex. Furthermore the per cent mortality of flies is considerably higher in the cages containing 25 flies than in those containing 50 flies.

Increasing the number of flies per cage reduced the relative number at any given moment on the under side of the petri-dish cover forming the top of the cage and on which the insecticide residue is deposited. An experiment was made to obtain quantitative data on this point. The same categories of flies shown in Table 2, and in the same number of lots per category, were confined to cages. Everything about the experiment was the same as for the one depicted in Table 2 except that the petri-dish covers forming the tops of the cages contained no insecticide residues. The two experiments were made simultaneously and with the same age mixture of flies, representing ages ranging from 3 to 16 days except that those 8 and 9 days of age were not used. The number of flies that could be seen on the tops of the cages at various times of the day were counted, with the results shown in Table 3.

It can be seen from Table 3 that the number of flies resting or crawling about on the tops of the cages was not in proportion to the numbers confined in the cages. There were only 72 per cent as high a percentage in the cages of \cdot

50 females as in those of 25, and only 54 per cent as high a percentage in the cages of 50 of mixed sexes as in those of 25. That this difference in the activity of the flies has an effect on mortality from insecticide residues can be seen from the fact, as shown in Table 2, that the kill was only about 75 per cent as high in the cages of 50 female flies as in those of 25, and only 63 per cent as high in the cages of 50 flies of mixed sexes as in those of 25.

TABLE 3

EFFECT OF SEX AND CROWDING ON PER CENT OF MELON FLIES ON THE CEILINGS OF CAGES

						Num	ber o	of flies	on ce	iling	of ca	ıge				
Time (Oct. 16, 1950)	w	ith 2	5 fem er lot	ales	W	ith 25 sex j	of n per lo	nixed ot	w	ith 5	0 fen er lot	ales	Wi	th 50 sex p) of m per lo	ixed t
	Lot 1	Lot 2	Lot 3	Av.	Lot 1	Lot 2	Lot	Av.	Lot 1	Lot 2	Lot 3	Av.	Lot 1	$_2^{\rm Lot}$	Lot	Av.
8:00 a.m.	15	9	12	12.0	16	11	13	14.7	10	13	14	12.3	14	12	20	15.3
9:30 a.m.	9	8	11	9.3	17	8	9	11.3	9	6	8	7.7	8	14	15	12.3
10:20 a.m.	11	5	9	8.3	10	7	5	7.3	12	15	12	13.0	10	-11	17	12.7
11:00 a.m.	6	6	9	7.0	12	5	7	8.0	10	15	16	13.7	8	8	12	9.3
11:30 a.m.	5	3	8	3.3	10	9	10	9.7	18	15	11	14.7	8	9	10	9.0
12:00 m.	4	4	11	6.3	12	9	6	9.0	9	8	17	11.3	6	9.	13	9.3
12:50 p.m.	9	9	10	9.3	11	7	5	7.3	10	11	16	12.3	8	12	13	11.0
1:30 p.m.	6	4	8	6.0	12	4	10	8.7	15	10	8	11.0	8	5	8	7.0
2:45 p.m.	4	6	5	5.0	12	10	9	10.3	5	8	16	9.7	9	6	10	8.3
4:00 p.m.	8	8	11	9.0	11	10	12	11.0	5	9	11	8.3	4	10	10	8.0
5:05 p.m.	4	6	8	6.0	11	7	9	7.3	9	8	5	7.3	13	11	7	10.3
Average number*				7.6				9.5				11.0				10.2
Average per cent				30.4				38.0				22.0				20.4

* L.S.D. of average number at P = 0.05, 1.48.

Weights of Three Species of Fruit Flies. Average weights of each sex of the three species of fruit flies are given in Table 1. The weights are averages of 200 flies of each sex for the melon fly and 100 of each sex for the other two species. The differences in the weights of the oriental fruit fly and of the Mediterranean fruit fly on different dates are due to differences in the amounts and kinds of food given the larvae. Those who reared these flies had not yet worked out a uniform system of rearing. Table 1 indicates that the melon fly is the largest and the Mediterranean fruit fly the smallest of the three species of fruit flies occurring in Hawaii.

EXPERIMENTS WITH THE SETTLING TOWER

The objective of the investigations with the settling tower was to obtain a comparative evaluation of contact insecticides—especially new synthetics—as wettable powders, emulsifiable concentrates, kerosene solutions, and dusts, in both space and residue treatments.

Deposit from Different Formulations. The amount of DDT deposited on the petri-dish covers resting on the turntable of the settling tower is shown in Table 4 for a suspension, an emulsion, a kerosene solution, and a dust.

The deposits were determined by calculation from gravimetric determinations of the amount of liquid or dust settling on the turntable. The following are the relative quantities of toxicant deposited by the various formulations, used at the quantity per charge indicated in Table 4, if the quantity deposited by the wettable powder is equated to 100: wettable powder, 100; emulsifiable concentrate, 96.6; kerosene solution, 29.2; and dust, 66.9. The kerosene aerosol not only descended to the bottom of the tower more slowly, because of the smaller droplet size, but was used at only 40 per cent as great a quantity per charge as were the other formulations. (In order to avoid mortality from the kerosene itself, only 2 ml of kerosene per charge was used.)

TABLE 4 DEPOSIT OF TOXICANT ON TURNTABLE IN SETTLING TOWER FROM FOUR FORMULATIONS OF DDT

Formulation	Quantity of charge	Deposit of DDT, mmg/cm ^{2*}
Suspension (wettable powder)	5 ml	14.59
Emulsion (emulsifiable)	5 ml	14.10
Kerosene solution	2 ml	4.26
Pyrophyllite dust	1 gram	9.76

 $\ ^{\bullet}$ One per cent in weight/volume for the liquids and weight/weight for the dusts.

Dosage-Mortality Regressions. In the settling-tower experiments, all of the insecticides listed on page 523 were tested in both space and residue treatments against the melon fly and the oriental and Mediterranean fruit flies. Many of them were tested in four formulations but some were not available in all four. All insecticides used in a given formulation, against a given species, and in a given treatment were tested the same day, in at least three concentrations. The charges were 5 ml for wettable powders and emulsifiables, 2 ml for kerosene solutions, and $\frac{1}{2}$ gram for dusts. An untreated control was used with each day's series, and tests on DDT as a wettable powder were repeated each day.

The data obtained are summarized in the form of dosage-mortality regression curves in Figures 12 to 14. It can be seen from these figures that the slopes of the regressions for a given species and with a given formulation are remarkably similar. Considerable variations in the slopes were obtained in preliminary experiments when different age mixtures of flies were used in testing the different groups of insecticides of a given formulation. Later work showed that these variations were caused primarily by the differences in the age mixture of the flies, and possibly by environmental factors as they varied from day to day. When all insecticides of a formulation were tested on the same day and with the same age mixture of flies, the differences in slopes of the curves tended to become reduced.

In comparing the different formulations it should be borne in mind that the kerosene solutions, at 2 ml per charge, deposited only 29.2 per cent as much toxicant as the wettable powders. The dusts were used in $\frac{1}{2}$ -gram



Fig. 12. Dosage-mortality regressions for the principal contact insecticides used against the melon fly in various formulations and as space sprays and residues. The dusts, at $\frac{1}{2}$ gram per charge, deposited about a third as much toxicant as the aqueous formulations.

charges, so they deposited only 33.45 per cent as much toxicant as the wettable powders. The wettable powders and the emulsifiable concentrates are the only formulations that can be directly compared, in Figures 12 to 14, for they deposited approximately equal quantities of toxicant.

LD-50 Values. For purposes of comparison, the LD 50's (least dosage for 50 per cent mortality), read from the dosage-mortality regression curves (Figs. 12 to 14), are shown in Tables 5 to 7 for nearly all insecticides used in the present investigation and in the various formulations in which they were





Fig. 13. Dosage-mortality regressions for the principal contact insecticides used against the oriental fruit fly in various formulations and as space sprays and residues. The dusts, at ½ gram per charge, deposited about a third as much toxicant as the aqueous formulations.

available. Since the dusts had been used at only $\frac{1}{2}$ gram per charge, the LD 50's for the dusts as read from the regression curves were divided by 2 to give ratings that would be expected from the relative deposits in Table 4. For this purpose it had to be assumed that doubling the charge halves the LD 50.

The various formulations were tested on different days, but a test on DDT wettable powder was made with each day's tests. It was thereby ascertained that the differences in the effectiveness of the formulations was not to any appreciable extent attributable to differences in the date of treatment, when



Fig. 14. Dosage-mortality regressions for the principal contact insecticides used against the Mediterranean fruit fly in various formulations and as space sprays and residues. The dusts, at $\frac{1}{2}$ gram per charge, deposited about a third as much toxicant as the aqueous formulations.

flies of the same general age group were used. Untreated controls were kept with each series of tests; but, as previously noted, natural mortality was ordinarily a negligible factor and no corrections were made for it. At one time the melon flies became infected with a spore-forming protozoan (*Nosema*) of undetermined species. This was first indicated by an increase in natural mortality and a significant increase in the mortality from the daily DDT wettable powder standard treatment. No further tests were made with melon flies until a completely new population could be reared from an uninfected stock of flies.

