THE EFFECT OF AREA DOSAGE, SOLUTION CONCENTRATION AND DROP SIZE OF SPRAYED SOLUTIONS AND EMULSIONS OF DDT AGAINST MOSQUITO LARVAE.

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(PLATES V–VIII.)

CONTENTS.

Introduction.

The work was carried out during the War and was intended to supplement the results of more practicable field trials, carried out in other parts of the world, where uncontrollable conditions and the inadvertencies associated with practical applications of insecticides would hinder precise assessment of certain variables. The object of the investigation was to assess the potential effectiveness of DDT spray against mosquito larvae living under natural conditions and to determine the characteristics of the most effective form of spray. The variables considered were (*a*) area dosage of DDT, (*b*) oil solutions compared with oil-in-water emulsions, (*c*) concentration of DDT in the spray liquid, and (*d*) drop size.

In this report the terms " area dosage " and " dose " denote the amount of DDT distributed per unit area of canal surface. The units of dosage employed throughout the paper are grams of DDT per acre. Various units have been used by other writers and the following conversion table is given to facilitate comparison of results :

10 gr./acre=2·5 mg./m².
=14 lb./sq.mi.
=0·176 quarts 5 per cent. solution/acre.

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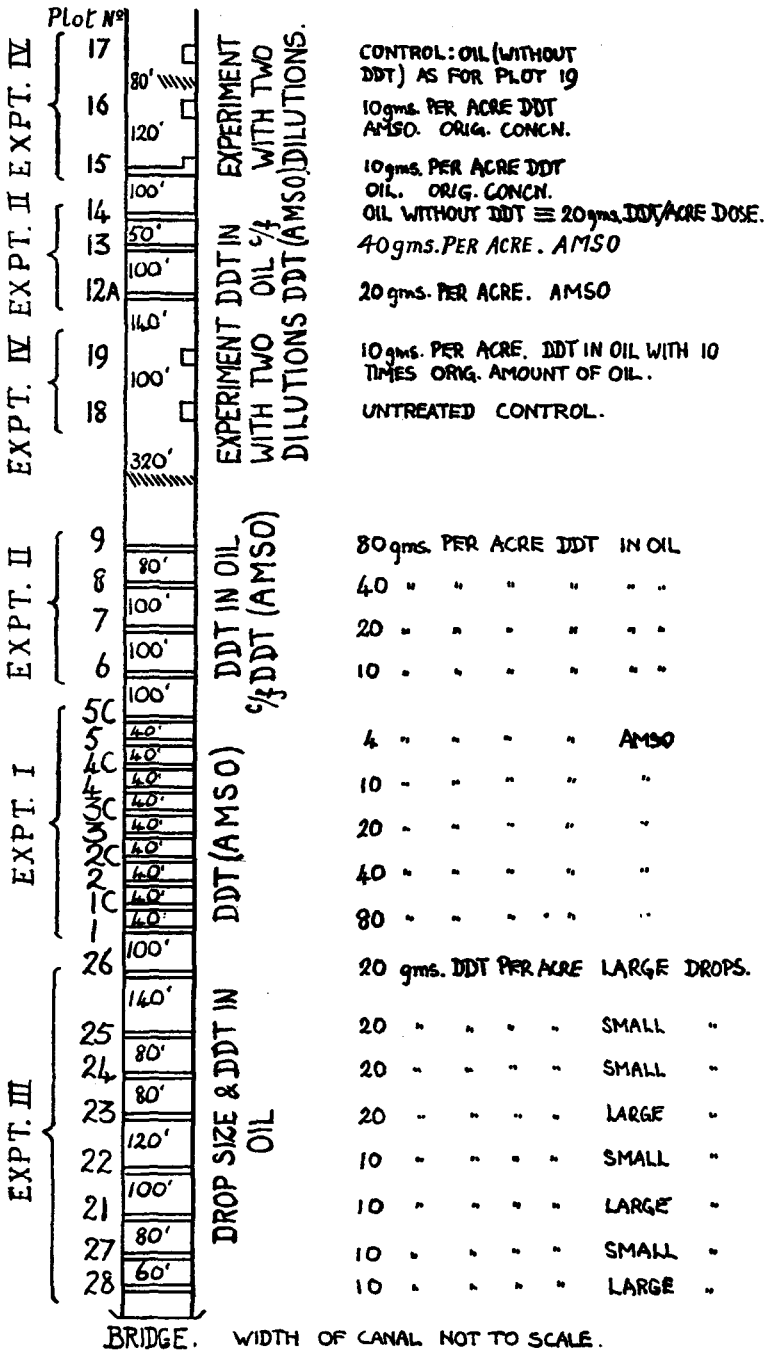

Fig. 1.—Layout of experimental sections, Basingstoke canal at Odiham, 1944.

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In actual aircraft trials it is difficult to apply spray of any desired characteristics to a small experimental area and so it was necessary to use a new spraying technique whereby, for experimental purposes, the spray characteristics could be accurately controlled. The choice of small experimental areas was necessary in view of the number of experiments planned and so that areas of similar uniformity in vegetation and larval population could be used.

Choice and Description of Site.

In order to make comparative experiments it was necessary to select a length of canal with a uniform type, distribution, and density of vegetation and mosquito larvae so that when divided up into experimental areas all would be as nearly identical as possible. A length of canal (approximately 1,100 yards) was chosen at Odiham (Ordnance Survey, Aldershot Command, 1 in. to mile, map ref. 175718–186713) where such conditions seemed to exist. When the canal was divided up, however, and the experimental areas were sampled, it was found that considerable variations in larval density existed from plot to plot and from day to day in the same plot, as well as slight but possibly important variations in the thickness of marginal vegetation. Thus before any differences in larval counts due to different treatments occurred there were differences due to these other factors to consider: these are discussed on pages 410–411. The experiments were made during August and September 1944. The width of the canal was about 40 ft. and each experimental area comprised a 20 ft. stretch of canal. The distance between successive areas was in no case less than 40 ft. and in most instances was considerably greater. The limits of each area were marked with numbered pegs in the bank. The disposition of the areas used in the various trials are shown diagrammatically in fig. 1. Both sides of the canal were accessible; there was a tow-path on one side, the other verge was rather marshy (see Pls. VI, fig. 2; VII, fig. 1). The canal was densely packed with vegetation. There was complete absence of water flow and no disturbance of the water surface due to human agency, *e.g.*, barges.

The central channel (sampling position 4 and 5, see fig. 3) was uniformly overgrown with *Potomogeton natans* so dense that there appeared to be more area of leaf than of open water surface (Pls. VI, fig. 2; VII, figs. 1 and 2). In places there were large masses of floating algae (a very coarse species of *Spirogyra*: Pl. VIII, fig. 1). Patches of open water in the sampling area free from either *Potomogeton* or *Spirogyra* were rare and when present were only a few inches or at the most a foot or two in extent (Pl. VIII, fig. 1).

This floating vegetation was continuous across the canal between the outer edges of the reed margins on opposite banks. At the outer edges of the reed margin there was sometimes a slight thinning out of *Potomogeton* (position 3). The reed margin varied in width from bank to outer edge from 1 to 5 ft. with an average width of about 4 ft. The dominant species here was *Sparganium ramosum* (Bur-reed). Sub-dominants were *Typha latifolia* ("Bull-rush"), *Juncus* sp., *Cyperus* and *Carex* spp. and, in places where the reeds were less dense, *Menyanthes trifoliata* (Buckbean) tended to become dominant.

The height of the vegetation of the reed margin varied between 1 and 4 ft. Except in a few places, mentioned specifically in the text, the extreme margin (position 1) where water and bank met, was completely obscured from above by densely packed reeds which had to be lifted or parted by hand to introduce the ladle when sampling.

The water surface among the standing reeds (position 2) was usually clear and free from floating vegetation, but it was much obscured from above by the standing rushes (Pl. VIII, fig. 2).

The species of mosquito were *Anopheles maculipennis*, Meigen, var. *messeae*, Flni., and some *Anopheles claviger*, Meigen.

Spraying Technique.

Spray requirements.—For the purpose of these experiments, it was necessary to devise a method of spraying known concentrations of liquid uniformly over the whole of each experimental area. Owing to the small size of the areas (approx. 800 sq. ft.) this necessitated the development of an apparatus capable of distributing a minimal dose of about 4 cc. uniformly over this area. Since it was required to investigate the effects of spray concentration and of drop size for solutions and emulsions of widely different concentrations, the volume of liquid and drop size delivered by the spray had to be independently variable over a wide range. The spray concentration required to be varied from 200 ml./acre corresponding to 10 g. DDT/acre from 5 per cent. solution, to 16 l./acre, corresponding to 80 g. DDT/acre from 0·5 per cent. emulsion, a range of 1 : 80. The smaller drops used were required to simulate aircraft spray, mean dia. c. 0·4 mm. and the larger drops to be of the order of 1 mm. in diameter.

Description of Apparatus.—The apparatus developed for the trial is illustrated in Plate V, fig. 1. It consisted of a rotating disc atomiser in which the liquid was fed from a syringe on to a motor-driven spinning disc and was thrown off from the periphery in the form of a fine spray by centrifugal force. This type of spray was chosen because the drop size was uniform, independent of the rate of liquid flow over a wide range, and was readily varied by altering the speed of the disc. The apparatus was carried on a cord stretched across the canal (Pl. V, fig. 2). The liquid delivery was determined by the movement of the syringe piston, which was carried on a screwed rod forced down by a rotating nut driven by a gear and pulley system. The carrying line passed round this pulley and the liquid was, therefore, delivered at a constant amount per unit distance travelled, independently of the speed at which the apparatus was towed across the canal. The flex carrying current to the motor was used for pulling the spray in one direction, and a separate cord was provided for pulling it back across the canal. The direction in which the cord passed round the pulley could easily be reversed for operation of the spray in either direction of travel. The apparatus was provided with interchangeable gears and pulleys giving a range of piston speeds in the ratio 1 : 2 : 4 : 8. Interchangeable syringes having cross sections in the ratio of 10 : 1 were also provided giving a total range of liquid deliveries in the ratio 1 : 2 : 4 : 8 : 10 : 20 : 40 : 80. The spray covered a band of width of from 5 ft. 3 in. to 6 ft. 8 in. according to the type of the disc used, so that four traverses of the canal were necessary to cover an experimental area 20 ft. wide.

