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Selected Insecticides in the Control of Invertebrates Possibly Associated With Avian Botulism

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SELECTED INSECTICIDES IN THE CONTROL OF INVERTEBRATES POSSIBLY ASSOCIATED WITH AVIAN BOTULISM

by

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J. Larry Haddock

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INTRODUCTION

Purpose and Scope

Avian botulism or "western duck sickness" has long been a major problem on duck marshes in the western part of the United States. This has been demonstrated to result from the ingestion of products of metabolism of bacterial cells of Clostridium botulinum type C. These products when ingested by waterfowl act as neurotoxins (Coburn, 1942). Studies have shown that many animal tissues are readily utilized as culture media by Cl. botulinum type C (Bell et al., 1955).

Since 1955 investigations have indicated a close relation between the occurrence of avian botulism and invertebrate population levels. It is thought that when invertebrates reach peak numbers during their most favorable reproductive season, a large die-off soon follows. Large numbers of birds show symptoms of avian botulism approximately one week after this die-off begins. The theory is that invertebrate carcasses provide a suitable medium for the rapid multiplication of Cl. botulinum, and hence, the development of high concentrations of toxin. These toxin-laden carcasses are readily ingested by waterfowl which succumb to the neurotoxins (Jensen and Allen, 1960).

The prime objective of this study was to find some means of preventing invertebrates from attaining high peak numbers during their most favorable reproductive season and thus possibly reduce botulism among waterfowl. Complete kill of the invertebrate population appeared to be undesirable for two reasons: (1) too large a kill would reduce waterfowl

below desirable levels; and (2) excessive kill would increase the number of decomposing carcasses and favor the outbreak of botulism. It was theorized that the kill should be between 30 and 60 percent of the invertebrates present prior to the time when peak numbers were reached.

Only those insecticides which have shown little toxicity to wildlife were used in this experiment. Attempts were made to estimate the value of LD 50 for each of the insecticides used in this study. LD 50 is the dosage causing 50 percent mortality in a test population and is considered the most reliable of the LD figures (Rudd and Genelly, 1956).

The specific objectives of this project were: (1) to evaluate the relative effectiveness of selected insecticides, (2) to determine the selectivity of the insecticides on several invertebrate groups, and (3) to determine the treatment most effective in reducing numbers of specific invertebrate groups.

Study Areas

The study was conducted at Bear River Migratory Bird Refuge, which is located in Northern Utah about 16 miles west of Brigham City, Utah. There are approximately 25,000 surface acres of Bear River water impounded on the Refuge. This water is divided by a system of dikes into five units of about 5,000 surface acres of water each (Kalmbach and Gunderson, 1934).

The Bear River delta area was one of the first in which botulism was reported and since has been one of the most consistent and spectacular in outbreaks of this disease.

Preliminary field investigations were conducted during 1960 in Unit 2. The full-scale investigations conducted during 1961 were run in

Unit 4 in an area where concentrations of sick and dead ducks had been found regularly in previous epizootics (Figure 1).

Botulism outbreaks rarely, if ever, affect birds on all parts of a marsh with equal severity. There are areas within each unit at Bear River Refuge where concentrations of sick and dead birds are found quite regularly and others which seldom show any evidence of the disease. Since data obtained by this investigation will be used in working out a possible control of invertebrates within areas where sick and dead birds are normally found, one of these areas was chosen for testing in order to obtain information of greatest value in future control efforts. The area chosen showed evidence of a botulism epizootic while the tests were being conducted.

The specific site of the preliminary field tests was selected mainly for convenience. It was the area closest to the laboratory and road which was large enough for experimental purposes and contained the right kinds of invertebrates. The location was a shallow channel with almost imperceptible water movement. The submerged vegetation was nearly all sago pondweed (Potamogeton pectinatus) and the banks were lined with alkali bullrush (Scirpus paludosus) and saltgrass (Distichlis stricta).

The full-scale investigations in Unit 4 were conducted in an open water area at least 200 yards from shore and any emergent vegetation. The submerged vegetation was mainly sago pondweed. This area was in slowly moving water about 6 inches in depth that was spreading out into the main impoundment from one of the larger channel openings (Figure 1).

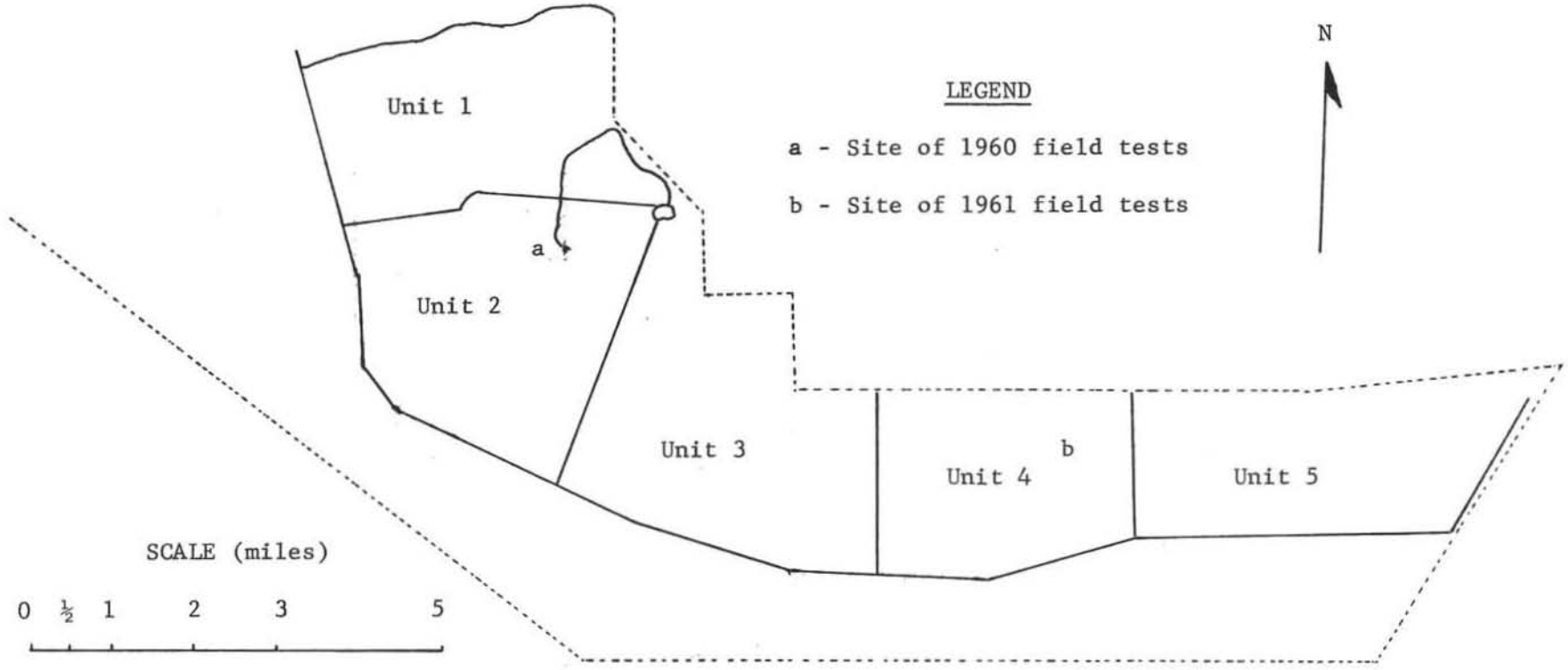

Figure 1. General map of Bear River Migratory Bird Refuge in northern Utah and location of the study sites used in this investigation

REVIEW OF LITERATURE

General

Botulism

The epizoology of wildlife diseases has never been a simple field of research. The history of the study of avian botulism shows that this investigation has been unusually complex. Since 1910 when "western duck sickness" became generally recognized as a serious menace to aquatic birds in the United States, 20 years elapsed before the first articles were published which presented conclusive evidence that the disease was a form of botulism. Although investigations have left no doubt that the disease in birds resulted from the ingestion of the toxin of Cl. botulinum type C, the substrates utilized by the bacterium for growth and elaboration of toxin under natural conditions are not known with certainty even today--32 years later (Jensen and Allen, 1960).