Based on the LD-50 Values with DDT in the Corresponding Formulation as the Standard (= 100) TOXICITY INDEXES OF INSECTICIDES USED AGAINST THE MELON FLY TABLE 5

				Space tre	atment*					Residue tı	reatment†	
Insecticide	Emuls	ifiable	Kerosene	solution	Wettable	powder	D	ıst	Emuls	ifiable	Wettable	powder
	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index
DDT	0.16	100	0.11	100	0.57	100	1.20	100	0.36	100	0.30	100
DDD.	0.21	26	0.18	61	1.30	44	1.20	100	2.20	16	1.60	19
DFDT	0.30	53	0.16	69	0.48	119	0.62	193	0.36	100	0.23	130
Methoxychlor	0.13	123	0.125	88	0.39	146	1.00	120	1.70	21	0.23	130
Dilan.	0.36	44	0.045	244	0.080	712	0.060	2,000	0.81	44	0.058	517
Lindane.	0.0135	1,185	0.007	1,571	0.0092	6, 195	0.035	3,428	0.019	1,895	0.021	1,428
Toxaphene.	0.0260	615	0.056	196	0.068	838	0.12	1,000	0.052	692	0.150	200
Chlordane.	0.0110	1,455	0.032	343	0.011	5,181	0.055	2,182	0.070	514	0.150	200
Heptachlor	0.0005	32,000	0.0030	3,667	0.0071	8,028	0.0055	21,818	0.070	514	0.007	4,286
Aldrin	0.0030	5,333	0.0061	1,803	0.0059	9,661	0.018	· 6,666	0.230	157	0.100	300
Dieldrin	0.0034	4,705	0.0110	1,000	0.0053	10,754	0.025	4,800	0.011	3,273	0.014	2,143
Paraoxon	0.0004	40,000	0.000	12, 222	:	:	:		0.0003	120,000	:	
Parathion	0.0007	22,857	0.0018	6,111	0.0005	114,000	0.0035	34,286	0.0008	45,000	0.0014	21,428
EPN.	0.0023	6,957	0.0038	2,895	0.0019	20,000	0.0090	13, 333	0.0024	15,000	0.0019	15,789
TEPP	0.017	941	0.0055	2,200	:		:	:	:	:	:	
Metacide	0.0021	7,619	0.0014	7,857			:		0.0053	6,792	:	:
No. 4018	0.0018	8,888	0.0023	4,783	:	:	:	:	0.002	18,000	:	:
E-838.	0.0052	3,077	0.0057	1,930	:	:	:	:	0.018	2,000	:	:
Rotenone	0.0080	2,000	0.0008	13,750			0.036	3,333	:	:		
Pyrethrins	0.0080	2,000	0.00035	31,429	:	:	0.010	12,000	:	:	:	:
Allethrin	0.080	200	0.0180	611	:	:	:	:	:	:	:	:
									-	-	-	

* The insecticides were applied to anesthetized adult flies in a settling tower. Counts were made 24 hours after treatment. † The insecticides were allowed to settle on petri-dish covers in a settling tower. The covers with their films of insecticide residue formed the ceilings of screen test cages. Adult flies were confined in these cages for 24 hours.

TOXICITY INDEXES OF INSECTICIDES USED AGAINST THE ORIENTAL FRUIT FLY Based on the LD-50 Values with DDT in the Corresponding Formulation as the Standard (= 100) TABLE 6

				Space tre	atment*					Residue	treatment	
Insecticide	Emuls	ifiable	Kerosene	solution	Wettable	powder	Ď	ıst	Emu	ılsifiable	Wettah	le powder
	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index
DDT	0.028	100	0.059	100	0.082	100	0.175	100	0.16	100	0.15	100
DDD.	0.040	2	0.070	84	0.540	15	0.255	69	0.22	73	0.61	25
DFDT.	0.046	61	0.095	62	0.062	132	0.175	100	0.22	73	0.34	44
Methoxychlor.	0.060	46	0.070	84	0.200	41	0.440	40	1.00	16	0.50	30
Dilan.	0.018	155	0.044	134	0.051	161	0.060	292	0.27	59	0.068	221
Lindane	0.0006	4,666	0.0015	3,933	0.004	2,050	0.0125	1,400	0.042	381	0.018	833
Toxaphene	0.0220	127	0.036	163	0.017	482	0.100	175	0.035	457	0.120	125
Chlordane	0.0110	254	0.014	421	0.013	631	0.035	500	0.096	167	0.074	203
Heptachlor	0.00016	17,500	0.0007	8,428	0.008	1,025	0.001	17,500	0.058	276	0.0072	2,083
Aldrin	0.00049	5,714	0.0009	6,555	0.0047	1,745	0.005	3,500	0.067	239	0.026	577
Dieldrin	0.00180	1,555	0.0027	2,185	0.002	4,100	0.0034	5,147	0.0052	3,077	0.0022	6,818
Paraoxon	0.00011	25,454	0.00015	39,333	:		:	:	0.0011	14,545		
Parathion	0.00016	17,500	0.00031	19,032	0.00042	19,524	0.0034	5,147	0.0032	5,000	0.0023	6,522
EPN.	0.0026	1,076	0.0033	1,788	0.0026	3,154	0.0185	946	0.0064	2,500	0.0037	4,054
TEPP.	0.0030	933	0.0052	1,135	:		:	••••••	:			
Metacide	0.0003	9,333	0.0015	3,933					0.013	1,231	:	•
No. 4018	0.00035	8,000	0.0012	4,917		•••••			0.0072	2,222	:	•••••
E-838	0.0013	2,153	0.0030	1,967						:	:	•••••
Rotenone.	0.011	254	0.00021	28,095			0.51	34	:			
Pyrethrins.	0.013	215	0.00011	53,636	:		0.023	260				•••••
Allethrin	0.072	39	:	:	:		:	:	:		:	
		-									_	

* The insecticides were applied to anesthetized adult flies in a settling tower. Counts were made 24 hours after treatment. + The insecticides were allowed to settle on peri-dish overs in a settling tower. The covers with their films of insecticide residue formed the ceilings of screen test cages. Adult fliss were confined in these cases for 24 hours.

TOXICITY INDEXES OF INSECTICIDES USED AGAINST THE MEDITERRANEAN FRUIT FLY Based on the LD-50 Values with DDT in the Corresponding Formulation as the Standard (=100)

				Space tres	atment*					Residue tr	eatment†	
Insecticide	Emulsi	fiable	Kerosene	solution	Wettable	powder	ñ	ıst	Emuls	ifiable	Wettable	powder
	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index	LD-50	Index
DDT	0.124	100	0.16	100	0.14	100	0.44	100	0.97	100	1.20	100
DDD	0.170	73	0.21	76	0.46	30	0.625	20	1.60	62	2.00	99
DFDT	0.160	78	0.34	47	0.18	78	0.160	275	0.70	139	0.70	171
Methoxychlor.	0.049	253	0.058	276	0.062	226	0.185	226	3.00	32	0.42	285
Dilan	0.031	400	0.031	516	0.056	250	0.046	957	0.34	285	0.12	1,000
Lindane	0.00095	13,050	0.0016	10,000	0.0047	2,979	0.0125	3,520	0.028	3,464	0.0064	18,750
Toxaphene.	0.0415	299	0.063	254	0.0350	400	0.125	352	0.45	216	0.250	480
Chlordane	0.0091	1,363	0.017	941	0.0128	1,094	0.056	786	0.11	882	0.060	2,000
Heptachlor	0.0009	13,778	0.004	400	0.0069	2,029	0.004	11,000	0.046	2,109	0.031	3,871
Aldrin.	0.0009	13, 778	0.0068	2,353	0.0062	2,258	0.0012	35,200	0.057	1,702	0.037	3, 243
Dieldrin	0.0013	9,538	0.0020	8,000	0.0022	6,364	0.009	4,889	0.013	7,462	0.0024	50,000
Paraoxon	0.00038	32,631	0.00067	23,880					0.0085	11,412	:	
Parathion.	0.0014	8,857	0.0020	8,000	0.0015	9,333	0.0065	6,769	0.0098	9,898	0.0051	23, 529
EPN.	0.0028	4,429	0.0033	485	0.0058	2,414	0.017	2,588	0.025	3,880	0.021	5,714
TEPP	0.0072	1,722	0.0100	1,600					:		:	
Metacide	0.00068	18,235			:	:			0.013	7,462	:	
No. 4018.	0.0020	6,200	0.0023	6,957	:				0.025	3,880	:	
E-838.	0.0080	1,550							0.22	441		:
Rotenone	0.0054	2,296	0.003	5,333	:		0.032	1,375	2.80	35		
Pyrethrins.	0.054	296	0.002	8,000			0.026	1,692	:	•••••		
Allethrin.	0.080	155			:				:	:		:
Nicotine	0.41	30	:		:	:		:	:	:	:	
NaDNOCHP		:	:	:	0.29	48	:	:	:	:		
	-								-			

* The insecticides were applied to anesthetized adult flies in a settling tower. Counts were made 24 hours after treatment. † The insecticides were allowed to settle on petri-dish covers in a settling tower. The covers with their films of insecticide residue formed the ceilings of screen test cages. Adult flies were confined in these cages for 24 hours.

TABLE 7

February, 1953] Ebeling: Laboratory Experiments on Control of Fruit Flies

A few insecticides that were tested, such as sabadilla, lethane, and OMPA, have been omitted from the tables because their toxicity to fruit flies was found to be extremely low. Two compounds, nicotine and the sodium salt of 2, 4, dinitro-6-cyclohexylphenol, are represented only in Table 7; these also have little toxicity to fruit flies.

TABLE 8 RELATIVE RATING OF THE THIRTEEN MOST EFFECTIVE INSECTICIDES IN EACH FORMULATION

Dala		Space t	reatment*	<u>a a 1112 kun a</u> nna an	Residue t	reatment †
tive tive rating	Emulsifiable	Kerosene solution	Wettable powder	\mathbf{Dust}	Emulsifiable	Wettable powder
1	paraoxon	pyrethrins	parathion	heptachlor	paraoxon	parathion
2	∫parathion‡	paraoxon	dieldrin	parathion	parathion	dieldrin
3	heptachlor	rotenone	EPN	aldrin	(EPN‡	EPN
4	Metacide	parathion	lindane	dieldrin	{No. 4018	heptachlor
5	∫No. 4018‡	No. 4018	aldrin	EPN	dieldrin	lindane
6	aldrin	Metacide	heptachlor	lindane	Metacide	aldrin
7	dieldrin	lindane	chlordane	pyrethrins	lindane	dilan
8	EPN	heptachlor	toxaphene	chlordane	E-838	chlordane
9	lindane	aldrin	dilan	dilan	heptachlor	toxaphene
10	E-838	EPN	methoxychlor	rotenone	toxaphene	methoxychlor
11	rotenone	dieldrin	DFDT	toxaphene	chlordane	DFDT
12	TEPP	TEPP	DDT	DFDT	aldrin	DDT
13	chlordane	E-838	DDD	methoxychlor	dilan	DDD
		1	•		· ·	

Based on Toxicity Indexes for Three Species of Fruit Flies in Tables 5 to 7

* The insecticides were applied to anesthetized adult flies in a settling tower. Counts were made 24 hours after treatment.