In the first experiments, a plain disc, concave side upwards, as shown in Plate V, fig. 1, was used, but it was found that this gave a somewhat higher concentration of drops at the edges of the sprayed band than in the middle (Pl. VI, fig. 1, A) an effect which was accentuated by any slight overlap of adjacent bands. A new disc with circumferential slots allowing drops to be thrown off at two different radii was next used and gave a satisfactory distribution for " small " drops as shown in Plate VI, fig. 1, B, but was unsatisfactory at larger sizes. A " disc " with a spiral edge expanding from 0·5 to 1·75 in. radius was finally employed and gave a satisfactory distribution for both small and large drops (Pl. VI, fig. 1, C, D). It will be seen that this disc gave a falling off in concentration at the edges of the band so that a slight overlap of adjacent bands was necessary in order to secure a uniform contamination. It also made the spacing of the bands much less critical.

The dimensions of the syringes, gears and pulleys were designed so that in various combinations concentrations of 4, 10, 20, 40 or 80 g. DDT/acre could be delivered, using either 5 per cent. solutions of DDT in oil or 0·5 per cent. emulsions. In practice these dosages could not be obtained exactly with solutions of these concentrations, mainly owing to variations in the width of the sprayed belt according to the type of disc employed. The concentrations of the solutions were therefore adjusted as

necessary to give the correct dosage of DDT per unit area. With the plain disc giving a 6 ft. 8 in. belt the solution concentrations required were 1·08 times the nominal values given above. For the 5 ft. 3 in. belt given by the spiral " disc", the concentrations were reduced to 0·86 of these values.

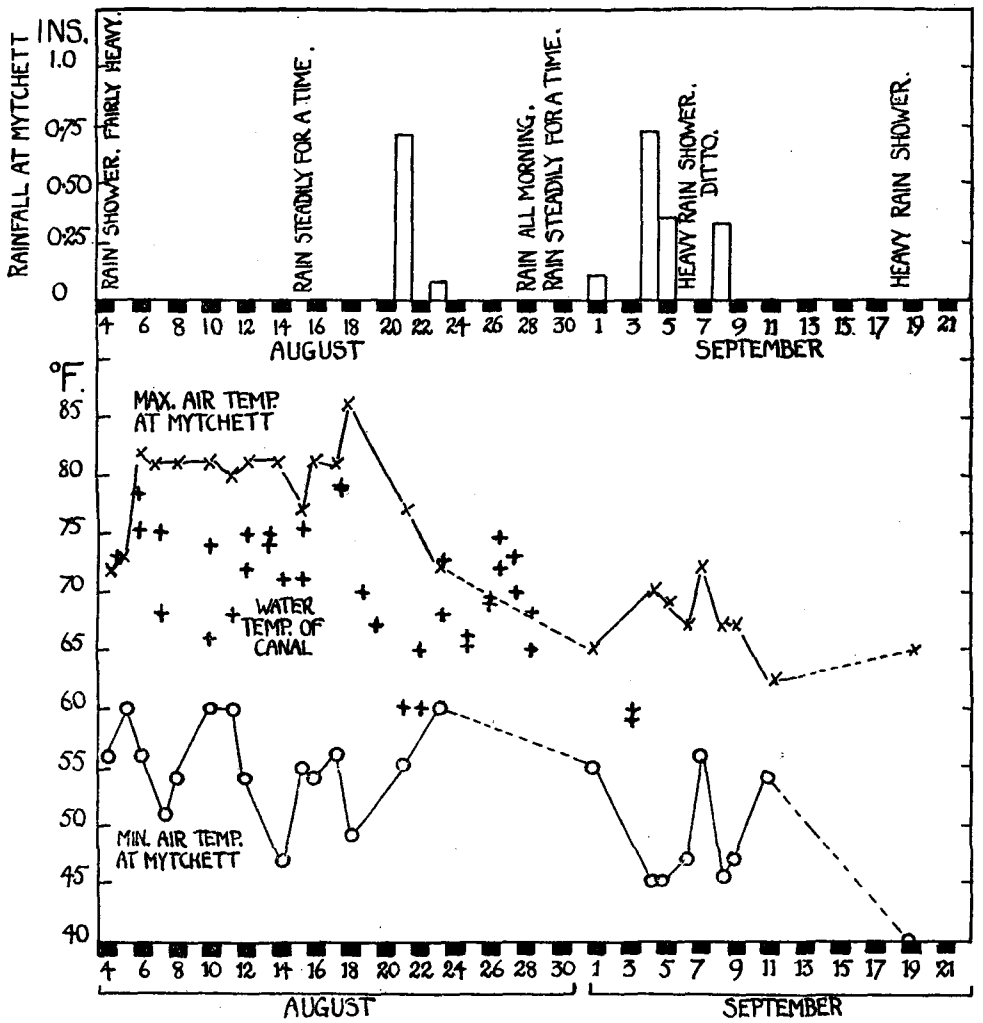

Fig. 2.—Diagram of rainfall and temperature during the experiments.

Spraying procedure.—Four men were required to operate the spray, two on each side of the canal. One man on each side supported a pole, 7 ft. high, to which the carrying line was attached. The tension of the line was adjusted to keep the spray at the correct height above the water surface. The other two men were responsible for towing the apparatus across the canal, changing the direction of the line round the pulley according to the direction of travel, and for refilling the syringe with solution when necessary. The 12 volt batteries and other electrical equipment for driving the motor and regulating its speed were situated on the tow-path side of the canal. The apparatus was moved along a measured distance equal to the band—width sprayed after each traverse of the area. It was observed that the fine spray in

particular was carried an appreciable distance downwind before reaching the canal surface and so an extra band was sprayed beyond the upwind edge of each experimental area. The apparatus was also kept at the minimum height, 3–4 ft., above the water necessary to ensure a full spread of the spray. During some of the trials the wind was rather gusty. Then the spraying was interrupted as far as possible during the gusts and carried out only during the periods of comparative calm.

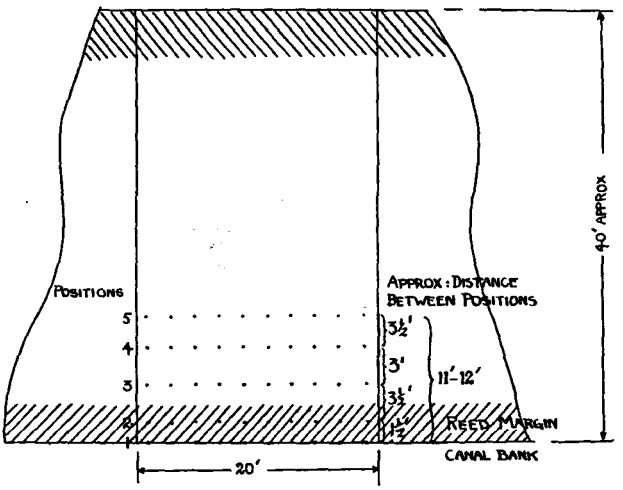

Fig. 3.—General arrangement of experimental area for sampling. Each dip is represented by a dot. Spraying was carried out over the whole width of the canal but samples were taken only from an area on one side.

Spray assessment.—The amount of solution delivered from the syringe per unit area of the experimental areas was known with accuracy, but blank runs over a sampling layout were made on a number of occasions in order to estimate the wind loss, uniformity of deposition, and drop size. The layout consisted of a row of eight floats equally distributed across the width of an area along a line parallel to the bank of the canal and about 10 ft. out between positions 3 and 4 (fig. 3). Each float carried a 9 in. × 10 in. sheet of filter paper (Whatman No. 1) on which the spray was received. For the purpose of the estimation, dyed oil or emulsion containing no DDT was sprayed. The number of drops on each paper was counted and the dye was then extracted and estimated colorimetrically. From these data the drop size and concentration of spray per unit area were calculated. The amount of spray intercepted by the reeds in the marginal areas of the canal was not determined.

Technique for sampling Larvae.

The ideal technique for estimating a population before and after spraying should be so arranged that one area is sampled several times each day. Then the fluctuations in numbers of larvae from plot to plot, day to day, and the " chance " variations in numbers counted due to random dipping, can be measured. Differences due to DDT treatments can thus be distinguished from differences due to the other factors. Unfortunately it was impossible to sample each area more than once a day, time and personnel being the limiting factors and for this reason the " sampling error " was not measured. In view of this it is idle to ask if a difference between two areas after treatment is statistically significant. For such a difference may be due, not to the treatments, but to one of the other factors mentioned above. No tests

for significance have therefore been made in the following results. Where differences do exist, they are surprisingly consistent and in accordance with expectation, or else are so slight that similarities rather than differences are outstanding.

The larval and pupal population in each experimental area was estimated by taking samples with a ladle fixed to a long handle. The total number of larvae and pupae for each dip and at the same time the numbers in each of the different developmental stages (1st, 2nd, 3rd and 4th instar and pupa) were recorded. The actual dipping was standardised and the ladle always put into the water in the same way. All larvae and pupae after counting were returned to the spot from which they had been taken.

Each experimental area was sampled at five different " positions " with respect to the bank, each position corresponding to the distribution of vegetation (fig. 3). Positions 4 and 5 were in the " open " canal, position 3 was at the outer edge of the marginal reeds, position 2 was among the marginal reeds and position 1 was at the very edge, among the reeds, as close to the bank as possible.

Ten dips were taken from each of the five positions at approximate intervals of 2 ft. (making a total of 50 dips for each area) on each day for sampling. In most experiments each area was thus sampled on each of three successive days immediately before spraying with DDT (days 1, 2 and 3) : spraying was carried out on day 4, and sampling was again made on the following three successive days (5, 6 and 7) and then on single days at approximately weekly intervals.

Experiments.

The experiment was carried out in four parts, priority being given to the more important items, as it was not known at the outset if the whole programme could be completed before the end of the mosquito season. Priorities were allocated as given below. It proved possible to carry out all the tests.

First Experiment.—Determination of the minimum lethal dose with DDT in AMSO, 0·5 per cent. (approx.) emulsion, droplets circa 0·4 mm. diameter.

Second Experiment.—Comparison of DDT in oil (5 per cent. solution) and in AMSO (0·5 per cent. emulsion), droplets as in 1st Experiment.

Third Experiment.—Effect of droplet size with DDT in oil (5 per cent. solution) at delivered dosages of 10 and 20 g. DDT per acre.

Fourth Experiment.—Effect of dilution. Equal dosages of DDT (10 g./acre) delivered in solutions of various dilution. Effect of oil without DDT.

Compositions of Spray Mixtures.