The idea that the tissues of invertebrate animals (such as are commonly found in epizootic areas) might satisfy the nutritional requirements of Cl. botulinum type C is not new. Kalmbach and Gunderson (1934, pp. 42-43) state:

> During the season of 1931 the many least and western sandpipers that perished at Tule Lake were feeding extensively on larvae and pupae of hydrophilid beetles in cocoons formed in the masses of algae on which these diminutive shore birds walked in search of food. The findings of the remains of many of these larvae in the stomachs of the dead, coupled with the demonstration of Clostridium botulinum, type C, in some of the dead larvae strongly suggests the particular medium through which these birds, and probably a few other species, obtained a lethal dose of toxin.

Until recently almost all reports concerning botulism seemed to

support a theory that has been called the "sludge-bed hypothesis." This is that: (1) Clostridium botulinum type C exists in the mud of lakes, marshes or flooded fields, (2) sometimes large quantities of decaying organic matter are made available to the bacteria, and (3) aerobic bacteria use up what oxygen there is after which the anaerobic clostridia propagate and secrete toxin. The temperature, pH and dissolved salts in the water are considered important corollary factors in this hypothesis (Bell et al., 1955).

A recent study by three bacteriologists at Bear River Refuge brought forth substantial evidence that a new theory, which they called the "microenvironment concept," should be given serious consideration. This concept states that: (1) Cl. botulinum type C germinates, reproduces, and synthesizes its toxin in small discrete particulate substances, possibly invertebrate carcasses, (2) these particulate substances are in no wise dependent upon the medium for nurture of the bacteria, but contain all of the requisites within them, and (3) the toxin is probably in the bacteria which reside in the particulate materials, rather than in the form of soluable, freely diffused toxin. Their experiments showed that beef liver and insect carcasses proved to be excellent media to cultivate Cl. botulinum, while no vegetable media gave positive results for them (Bell et al., 1955).

The latest investigation has been carried on at Bear River Refuge for the past seven years with the ultimate aim of identifying the natural substrates utilized by the bacterium. By establishing or disproving a relationship between aquatic invertebrates and avian botulism, a big step forward could be made toward solving the main problem of the epizoology of the disease.

This was attempted by sampling the water and bottom mud in areas that have a history of botulism and those without. These samples were examined both for numbers and kinds of invertebrates through the botulism season, and these figures were then compared to the time of major outbreaks of avian botulism. In this experiment the outbreaks of botulism seem to correlate with a sharp decrease in invertebrate numbers after the invertebrates have reached a peak. This decrease would make invertebrate carcasses available to the bacteria for toxin production and also make them more available to the birds which seem to take dead invertebrates quite readily. This study is still in progress; therefore, final evaluation of the data cannot be made until the work is completed. It appears that the evidence is strong enough to continue the investigation until definite conclusions can be reached (Jensen and Allen, 1960).

It has been shown that botulism is not the result of an infection in which bacteria invade the tissues of the host organism. Instead, it involves intoxication resulting from the absorption of products of metabolism of bacterial cells (Cl. botulinum type C). The toxin of Cl. botulinum type A--and presumably that of type C also--is a protein. It is further thought to be a particular type of protein termed a globulin. The molecular weight of this complex compound has been calculated to be between 1,000,000 and 2,000,000. The molecular weight of a simple molecule of water by comparison is 18 (Lamanna et al., 1946).

The bacteria which form this toxin are saprophytes, anaerobes, and spore-formers. As such, they apparently are almost everywhere (Kalmbach and Gunderson, 1934). They have been found in every "duck-sickness" location where an effective search has been made, as well as in other locations in the western United States where outbreaks of avian botulism

have not occurred to our knowledge. The microscopic spores formed by these bacteria have such resistance to climatic conditions that long periods of the most adverse weather do not destroy them. The spores are resistant to boiling for hours. If favorable conditions for growth are provided, a good population of vegetative forms of the bacteria can be established quite rapidly. The toxin produced by these bacteria consists of two fractions. One is toxic when taken orally and the other is toxic when injected into the peritoneal cavity. Both fractions are rapidly-acting neurotoxins (Coburn, 1942).

Several studies of the stomach contents of sick and dead birds have revealed little information. The birds stop feeding when they become sick. Generally, food eaten by birds is completely digested within 18 hours. However, small amounts of material have been found in sick birds which have been identified as invertebrate carcasses. Support for this concept was provided by laboratory experiments in which the instars of two orders of insects suspended in distilled water supplied all of the requirements of the bacterium for growth and toxin production. The experiments showed that enough toxin could be produced in one gram of insect tissue in one day to kill a duck (Bell et al., 1955).

Insecticides

In choosing insecticides to test as possible means of control for the invertebrates in question (principally Tendipedidae), it was thought advisable to use only chemicals which have shown little toxicity to wildlife in past usage. In keeping with this idea, the publication, Pesticides: Their Use and Toxicity in Relation to Wildlife (Rudd and Genelly, 1956), was used as a guide in determining those chemicals which should be relatively harmless to wildlife in moderate amounts. In no case can it be assumed that any material is completely safe to use under all conditions.

Theoretically, the organic phosphate insecticides should be the safest with respect to wildlife loss as they hydrolyze in water after a relatively short time to harmless products (Carlson, 1961). There are, however, some hazards from dermal contact or respiratory exposure in dealing with some of these agents. Malathion is considered as the safest material in this group (Davis, 1958), and was chosen for testing. Malathion passes through the insect cuticle readily and thus acts as a contact poison (Metcalf, 1955).

A number of the chlorinated hydrocarbon insecticides have shown bad effects on wildlife. TDE and DDT have been two of the least harmful chemicals in this respect. TDE (DDD) is probably the safest of this group (Davis, 1958), but was not readily available and so was not tested. DDT is a contact poison that enters through the cuticle and works best in cooler temperatures. DDT poisoning in insects causes a marked increase in oxygen consumption (Metcalf, 1955).

According to Shepard (1951) the effectiveness of DDT depends often upon the type of formulation employed. Rudd and Genelly (1956, p. 5) state:

> The early development of DDT was accompanied by spectacular claims for its insecticidal efficiency. No pesticide before or since has been given such thorough testing or such widespread publicity. Soon after its announcement, studies were initiated to determine the effects of DDT on the natural fauna. At the conclusion of these and other studies it became possible to appraise both the efficiency and the hazards of DDT. Considered in retrospect, it is clear that DDT is indeed an unusually effective insecticide whose use involves some risk. However, neither the sensational claims made for its insecticidal effectiveness nor the dire consequences predicted for our native fauna, should it be used, seem now to be correct.