† The insecticides were allowed to settle on petri-dish covers in a settling tower. The covers with their films of insecticide residue formed the ceilings of screen test cages. Adult flies were confined in these cages for 24 hours. ‡ The bracketed insecticides had equal effectiveness.

Toxicity Indexes of the Insecticides. The "toxicity index" was suggested by Sun (1950) as a method of comparing the relative toxicity of insecticides. It is the ratio between the LD 50 value of a standard insecticide and the LD 50 of the test sample, multiplied by 100. According to Sun, many factors that changed the LD 50 values of insecticides tested on house flies did not appreciably affect their toxicity indexes.

It can be seen from the toxicity indexes in Tables 5 to 7 that the fruit flies are extremely susceptible to some insecticides, notably paraoxon, parathion, No. 4018, metacide, EPN, heptachlor, dieldrin, and aldrin, and, in kerosene solutions, pyrethrins and rotenone. It should be borne in mind, however, that the data in these tables are based on counts made 24 hours after space treatment or after the confinement of the flies to the residues. If the period between treatment and counting had been substantially changed, the order of effectiveness of the insecticides might have been different.

The relative effectiveness of the insecticides in their various formulations can be more easily seen in Table 8. Here the thirteen most toxic insecticides have been arranged in descending order of their average effectiveness against all three species of fruit flies. In the emulsifiable concentrates and in the kerosene solutions certain insecticides are represented that were not avail-

able as wettable powders. Among the residue treatments, certain insecticides that decompose rapidly in sunlight and are consequently of little practical value as residues in the field, were not included, although under the conditions of the experiment they might have shown considerable toxicity.

It can be seen from Table 8 that the order of effectiveness of the insecticides was not the same in the different formulations. In solution in kerosene,

TABLE 9

TOXICITY INDEXES OF VARIOUS FORMULATIONS OF 13 INSECTICIDES USED AGAINST THE MELON FLY

Based on the LD-50 Values (Those Not Given Here Are Given in Table 5) with the Emulsifiable Concentrate of the Corresponding Insecticide as the Standard (=100)

	,	Space tr	eatment*				Residue	treatmen	t†	
Insecticide	Emulsi-	Kerosene	Wettable	Durt	Emulsi-	Wettable	Kerosene	solution	Du	ıst
	fiable	solution	powder	Dust	fiable	powder	LD 50	Index	LD 50	Index
DDT	100	145.5	28.1	13.3	44.4	53.3	1.8	8.9	0.57	28.1
DDD	100	116.7	16.2	17.5	9.6	13.1	3.6	5.8	1.20	17.5
DFDT	100	187.5	62.5	48.4	83.3	130.4	1.45	20.7	0.55	54.6
Methoxychlor	100	104.0	33.3	13.0	7.6	56.5	1.9	6.8	0.90	14.4
Dilan	100	80.0	45.0	60.0	4.4	62.1	0.52	6.9	0.115	31.3
Lindane	100	192.9	146.7	38.6	71.1	64.3	0.0019	71.1	0.030	34.6
Toxaphene	100	46.4	38.2	21.7	50.0	17.3	0.150	17.3	0.130	20.0
Chlordane	100	34.4	100.0	20.0	15.7	7.3	0.056	19.6	0.280	3.9
Heptachlor	100	16.7	7.0	9.1	0.7	7.1	0.017	2.9	0.032	1.6
Aldrin	100	49.2	50.8	16.7	1.3	3.0	0.023	13.0	0.021	14.3
Dieldrin	100	30.9	64.1	13.6	30.9	24.3	0.0032	106.2	0.017	20.0
Parathion	100	38.9	140.0	20.0	87.5	50.0	0.00081	86.4	0.011	6.4
EPN	100	60.5	121.1	23.0	95.8	121.1	0.0017	135.3	0.0041	56.1
Average	100	84.9	65.6	24.2	38.6	46.9		38.5		23.3

* The insecticides were applied to anesthetized adult flies in a settling tower. Counts were made 24 hours after treatment.

[†] The insecticides were allowed to settle on petri-dish covers in a settling tower. The covers with their films of insecticide residue formed the ceilings of screen test cages. Adult flies were confined in these cages for 24 hours.

pyrethrins and rotenone were first and third, respectively, in effectiveness, but in emulsions or dusts they were relatively ineffective. Heptachlor was particularly effective as a dust and as an emulsion.

As will be shown later, the order of effectiveness of the insecticides as residues changes continually as the period of exposure of the residue to the weather increases, particularly when the residues are exposed to sun or rain. The relative value of insecticides such as dieldrin, DDT, toxaphene, and EPN, which volatilize or decompose relatively slowly, increases with respect to such insecticides as heptachlor, lindane, chlordane, and parathion, which volatilize or decompose relatively rapidly or are readily removed by rain.

Toxicity Indexes of the Formulations. Tables 9 to 11 give the toxicity indexes of the various formulations, with the LD 50's for the emulsifiable concentrates in the space treatments as the standard (=100).⁵

⁵ As previously noted, DDT wettable powder was included in each day's tests, and this could have been used as a standard insecticide, the basis for toxicity indexes, in accordance

For example, comparing only the emulsifiable concentrates and the wettable powders as space treatments, note in Table 9 that against the melon fly heptachlor was only 7 per cent and aldrin only 50.8 per cent as efficient in the latter formulation. In Table 10 it can be seen that against the oriental fruit fly heptachlor was only 2 per cent and aldrin only 10.4 per cent as efficient in the wettable-powder as in the emulsifiable-concentrate formulation.

TABLE 10

TOXICITY INDEXES OF VARIOUS FORMULATIONS OF 13 INSECTICIDES USED AGAINST THE ORIENTAL FRUIT FLY

Based on the LD-50 Values (Table 6) with the Emulsifiable Concentrate of the Corresponding Insecticide as the Standard (= 100)

		Space tre	eatment*		Residue t	reatment†
Insecticide	Emulsi- fiable	Kerosene solution	Wettable powder	Dust	Emulsi- fiable	Wettable powder
DDT.	100	47.4	34.1	16.0	17.5	18.7
DDD	100	57.1	7.4	15.7	18.2	6.6
DFDT	100	48.4	74.2	26.3	20.9	13.5
Methoxychlor	100	80.5	30.0	13.6	6.0	12.0
Dilan	100	40.9	35.3	30.0	6.7	26.5
Lindane	100	40.0	15.0	30.4	9.0	21.1
Toxaphene	100	61.1	129.4	22.0	62.8	18.3
Chlordane	100	78.6	84.6	31.4	11.5	14.9
Heptachlor	100	17.7	2.0	16.0	0.3	2.2
Aldrin	100	54.4	10.4	9.8	0.7	1.9
Dieldrin	100	66.6	85.0	52.9	34.6	81.8
Parathion	100	51.6	38.1	4.7	5.0	7.0
EPN	100	78.8	100.0	13.9	40.6	70.3
Average	100	55.6	49.7	21.7	18.0	22.7

* The insecticides were applied to anesthetized adult flies in a settling tower. Counts were made 24 hours after treatment.

[†] The insecticides were allowed to settle on petri-dish covers in a settling tower. The covers with their films of insecticide residue formed the ceilings of screen test cages. Adult flies were confined in these cages for 24 hours.

In Table 11 the corresponding figures for the Mediterranean fruit fly are 13.0 and 14.5 per cent, respectively. Both these insecticides are formulated with difficulty as wettable powders, and particle sizes are far too large for optimum performance.

In general, when used as space sprays, emulsifiable concentrates were superior to the wettable powders 11 times (out of 13) for the melon fly, 11 times (and tied once) for the oriental fruit fly, and 12 times for the Mediterranean fruit fly (Tables 9 to 11).

As residues, in contrast, the wettable powders were superior to the emulsifiable concentrates 8 times (out of 13) for the melon fly, 10 times for the oriental fruit fly, and 10 times (and 1 tie) for the Mediterranean fruit fly (Tables 9 to 11).

with Sun's (1950) method. Results with this insecticide were so consistent, however, as to indicate that there were no differences in environmental conditions from day to day that appreciably affected the kills obtained. It was therefore considered sound to use the emulsifiable formulation of each insecticide as the basis for toxicity indexes of other formulations, even though the tests with the different formulations were made on different dates.

Despite the relatively small quantity of kerosene deposited in the settling tower, as space sprays the kerosene solutions were superior to the wettable powders 8 times out of 13 with both the melon fly (Table 9) and the oriental fruit fly (Table 10), and 6 times out of 13 with the Mediterranean fruit fly (Table 11). The emulsifiable concentrates proved to be significantly superior to the kerosene solutions as space sprays at the quantities per charge indicated in Table 4. If, however, one calculates the effectiveness of the formulations per unit of toxicant deposited, the kerosene solutions were, on the average, the most efficient of them all, both as space sprays and as residues.