AMSO Emulsion* was an oil-in-water emulsion, the oil phase of which was substantially a 5 per cent. solution of pure DDT (active isomer only) in a heavy lubricating oil.

The spray mixtures referred to in the text had the following compositions :—

(i) *AMSO Emulsion.*—The emulsion was prepared by adding distilled water to a self-emulsifying oil solution of DDT (AMSO-1) *containing* :—

30 g. pure DDT : cryst. m.p. 108°C.
400 ml. pool 3 oil
200 ml. Amoa A5X

*Anti-Malaria Soluble Oil. Code name given to the self-emulsifying oil solution of DDT used in preparing the emulsion.

The emulsifying agent (Amoa A5X) was a proprietary mixture, consisting substantially of sodium oleate and cresol, which was miscible with the heavy oil, and brought about the immediate emulsification of the latter when the mixture was stirred into cold water.

The standard 0·5 per cent. AMSO emulsion was made by diluting 1 volume of AMSO-1 with 9 volumes of distilled water. For the experiment, the dilution with water was varied slightly according to the band width sprayed (see p. 408) in order to give the desired delivery of DDT per unit area. In the 1st experiment where the spray-band width was 6 ft. 8 in., the emulsion concentration was 0·54 per cent. DDT. In the others, the spray-band width was reduced to 5 ft. 3 in., and so the concentration was reduced to 0·43 per cent. in order to maintain the same area, dosage of DDT.

Distilled water was used in the preparation of these AMSO emulsions in order to secure high stability in the sprayed mixture and to avoid any possibility of bulk lack of homogeneity due to curding.

In this way it was possible to disperse on a water surface fine droplets of DDT in oil (of diameter of the order of 10μ). Small scale laboratory experiments showed that the dispersion of an emulsion in this manner gave, more rapidly, a much more even covering of a water surface with a DDT preparation than could be achieved by the direct use of a solution of DDT in oil (as below). It was felt that this use of an emulsion might be of particular advantage in dealing with water surfaces broken up by foliage which are the normal breeding places of mosquito larvae.

A subsequent mathematical investigation, however, indicated that there was little to be gained in spraying an aqueous emulsion from an appreciable height above ground, since evaporation of the water phase occurred in the falling drops.

(ii) *Emulsion of Oil Yellow.*—Used for spray assessment in 1st experiment. Composition similar to 0·5 per cent. AMSO emulsion, but containing 0·23 per cent. Oil Yellow dye dissolved in the oil phase; no DDT.

(iii) *Oil Solution of DDT.*—Since it is difficult to dissolve technical DDT directly in heavy oil without heat or mechanical stirring, the oil solution of DDT was made by diluting a 20 per cent. concentrate of pure DDT (active isomer only) in solvent naphtha with a heavy lubricating oil to give finally a 5 per cent. solution of DDT in oil. This represented, after spraying, a saturated solution of DDT in the heavy oil, since solvent naphtha was found to evaporate rapidly when spread in a thin film on a water surface. To ensure that the heavy oil would spread on dirty stagnant water ½ per cent. of oleic acid was added to it. Thus the standard 5 per cent. solution contained:—

10 g. pure DDT: cryst. m.p. 108°C.
50 ml. heavy solvent naphtha
150 ml. HD50 lubricating oil
1 ml. oleic acid.

It is inadvisable to spray naphtha concentrate alone. It evaporates on water, and the crystals of DDT which then separate soon sink, since their density is so much greater than that of water. The density of the 5 per cent. solution of DDT in heavy oil is less than unity, and consequently the latter persistently contaminates the water surface. The concentration of DDT relative to the other constituents was adjusted slightly, as for the AMSO emulsion, according to the band width sprayed.

(iv) *Dyed Oil Solution.* Used for spray assessment in 3rd experiment. Composition as (iii) but containing 1·35 per cent. Oil Yellow dye; no DDT.

Characteristics of the Sprays delivered.

Delivered dosages.—By a suitable choice of gears and pulleys (see p. 408), it was possible to deliver from the spray apparatus the exact area dosage of DDT required in each test. Some spray was blown away by the wind and lost, however, particularly the finer droplets, in spite of the precautions described on p. 409. There was therefore some uncertainty in the exact concentration of DDT reaching the water surface in any given experimental area. Some indication of the error was obtained by assessment trials in which dyed solutions were sprayed. The results of these experiments are given below. *For the purpose of simplifying the discussion of the entomological results throughout this Report, the "delivered dosages" of DDT will be quoted,* i.e. *the area dosage of DDT delivered from the spray. It should be borne in mind that the actual dosage on the water surface may have been about 30 per cent. less than the quoted value in the case of the fine spray; the error was probably negligible for the coarse spray* (*p.* 414).

An error was made in making up the oil solution of DDT used in the 2nd experiment. The concentration of the solution was 25 per cent. too high (5·4 instead of 4·3 per cent.), and so the dosage actually delivered was 25 per cent. greater than the delivered dose indicated in the diagrams and text of this report. The delivered dosage of DDT in AMSO in these tests was as stated.

Drop size.—The drop-sizes of the sprays used in the various tests are given below. The assessments were made with dyed solutions sprayed over a float layout (p. 410). The number of drops and total amount of dye falling per unit area were determined as described on p. 410 and hence the drop size was calculated.

Experiments	Drop size (dia.)	Remarks
1st	0·42 mm. (" Small drops ") ...	By assessment of dyed emulsion. Plain spray disc.
2nd	0·42 mm. (" Small drops ") ...	No direct assessment. Disc speed similar to previous tests. Slotted disc.
3rd	0·41 mm. (" Small drops ") ... 0·95 mm. (" Large drops ") ...	By assessment of dyed oil solution. Spiral disc.
4th	0·95 mm. (" Large drops ") ...	No assessment. Spraying conditions as for large drops in 3rd tests.

In all cases the drops were predominantly of uniform size, as shown by the stain diameters given in the tables below. The sprayer always produced a quantity of very small drops of uniform size in addition to the main spray output of larger uniform drops. These smaller drops were the satellites formed when the parent drops detached themselves from the spinning disc. They constituted only a negligible proportion of the total mass of the spray.

Wind loss and uniformity of deposition.—In the assessment trial of the 1st experiment, three bands, 6 ft. 8 in. wide, were sprayed over the experimental area only and spraying was not interrupted during the frequent gusts of wind. Examination of the layout showed that there was a low concentration of spray over the upwind half of the area. Therefore, in subsequent runs with DDT spray, an extra band was sprayed outside the upwind edge of the area in order to allow for the spray being blown down-wind and spraying was stopped when the wind velocity increased. The assessment results with spray containing no DDT, therefore, did not accurately represent the area dosage of the DDT sprays. The average value obtained over the whole width of the plot was 38·6 per cent. of the delivered dose. When only the down-wind half of the layout is considered the spray recovery comes out at 60 per cent. of the delivered dose. The actual value for the DDT spray was, therefore, probably rather greater than 60 per cent.

Detailed assessment results for the 3rd experiment are given below. Here the dye solution was sprayed under similar conditions to the DDT spray on the experimental area, and the results therefore give a more reliable indication of the wind loss and uniformity of deposition than in the 1st experiment. It will be seen that with large drops there was no loss of spray due to wind within the limits of experimental error. About 30 per cent. of the small drops were lost.

(i) *Large drops.*

Envelope No.	No. of stains on envelope	Dia. of stains	mg. dye per m²
A1	9	6 mm.	1·48
	3	10 ,,	
A2	16	6·5 ,,	1·81
A3	10	7 ,,	0·94
A4	11	7 ,,	1·15
	16	2 ,,	
A5	28	7 ,,	2·96
A6	5	7 ,,	0·63
A7	25	7 ,,	2·43
A8	18	7 ,,	1·81

Delivered dosage equivalent to 20 g. DDT/acre in 4·3 per cent. solution.	
Average droplet concentration	273 per m²
Average dye concentration	1·65 mg./m²
Drop diameter	0·95 mm.
Recovery. Per cent. of delivered dose	106 per cent.
Wind loss	nil
Uniformity of deposition on envelopes. (average deviation from mean value)	36 per cent.
Mean distance apart of drops	6·1 cm.

(ii) *Small drops.*

Envelope No.	No. of stains	Av. size of stains	mg. dye per m²
B1	48	3 mm.	0·42
B2	74	3 mm.	0·63
	110	1 mm.	
B3	159	3 mm.	1·36
B4	67	3 mm.	0·59
B5	181	3 mm.	1·57
B6	128	3 mm.	1·30
B7	120	3 mm.	1·22
B8	167	3 mm.	1·48
	202	1 mm.	

Delivered dosage equivalent to 20 g. DDT/acre in 4·3 per cent. solution	
Average droplet concentration	2,130 per m²
Average dye concentration	1·07 mg./m²
Droplet diameter	0·415 mm.
Recovery per cent. of delivered dose	69 per cent.
Wind loss	31 per cent.
Uniformity of deposition on envelopes (average deviation from mean value)	37 per cent.
Mean distance apart of drops	2·2 cm.

It was noticed that the oil in particular, and also the AMSO, even in the lowest dosages, spread right up to the bank of the canal, *i.e.* to position 1. Even the smallest corners, much obscured by reed from above, were covered with a film within 24 hours which intimately penetrated the many irregularities where the bank shelved into the water (see p. 407).

Entomological Results.

First Experiment.

Determination of the minimum lethal dose with DDT in AMSO emulsion. Small drops.

AMSO emulsion concentration ...	0·54 per cent. DDT.
Delivered dosages	80, 40, 20, 10 and 4 g. DDT/acre.
Drop size	0·42 mm. diameter.

Ten experimental areas were chosen each with a space of 40 ft. between it and the next area. Alternate areas were sprayed with DDT in AMSO delivered at the rates of 80, 40, 20, 10 and 4 g. of DDT per acre. The remaining areas (with one exception) were left unsprayed, as controls. The exception was the area used for drop assessment next to that sprayed at the 80 g. rate. This area was sprayed with dyed AMSO *without DDT* at the rate equivalent to 40 g. DDT per acre. The layout of the experiments is shown in fig. 1. The total larval and pupal numbers for all dipping positions—*i.e.* from bank to central region of the canal—are shown in fig. 4. On the three successive daily counts before spraying the larval numbers varied between 47 and 93 per 50 dips. The areas were sprayed on day 4 and counts on the following days (5, 6 and 7) showed that the population had dropped in order of dosage—*i.e.* the heaviest dose, 80 g. per acre, gave the greatest kill, the smallest dose delivered, 4 g. per acre, gave the least kill (see also fig. 5).