Cottam and Higgins (1946) reported that one pound of DDT per acre has little or no effect on bird or mammal populations. Application of more than two pounds per acre gave more unfavorable results.

Rotenone (derris), though deadly to fish, has been shown to have little effect on warm-blooded animals. It can act either as a stomach or contact poison. The time it takes to kill insects is reduced as the temperature increases, even though it deteriorates more rapidly with heat and/or sunlight (Metcalf, 1955).

Literature Specific to this Problem

General Biology of Midges

Most of the female midges apparently lay their eggs while resting on the surface of the water, as was observed by Fellton (1940) for Procladius sp. and Tendipes tenuicaudatus. The egg mass is somewhat denser than water and sinks to the bottom. Some of the gelatinous masses are attached to vegetation, rocks, etc., by means of an involuted stalk-like process. They are found at or just below the surface of the water. Jamnback (1954) found an average of 200 eggs per mass for Tendipes decorus while Fellton (1940), in counting undifferentiated egg masses, found them to average 1768 eggs per mass. These egg masses were cylindrical, spherical or irregular depending on the species involved and from 3 to 5 millimeters in diameter. Jamnback (1954) and Fellton (1940) found that the eggs hatched 2 to 3 days after being laid.

The color of the larvae is variable according to the species involved. Some are white (colorless) or yellow, others are greenish, while still others are dark red (commonly called "bloodworms") or red mottled with green (Needham et al., 1905). The larvae at Bear River Refuge are almost all the white or dark red colored groups with an occasional larvae of a different color.

The larvae of the midges found at Bear River Refuge do not have to come to the surface to breathe, but obtain their oxygen from the water

by gills usually found in the anal opening. These larvae construct tubes or burrows in the mud within which they generally spend almost all of their larval life. The larvae generally obtain their food by means of an induced flow of water through their tubes. This current of water, which is caused by the rhythmic undulatory movements of the larvae within their tubes, is probably of primary importance in supplying the oxygen requirements of these animals. The larvae emerge, periodically, from their tubes and eat any food encountered. One end of the body is kept well anchored in the tube when feeding in this manner (Fellton, 1940).

The duration of the larval stage is apparently quite variable. Tests by Fellton (1940) indicate that the availability of food can strongly affect the duration of this stage. He was able to postpone pupation for five months by reducing the food available to the larvae. By comparison he found that the duration of the life cycle, adult to adult, of four species of Tendipeds was 21 to 30 days in midsummer.

Jamnback (1954) and Fellton (1940) found the pupal stage of the tendipedids to be quite short. In most cases it did not exceed 24 hours in duration. They were also in close agreement on the life span of the adult stage. The life span of adults in the laboratory varied from 2 to 9 days. It was not determined whether there was any difference between those figures and the life span of adults under natural conditions.

Sadler (1934), Jamnback (1954), and Fellton (1940) noted a positive correlation between the degree of water pollution and the numbers of midge larvae. They found that breeding of the midges was apparently favored by sewage and/or organic pollution. If a pond was treated with fertilizer, midges would be attracted to it in a day or so and deposit enormous numbers of eggs. Needham et al. (1905) found that Chironomus

larvae feed on dead leaves and other vegetable refuse.

It was found by Jamnback (1954) that 93 percent of the larvae of Tendipes decorus were in the top 2 inches of mud. None were found deeper than 3 inches.

Control

Tendipedids have been controlled in other areas of the United States and the world, but the conditions were sufficiently different so that this previous work could not be applied directly to the Bear River Refuge problem. Most of these treatments involved rivers, tidal creeks and bays, or deeper lakes, whereas, Bear River Refuge is a shallow, fresh water area with little water movement. The ultimate aim of past studies has been complete eradication of the midge population. In this problem only a partial kill was sought.

Fellton (1940, 1941) reported successful control of midge larvae, especially Chironomus sp., in 1939 using 6 p.p.m. and 10 p.p.m. rotenone in two small lakes in New York aggregating about 150 acres. In conjunction with the application of the rotenone powder mixed with lake water, copper sulfate was dissolved in the lakes at the rate of 1.0 p.p.m. to kill most of the algae which served as a source of food and shelter for the larvae, and also to increase the potency of the rotenone by reducing the alkalinity of the lake water. The following year chlorinated benezenes were applied in five treatments at intervals of about one month. Orthodichlorbenzene was used in the first three treatments at the rate of 275, 242 and 231 pounds per acre, while in the last two treatments trichlorbenzene was applied at the rate of 192 pounds per acre. These materials were distributed by subsurface application from a motor boat.

The report of Lindquist et al. (1951) on control of the Clear Lake

gnat, Chaoborus astictopus, is worthy of mention, even though it is a culicid rather than a tendipedid. A total of about 14,000 gallons of TDE emulsion was applied by boat to the 41,600 acre lake in California. This gave a concentration of about 0.014 p.p.m. Good dispersion was obtained due to wind action and water currents. Chaoborus larvae were completely eliminated. This was probably due to their habit of nocturnal migration into upper water which takes them out of the mud where they are almost impregnable. There was no effect on oligochaetes and, though reduced in numbers, many tendipedid larvae survived. At least some of the insecticide settled to the bottom as shown by colorimetric analysis of mud samples taken 28 days after treating. The top layer of mud showed 0.61 p.p.m. TDE while there was 0.15 p.p.m. found in a deeper layer.

Jamnback (1954) dealt with the control of Tendipes decorus. He found that airplane applications of heptachlor and TDE emulsions, applied at rates of 0.2 and 0.5 pound per acre respectively, were not effective. An airplane application of 10 percent granular TDE applied at the rate of 1.0 pound per acre gave good results which lasted at least 31 days. DDT, heptachlor, and dieldrin emulsions were all very effective in small scale tests, with 0.12, 0.05, and 0.03 pound per acre, respectively, giving good control. In these same small scale tests TDE-coated granules and TDE emulsion showed promise at dosages of 0.8 and 0.3 pound per acre respectively.

In a report on the control of colorless tendipedids in the water supply of a city, Flentje (1945) mentions that copper sulfate and chlorine had proved ineffective. He reported successful treatment of 165 million gallons of water using 25 percent DDT emulsion applied at the rate of 0.01 p.p.m.

The use of DDT in the control of midge larvae has been reported by Eide et al. (1945), and Brown et al. (1961). Eide found that in 4 inches of water, an airplane application of 2 quarts per acre of 5 percent DDT emulsion caused tendipedids to die in 3 to 4 hours. By using airplane application, Brown et al. (1961) reported their best results with TDE. DDT was found to have little effect in their control efforts along the Blue Nile.

At the time of this writing, a study is underway (Carlson, 1961) to obtain chemical control of mayflies and caddisflies in the Mississippi River. The use of organophosphates, particularly malathion, is being studied. This information, when obtained, may be useful in the continuation of this problem.