TABLE 11

TOXICITY INDEXES OF VARIOUS FORMULATIONS OF 13 INSECTICIDES USED AGAINST THE MEDITERRANEAN FRUIT FLY

Based on the LD-50 Values (Table 7) with the Emulsifiable Concentrate of the Corresponding Insecticides as the Standard (= 100)

		Space tre	eatment*		Residue t	reatment†
Insecticide	Emulsi- fiable	Kerosene solution	Wettable powder	Dust	Emulsi- fiable	Wettable powder
DDT	100	77.5	88.6	28.2	12.8	10.3
DDD	100	84.8	36.9	27.2	10.6	8.5
DFDT	100	47.1	88.9	100.0	22.8	22.8
Methoxychlor	100	84.5	79.0	25.2	1.6	11.7
Dilan	100	100.0	55.4	67.4	9.1	25.8
Lindane	100	59.4	20.2	7.6	3.4	14.8
Toxaphene	100	65.9	118.6	32.8	9.2	16.6
Chlordane	100	53.5	71.1	16.2	8.3	15.2
Heptachlor	100	22.5	13.0	22.4	1.9	2.9
Aldrin	100	13.2	14.5	7.2	1.6	2.4
Dieldrin	100	65.0	59.1	32.4	10.0	54.2
Parathion	100	70.0	93.3	21.6	14.3	27.4
EPN	100	84.8	48.3	16. 4	11.2	13.3
Average	100	63.7	60.5	31.2	9.0	17.4

* The insecticides were applied to anesthetized adult flies in a settling tower. Counts were made 24 hours after treatment.

t The insecticides were allowed to settle on petri-dish covers in a settling tower. The covers with their films of insecticide residue formed the ceilings of screen test cages. Adult flies were confined in these cages for 24 hours.

Table 9 shows that the residues deposited by the kerosene solutions were, on the average, as effective against the melon fly as those deposited by the emulsifiable concentrates, despite the fact that they were present on the treated surfaces in only 30.2 per cent as great a quantity. On the basis of the relative quantities deposited, they were also found to be more efficient against the melon fly than the wettable powders. These results agree with those obtained when comparing the residues of kerosene solutions and wettable powders against the motile young (crawlers) of the California red scale, *Aonidiella aurantii* (Maskell) (see Ebeling, 1945).

The dusts, unlike the other formulations, were as effective as residues as they were as space treatments (see Table 9). As stated previously, because of the fact that the dusts were used in charges of only $\frac{1}{2}$ gram per treatment, the LD 50's were decreased by one half so that the tables would indicate the relative effectiveness of all the formulations at 1 per cent concentration of toxicant. For this purpose it had to be assumed that doubling the dosage of dust would decrease the LD 50 by one half. Even when considering the lesser quantity of toxicant deposited by the dusts, it must be concluded from the data shown in Tables 9 to 11 that they are considerably less efficient than the liquid formulations, particularly as space treatments.

Effect of Activity of the Insect Species. Average figures for each formulation are presented in Tables 9 to 11 to show how the activity of the insect species affects the relative value of the residues. It might be expected that a given deposit of insecticide would be less effective if the flies contacted it only by means of their feet than if the insecticide were distributed over their entire body. Nevertheless the tables show that for the melon fly some insecticides proved to be about as efficient or even more efficient as residues than as space treatments. On the other hand, against the Mediterranean fruit fly the same insecticides were singularly inefficient as residues. Against the oriental fruit fly the residues were on the average between the other two species in insecticidal efficiency.

DDT may be used as an example. It can be seen from the LD 50's in Tables 5 and 7 that with the wettable powder as a space spray it required four times as high a concentration of DDT for a 50 per cent kill of the melon fly as of the Mediterranean fruit fly. Yet when the flies were allowed to crawl over the wettable-powder residues, the opposite was found to be true.

The reason for the difference in the average insecticidal efficiency of residues of the 13 insecticides in Tables 9 to 11 is to be found, at least in part, in the differences in the habits of the three species of flies. It has already been shown that the greater the per cent of flies crawling about on the ceiling of the test cage, on which the insecticide residue was deposited, the greater the per cent kill. An experiment was made to determine the difference in the percentage of flies of the three species found on the ceilings of test cages at various periods of the day. Since the flies are relatively inactive at night, it was considered that a record of their behavior during the daylight hours would suffice for the purposes of the present experiment.

One hundred and twenty-five flies of each species were anesthetized and 25 were placed in each of 5 laboratory test cages. Also 25 flies of each species were placed in a 1-cubic-foot screen cage such as is used for rearing the flies. Although it is the laboratory test cages that are of special interest in the present connection, it was considered advisable to check the results in cages of much larger dimensions. The number of insects standing or walking on the ceiling of each cage was recorded once every hour from 7 a.m. to 6 p.m. on May 15, 1951, and are expressed as percentages of the total number of flies per cage in Table 12.

It can be seen that there is a tendency for the melon flies to spend a high proportion of their time on the insecticide-covered ceilings of their cages. Flies are moving back and forth from the ceilings to the sides and bottom of the cages, but all flies eventually come in contact with the residue. If a highly toxic residue is used, all flies are soon paralyzed, showing that none avoid the ceiling of the cage. In fact, the reason the insecticide is deposited on the petri dish that is to form the ceiling of the test cage is that fruit flies

have a greater tendency to rest or crawl about on the under side of horizontal surfaces than on the upper side. (This habit should be remembered when sprays are applied as residue treatments in the field.)

In Table 12 the percentage of flies on the ceilings of the small test cages was numerically, but not significantly, greater for the melon fly than for the oriental fruit fly. In the large rearing cages, there were significantly more melon flies than either oriental fruit flies or Mediterranean fruit flies on the ceilings. In the small cages both the melon fly and the oriental fruit fly show a significantly greater tendency to rest or crawl on the ceiling of

TABLE 12							
THE	AVER	RAGE	\mathbf{PER}	CENT	\mathbf{OF}	THREE	2
\mathbf{SPI}	ECIES	OF F	LIES	FOUNI	D ON	THE	
	CE	EILIN	[GS O]	F CAGI	ES*		

Species	Average per cent of flies found on ceiling of			
	Small test cages	Large rearing cages		
Melon fly	42.0	29.3		
Oriental fruit fly	35.1	12.8		
Mediterranean fruit fly	22.7	12.0		
L.S.D. at $P = 0.05$	9.6	8.8		
	1	1		

* Counts of the number found on the ceiling of each cage were made once every hour from 7 a.m. to 6 p.m. on May 15, 1951.

their cages than the Mediterranean fruit fly. This tendency alone would bring the former two species into greater contact with the insecticide residues than the latter species.

In addition the activity of the flies must be taken into consideration. The flies are anesthetized in order that they may conveniently be placed within the testing cages. The melon flies are the first to recover from the anesthetization and are therefore the first to begin crawling over the insecticide deposits. After the oriental fruit flies have recovered from the CO_2 they crawl over the interior of their cages about as actively as the melon flies do on first recovering. The melon flies, however, become continuously more active while the activity of the oriental fruit flies remains more or less constant. The difference in the activity of the two species becomes greater as the period of their confinement increases. During the second 24 hours of confinement the activity of the period of confinement, the Mediterranean fruit flies are conspicuously more sluggish than the other two species.⁶ The comparative sluggishness of this species would also be a factor in reducing its susceptibility to insecticide residues.

⁶ Mr. K. L. Maehler, who has been conducting extensive investigations on the ecology of the fruit flies in Hawaii, informs the writer that according to his observations of the three species of flies in the field, the melon fly crawls about the most actively, followed by the oriental fruit fly and the Mediterranean fruit fly in the order named. The Mediterranean fruit fly is by far the most sluggish.

Time \times **Concentration.** The dosage-mortality regressions shown in figures 12 to 14 are based on much lower concentrations of toxicant than would ever be used in field operations with aerosols. In the field an insect might be exposed to the aerosol for only a few seconds and it is important that the insecticide be sufficiently toxic and at a sufficiently high concentration to result in the death of the insect despite the short period of exposure.

The operation of the settling tower used in the present experiments was modified so as to make possible an evaluation of insecticides at high concentrations for short periods. The base upon which the tower rests was raised $1\frac{1}{2}$ inches above the turntable. The anesthetized flies were placed in petridish covers and on the turntable as usual. A sheet of tin was inserted in grooves attached to the under side of the base of the tower and pushed in so as to prevent any aerosol from reaching the flies as the liquid was injected into the tower. Thirty seconds after the liquid was injected into the tower, and after the turbulence of the clouds of aerosol had subsided, the sheet of tin was pulled out from under the base upon which the tower rested. The aerosol was allowed to fall on the flies on the turntable for periods varying from 1 to over 200 seconds. The turntable was revolving as usual. The period of exposure was timed with a stopwatch and the sheet of tin was pushed back under the baseboard at the proper instant so as to prevent any further deposit of insecticide on the flies. The down draft of air during the 6 seconds required for the injection of the liquid causes the greatest deposit of aerosol on the turntable during that period. By waiting 30 seconds before allowing the aerosol to fall on the flies, the deposit of insecticide per second was much reduced. Since the density of the aerosol cloud was gradually reduced with increasing period of exposure, when more than 50 seconds of exposure was required, the flies were exposed to a new charge of aerosol every 50 seconds.

Emulsifiable concentrates were diluted so as to make emulsions of 2 per cent actual toxicant for experiments with the oriental fruit fly, 4 per cent for the Mediterranean fruit fly, and 5 per cent for the melon fly. At these concentrations, paraoxon and parathion aerosols resulted in 100 per cent kills in 1 second or less of exposure for all three species of flies. Heptachlor gave 100 per cent kills in 1 second or less for the oriental fruit fly and melon fly, Metacide for the Mediterranean fruit fly and melon fly, No. 4018 for the melon fly, and lindane for the Mediterranean fruit fly. All of the above insecticides and E-838, aldrin, dieldrin, lindane, and, except for the oriental fruit fly, rotenone and TEPP, gave 100 per cent kills in 10 seconds or less. Two hundred or more seconds of exposure was required for a 100 per cent kill of the flies when DDT, DFDT, DDD, methoxychlor, or allethrin was used.

Approximately the same toxicity indexes were found for the various insecticides as when the lower concentrations were used for a 3-minute period. As might be expected, the mortalities in either case are correlated with the time \times concentration factor.