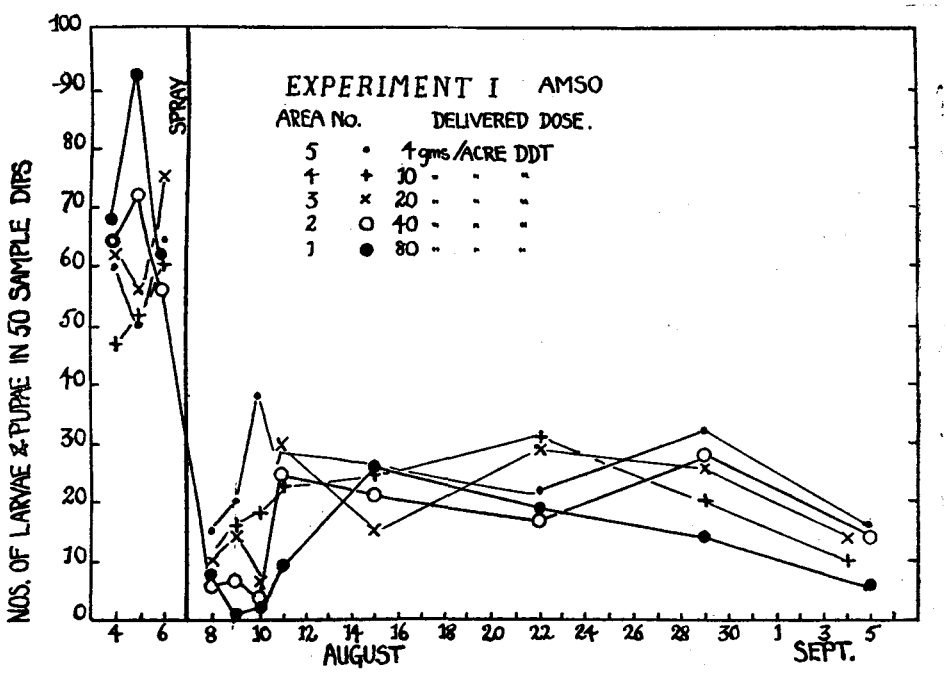

FIG. 4.—Experiment No. 1. DDT in AMSO.

The general situation is seen more clearly if the figures are put on a proportional basis. If the daily average for the three successive counts before spraying be taken as 100 per cent. and each subsequent daily count expressed as a percentage of this, we get the graphs shown in fig. 5. The degree of recovery is of the same order in each area, but the lower dosages recover to a slightly higher population level than the heavier ones because the initial kill is not so great.

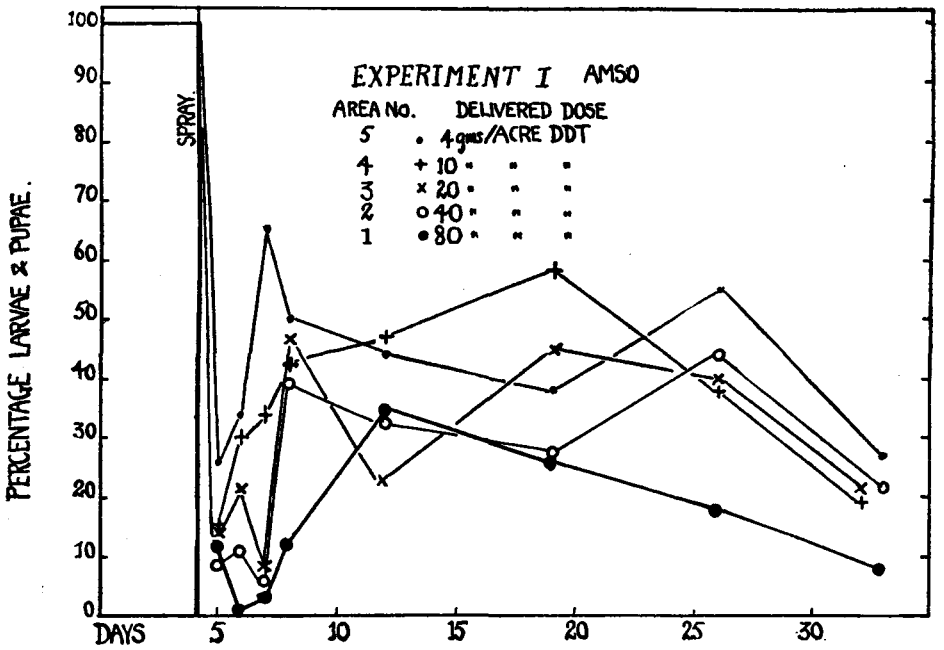

FIG. 5.—Experiment No. 1. DDT in AMSO. The numbers of larvae and pupae have been expressed as a percentage of the daily average for the three successive days before spraying.

After the 3rd day from spraying, the larval population showed an increase in all the areas, the recovery starting within 48 hours of spraying with the two lowest dosages. This recovery, due no doubt to the hatching of eggs not killed by the spray, does not continue beyond the 4th day after spraying except at 80 g. per acre where the peak of recovery is reached 8 days after spraying. The results show quite clearly that initial kill and speed of recovery are correlated with dosage. This recovery is, however, incomplete and the population never regains its original state but continues at a much reduced level until it declines at the end of the season.

This stabilisation of the population at a low level may be due to several factors and not at all to the residual effect of DDT. For example :—

(i) The reproductive potential of the population is probably insufficient to allow for complete recovery at that time of year (August–September). If the recovery seen in fig. 4 is due to the hatching of eggs already laid at a time of spraying, then subsequent egg production must have been low, otherwise a greater increase would have occurred. This is also suggested by the behaviour of the population in the control areas (see below), whose general decline after August 16th is associated with a drop in temperature (*cf.* figs. 6 and 2).

(ii) This low egg production may have been partly seasonal and partly due to some adults being killed off either by the spray while they were resting in the reed margin or due to a kill of adults when they visited the area to oviposit.

In the case of the untreated control areas (fig. 6), areas 4c and 5c, those next to 10 and 4 g. per acre areas respectively (fig. 1) show a fairly high population level over a period beyond the spraying date. Area 5c was at the end of the series and could have been influenced only by the treated area to one side of it (and this at only 4 g. per acre). It is highly unlikely that fluctuations in 4c and 5c are due to DDT spreading from neighbouring areas and all variation is almost certainly due to natural

causes. Both areas 4c and 5c show a decline in larval numbers corresponding to the stabilisation level in the treated area and to a drop in temperature (fig. 2) and this supports the view that the stabilisation was due rather to natural causes than to DDT.

All the other control areas, 1c to 3c, show a decline about five days after the neighbouring areas were sprayed, and it seems almost certain that these control areas were affected by the DDT deposited 40 ft. on each side of them. But in every control area there is a slight upward trend in the population which is reflected in the recovery of the treated areas which took place at about the same time. There was evidently a natural tendency for the larval population of the canal to increase just then.

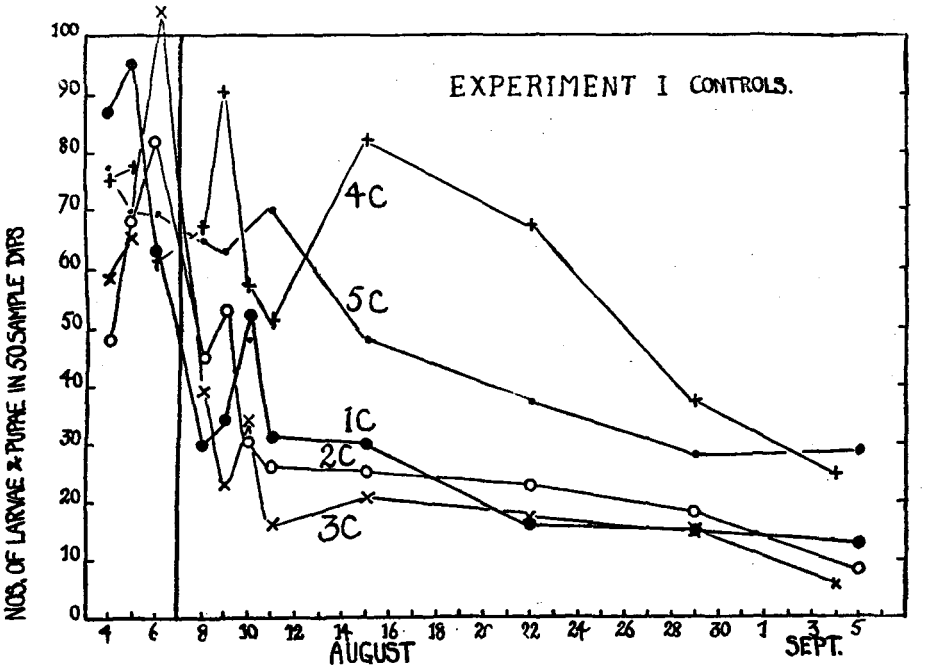

FIG. 6.—Experiment No. 1. Larval and pupal population in untreated control areas.

Thus the evidence for prolonged residual effect of DDT as a larvicide is inconclusive. The dosage for initial kills is best seen if the average daily count for the two days immediately after spraying is expressed as a percentage of the daily average for the three days before spraying (it is better not to include the 3rd day after spraying since by then recovery has started at the lower dosages). The results are shown in fig. 7 A, and are discussed below.

SECOND EXPERIMENT.

Comparison of DDT in oil and in AMSO. Small drops.

AMSO concentration ...	0·43 per cent. DDT.
Oil solution concentration	5·4 per cent. DDT.
Delivered dosages AMSO...	20 and 40 g. DDT/acre.
,, ,, Oil ...	10, 20, 40, and 80 g. DDT/acre (plus 25 per cent. see p. 413).
Drop size	0·42 mm. diameter.

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Seven areas were chosen : Nos. 6–9, to be treated with DDT in oil. They were arranged with 100 ft. of canal between each area except between 8 and 9 where only 80 ft. could be allowed if vegetation was to be kept fairly uniform.

The areas to be treated with AMSO and the control area (12A, 13 and 14) were 100 and 60 ft. apart and there were 320 ft. between area 9 and 12A. The general layout is shown in fig. 1.

Thus for purposes of comparison we have not only oil and AMSO treated areas in the 2nd experiment but also AMSO areas from the 1st experiment.