METHODS AND PROCEDURE

Laboratory

The purpose of the laboratory experiments was to test the effectiveness of several forms and kinds of insecticides in the control of invertebrates easily and cheaply in order to eliminate those which proved ineffective before the more time-consuming field testing stage was reached.

Preliminary studies were carried out in shallow, white enameled pans (9" x 13" x 2") within the laboratory. In all tests controls were used and temperatures recorded. The influence of malathion and DDT on the control of dipterous larvae (chiefly Tendipedidae), oligochaetes, Corizidae and Notonectidae was observed. Most of the experiments were conducted using DDT and malathion in emulsion form. Other studies were made using DDT as a wettable powder, granular toxaphene, pyrethrum emulsion, dibrom 8 emulsive and rotenone powder.

Distilled water was the first medium used, as it was thought that results could be easily duplicated. One liter of water was placed in each pan. A specific number of invertebrates were picked at random from a population and evenly spaced throughout each pan. A base solution of the insecticide to be tested was prepared at a 1:1,000 dilution (one part insecticide to 1,000 parts distilled water). Each pan received the amount of this base solution needed to obtain a previously specified p.p.m. concentration of the insecticide. Each pan, including the control, was stirred to evenly distribute the insecticide. The numbers of invertebrates dead after various intervals were recorded until all were dead or 24 hours had lapsed.

Contrary to expectations there was little consistency in duplicate tests. Therefore, another test was devised. The same procedure as outlined above was followed using 1 liter of river water, such as flows into the impoundments, as a medium. This gave better results than the distilled water tests, but still the desired degree of consistency was not obtained. An additional test was devised with the idea that natural conditions might be better than the unnatural water medium by itself.

To obtain as natural conditions as possible within the laboratory, the same general procedure as outlined above was followed using river water and mud from the impoundments known to contain the invertebrates. A large amount of mud was mixed thoroughly by hand in a metal pan to approach homogeneity. A sample 4" x 4" x 2" was taken, placed in each pan, and smoothed out by hand. A liter of river water poured gently on top of the mud. Controls were used in each experiment. The treated pans were allowed to stand for 24 hours, after which the combination of mud and water was placed in a Tyler Standard 42 mesh Sieve. A gentle stream of running tap water washed the soil particles through the screen, leaving only the solid debris and the living and dead invertebrates. This material was then washed out of the screen into a shallow white enameled pan to be counted. The number alive and dead of each kind were counted and a percent kill calculated. With this test it was possible to duplicate experiments with a high degree of consistency. This test then became the basis for further laboratory experiments.

Field

Preliminary experiments

An attempt was made in the field experiments to keep conditions as natural as possible. The experiments conducted in 1960 were not designed for statistical analysis, but were set up on fairly sound design principles in accord with equipment already on hand. Three galvanized steel rings 18 inches high and 7 feet 6 inches in diameter (Figure 2) were placed in the water and pushed about 6 inches into the mud sealing off that portion of the environment enclosed by them. This prevented contamination or dilution within the rings (unless carried in by wind or animals). The insecticides and invertebrates were effectively contained within the plots.

The rings were placed in position and given 24 hours to stabilize before being treated. Ten points were picked at random in each ring. The water depth at each point was measured and the 10 averaged to calculate the volume of water contained within each ring. Water and mud samples were taken and the invertebrates were counted before and after each 24 hour test period. The water samples consisted of 10 liters taken at random in five 2-liter samples within each ring. The water was strained through a small plankton net and the invertebrates transferred to a small jar for transporting to the laboratory for counting. Two mud samples were taken in each ring. Each sample consisted of .01 m^2 of mud surface two inches deep (10 cm x 10 cm x 2 in.). These samples were taken with a mud sampler with a sliding metal bottom designed and used by the Bear River Research Station (Figure 4). The mud was transferred to plastic containers and transported to the laboratory for washing and counting of the invertebrates by the sieve and pan method already described. The

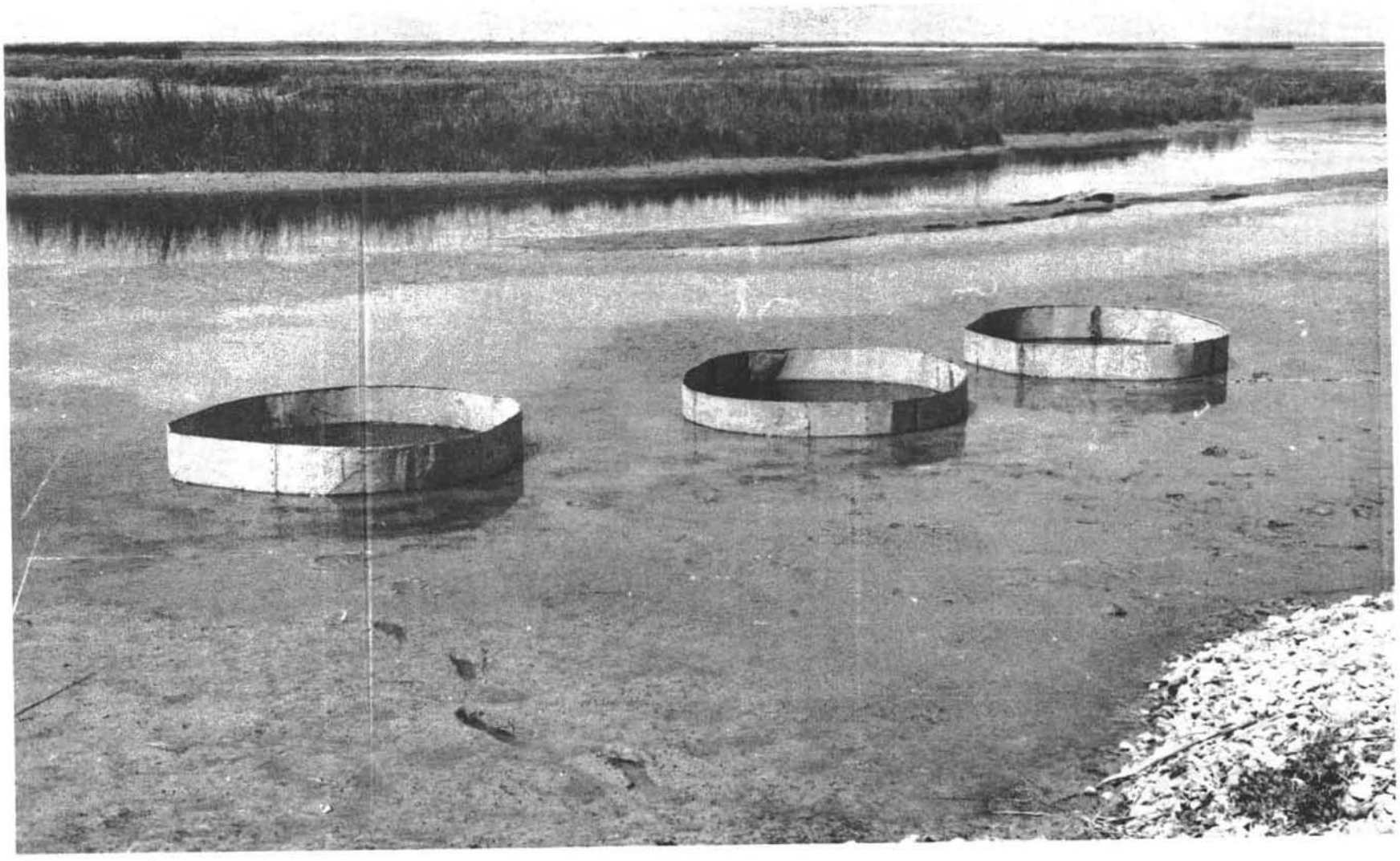

Figure 2. Galvanized steel rings used in preliminary field experiments on the effects of insecticides on midge larvae, Bear River Refuge, Utah, 1960

water samples were placed in petri dishes and the invertebrates counted under a dissecting microscope. A maximum-minimum thermometer was placed in the water within one ring for each test; pH readings were taken before and after each treatment. Each test consisted of one control ring with no insecticide and two rings with different concentrations of the same chemical. The chemical and concentrations used were determined by the laboratory tests. Three tests, of both malathion and DDT, and one using the two in combination, were conducted. Numbers of living and dead were recorded 24 hours after application and percent kill calculated.