Effect on the Flies of Sublethal Doses of Insecticide. On April 12, 1951, 24 flies, representing a 16 per cent survival from a 24-hour confinement with residue from a 1 per cent DDT suspension, were placed in a clean rearing cage of a type described earlier in this paper. The flies were given food and water in accordance with the best known practice. Fifty-eight untreated

melon flies of the same age were confined in an adjoining cage under identical conditions. Of the 24 DDT-treated flies, 66.7 per cent were still alive on April 30, as compared with 79.1 per cent of the untreated flies.

A section of cucumber was placed in each cage. On the day the flies were placed in the clean cage and allowed to oviposit, only 0.6 egg per fly was found in the cucumber in the cage containing the treated flies, as compared

TABLE 13 MORTALITY AND EGG-LAYING CAPACITY OF MELON FLIES AFTER SUBLETHAL DOSAGES OF DDT AND PARATHION*

Number of days after treatment	Nur	mber of dead	flies	Number of eggs laid			
	DDT	Parathion	Untreated	DDT	Parathion	Untreated	
5	46	76	29	144	151	227	
6	3	0	5	927	953	759	
7	3	1	2	477	563	558	
9	9	5	4				
12	3	4	6	513	440	611	
14	5	3	1				
15	8	1	0				
Total	77	90	47	2,061	2,107	2,155	
Per cent	10.1	9.9	4.9				

* One group of 1,000 melon flies was treated with 0.2 per cent DDT, with a 41-hour mortality of 24.2 per cent; another group was treated with 0.0008 per cent parathion, with a mortality of 8.7 per cent. The survivors were placed in clean rearing cages 2 days after treatment and counts were made at various intervals of the number that had died and the number of eggs that were laid in portions of cucumbers.

to 4.8 in the check cage. Sections of cucumber were placed in the two cages again on April 15, 2 days after confinement of the flies in the clean cages and 3 days after the treated flies were exposed to the DDT residues. This time 11.0 eggs per fly were found in the cucumber in the cage containing the treated flies and only 1.2 in the check cage.

In another experiment 1,000 flies were treated with 0.2 per cent DDT emulsion and another group of 1,000 flies were treated with 0.0008 per cent parathion emulsion. The insecticides were allowed to settle on anesthetized flies in the settling tower. In 41 hours it was found that the DDT had given a 24.2 per cent kill and the parathion an 8.7 per cent kill. The natural mortality in check lots during this period was 4.0 per cent. Thus while all the flies had insecticide on their bodies, it was not sufficient to kill more than small percentages of them.

The survivors from each treatment were divided into three groups, placed in three clean rearing cages, and given food and water as in the previous experiment. A thousand untreated flies were cared for in the same manner. Beginning 5 days after treatment and 3 days after confinement of the flies in the clean rearing cages, the number found dead in the cages was recorded, and this was continued at intervals up to 15 days after treatment. At 5, 6, 7, and 12 days after treatment a count was made of the number of eggs the flies had deposited in freshly cut sections of cucumber. The results of the experiment are shown in Table 13. Note that for a period of 15 days after the treated flies had suffered their initial mortality from the treatment and had been placed in clean rearing cages, they suffered an additional mortality of only about 10 per cent, approximately twice that of the untreated flies. The oviposition records show that the flies that had obtained the sublethal dosage of DDT or parathion laid on the average about as many eggs as the untreated flies.

The conclusion to be drawn from the above experiment is that an insecticide treatment has little value aside from the elimination of the flies killed outright. Little is gained by exposing the flies to a sublethal concentration of insecticide, at least as far as DDT and parathion are concerned: the flies that survive for 48 hours have nearly as high a survival potential and oviposit about as readily as untreated flies.

EXPERIMENTS WITH SPRAYS

Experiments with the spray apparatus were designed to test the insecticides when deposited in a manner simulating field applications. In the preliminary phase an attempt was made to gain rapidly an estimate of the relative effectiveness of all insecticides, as a basis for selection of materials and dosages for the field experiments. Later spray experiments were especially directed toward determining the relative effectiveness of insecticide residues after weathering for various periods and under various conditions of exposure.

Insecticide-depositing Properties of the Sprays. Nearly all the insecticides used in the spray experiments were formulated as wettable powders. The emulsifiable concentrates were usually superior as space sprays, but were inefficient when applied to the waxed cards according to the method of spraying employed in the present experiments. The spray stream was applied for 5 seconds. During that period the wettable powders had a chance to build up a heavy deposit, while the emulsifiables, since they wet excessively, had little tendency to do so. The writer does not wish to imply that the same difference would prevail in ordinary spraying in the field.

The deposits with the spray apparatus were much heavier than those obtained with the mist spray in the settling tower: for example, in one experiment with 50 per cent DDT wettable powder at 0.5 per cent concentration applied to waxed cards, the spray apparatus deposited 30.95 micrograms per square centimeter, whereas the settling tower deposited only 7.30.

Although with the majority of proprietary wettable powders a spray wets and spreads rather poorly, it can be demonstrated that these powders are usually prepared with the optimum amount of wetting agent, from the standpoint of their insecticide-depositing properties. In one test in which the waxed cards described on page 519 were sprayed, Triton B-1956 emulsifier was added to spray containing 2 pounds of actual DDT to 100 gallons of spray, made from 50 per cent DDT wettable powder. Emulsifier at $\frac{1}{2}$ ounce to 100 gallons caused no reduction in per cent kill of melon flies confined in laboratory test cages with the DDT residues. However, 1 ounce to 100 gallons caused a 22 per cent reduction, and 4 ounces to 100 gallons a 30 per cent reduction, in per cent kill, although the wetting was "improved" from a "beading" to a continuous film of spray. **Comparison of Freshly Deposited Residues.** The results of an experiment designed to give a rapid survey of the relative effectiveness of insecticides under conditions simulating field application are shown in Table 14. This table reports the per cent kill of melon flies confined for 4, 6, 10, and 24 hours with cards upon which various insecticides, used at 0.12 or 0.24 per cent of actual toxicant, had been freshly deposited. A 0.12 per cent concentration is equivalent to 1 pound to 100 gallons.

In this table the arc-sine transformation was employed in the analysis of variance—that is, "arc sine $\sqrt{\text{per cent}}$ " was employed instead of per cent

INSE	CTICIDA	AL SPRA	AY RES	IDUES	ON WA	XED PA	APER-C	UP LID	S* ·
	4 hours		6 hours		10 hours		24 hours		
Insecticide	conc.†	Per cent dead	Angle‡	Per cent dead	Angle‡	Per cent dead	Angle‡	Per cent dead	Angle‡
DDT	0.24	11.2	19.2	20.0	26.8	83.2	66.2	87.2	69.4
Methoxychlor	0.12	18.4	25.2	26.0	26.8	89.8	71.0	92.0	75.6
Methoxychlor.	0.24	42.4	40.6	57.6	49.2	92.8	76.6	96.0	82.8
Lindane	0.24	98.4	86.8	100.0	90.0	100.0			
Toxaphene	0.24	39.2	38.6	68.8	56.4	100.0			
Chlordane	0.24	56.8	49.0	96.0	81.2	100.0			
Dieldrin	0.12	28.0	31.8	88.0	71.4	100.0			
Aldrin	0.12	27.2	31.2	65.6	54.4	100.0			
Parathion	0.12	92.8	76.6	100.0	90.0	100.0			

TABLE 14	f
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MORTALITY OF MELON FLIES EXPOSED FOR VARIOUS PERIODS TO INSECTICIDAL SPRAY RESIDUES ON WAXED PAPER-CUP LIDS*

LSD for angle at P = .05 for 4 and 6 hours = 5.49°, for 10 and 24 hours = 7.33°

85 2

70 6

60.6

100.0

100.0

100.0

* The insecticides were all in the form of wettable powders, except aldrin, which was in the form of an emulsifiable concentrate. The sprays were applied by means of a venturi-type laboratory precision sprayer. † The per cent concentration refers to actual toxicant.

98 4

04 4

75.2

+ And sing (and some first

0.12

0.12

0.12

64 8

74 4

49.6

53 8

50 8

44.8

 \ddagger Arc sine $\sqrt{\text{per cent}}$; see text.

EPN

No. 4018....

No. 4049....

in testing the significance of the data. Enumeration data expressed as frequencies or percentages tend to have the mean and the variance closely related. If the means differ too widely, as in this experiment, the variances are not homogenous and thus violate a condition for the analysis of variance. Snedecor (1946) suggests that if the percentages range between 20 and 80 and if they are based on counts of 100 or more *in the numerator*, they may be analyzed without transformation. In other cases tests for significance can be made only with the transformed means. In the table, the word "angle" is used in place of "arc sine $\sqrt{per cent}$," in accordance with the usual practice.

The data indicate that the freshly deposited residue of a number of chlorinated hydrocarbons and organic phosphates will give a 100 per cent kill of melon flies confined to the residue for a day. Dieldrin and aldrin at 0.12 per cent and toxaphene at 0.24 per cent resulted in a lower kill in 4 hours than methoxychlor at 0.24 per cent, but in 10 hours the first three insecticides had resulted in 100 per cent kill, while methoxychlor at 0.24 per cent gave only a 96 per cent kill in 24 hours. Parathion was the only insecticide that gave a 100 per cent kill in 6 hours at 0.12 per cent concentration of toxicant.

Later tests in the field showed that the residues from the concentration of toxicant given in Table 14 result in approximately equivalent kills of the melon fly on corn foliage.