	Area No.		*Delivered dose of DDT in g./acre.*			
Expt. II	6–9	Oil ...	10	20	40	80
	12A and 13	AMSO ...		20	40	
	14	Control without DDT but oil≡20 g./acre area.				
I	1–4	AMSO ...	10	20	40	80

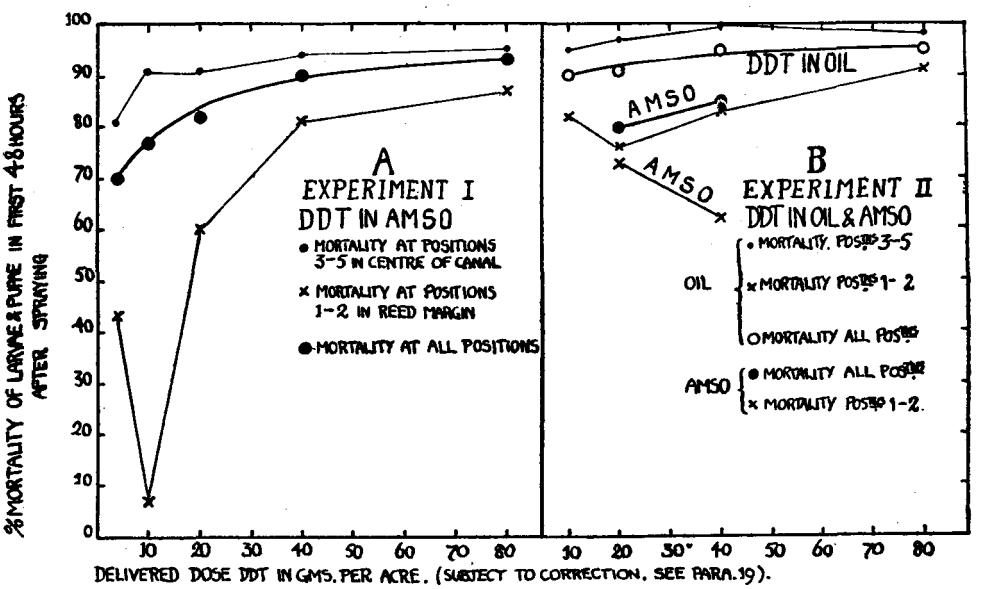

FIG. 7.—A. Experiment No. 1. AMSO ; percentage mortality of larvae and pupae in the first 48 hours after spraying.

B. Experiment No. 2. DDT in oil and AMSO ; percentage mortality of larvae and pupae in the first 48 hours after spraying.

Consideration of initial kill.—If figs. 7 A and B, showing the percentage mortality in the first 48 hours after spraying are compared it will be obvious that :—

(*a*) 10 and 20 g. of DDT in oil give a higher mortality than the same doses in AMSO. This superiority is seen not only in the experiments carried out with the oil and AMSO simultaneously as 2nd experiment (fig. 7 B) but also when the DDT in oil is compared with DDT in AMSO (First expt. : fig. 7 A *cfd.* with fig. 7B). The results at 40 and 80 g. DDT per acre also show a superiority of the oil over the AMSO although the differences are not so marked as at the lower dosages.

(*b*) The superiority of the oil spray is seen clearly in the kill obtained in the reedy margins (Positions 1 and 2. Figs. 7 A and B).

This higher kill with DDT in oil in the reedy margin may be due to a more satisfactory penetration of the oil compared with AMSO on the water surface in between the reed stems from spray deposited at the outer edge of the reed margin.

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The generally higher kill with the oil will, at any rate in part, be due to the slightly greater amounts of DDT inadvertently delivered (as described above, p. 413). But it would seem from a comparison of the graphs that a 25 per cent. increase in dosage of DDT is unlikely to account for the much higher mortality that is shown in the reedy margins with the oil spray (see also p. 427).

Another factor to be considered is the slightly more open type of vegetation in the margins of the 2nd experimental areas. This would favour a higher mortality whatever the spray. A comparison of results for AMSO in the first and second experiments does not indicate, however, that this was of great significance (*cf.* AMSO in figs. 7 A and B).

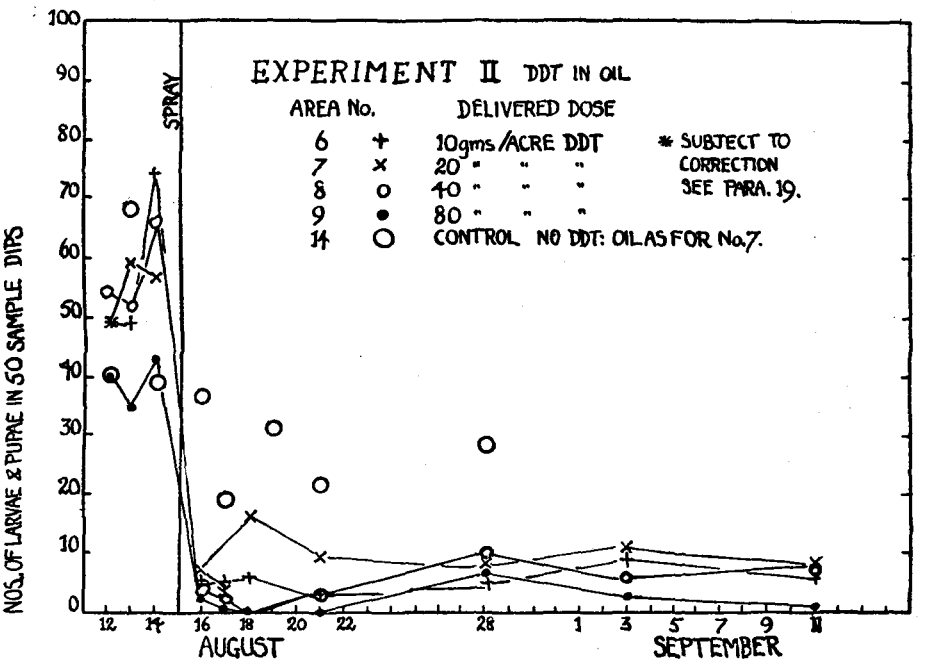

FIG. 8.—Experiment No. 2. DDT in oil. Larval and pupal counts in treated and control areas.

The population after initial kill.—If the graphs in figs. 8–10 (oil and AMSO, 2nd experiment) are compared with those in fig. 4 (AMSO 1st experiment) it will be seen that in the second trials both with oil and AMSO (figs. 8 and 10) the sharp recovery in the larval population so characteristic of the first AMSO experiments (fig. 4) does not occur to the same marked extent. A consideration of the control areas (figs. 6 and 8) bears out the suggestion put forward on p. 417 that the recovery in the first AMSO experiments was due to a general tendency for the mosquito population in the canal to increase at that time. In the 2nd experiment, the control (fig. 8) shows only a slight increase between 16th and 18th August and thereafter there is a general decline in numbers. This downward trend is shown also in the controls after 16th August in fig. 6 and in both cases corresponds to a general drop in the temperature (fig. 2). The failure of areas in the 2nd experiment to show a recovery is probably due to a general, natural decline in the population.

It is very interesting to note that with a declining population, as compared with one on the increase, the kill with a small dosage is more marked and a small dose may be almost as effective as a large one. If the population tends to increase this is not the case (*cf.* figs. 5 and 9).

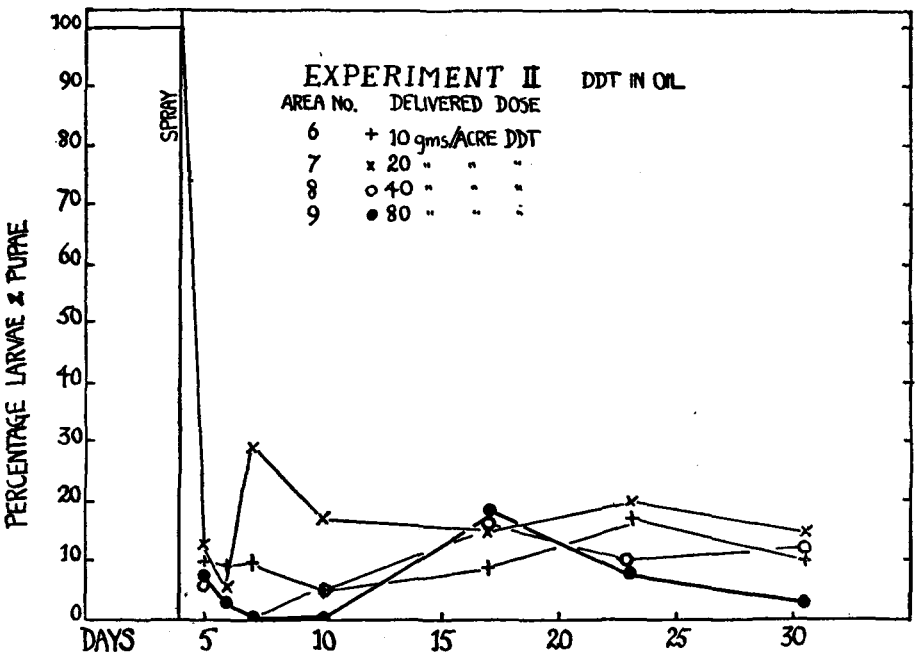

FIG. 9.—Experiment No. 2. DDT in oil. The numbers of larvae and pupae (as in Fig. 8) after spraying expressed as a percentage of the daily average of the three successive days before spraying.

To the above results can be added those obtained from two other areas sprayed at a later date (Nos. 15 and 16 of 4th experiment), where the superiority of DDT in oil as against DDT in AMSO was demonstrated.