Principal experiments

Four experiments were designed for statistical analysis, each being set up in a randomized block design and conducted within an area which appeared homogeneous with respect to type and amount of aquatic plants, soil composition and water depth. The treatments were randomized within each block. The assumption was made that a replication (or block) was a space in which the invertebrate population had a homogeneous distribution within a block but not between blocks. A plot was a homogeneous unit within a block.

In these experiments polyethylene rings were used to enclose each plot. Each ring was 18 inches high and 4.08 feet in diameter covering an area of .0003 acre. Each block consisted of one control ring and two rings with different concentrations of the chemical to be tested. It was not feasible to count all the invertebrates within a ring; therefore, samples were taken.

The rings were set up in a three by three manner (Figure 3) and allowed to stabilize for 24 hours before treatment. Four water-depth measurements were taken and averaged within each ring for volume

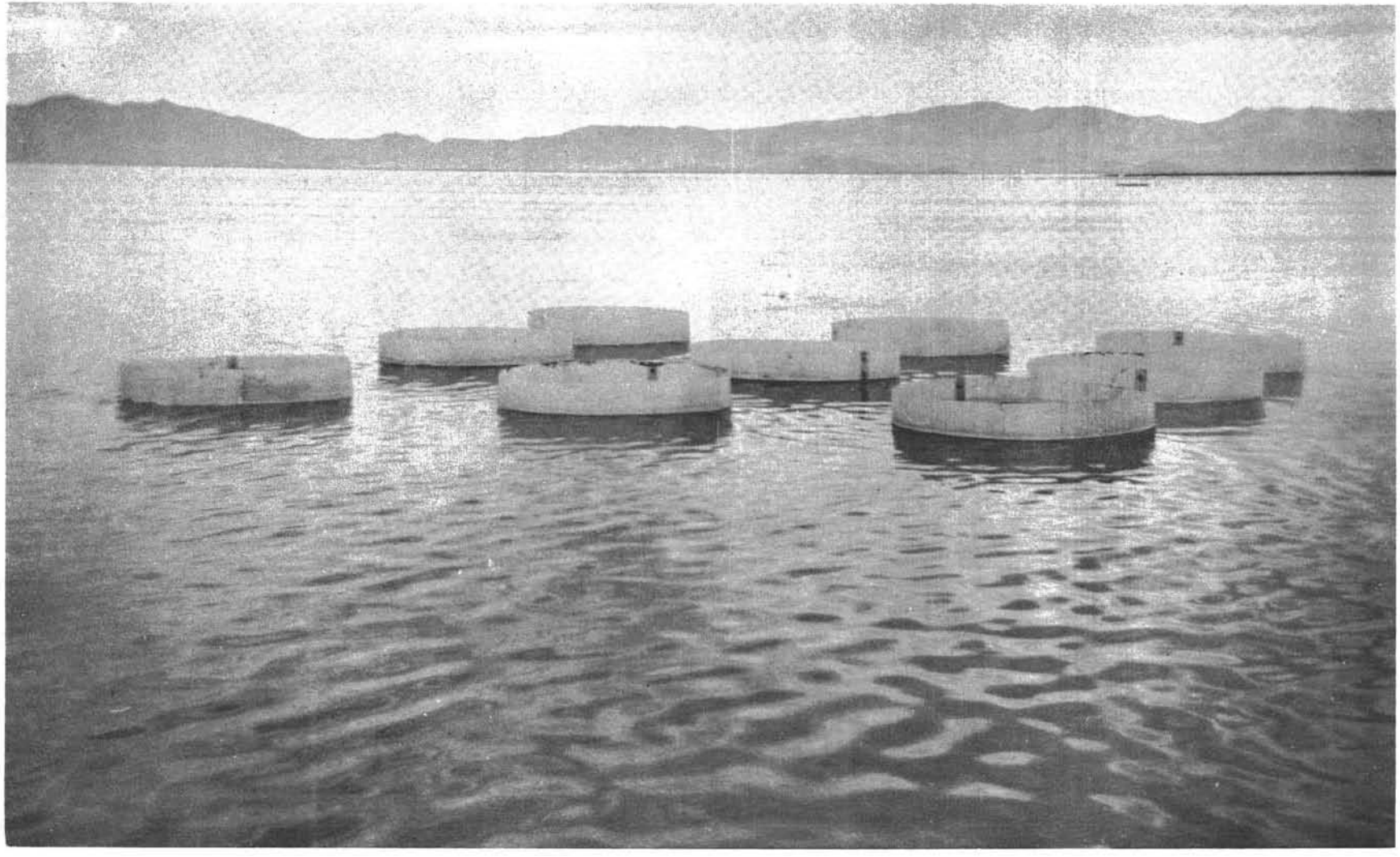

Figure 3. Polyethylene rings used for insecticide testing, Bear River Refuge, Utah, 1961

calculations. After treating, the rings were left for 24 hours before sampling. They were sampled again after 48 hours and 72 hours had lapsed from the time of treatment. This allowed an evaluation of the lasting effects and the type of kill curve obtained (linear or quadratic).

Samples of the water fauna such as corixids and notonectids, were not taken as there were not enough animals in each ring to obtain a good sample. Samples taken in the previous field tests had shown inconclusive results. The main reason for not sampling water fauna, however, was that the water animal population had not shown a good relation to botulism outbreaks, while the macro-bottom fauna had shown evidence of correlation (Jensen and Allen, 1960).

Many species of invertebrates were found in the mud samples, but the predominant groups were the dipterous larvae (chiefly Tendipedidae and oligochaetes). These groups are the ones which appear to show a relationship to botulism outbreaks (Jensen and Allen, 1960). Because oligochaetes showed a remarkable resistance to insecticides in the laboratory tests and there was no observable kill of them in the preliminary field tests, the main experiments were narrowed down to include only the Tendipedidae.

Before sampling the mud in the rings, a table of random numbers was consulted. A number between 0 and 360 was obtained for each ring. This number would correspond to a line through the center of each ring representing one of the 360 degrees in a circle. This was called the base line and was determined in the field by the use of a compass. In the case of the control rings (and treated rings in the first malathion

experiment[1]), where only two samples were taken each sampling period, 90 degrees was added to the random number to obtain the second sampling line. The treated rings were sampled three times for each period. To obtain the second and third sampling lines, 60 and 120 degrees respectively were added to the base number. Since the base line was chosen at random, each additional line was also randomly chosen, being a constant added to a random.