Period Required for 50 Per Cent Knockdown from Various Insecticide Residues. Insects exposed to residues. such as on the corn borders to be described in the companion paper, may have only a limited period of exposure

TABLE 15 PERIOD REQUIRED FOR 50 PER CENT AND 100 PER CENT KNOCKDOWNS OF MELON FLIES CONFINED WITH THE RESIDUES OF 14 INSECTICIDAL SPRAYS*

	Number of 50 per cent	minutes for knockdown†	Number of minutes for 100 per cent knockdown†		
Insecticide	At 2 lbs./100 gals.	At 5 lbs./100 gals.	At 2 lbs./100 gals.	At 5 lbs./100 gals.	
Parathion	34	18	59	46	
EPN	88	41	190	85	
Lindane	109	49	218	107	
Aldrin	90	76	207	113	
Heptachlor	112	89	207	175	
Dieldrin	231	176	—‡§	298	
Chlordane	268	137	_	264	
Dilan	501	95	·	213	
DFDT	‡	177		—t	
Toxaphene		260			
Methoxychlor		321		_	
Neotran		370	· _		
DDT		635		_	
DDD		-‡	—	_	

* Suspensions were applied to waxed papar-cup lids by means of the laboratory sprayer. † A fly that is "knocked down" is lying on the bottom of the test cage, usually on its back, but in all cases in an obviously paralyzed condition from which recovery is impossible. ‡ Dashes indicate that the period was too long for accurate comparisons. After nightfall the flies do not move about, and a given period on a residue would not have as much effect as the same period during the day. § In 20 hours the per cent mortality for the remaining insecticides was as follows: dieldrin, 100; chlordane, 100; dilan, 100; DFDT, 94; toxaphene, 100; methoxychlor, 89; neotran, 60; DDT, 87; DDD, 72.

under certain circumstances. The speed with which an insecticide kills may have a bearing on the ultimate success of a treatment in which control is dependent mainly on the contact the flies have with insecticide residues. Some information on speed of action of a number of insecticides is furnished by the preceding experiment. To gain further information on this point, an experiment was performed to determine the period required for a 50 per cent knockdown.

The per cent of knockdown was based on the number of flies lying paralyzed, moribund, or dead on the bottoms of the test cages with insecticide residues on the ceilings. This is the same criterion as was used for "per cent dead" in the other experiments of the investigation. In the present experiment, however, the majority of the flies were paralyzed or moribund rather than dead when the 50 per cent knockdown point was reached; whereas in the usual mortality tests, in which counts were made after the flies were in the laboratory test cages for 24 hours, the great majority of those on the bottom of the test cages were dead.

The results of the experiment are shown in Table 15. The concentrations of insecticide indicated in the table refer to actual toxicant and may be much higher—particularly the dosage of 5 pounds to 100 gallons—than any that would ever be used in the field. Such concentrations were necessary. however, when only residual effect was to be investigated, in order that the 50 per cent knockdown might occur within a reasonably short period. If this period is too prolonged the experiment may extend beyond the daylight hours. The resulting decrease in the activity of the flies would then unduly prolong the period required for a 50 per cent knockdown with the less toxic insecticides as compared with those insecticides that resulted in an earlier knockdown. Even with 2 pounds of actual toxicant to 100 gallons, the period required for 50 per cent knockdown with the less toxic insecticides was too long for the purposes of this experiment. Only with 5 pounds of actual toxicant to 100 gallons would the less effective insecticides result in a sufficiently rapid knockdown to make possible a comparison of all the insecticides used (except DDD). DDT, the slowest acting of all the insecticides with the exception of DDD, required 10 hours and 35 minutes for a 50 per cent knockdown, as compared with 18 minutes for parathion.

With some of the more rapidly acting insecticides, it was possible to determine the period required for a 100 per cent knockdown. These data also are shown in Table 15.

When large areas are to be treated and the flies must remain in constant contact with the insecticide for days, the rapidity of knockdown may not be important. The flies will probably pick up enough insecticide to result in their death before they leave the treated area. In an area of limited extent, such as the corn borders that are sometimes used as trap crops, it is likely that many flies may have a relatively limited period of contact with the residues before moving on to the crop field to oviposit. A rapidly acting insecticide might result in the earlier death of some insects that would cause a certain amount of damage before receiving sufficient toxicant from the slower-acting insecticides.

The data shown in Table 15 would argue for a change from DDT to parathion in Hawaii if growers are attempting to control flies by treating the crop field itself. The short period of contact of the flies with the crop plants before oviposition begins makes it especially important that the insecticide used be rapid in its action. However, as is shown in the companion paper, for melon flies the treatment of the crop field itself is not an efficient way to use any insecticide. In addition, parathion should not be applied to any crop that is to be consumed within a month after the last application.

As has been previously stated, a given concentration of insecticide when sprayed onto the waxed cards, as in this experiment, will deposit a much greater quantity of residue than is deposited by the same concentration used in the settling tower. However, an experiment on the period required for 50 per cent knockdown was made with the settling tower. At the concentration of 5 pounds of actual toxicant to 100 gallons, parathion required 1 hour and 55 minutes for a 50 per cent knockdown of melon flies. EPN required

TABLE 16

EFFECTIVENESS OF RESIDUES OF 18 INSECTICIDES AGAINST MELON FLIES AFTER WEATHERING IN SHADE AND PROTECTED FROM RAIN FOR 1 TO 6 WEEKS*

Per cent mortality after residues weathered for: Per cent No. Insecticide concentra tion 0 weeks 1 week 2 weeks 3 weeks 4 weeks 6 weeks DDT..... 0.125 DDT†..... 0.125 DDT..... 0.5 DDT (Paste)..... 0.5 DFDT..... Λ Methoxychlor..... 0.5 Lindane..... 0.125Lindane..... 0.5 n n Chlordane..... 0.5 Aldrint 0.125 Aldrin‡..... 0.5 0.125 Dieldrin[‡]..... Dieldrin[‡]..... 0.5 0.125 Heptachlor n Heptachlor 0 5 A Toxaphene..... 0.2 Toxaphene..... 0.5 0.125Parathion..... Parathion †.... 0.125 Parathion..... 0.5 Parathion †.... 0.5 QÅ EPN..... 0.125 EPN..... 0.5 Dilan‡..... 0.5 No. 3975..... 0.125 No. 3975.... 0.5 No. 4018..... 0.125 No. 4018..... 0.5 No. 4049..... 0.125 No. 4049..... 0.5 No. 4124..... 0.125 Λ Λ No. 4124..... 0.5 Genitol 923..... 0.5 Ditolyl tri-0.5 n chloroethane.....

Suspensions of Wettable Powders Used Except as Otherwise Indicated

* Waxed paper-cup lids were sprayed and tacked on to Canec boards in a vertical position in continuous shade and sheltered from rain. Flies were confined with the residues for 24 hours. † These are each different proprietary brands than the preceding, and have a lower insecticide-depositing ability on waxed cards because they are emulsifiable concentrates that result in excessive "drain-off."

‡ Emulsifiable concentrates of low insecticide-depositing ability.

2 hours, 15 minutes; lindane 2 hours, 29 minutes; aldrin 3 hours, 39 minutes; heptachlor 3 hours, 45 minutes; dieldrin 4 hours, 23 minutes; and chlordane 4 hours, 23 minutes. The other insecticides killed too slowly for the purposes of the experiment.

It must be borne in mind that the above figures refer to the period that the insects must crawl about on the residues for a 50 per cent knockdown. It will be remembered from a previous experiment, however, that only 1 second of exposure of the entire insect body to the aerosol itself will result in a 100 per cent kill, with some insecticides, although the mortality may not be complete for several hours (see p. 545).

Effect of Weathering of Spray Residues in the Shade and Sheltered from Rain. In experiments designed to test how weathering in the shade and sheltered from rain affects the per cent kill from insecticide residues, the sprayed cards were pinned by means of thumb tacks to Canec' boards located in a vertical position on the east side of the laboratory building under the wide eaves of the building and a large shade tree. The cards were thus pro-

TABLE 17 EFFECTIVENESS OF RESIDUES OF 3 INSECTICIDES AGAINST MELON FLIES AFTER WEATHERING IN SHADE AND PROTECTED FROM RAIN FOR 126 DAYS* Suspensions of Wettable Powders Used Except as Otherwise Indicated

Insecticide	Residues, mmg/cm ²	Concentration of toxicant, per cent	Mortality, per cent
Parathion, brand A	23.4	0.12 0.24 0.36	7 56 99
Parathion, brand B	22.2	0.12 0.24 0.36	47 84 100
Parathion, brand C†	10.0	0.12 0.24 0.36	9 28 43
Parathion, brand D	17.4	0.12 0.24 0.36	17 35 93
Parathion, brand E	15.1	0.12 0.24 0.36	7 41 55
EPN	23.5	0.12 0.24 0.36	17 95 100
Dieldrin	30.0	0.12 0.24 0.36	93 99 100

* Waxed paper-cup lids were sprayed by means of a venturi-type laboratory sprayer on November 9, 1950, and were kept outdoors in the shade, but sheltered from rain, until March 16, 1951. † Emulsifiable concentrate.

tected from both sun and rain. It was believed that this location might provide a degree of weathering that might be somewhat comparable to that which occurs on the under side of corn leaves, on which these residues were later extensively tested.

Table 16 shows the performance of 18 insecticides at one or two concentrations and with different formulations. The insecticides referred to in the second footnote of the table, as well as aldrin, dieldrin, and dilan, were used in the form of emulsifiable concentrates. All the others were used as wettable powders. Those used as emulsifiable concentrates did not deposit as much

⁷ Fiberboard made from sugar-cane waste.

insecticide as if they had been used as wettable powders and consequently the results were relatively poor. Later work showed that the wettable powders of aldrin, dieldrin, and dilan, when used as sprays, have good residual effect.