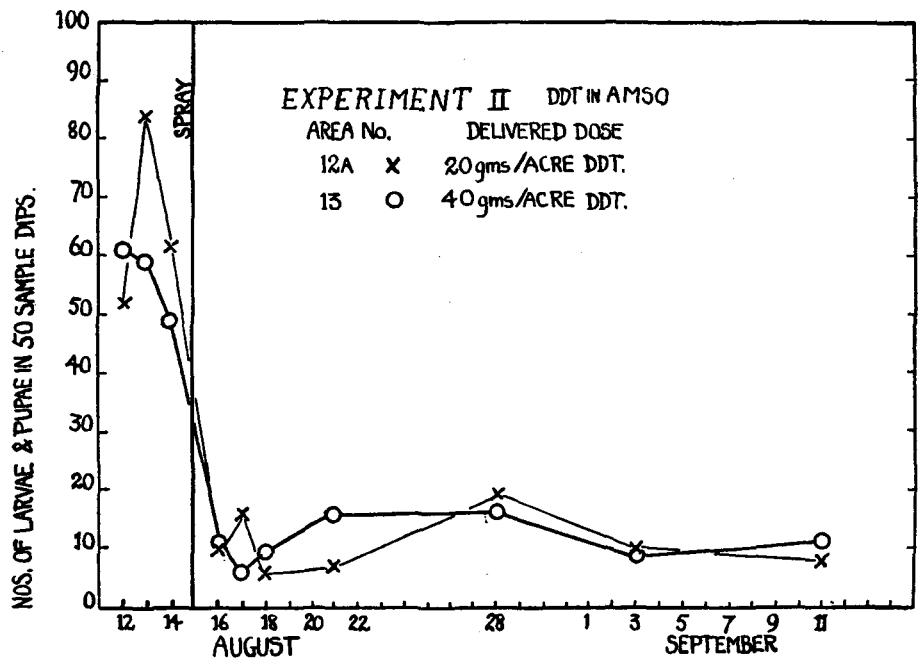

FIG. 10.—Experiment No. 2. DDT in AMSO.

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Early in September it was impossible to find areas with a sufficient larval and pupal population extending right across the canal and it was therefore necessary to work entirely on the population in the reedy margin. A full description of the tests follows on pp. 423–424. It is sufficient here merely to refer to the effect on the marginal population of a delivered dose of DDT at 10 g. per acre in oil and in AMSO. From the graphs (fig. 13) it will be seen that DDT at 10 g. per acre in oil gave a substantial kill during the three days after spraying, whereas in the area sprayed with DDT at the same rate in AMSO the population actually increased. There may in fact have been a certain mortality with the AMSO since there was a much greater increase in numbers in the control areas. But the success of the treatment with AMSO bears no comparison with that of oil and in this experiment there was no excess of DDT sprayed inadvertently as had happened previously.

Third Experiment.

The effect of droplet size with DDT in oil.

Oil solution concentration	4·3 per cent. DDT.
Delivered dosages	10 and 20 g. DDT/acre.
Drop size, small	0·41 mm. diameter.
Drop size, large	0·95 mm. ,,

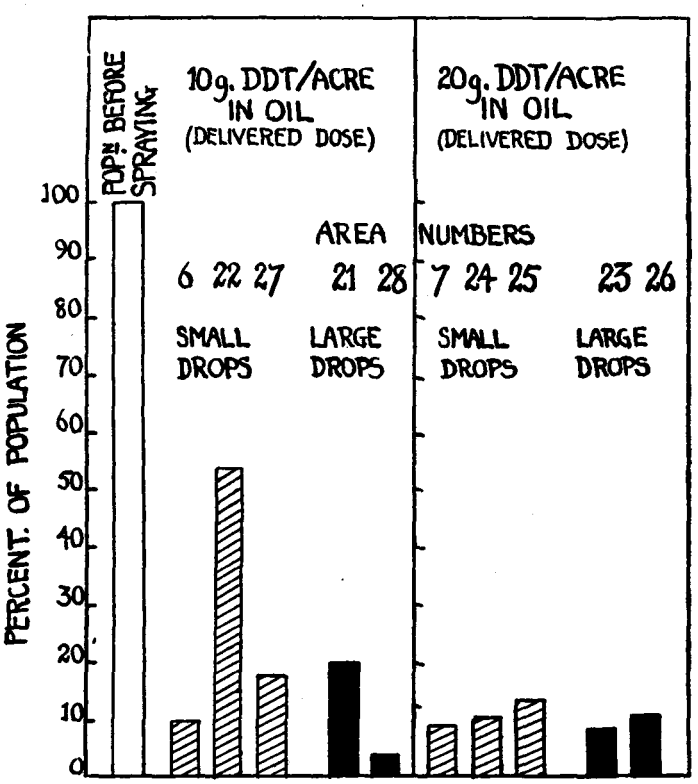

Fig. 11.—Experiment No. 3. The effects of droplet size. Percentage of population (larvae and pupae) remaining in first 48 hours after spraying. Percentage is based on mean of three days' counts before and two days after spraying.

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It was thought that small drops might penetrate the vegetation or hit the small interstices of water surface between floating leaves more frequently and thus give a higher kill than large drops. For, although the diameter of the small drops was only half that of the large ones, the relative numbers were approximately 8 to 1.

Duplicate plots were selected since by this time larval and pupal counts were rather low on the remaining sections of the canal. The results are given in Table I and figs. 11 and 12.

With a delivered dosage of 20 g. per acre the results were very consistent on all four plots and drop size was without effect. This is shown clearly in the mortality for the first 48 hours after spraying (fig. 11). The similarity of all the graphs for the general level of population over the succeeding days is also striking (fig. 12).

At a delivered dosage of 10 g. of DDT per acre differences in results with two different drop sizes are scarcely more noticeable. For the higher initial mortality with the spray of larger drops is associated with higher kill in one experiment although there is little to choose between the results in the other four (fig. 11). If the initial kill for the first *three* days is taken both the areas sprayed with large drops have a higher mortality than those sprayed with small drops. The results are seen also in the population graphs (fig. 12) where a larger drop-size gives a better kill in both experiments.

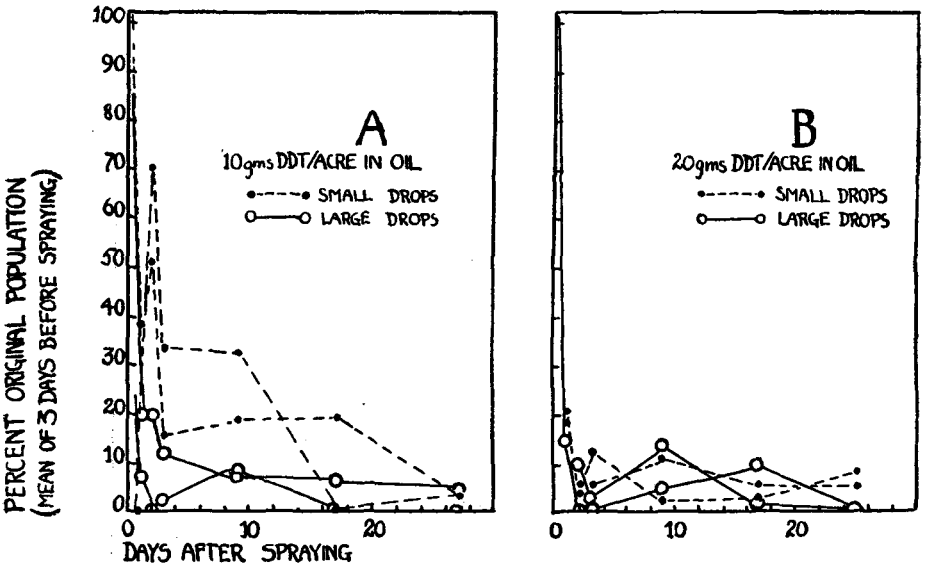

FIG. 12.—Experiment No. 3. The effects of droplet size. Population during the weeks following spraying with DDT in oil.

It is known (p. 413) that the loss of spray by slight breezes during spraying is more pronounced with small droplets than with larger ones and that consequently a smaller quantity of spray reaches the water surface if the droplets are small. This would tend to make itself shown when the dosage delivered is sufficiently low (*e.g.* 10 g. DDT per acre). With a higher rate of DDT delivery evidently enough can get down on to the water (under the conditions of these experiments) always to produce the same general level of mortality.

FOURTH EXPERIMENT.

Effect of dilution of the spray liquid.

Oil solution concentrations ... ... 4·3 per cent., 0·43 per cent. and 0 per cent. DDT.

AMSO concentration ... ... 0·43 per cent. DDT.

Delivered dosage, oil and AMSO ... 10 g. DDT/acre.

,, ,, pure oil, no DDT As for 0·43 per cent. solution, 2300 ml./acre.

Drop size ... ... 0·95 mm. diameter (large).

TABLE I.

DDT in oil. The effect of droplet size. See figs. 11 and 12.

Area No.	22	27	21	28	24	25	23	26
Dosage DDT/Acre	10 gms.		10 gms.		20 gms.		20 gms.	
Droplets	small		large		small		large	
Day	Total larvae and pupae in 50 sample dips							
1	34		31		43	49	19	65
2	26	39	52	49	28	36	28	43
3	33	24	35	59	39	23	17	37
4 sprayed								
5	12	0	8	3	7	7	3	7
6	22	11	8	0	1	2	0	5
7	5	7	5	1	5	2	0	1
13	6	7	3	3	1	4	1	7
21	6	0	3	0	1	2	2	1
29	1	1	2	0	3	2	0	0
Mean of 2 days after spraying as percentage mean of 3 days before spraying								
	55	17	20	3	11	4	7	12

Owing to a general decrease in larval numbers towards the end of the season it was necessary to work entirely in the reed margin (Positions 1 and 2). In order to increase the accuracy of the method, strips along the canal side of twice the usual length (*i.e.* 40 instead of 20 ft.) were marked out and 40 dips were taken along this length instead of 10 per 20 ft. In order to avoid undue disturbance of the water surface by dipping too closely, 20 dips, approximately 2 ft. apart, were taken along the whole length of the plot at each position. This was then repeated immediately so that each plot was sampled with 80 dips.

In order to avoid undue leakage of the dose from the margins outwards due to spreading of oil, the whole width of the canal was sprayed, although samples were taken only from the reedy margin (Positions 1 and 2).

All areas were sprayed at a delivered dose of 10 g. DDT per acre since it was thought that an effect due to dilution most likely would be seen if there was an incomplete kill.

Area 15 was sprayed with DDT in oil at the original concentration, *i.e.* that used on all other plots previously sprayed at this dose.

Area 19 was sprayed with the same amount of DDT but in ten times as much oil.

Area 17 was sprayed with the same amount of oil as in Area 19 but without DDT (Oil Control).

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Area 18 was left unsprayed as a blank control.

Area 16 was sprayed with AMSO in original dilution.

Consideration of Fig. 13 shows clearly that :—

(*a*) Following spraying there was a similar increase in population in both controls and that oil alone even in ten times the quantity as in the original spray appears to be non-lethal.

(*b*) The rise in the area sprayed with AMSO is not so great as in the control areas and there has probably been a mortality. This is evidently very small, however.

(*c*) Both areas sprayed with oil solution show a very much higher mortality than the AMSO area.