For each sampling line there were ten possible sampling sites. Each site was assigned a number and a table of random numbers was again consulted to obtain the three specific sampling sites on each line. The first number chosen for each line was sampled after 24 hours. The second and third numbers obtained were sampled after 48 and 72 hours respectively. As each possible sampling site was selected and sampled, it was removed from the possible choices for the second or third sampling periods. Nothing could be discovered by sampling a site twice, as about 90 percent of the invertebrates and all of the top 2 inches of mud and organic matter would have been removed in the first sample.

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[1]The first malathion experiment (30 percent plant cover) was used as a learning process for techniques and time required for each step. All rings were sampled twice for each time period. After observing the variation between samples taken from the same ring during the same sampling period, it was thought advisable in future tests to take more samples where possible. Thus, a more accurate mean value could be obtained. The time required to wash and count these additional samples was a big factor.

It was decided to continue taking two samples from the control rings and to take three samples from each treated ring. The control ring counts, being high, would be affected little percentage-wise, by the variation, but the low counts from the treated rings could have a large percentage difference even though the actual number difference would be slight. It was also possible to count the few live invertebrates in the samples from treated rings more rapidly than the large numbers found in control samples.

The mud samples were taken in a sampler (10 centimeters square, 2 inches deep) with a sliding metal bottom as previously described (Figure 4). This sliding bottom prevented loss of the sample which was placed immediately in a 32 mesh Tyler Sieve (8 inches in diameter, 2 inches deep) (Figure 4). Another sieve was placed over the sample, completely enclosing it, and the combination thus formed was then moved rapidly a number of times through the water to remove the soil particles. After washing the samples, the sieves were separated and the organic matter and invertebrates were removed by use of a pair of forceps and an aspirator bottle designed for this purpose (Figure 4). This material was then placed in a small bottle along with some fresh clear water to keep the invertebrates alive until they were counted. After all samples (24) had been taken for a particular time period, they were transported to the laboratory. There they were transferred to shallow, white enameled pans for counting and classification.

In counting the Tendipedidae larvae, distinction was made according to their color. Red larvae and white or yellowish colored animals were easily distinguished. The two groups, thus formed superficially, appeared to react to the insecticides as if they were correctly grouped. Sometimes there were marked differences between the reactions of the two groups. The red larvae are almost entirely from the sub-family Tendipedinae and genus Tendipes. The white group comes from two sub-families, Pelopiinae and Hydrobaeninae. In Pelopiinae the main genera represented are Pentaneura and Coelatanypus, with an occasional specimen from two other genera. The genus Cricotopus represents Hydrobaeninae. Classification follows Usinger (1956) and Pennak (1953).

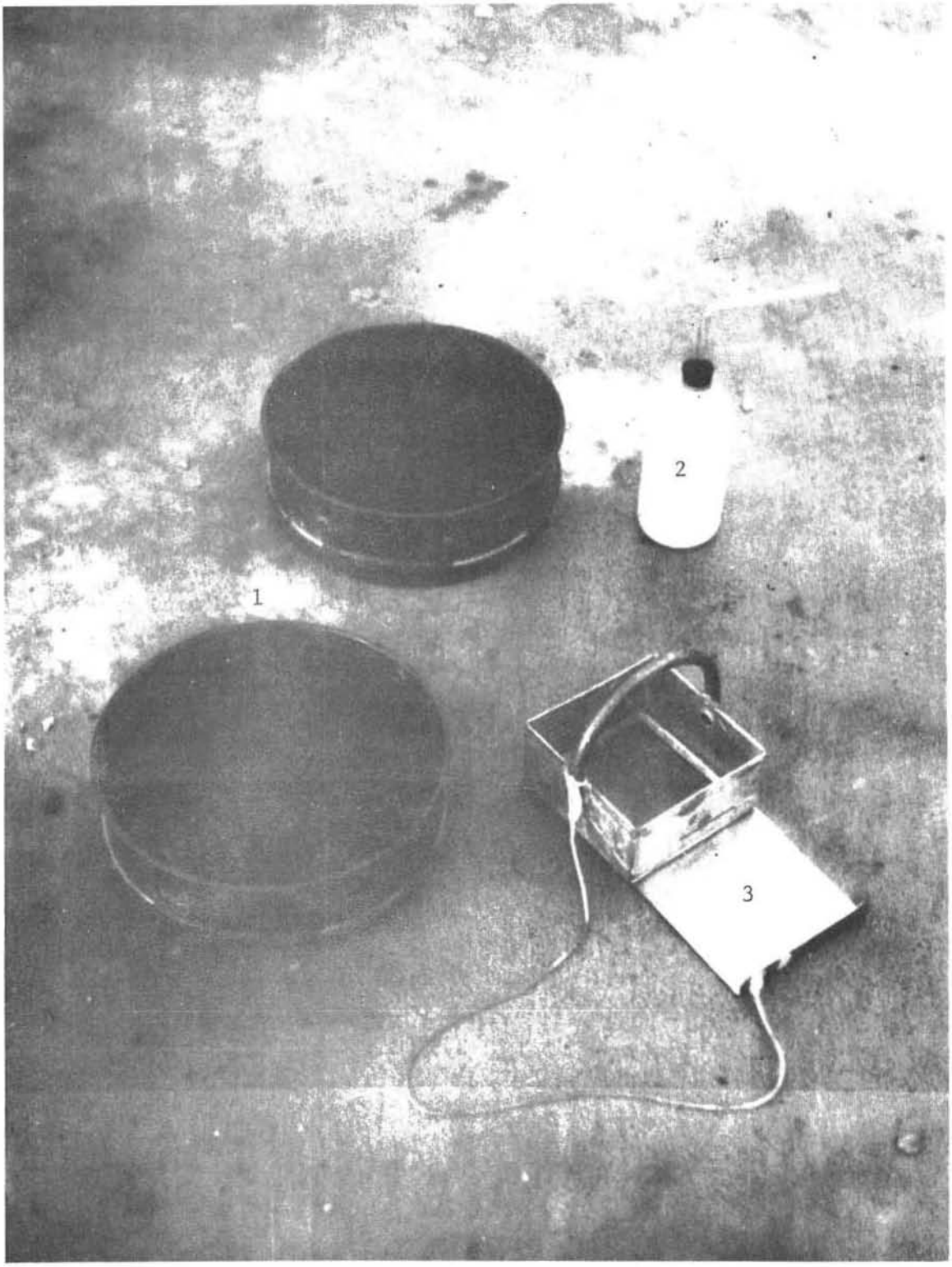

Figure 4. Equipment used in invertebrate sampling, Bear River Refuge, Utah, 1961

1. Tyler Sieve, 2. Aspirator bottle, 3. Mud sampler

As the early field experiments progressed, different results were observed using the same insecticide with various amounts of vegetation. In the areas where control of the invertebrates is sought, there are varying degrees of the bottom covered by the submerged aquatic plants. To discover the effects, if any, of this cover on the insecticidal action of the chemicals, two complete experiments were conducted with both malathion and DDT. Each insecticide was tested in two different plant cover situations. Malathion was tested in 30 percent and 100 percent plant cover and DDT in 30 percent and no cover at all. These situations are common in and around channel openings and in the main water areas of the refuge.