It appears from Table 16 that dieldrin, parathion, EPN, and No. 4018 at 0.125 per cent actual toxicant (about 1 lb. to 100 gallons) and DDT,

TABLE 18

EFFECTIVENESS OF RESIDUES OF 3 INSECTICIDES AGAINST TWO SPECIES OF FRUIT FLIES AFTER WEATHERING IN SHADE AND PROTECTED FROM RAIN FOR 85 DAYS* Suspensions of Wettable Powders Used Except as Otherwise Indicated

	Concentration	Per cent mortality		
Insecticide	of toxicant, per cent	Mediterranean fruit fly	Oriental fruit fly	
	0.12	31	49	
Parathion, brand A	0.24	72	86	
	0.36	94	99	
	0.12	34	58	
Parathion, brand B.	0.24	77	88	
	0.36	92	100	
	0.12	0	23	
Parathion, brand Ct	0.24	16	41	
	0.36	67	86	
	0.12	12	51	
Parathion, brand D.	0.24	52	69	
	0.36	90	100	
	0.12	18	46	
Parathion, brand E	0.24	72	74	
	0.36	86	96	
	0.12	43	98	
EPN	0.24	76	100	
	0.36	96	100	
	0.12	82	100	
Dieldrin	0.24	100	100	
	0.36	100	100	

* Waxed paper-cup lids were sprayed by means of a venturi-type laboratory sprayer on November 9, 1950, and the flies were exposed to the residues on February 2, 1951. Five lots of 25 flies were exposed to the cards sprayed with each concentration. † Emulsifiable concentrate.

methoxychlor, chlordane, and toxaphene at 0.5 per cent actual toxicant, resulted in 100 per cent kill of melon flies confined with the residues for 24 hours 1 week after the cards were sprayed. Residues of 0.125 per cent EPN and parathion used as wettable powder continued to give 100 per cent kills 6 weeks after the cards were sprayed.

In the experiment shown in Table 16, the dieldrin residues were not as effective as those from EPN and parathion after 6 weeks of weathering. This was due to the fact that the emulsifiable concentrate that was used gave a

low deposit, at least on the type of surface and with the type of spraying apparatus employed in this experiment. The usual superiority of dieldrin in prolonged residual effectiveness when a formulation of good insecticidedepositing properties is used is unmistakably demonstrated with respect to the melon fly in Table 17 and with respect to the Mediterranean and oriental fruit flies in Table 18. These tables also refer to residues that had been kept outdoors in the shade and out of the rain. Only dieldrin, EPN, and various formulations of parathion were used in these experiments. All were wettable powders except parathion brand C.

It can be seen from Table 17 that 126 days after spraying the residues from dieldrin were more effective against melon flies than those from EPN, and the latter were more effective than those from parathion. In Table 18 dieldrin again shows marked superiority as a residue, this time against the Mediterranean and oriental fruit flies after 85 days of weathering; and EPN was again superior to parathion against the latter species.

Note in Tables 17 and 18 that with the various parathion formulations there was a considerable difference in the amount of insecticide deposited, with a corresponding difference in the per cent mortality of the flies confined to the residues months after treatment. Brand C was an emulsifiable concentrate and deposited less parathion on the sprayed cards, at a given per cent of actual toxicant in the spray, than the other formulations, which were all wettable powders. Among the wettable powders, brands A and B deposited more parathion on the cards than brands D and E.

Effect of Weathering of Residues in Sun and Rain. In an experiment with melon flies in which the same insecticides and formulations were used as in the previous experiments (Tables 17 and 18) but in which the cards were exposed to the sun and rain, the influence of these factors in the decomposition and removal of insecticide residues was strikingly demonstrated.

In the sunny location the cards were pinned to Canec boards attached to a frame that supported the boards at an angle of 60 degrees from the horizontal (Fig. 15). They were thus exposed to the sun during all daylight hours and had no protection from the rain.

Temperature and rainfall data from a weather station only a few hundred feet from the site of the above experiment were obtained through the courtesy of the Pineapple Research Institute. These data for the period of the above experiment, which was begun on October 27, 1950, were as follows:

Period in experiment	Mean max. temperature, °F	Mean min. temperature, °F	Rainfall, inches
First week		67.5	0.47
Second week	85.0	66.6	0.15
Third week	83.4	65.9	0.58
Fourth week	83.6	65.4	0.91
Fifth week		65.8	2.86

The results of the experiment are shown in Table 19. Dieldrin residues after 4 weeks of weathering resulted in a greater per cent kill of melon flies than EPN residues after 1 week. The EPN in turn was $1\frac{2}{3}$ times as effective 1 week after treatment as the two most effective parathion formulations. Among the parathion brands, the variation in effectiveness is even more February, 1953] Ebeling: Laboratory Experiments on Control of Fruit Flies

strikingly demonstrated than in Table 17. The emulsifiable concentrate (brand C) again gave the lowest per cent kill. The high-depositing brands (A and B) were 2 times as effective as the low-depositing brands (D and E) at 0.24 per cent concentration, and about $2\frac{1}{2}$ times as effective at 0.36 per cent concentration.

TABLE 19

EFFECTIVENESS OF RESIDUES OF 3 INSECTICIDES AGAINST MELON FLIES AFTER WEATHERING IN SUN AND RAIN FOR 1 TO 5 WEEKS*

Suspensions of Wettable Powders Used Except as Otherwise Indicated

T	Per cent	Deposit, mmg/cm ²	Per cent mortality after residues weathered for:				
Insecticide	tion		1 week	2 weeks	3 weeks	4 weeks	5 weeks
	0.12		5	0	0		
Parathion, brand A	0.24	23.4	26	. 8	0		
	0.36		60	15	0		
	0.12		8	0	0		
Parathion, brand B	0.24	22.2	28	10	0		
	0.36		60	14	0	••	
	0.12		2	0	0		
Parathion, brand C†	0.24	10.0	5	0	0		
	0,36		5	· 0	0		'
	0.12		3	0	0		
Parathion, brand D	0.24	17.4	11	0	0		
	0.36		25	8	0		
	0.12		5	0	0		
Parathion, brand E	0.24	15.1	16	0	0		
•	0.36		24	6	0		
······	0.12		13	0	0		
EPN	0.24	23.5	43	16	0		
	0.36		92	25	0		
	0.12		100	97	80	73	31
Dieldrin	0.24	30.0	100	100	77	80	41
	0.36		100	100	91	96	85

* The waxed paper-cup lids were sprayed by means of a venturi-type laboratory spray on October 27, 1950. Three lots of 25 flies each were exposed to each concentration of each insecticide. † Emulsifiable concentrate.

In the above experiment the addition of 1 per cent light medium emulsive oil in the trials with 0.24 per cent of parathion, EPN, or dieldrin did not increase the effectiveness of the sprays. (The results with oil are not shown in Table 19.)

The data presented in Table 19 do not give us any information as to the relative effect of sun and of rain in causing the deterioration in the insecticidal effectiveness of the parathion and EPN residues. It will be noted from the weather data, however, that less than $\frac{1}{2}$ inch of rain fell during the first week that the cards were exposed.

The fact that the reduction in effectiveness was due primarily to either sunlight or high temperatures, or both, was demonstrated in a later experi-

ment in which eards similarly treated with parathion, EPN, and dieldrin were exposed to 5 days of an almost continuous period of rainfall and overcast, with practically no sunshine. The meteorological data for the 5-day period of the experiment, which was begun on November 30, 1950, are as follows: mean maximum temperature, 75.8° F; mean minimum temperature, 65.0° F; and rainfall, 5.23 inches. It will be noted that the mean temperatures were lower than in the earlier experiment.



Fig. 15. Canec boards on which the circular waxed cards (paper-cup lids) sprayed in the laboratory were attached, by means of thumb tacks, in order to expose them to the weather.

The results of the experiment are shown in Table 20. Whereas after 7 days of exposure to sunshine and very little rain the parathion wettable powders (brands A and E) at 0.24 per cent concentration of toxicant gave only 26 and 16 per cent kill respectively, and EPN 43 per cent kill (see Table 19), after 5 days of practically no sunshine but high rainfall, the same suspensions gave, respectively, 100, 99, and 100 per cent kill. Rainfall was thus shown to be less important than sunlight and high temperature in the weathering of insecticide wettable powders on waxed cards. However, the same period of rain resulted in the almost complete removal of the insecticide residues on the upper sides of corn leaves in a field only a half mile distant from the site of the above experiment. Mature corn leaves are readily wet by water, consequently solid material is more easily removed than from the waxed cards, on which the contact angle of water is about 90° . 'February, 1953] Ebeling: Laboratory Experiments on Control of Fruit Flies

The first column of percentages in Table 20 gives the per cent kill only 4 hours after the flies had been allowed to crawl over the waxed cards containing the insecticide residues. The slow action of dieldrin, as compared with EPN and especially as compared with parathion, is shown by the fact that after 4 hours of exposure to dieldrin residue only 4 per cent of the

TABLE 20

EFFECTIVENESS OF RESIDUES OF 3 INSECTICIDES WITH AND WITHOUT ADJUVANTS, AGAINST MELON FLIES AFTER 5 DAYS OF HEAVY RAINFALL*

Suspensions of Wettable Powders

	Per cent mortality after residues weathered:				
Insecticide	24 h	ours	5 days:		
	Flies exposed 4 hours	Flies exposed 24 hours	flies exposed 24 hours		
With no adjuvant:					
Parathion, brand A	100	100	100		
Parathion, brand E.	100	100	99		
EPN	91	100	100		
Dieldrin	4	100	99		
With 0.24 per cent Kolofog, 0.04 per cent Z-1:					
Parathion, brand A	100	100	99		
Parathion, brand E.	100	100	95		
EPN	77	100	99		
Dieldrin	0	100	100		
With 0.25 ml boiled linseed oil per 100 ml:					
Parathion, brand A.	100	100	88		
Parathion, brand E	100	100	89		

* Waxed paper-cup lids were sprayed by means of a venturi-type laboratory sprayer on November 30, 1950, and exposed to the weather on December 1, 1950. The insecticides were used at a concentration of 0.24 per cent (21bs. to 100 gals.).

† Kolofog is a bentonite-sulfur product and Z-1 refers to Colloidal Z-1 Spreader. These adjuvants have been used as deposit builders and stickers.

insects had died, although all were dead in the usual 24-hour period allowed before the per cent kill is ordinarily determined. This slow action of dieldrin would, of course, be disadvantageous in field spraying if melon flies were to move from a sprayed surface after resting there only a short period, for they would not have time to pick up a lethal dosage. This quality of slow knockdown possessed by dieldrin must be balanced against its superior adhesiveness on foliage and the longevity of the residue in sun and rain. EPN is some place between parathion and dieldrin in the above characteristics, having neither as slow knockdown nor as great a resistance to weathering and decomposition as dieldrin. EPN is not as toxic to melon flies as parathion, but it has longer residual effectiveness.