(*d*) Although the difference in mortality in the two areas sprayed with DDT in oil at different dilution is not great and cannot be definitely attributed to dilution of the spray, the smaller quantity of more concentrated solution is, if anything, slightly more effective than the larger quantity of dilute spray. This may be due to a greater loss of oil and DDT because the larger quantity would tend to spread over a wider area than the smaller amount of more highly concentrated spray.

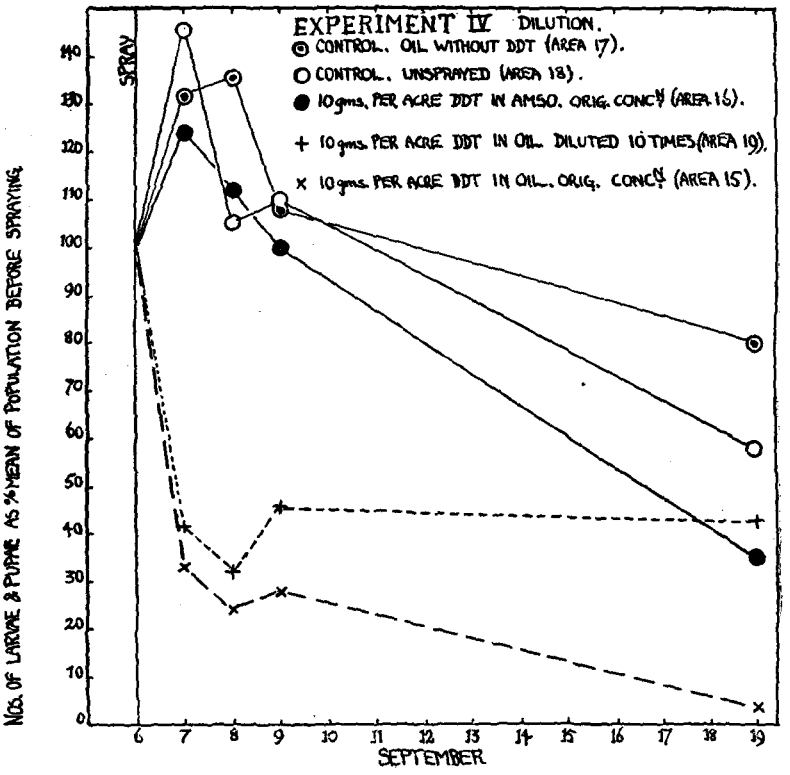

FIG. 13.—Experiment No. 4. The effects of dilution of spray. Population after spraying expressed as a percentage of original population (mean of three days before spraying).

It may be supposed that even if there was not such a loss, much of the DDT in the more dilute spray would be in the upper layers of a thick oil film and therefore fewer molecules would be available to the mosquito larvae coming up beneath.

The susceptibility of the different developmental stages to DDT and its bearing on residual effect.

There is the possibility that DDT affects the larvae by delayed action so that, although they hatch from the egg, they fail to develop beyond the third larval stage.

While it is difficult to investigate this matter with the present data and in view of the general decline of the population, it can be stated that :—

(*a*) at least some 3rd instars and later stages do develop subsequently to DDT treatment.

(*b*) That the first two larval stages are more easily killed than the 3rd and 4th stages and also more easily killed than the pupae.

(*c*) Although it is not known what proportion of later stages do succeed in developing in spite of DDT previously put down it seems unlikely that there is a strong residual effect. For although there is a general decline of the population, 3rd instars and later stages do develop and the population eventually reaches its original composition, though not its original level, about three weeks after treatment. This would be expected if there was no residual effect.

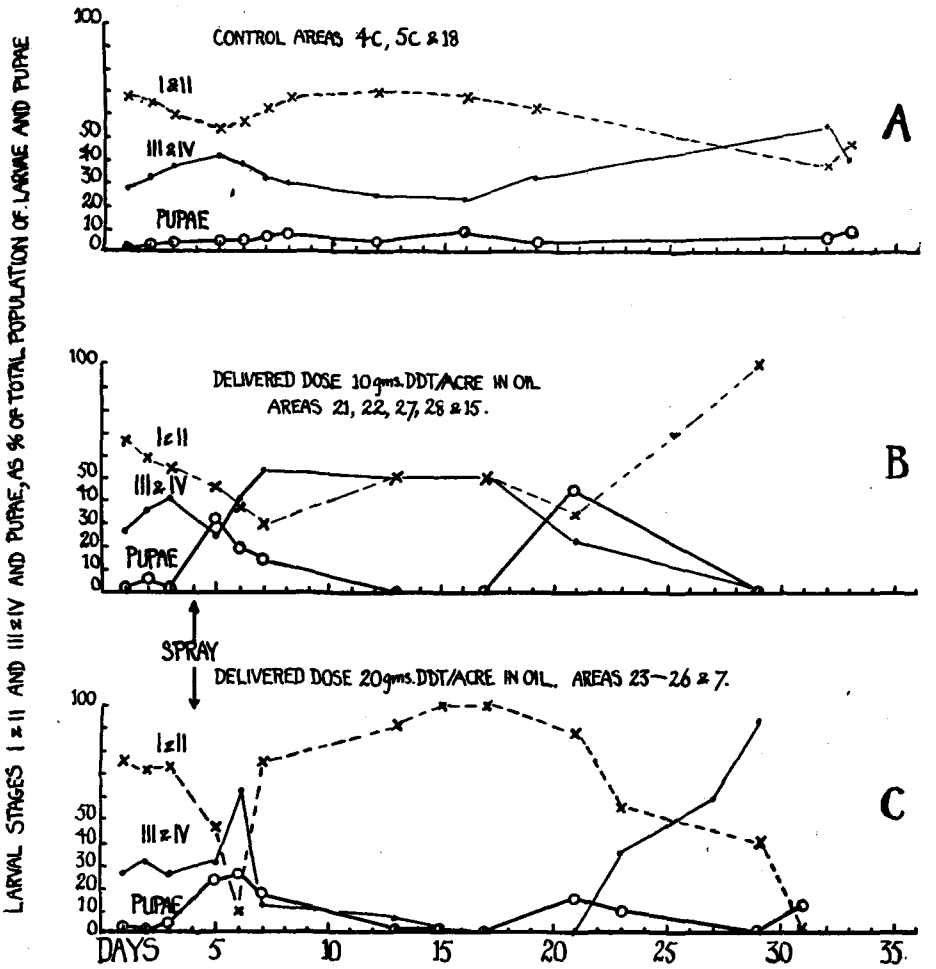

FIG. 14.—Proportion of larval instars (I & II and III & IV) and pupae in the populations before and after spraying.

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Each treated and control area has been analysed in the following manner. The 1st and 2nd instars were grouped, the 3rd and 4th instars grouped and the total, together with the total pupae, were tabulated for each sampling day. The proportion of 1st and 2nd, 3rd and 4th and pupal stages in the total count was calculated and the figures graphed. This gives *not the rise or fall* of the population but the proportion of the different stages in it before and after spraying. Actually the *total* population was usually falling.

Since all areas treated with DDT gave exactly similar graphs, only a few selected ones and graphs of the totals for several similar areas are shown in figs. 14 and 15.

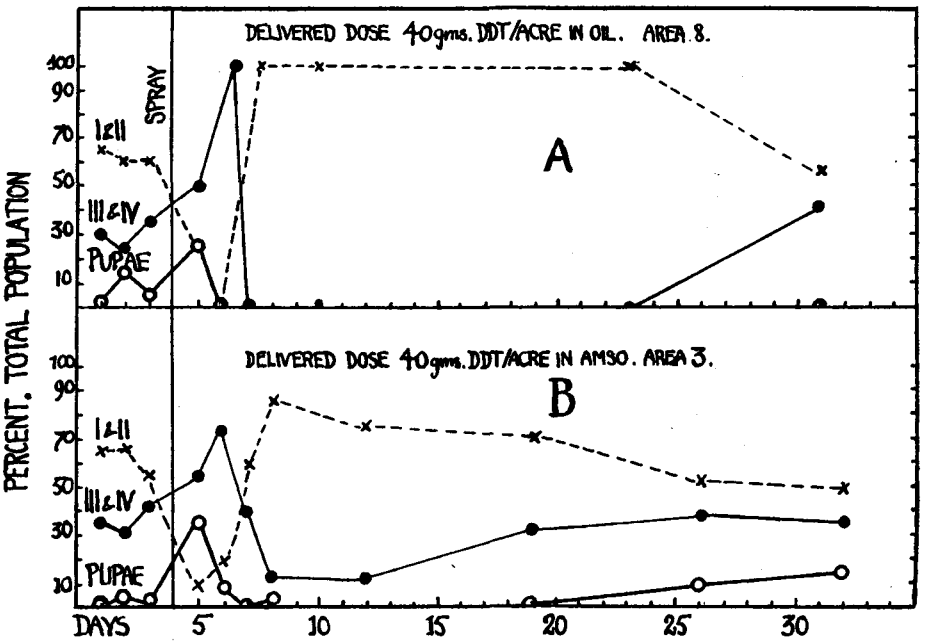

FIG. 15.—Proportions of larval instars and pupae in the populations before and after spraying.

Fig. 14A, where the control (untreated) areas are analysed, shows that the proportion of 1st and 2nd, 3rd and 4th and pupae remain fairly constant over a long period and that the early stages predominate.

When an area is sprayed (fig. 14 C) the first two stages suffer a higher mortality compared with the later larval stages and the pupae, and the proportion of pupae and later instars thus rises sharply. Soon after spraying, however, the proportion of early stages recovers and after about three weeks again declines because the young are developing into the later instars: these later stages show a corresponding increase. This is well seen in fig. 14 C and also in fig. 15. It occurs with DDT either in AMSO or in oil and it happens at all dosages.

It is suggested that if a fairly strong residual effect were present the alteration in proportions of different stages following spraying would be maintained—instead of reverting to the proportions before spraying.

To these data may be added some observations made on the persistence of oil films. It was often seen in the areas which had received a delivered dosage of 10 g. per acre of DDT in oil that the oil persisted for as long as three weeks after spraying.