As the experiments progressed the lasting effects of DDT could not be observed as the insecticide was still showing toxic effects on the invertebrates at the end of the 72 hour test period. It was thought advisable to learn just how long the effects of DDT would hold the invertebrate populations below their original level. Two experiments were set up to determine this.

Experiment A involved three of the polyethylene rings already described. They were set up, measured, treated with 0.1 p.p.m. DDT, and sampled (by the method previously described) periodically for 9 days, which was as long as time would permit.

Experiment B consisted of marking off three 5' x 5' areas which differed with respect to amount of vegetational cover, exposure to wind and currents, and water depth. These areas were sampled and treated with DDT. The concentration could not be specified as the areas were not enclosed by any container. The only desired result was to obtain a reduction in invertebrate numbers. Three samples were taken at random

in each plot at one sampling time until the numbers returned to the level at which the experiment started (as found by control samples taken before treatment). The first plot was located in a shallow channel about 6 inches deep. The second was in 100 percent plant cover, also about 6 inches deep, and the third was in a calm water area (no current and little wind action) with a water depth of about 3 inches.

All samples were averaged for each sampling period in the ring experiments and the three samples for each sampling time in each of the three plots were averaged in the open test. From these averages, graphs were drawn to obtain a clearer picture.

Analysis

The laboratory data were classified as to their acceptability for further testing in the field without statistical analysis (Table 1). The samples of the preliminary field experiments were averaged and put in a table (Table 2). These data were used in setting up the main field tests. The information on the lasting effects of DDT was tabulated and put on graphs for simplified interpretation.

Values of LD 50 for the effect of malathion and DDT on midge larvae were estimated by the method of probit transformation. In this method the percentage mortality is transformed to probits and the dose is changed to logarithms. Thus, when plotted on a graph, the regression line is straight and the LD 50 estimation can be made directly from the graph.

Statistical analysis was made on the main field tests. The analysis proposed in this case was a modified Karl Pearson Minimum Chi-square Test. When the distribution of the population is discrete, analysis of variance test, popularly known as F-test, yields only approximate results.

If the environmental disturbances are partially unfavorable, it might even lead to wrong conclusions. The proposal of modified Karl Pearson Minimum Chi-square Test was thought to be appropriate because in these experiments the attribute involved was survival and so the data were discrete. The attribute data fall into certain definite categories and form a binomial distribution.

The following formula was proposed for this case in which there are unequal numbers of insects (n_1) per treatment:

$$X^2_{(t-1)d.f.} = \frac{\frac{\sum r_i^2}{n_i} - \frac{\left(\sum r_i\right)^2}{T}}{\left(\frac{\sum r_i}{T}\right)\left(1 - \frac{\sum r_i}{T}\right)}$$

where r_i is the number of kills associated with the i^{th} treatment, t is the number of treatments, T equals $\sum n_i$ which is the total number of insects involved in the whole experiment and n_i is the number of insects subjected to the i^{th} treatment (Shabbir, 1961 and Snedecor, 1956).

By using the above named test statistic, the following was obtained by X^2 values:

(1) Detection of treatment differences in the attribute data (Appendix Tables 7 and 8).

(2) Homogeneity of replicates (Appendix Tables 9, 10, 11 and 12).

Due to the variability in the samples, it was decided to accept as significant only those values that were significant at the .01 level of probability. This would reduce the chance of a sampling error affecting the interpretation of the data.

RESULTS

Laboratory Experiments

General

The laboratory experiments were used as a means of determining the concentrations of each insecticide that should give the desired range of kill when used in later field tests. Chemicals which, by their physical properties or insecticidal qualities, were found to be undesirable for further field testing were quickly eliminated.

Insecticides in relation to test media

Three media were used in the laboratory tests to determine the effects of insecticides on certain invertebrates. These were: (1) distilled water, (2) river water, and (3) mud and river water. Contrary to expectations, the results of the tests using distilled water were inconsistent. Approximately 10 times as much insecticide was required in distilled water to obtain a certain kill of midge larvae as to obtain the same results under more natural conditions of mud and river water. The reason for this difference was not determined by this study. It was theorized, however, that since the larvae had no soil in which to build their tubes they were unable to set up the water currents which normally bring food, oxygen, and insecticides into contact with their bodies. Insecticides sometimes accumulate in organic matter and are ingested in larger quantities by the invertebrates. This was not possible in this case.

The use of river water as a medium was tried following the tests

with distilled water. It was possible to obtain a good kill of water fauna such as corixids and notonectids in river water. Midge larvae, however, reacted much as they had in distilled water. The testing of corixids and notonectids in insecticides was soon halted as these animals were not considered serious in botulism outbreaks.

The next medium used was a combination of mud from the impoundments and river water. In this medium it was possible to obtain good kills of midge larvae with DDT, malathion and rotenone at concentrations of 0.1 to 0.5 p.p.m. Consistent, reasonable results were obtained and verified by duplication.

Most of the insecticides tested were run in all three of the media to see their reaction. The conclusions drawn from these laboratory tests were taken almost completely from the mud and water test phase. These results were apparently quite accurate inasmuch as the concentrations found effective by this means were close to those later found by field tests.

The Tendipedidae larvae would leave their tubes when affected by an insecticide. It was impossible to observe the effects of an insecticide on red colored midge larvae and corixids in the same pan. The larvae would be affected first by the chemical, and when the larvae were in the open the corixids would grasp them and hold fast. The corixids were then observed to "suck" out all the red hemoglobin-like substance which gives these larvae their distinctive color.

Summary of laboratory results

Malathion gave a good kill of midge larvae in the laboratory tests at 0.1 to 0.5 p.p.m. No other references to its effectiveness on these animals could be found. DDT also proved effective in the control of

tendipedids at concentrations of 0.1 to 0.5 p.p.m. This was in agreement with the findings of Jamnback (1954), Flentje (1945), and Eide et al. (1945). Rotenone gave good control of larvae at 0.1 to 0.5 p.p.m. No reference as to its use for this specific purpose was discovered.

Toxaphene reacted poorly in the tests. This insecticide has one unstable chlorine atom that is attacked by alkaline materials (Shepard, 1951) which may account for this poor reaction. Gerry (1951) reported having to use concentrations of pyrethrum as high as 600 and 667 p.p.m. to control the larvae of two different species of Tendipedidae. This agrees in general with the findings reported herein, although no attempt was made to test concentrations greater than 10.0 p.p.m. Dibrom is a newer insecticide and no reference as to its use in controlling midge larvae could be found. For effective control in the laboratory tests, dibrom required a concentration too great to be considered safe to use in the field. Results of the laboratory tests are summarized in Table 1.

Field Experiments

Preliminary experiments

Although other invertebrates were counted, the Tendipedidae larvae were the only ones considered in analyses of the effectiveness of the insecticides. The other invertebrates were too few in number and inconsistent in their reactions. It was found that even though the tendipedid carcasses disintegrated rapidly after death, a sufficient portion remained for accurate counting at the end of the 24-hour test period. Since the experiments were terminated after 24 hours, it was impossible to obtain any estimate of the lasting effects of the insecticides.