It will be noted from Table 20 that after 5 days of heavy rainfall the insecticide residues containing no "stickers" generally resulted in a higher per cent kill of melon flies than those containing these adjuvants. Linseed oil (raw or boiled) at 0.25 per cent detracted considerably from the effec-

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tiveness of 5-day-old parathion residues, despite the fact that the oil is very effective in preventing the removal of residues by rain. The linseed oil covers the insecticide particles with a varnishlike layer that sheds water, but also

TABLE 21 EFFECTIVENESS OF RESIDUES OF 17 INSECTICIDES AGAINST MELON FLIES AFTER WEATHERING IN SUN AND RAIN FOR 1 TO 3 WEEKS*

Insecticide	Per cent	Per cent mortality after residue weathered:			
	concentration	1 week	2 weeks	3 weeks	
Wettable powders:					
	0.12	12	0	0	
DDT	0.24	28	30	0	
	0.36	56	47	0	
DDD	0.24	11			
DFDT	0.24	0			
	(0.12	0			
Methoxychlor	0.24	11			
-	0.36	24			
Dilan	0.24	10			
Lindane	0.24	0			
Toxaphene	0.24	20	50	0	
Chlordane	0.24	0			
Heptachlor	0.24	0			
Aldrin	0.24	15			
Dieldrin	(0.12	63	99	0	
	0.24	97	100	15	
Parathion	0.24	15			
Emulsifiables:					
Paraoxon	0.24	0			
EPN	0.24	9			
No. 4018	0.24	15			
Metacide	0.24	17			
E-838	0.24	0			
			1		

* Waxed paper-cup lids were sprayed on March 6, 1951. During the first week 8.84 inches of rainfall was measured in the near-by gauge. There was little sunlight. The next week there was only 0.52 inch of rain and much sunlight. During the third week there was 8.45 inches of rain, some of very high intensity, which appeared to have removed practically all the insecticide residue.

to some extent prevents adequate contact of the tarsi of the flies with the insecticide. Likewise a mixture of Kolofog, a bentonite sulfur product, at 2 pounds to 100 gallons, and Colloidal Z-1 Spreader, at $\frac{1}{3}$ pound to 100 gallons, contributed nothing to the effectiveness of the insecticides to which they were added and, in mortality counts made 4 hours after exposure to residue, decreased the effectiveness of both EPN and dieldrin. This combination is ordinarily added to spray mixtures to increase deposit and adhesiveness.

In another experiment a study was made of the effect of sun and rain on 17 insecticides. During the first week of exposure of the insecticide residues there was little sunlight and an exceedingly heavy rain.

The results of this experiment are shown in Table 21. During certain periods the rain was of such intensity that its effect on the insecticide residues was much greater than during the 5-day period of the previous experiment. Of the 17 insecticides tested, only DDT at 0.36 per cent of actual toxicant and dieldrin at 0.12 and 0.24 per cent gave better than a 50 per cent kill of flies exposed to the residues after they had weathered 1 week. The reason for the apparently improved performance of toxaphene and dieldrin residues after 2 weeks of weathering, as compared with 1 week, is not known. During the second week there was little rain and almost continuous sunshine. Among the three insecticides for which observations continued to be made—DDT, toxaphene, and dieldrin—there was no decrease in insecticidal effectiveness. During the third week of the experiment there was again an exceedingly heavy rainfall (8.45 inches) and practically all residues were removed except that of dieldrin at 0.24 per cent concentration, which gave a 15 per cent kill.

SUMMARY

The equipment constructed for laboratory investigations included a settling tower and an apparatus for spraying. All equipment was based on the venturi principle. It was easily decontaminated and lent itself well for rapid manipulation of large series of insecticide evaluations.

Laboratory test flies were anesthetized in carbon dioxide to facilitate their subsequent manipulation, and flies 7 days or less in adult age were mixed and then separated into lots of 25 and placed in test tubes. They were again anesthetized prior to placing them in the settling tower or confining them either with treated petri-dish covers or circular waxed cards (paper-cup lids) that had been sprayed in the laboratory.

When flies were confined with insecticide residues the carbon dioxide anesthetization greatly decreased the per cent kill because it retarded the activity of the flies for the entire period they were confined with the residues, so that they crawled about less actively and absorbed less toxicant through their tarsi. However, the carbon dioxide anesthetization did not affect the results of space treatment.

If 25 untreated flies were placed in a cage containing insecticide residue, the per cent kill was higher than if 50 flies were placed in the cage because in the latter case a lower percentage of the flies were found at any one time crawling about on the ceiling of the cage, which contained the residue.

With the melon fly and the Mediterranean fruit fly the males were found to be more susceptible to insecticides than the females; with the oriental fruit fly the opposite was true.

The effect of adult age on per cent kill from a given concentration of DDT was determined for each of the three species of fruit flies. In repeated experiments a definite pattern of susceptibility of the insects of successively greater age was found when flies varying from 1 to 17 days of age were treated simultaneously and the per cent kill was plotted against age.

The toxicity indexes for all insecticides in each of the above categories were calculated, first with the LD 50's of the corresponding DDT formulations as the standard (Tables 5 to 7) and then with the emulsifiable concentrates of each insecticide as the standard (Tables 9 to 11). The order of effectiveness of the insecticides was different in the various formulations. For example, pyrethrins and rotenone were first and third, respectively, in effectiveness when in kerosene solution, but were among the less effective insecticides in other formulations.

The relative effectiveness of the insecticides varied in the different formulations. The most toxic insecticides in emulsifiable concentrates, kerosene solutions, wettable powders, and dusts were, respectively, paraoxon, pyrethrins, parathion, and heptachlor.

The kerosene solution was the most efficient type of formulation, both as a space spray and as a residue. In average performance, the emulsifiable concentrate was superior to the wettable powder as a space spray, but inferior as a residue. The dust was the least efficient formulation, but, unlike the others, it was as effective in residue as in space treatment.

Both the period a group of insects spend in contact with a residue and the speed with which they crawl about on the residue have an effect on the per cent kill. Among groups of insects of the same species, anything that causes a variation in the above factors will cause a difference in the per cent kill between the groups. Between two species, the difference in their susceptibility to a residue as compared with a topical application will also depend on the inherent differences between the two species with regard to the above factors.

As between the three fruit-fly species investigated, residues are most efficient against the melon fly and least efficient against the Mediterranean fruit fly. The activity of the flies in the laboratory likewise decreases in the following order: melon fly > oriental fruit fly > Mediterranean fruit fly. Investigators who have had much experience with the three species in the field report that the same difference in their activity is noticed under natural conditions.

The settling tower used in the present investigation may be modified so as to make possible an investigation of insecticides at high concentrations for short periods. At concentrations of emulsifiable concentrates varying from 2 to 5 grams per 100 ml of actual toxicant, depending on the fruit fly species, paraoxon, parathion, heptachlor, Metacide, No. 4018, and lindane gave 100 per cent kills of at least one of the species in 1 second or less. Aldrin, dieldrin, E-838, lindane, TEPP, and rotenone gave 100 per cent kills in 10 seconds or less. Two hundred seconds or more of exposure was required for a 100 per cent kill of the flies when DDT, DFDT, DDD, methoxychlor, or allethrin was used.

Melon flies that survived a 24-hour contact with DDT residue or that had survived for 48 hours after exposure to a DDT or parathion residue, did not differ much from untreated flies in their subsequent mortality or in their capacity for egg laying.

The period required for 50 per cent knockdown from various insecticide spray deposits varied from 18 minutes for parathion to 10 hours and 35 minutes for DDT.

Insecticide residues in amounts sufficient to give a 100 per cent kill when first applied were tested with regard to their ability to withstand weathering. In the shade and protected from rain, the residues of dieldrin, EPN, and parathion sprays gave 100 per cent kills as long as 137 days after application. In the sun and exposed to rain, EPN and parathion residues were reduced in effectiveness to such an extent that they no longer gave a 100 per eent kill after 1 week of weathering, but dieldrin gave 100 per cent kills for as long as 2 weeks under similar conditions. When DDT was used at sufficient concentration to result in a 100 per cent kill immediately after application, it also retained a high degree of effectiveness for prolonged periods in the sun and rain.

ACKNOWLEDGMENTS

The writer's participation in the melon-fly investigation was in connection with a joint project coördinated by Professor Harry S. Smith, who was in charge of the fruit-fly research program of the University of California in Hawaii. Mr. Glenn L. Finney, in charge of the rearing of fruit flies and parasites for all phases of the fruit-fly investigations, supplied the flies for the investigations described in this report. This made possible an investigation of much broader scope than would otherwise have been possible.

The University of Hawaii provided office and laboratory space. Dr. H. A. Bess, Dr. Martin Sherman, Mr. W. C. Mitchell and Mr. T. Nishida, all of the University of Hawaii, and Dr. P. S. Messenger, of the University of California, offered advice and helpful suggestions and aided in many ways. Dr. Martin Sherman, Dr. P. S. Messenger, and Mr. T. Nishida, as well as Dr. R. L. Metcalf, Dr. A. E. Michelbacher, and Dr. Ray F. Smith of the University of California read the manuscript and offered valuable criticism.

Many formulations of insecticides for the laboratory investigations were supplied by American Cyanamid Company, California Spray-Chemical Corporation, Commercial Solvents Company, Dow Chemical Company, E. I. du Pont de Nemours and Company, Eston Chemicals, Inc., Geigy Company, Hercules Powder Company, John Powell and Company, Julius Hyman and Company, Niagara Chemical Division of Food Machinery and Chemical Corporation, Pennsylvania Salt Manufacturing Company, and Velsicol Corporation.

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