This was seen particularly close to the bank (Position 1). For example, in Area 22 oil was still evident at the extreme edges of the canal among the reeds seventeen days after spraying. This oil occurred in patches of varying size up to 3–4 in. across, each patch having an irregular but discrete edge. The film therefore did not break into lenses—or if lenses did exist they were exceedingly small and inconspicuous. It was common, moreover, to find larvae of all stages in this position, so that when ladling out a sample both oil and larvae would be collected together. Over the seventeen days subsequent to spraying, 34 larvae and six pupae were collected from Position 1 in Area 22. These larvae must have been in contact with the film at some time during this period yet they were apparently tolerant of it at least after many days. This suggests that the film loses its insecticidal potency although considerable quantities of oil remain. Whether the DDT available to the larvae is reduced by spreading of the film to a point where it is rarely encountered in a sufficient concentration or whether the DDT itself deteriorates is not known.

Discussion.

The objects of the tests were to determine :—

(i) The minimum lethal dose (MLD) of DDT in oil and in AMSO.

(ii) The effects of drop size.

(iii) The effects of sprays of different dilutions in relation to amount of spray delivered.

For ease of discussion some factors can now be eliminated fairly easily. Except for very small dosages (< 10 g. DDT per acre), drop size within the range 0·4 mm. to 1 mm. diameter is of no account under the conditions of these experiments and with the same doses a large number of small drops is no more effective than a small number of large ones in covering the water surface between vegetation. With solutions of DDT in oil the concentration of the DDT in solution appears to be a minor matter as far as larvicidal action is concerned provided the surface dose of DDT is the same.

We are left therefore with a discussion of the MLD of DDT in AMSO and in oil. At the same time we may discuss the assessment of actual surface dosage in relation to the delivered dose.

A consideration of the MLD with oil solution and AMSO, account being taken of the actual surface dosage in relation to the delivered dosage.

It was stated on p. 413 that with small drops the surface dosage was probably about 70 per cent. of the delivered dosage, a certain loss being caused by wind. This estimate was derived from the assessment tests in the 1st and 3rd experiments (pp. 413–414). With large drops there was evidently no loss and delivered and surface dosages are the same. This is shown by the 106 per cent. recovery of delivered dose in the assessment trial (p. 414).

With AMSO the overall mortality curve (*i.e.* at all sampling positions) drops rather sharply at delivered dosages below 40 g. DDT per acre (fig. 7 A). This drop in mortality occurs most in the reed margins (which have greater cover) and least in the unobscured central regions of the canal. It would seem that to increase the delivered dosage above 40 g. per acre would be uneconomical in the use of DDT, while to drop below 40 g. per acre would definitely result in a much lower kill—particularly where there is cover, as in the reed margin. Thus 40 g. per acre may be taken as the most economical delivered dose. This is estimated at 28 g. DDT per acre surface dose, since small drops were used.

It is evident from fig. 7A and B that the tests with oil show a more satisfactory kill than those with AMSO. The overall mortality at a delivered dose 20 g. per acre on the graph (actually 25 g. per acre due to error, see p. 413), is 91 per cent. as com-

pared with 90 per cent. at the 40 g. per acre point on the AMSO graph (fig. 7 A) Moreover the mortality at 10 g. per acre (12·5 g. per acre actual delivered dose) was also 90 per cent.

Thus for an actual *surface dosage* estimated at 9 g. DDT per acre a 90 per cent. kill was obtained in the trials with an oil solution and a 91 per cent. kill with an estimated surface dose of 17·5 g. per acre. It is true that at a delivered dose of 40 g. per acre (50 actual) a 95 per cent. mortality was obtained (25 g. delivered in oil seems to be equivalent to 40 g. in AMSO).

AMSO is definitely inferior to oil where there is reed cover as shown in figs. 7A and B and in fig. 13 where a satisfactory minimum dose is in the neighbourhood of 56 g. per acre, surface dosage (80 g. delivered dosage). It is difficult to say with certainty that this result was not due in some way to a greater amount of reed cover in the AMSO areas. But judging qualitatively, the reed cover seemed to be about the same with all plots in this trial.

The following hypotheses may be advanced tentatively to suggest why, at low area dosages, DDT is more effective when applied in oil solution than when applied in a finely dispersed oil-in-water emulsion :—

(*a*) Some of the fine oil drops of AMSO emulsion penetrate below the water surface, thus distributing some of the DDT through the bulk of water ; solutions of DDT in oil remain on the water surface.

(*b*) The laboratory tests on emulsions, which indicated them to be superior to oils, were carried out on Culicine larvae which live largely below the surface. Since *Anopheles* larvae spend a high proportion of their active lives on the water surface they may be able to pick up a greater dosage of DDT from the localised oil film than can the Culicine larvae.

(*c*) The soap and phenolic components of the emulsifier, AMOA, containing cresols and sulphonated fats may be mild repellents for the larvae, which make them seek only the uncontaminated areas when breathing at the water surface.

From the point of view of larvicidal action, the experiments have not shown that it is an advantage to distribute DDT in high dilution, either in the form of a dilute solution in oil, or in a dilute emulsion. The efficacy of DDT is primarily dependent upon the weight dispersed per unit area rather than on the means of distribution. Drop size and solution concentration should not be increased, however, to the point where the drops are so widely separated that small breeding areas may fail to be " hit ". This limiting condition is unlikely to be approached with petroleum oil solutions of DDT (Saturation concentration c. 5 per cent.) dispersed as aircraft spray. Considerations of oil and transport economy argue the use of concentrated rather than of dilute solutions.

The actual dosage to aim at will depend on the frequency with which spraying is carried out. If, as now seems possible, the residue of spray falling on to water surfaces after anti-adult spraying is used under certain circumstances as a bonus for larvicidal effects and spraying is done at fairly frequent intervals, to aim at 100 per cent. kill of larvae may not be compatible with economy in use of insecticide; a surface dosage of 7–18 g. DDT per acre may be sufficient to produce a periodic and accumulative 90 per cent. larval mortality. This view is supported by the results graphed in figs. 4, 5 and 8 where at the end of the breeding season and with a falling larval population the slightly increased kill at a high dosage may be uneconomical in view of the similarity of the subsequent low population levels at all dosages.

It must be remembered that the minimum lethal dosages discussed here refer to the dose laid down on a very narrow strip of water and that leakage from either side was relatively large, at any rate after the first day (viz. effect on controls in 1st

experiment). It is, of course, probable that all the lethal effect is produced in the first few hours after spraying and that the effect of subsequent leakage by spreading from each edge of the experimental area was negligible. But it would be supposed that on a wide area where leakage was proportionately less the MLD might even be lower than that discussed here.

In all the tests of the present series, the lethal effect of the DDT was noticeably reduced after a few days. This contrasts strongly with laboratory experiments and with known data concerning the chemical stability of DDT. It is possible that leakage of the oil films from the experimental areas may have contributed to the lowering of residual effects, but, nevertheless, the observations reported on p. 426, that both visible oil films and larvae were to be found some days after spraying, strongly suggests that the DDT has been selectively removed from the oil. An absorption of DDT by vegetation is a possible explanation of the effect. Another possible source of loss is via aqueous solution into fatty parts of various organisms in the water.

The minimum lethal dose of DDT as a larvicide has not been defined exactly : but it has been narrowed down to 40 *g*. DDT per acre and below. The amount of DDT to use at one spraying will depend on the frequency of spraying, whether the aim is purely larvicidal or larvicidal and adulticidal. It will also depend on the vegetation and perhaps leakage of the surface film by spreading. These points still require study. The residual lethal effect of persistent oil films appears to be low. It is clear that work is required to determine how long DDT retains its potency as a larvicide on natural water surfaces or as an adulticide to emerging or ovipositing adults coming in contact with such surfaces.

Summary.

In order to assess the potential effectiveness of DDT spray against mosquito larvae living under natural conditions and to determine the characteristics of the most effective form of spray, the variables considered were, area dosage of DDT, oil solutions compared with oil-in-water emulsions, concentration of DDT in the spray liquid, and drop size.

Spraying was carried out over small (20 ft. $\times$ 40 ft.) areas of canal harbouring larvae of *Anopheles maculipennis* var. *messeae*, and *A. claviger*. The canal was densely packed with vegetation. The central channel was overgrown with *Potomogeton natans*, so dense that in some regions there was more area of leaf than of open water surface, and in places there were large masses of floating algae (a coarse species of *Spirogyra*). At the edge of the canal near the bank there was a margin of reeds averaging about 4 ft. wide, and from 1 to 4 ft. in height.

Spraying was carried out with a specially designed spinning disc atomiser giving drops of nearly uniform size and enabling drop-size and dosage delivered to be independently varied over a wide range. The range of DDT dosages investigated was from 4 g./acre to 80 g./acre, delivered in solutions or emulsions of approximately 0·5 per cent. and 5 per cent. concentration and drop sizes of 0·4 and 1 mm. diameter. Assessment of surface dosage in relation to delivered dosage, by spraying dyed solutions, showed that about 30 per cent. of the smaller drops were lost in the wind, but that there was no appreciable loss of the coarser spray.

For 5 per cent. oil solutions of DDT drop size in the range 0·4 to 1 mm. diameter had no effect on the kill, except at low dosages ($<$ 10 g. DDT/acre) when the greater wind loss of small drops produced variable results. Tests on the effect of drop size were not carried out with emulsions.

From the point of view of larvicidal action, the experiments have not shown that it is advantageous to distribute DDT in high dilution. 4·3 per cent. DDT oil solution sprayed at 10 g. DDT/acre actually gave a slightly higher mortality than

0·43 per cent. solution at the same dosage area of DDT, but the difference may not be significant owing to uncontrollable variables. Under the conditions of these trials, both oil solutions were superior to 0·43 per cent. AMSO emulsions. Experiments on the effect of concentration at constant area dosage were made only in the reed margin of the canal and data were not obtained for open water.

In the centre of the canal, mortalities greater than 90 per cent. were obtained for both oil solutions and AMSO emulsions at delivered dosages greater than 10 g. DDT/acre, the oil solution being slightly superior. In the reed margin the kill dropped sharply with AMSO emulsions at dosages below 40 g. DDT/acre, the fall-off was much less marked with oil solution. From considerations of the average mortality in all areas, a delivered dosage of 40 g. DDT/acre (small drops) or estimated surface dosage of approximately 30 g. DDT/acre, appears the optimum for economical usage of DDT.

When areas were sprayed the first two larval stages suffered a higher mortality compared with the later larval stages and pupae, and the proportion of pupae and later instars thus rose sharply.

There was no evidence that residual lethal effects of oil films extended beyond three days. The initial kill in these trials was followed by a very low population level, due, it is thought, to a natural decline in the reproductive potential of the population.

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