Because of the lack of equipment, replications were not made since

it was not possible to replicate without having time differences between the replications. For this reason the results of these preliminary tests were not analyzed statistically. The results were averaged and used as guides for additional tests.

Table 1. Summary of laboratory test results of insecticides used at Bear River Refuge, 1960-1961

Insecticide and form	Acceptability for field testing	Explanation
Malathion 55% emulsion	Excellent	Used in field tests.
DDT 25% emulsion	Excellent	Used in field tests.
Toxaphene granular	Poor	Required too heavy a dose. Dissolved very slowly.
Pyrethrum 2% emulsion	Poor	Required too heavy a dose. (Given very little testing)
DDT 50% wettable powder	Good	Emulsion easier to handle and about same results. Not field tested due to lack of time and possible duplication.
Dibrom 8 emulsion	Poor	Required too heavy a dose. (Given very little testing)
Rotenone 5% powder	Excellent	Not field tested due to lack of time.

Malathion was found to be effective in reducing numbers of midge larvae. Concentrations of 0.1 and 0.5 p.p.m. malathion gave an average reduction of 64 and 96 percent, respectively, of the tendipedid larvae. Dead larvae in the control rings averaged only 1.2 percent of the animals sampled. Temperature appeared to have no effect on the kill (Table 2).

DDT was less effective in the preliminary tests than malathion.

The reduction of midge larvae averaged 36.7 and 66.7 percent for DDT concentrations of 0.1 and 0.5 p.p.m., respectively. Normal mortality in the control rings averaged 7.6 percent. The kills in the treated rings appeared to be negatively correlated with the minimum temperatures and positively correlated with the maximum temperatures. The highest kills were obtained with the lowest minimum temperatures and the highest maximum temperatures (Table 2).

Table 2. Results of preliminary field experiments on the effects of insecticides on midge larvae, Bear River Refuge, 1960[a]

	Percent dead	Percent kill		Temperature °F	
	Controls	0.1 p.p.m.	0.5 p.p.m.	Max.	Min.
		Malathion			
Test 1	0.6	56.0	100.0	88	64
Test 2	1.0	78.0	89.0	91	68
Test 3	2.0	58.0	99.0	86	64
Average % dead	1.2				
Average % kill		64.0	96.0		
		DDT			
Test 1	19.0	26.0	48.0	80	70
Test 2	3.0	30.0	63.0	84	62
Test 3	0.7	54.0	89.0	92	63
Average % dead	7.6				
Average % kill		36.7	66.7		
		Malathion and DDT			
Test 1	0.8	13.0[b]	94.0[c]	97	71

[a]Figures given are percent dead tendipedids found in samples taken 24 hours after treating.
[b]Treated with 0.1 p.p.m. malathion and 0.1 p.p.m. DDT
[c]Treated with 0.5 p.p.m. malathion and 0.5 p.p.m. DDT

A test was conducted to determine whether the effects of a mixture of DDT and malathion would give better results than either one alone.

The results of this test were not satisfactory. At a concentration of 0.1 p.p.m. of each insecticide, the chemicals apparently had a neutralizing effect on one another as the kill of larvae was only 13 percent. A 94 percent kill of the midge larvae was obtained using 0.5 p.p.m. of both chemicals. The kill was not as high as the average for malathion alone, which was 96 percent for 0.5 p.p.m., but it was higher than any of the tests of DDT by itself which averaged 66.7 percent for 0.5 p.p.m. (Table 2).

Principal experiments

General

In the 1960 field experiments where samples were taken only after 24 hours, it was possible to make a count of the number of dead invertebrates in the mud samples as well as those still alive. In the 1961 field experiments, however, samples were taken after 48 and 72 hours as well as after 24 hours. The bodies of the dead midge larvae were decomposed too much to count accurately after 48 and 72 hours. To keep counts consistent, it was decided to count only live invertebrates in all samples taken and relate the numbers remaining alive in the treated plots to those found in the controls (assuming natural mortality in controls and treated plots to be equal).

In estimating LD 50's for malathion and DDT on midge larvae, the results obtained were rounded off to the nearest one-hundredth p.p.m. Estimation of LD 50 for malathion was made after the 24 hour sampling period since it was found that the effect of the insecticide lasted no longer than 24 hours, and the larval midge population either remained the same or increased after that time. By combination of the results of both experiments using malathion, LD 50 for this insecticide was estimated to be 0.04 p.p.m.

The results of the two experiments in which DDT was used were combined to estimate the LD 50 for this chemical. DDT was still reducing the midge larvae population at the end of the 72 hour test period. The LD 50, 0.03 p.p.m. for DDT on midge larvae, was estimated from data obtained from the 72 hour sampling period.

Problems were encountered in the analysis as it was found that the test sites were heterogeneous. This had an effect on the results since there might be large variations in the density of the midge larvae from the location of one study plot (ring) to that of another. Not only was there a great deal of variation between rings, but also within each ring. These variations might have been from differences in micro environments such as mud consistency, soil composition, amount or type of vegetation, organic matter accumulation, or a combination of these factors or several others affecting the distribution of tendipedid larvae.

Chi-square analysis was omitted on one replicate of two experiments (malathion in 100 percent vegetation cover and DDT in no plant cover) because one of the treated rings had a higher count of live invertebrates after treating than the control ring of that replicate. In Appendix Tables 9, 10, 11 and 12 results are shown of computations for Pooled X^2, Sum X^2, and Heterogeneity X^2. Pooled X^2 takes into account the replicate effects and shows whether the sum of the chi-squares of the three replicates is significant. Sum X^2 considers all three replicates as one and in doing so ignores the replicate (location) effect. Heterogeneity X^2 shows whether there is consistency among the replicates in detecting the treatment difference.

Malathion experiments

Effects of insecticide on midges in 30 percent vegetative cover. The concentrations of malathion used in this experiment were 0.05 and 0.10 p.p.m. The reduction in the numbers of midge larvae by both of these concentrations was approximately 67 percent in the first 24 hour period of this test. The reduction of larvae in both treated rings as compared to the controls was still about 65 percent after 48 hours. By the end of 72 hours the reduction of larvae in the 0.10 p.p.m. treated plots was down to 53 percent and the larvae in the rings treated with 0.05 p.p.m. malathion had decreased to 35 percent as compared to the controls (Figure 5).

The samples from the controls showed a majority of the midge larvae present to be red in color. After treatment with malathion, the red larvae in the treated rings were greatly reduced in numbers, while the white larvae were not reduced at all, but in fact, increased two to three fold over the 72 hour period. The reason for this is not known, although the white group probably had greater resistance to malathion than the red group. Further, the insecticide may have stimulated the early hatching of eggs which had been previously deposited. Another possibility is that some limiting factor was eliminated such as a predator or competitor for food or oxygen.

The statistical analysis showed no significant difference between the effects of 0.10 and 0.05 p.p.m. malathion on midge larvae after 24 and 48 hours. The difference did become significant after 72 hours (Appendix Table 7). The difference between the effects of the treatment in different rings treated with the same concentration of insecticide was not significant after 24 or 48 hours but was significant at the 72 hour sampling period (Appendix Table 9).

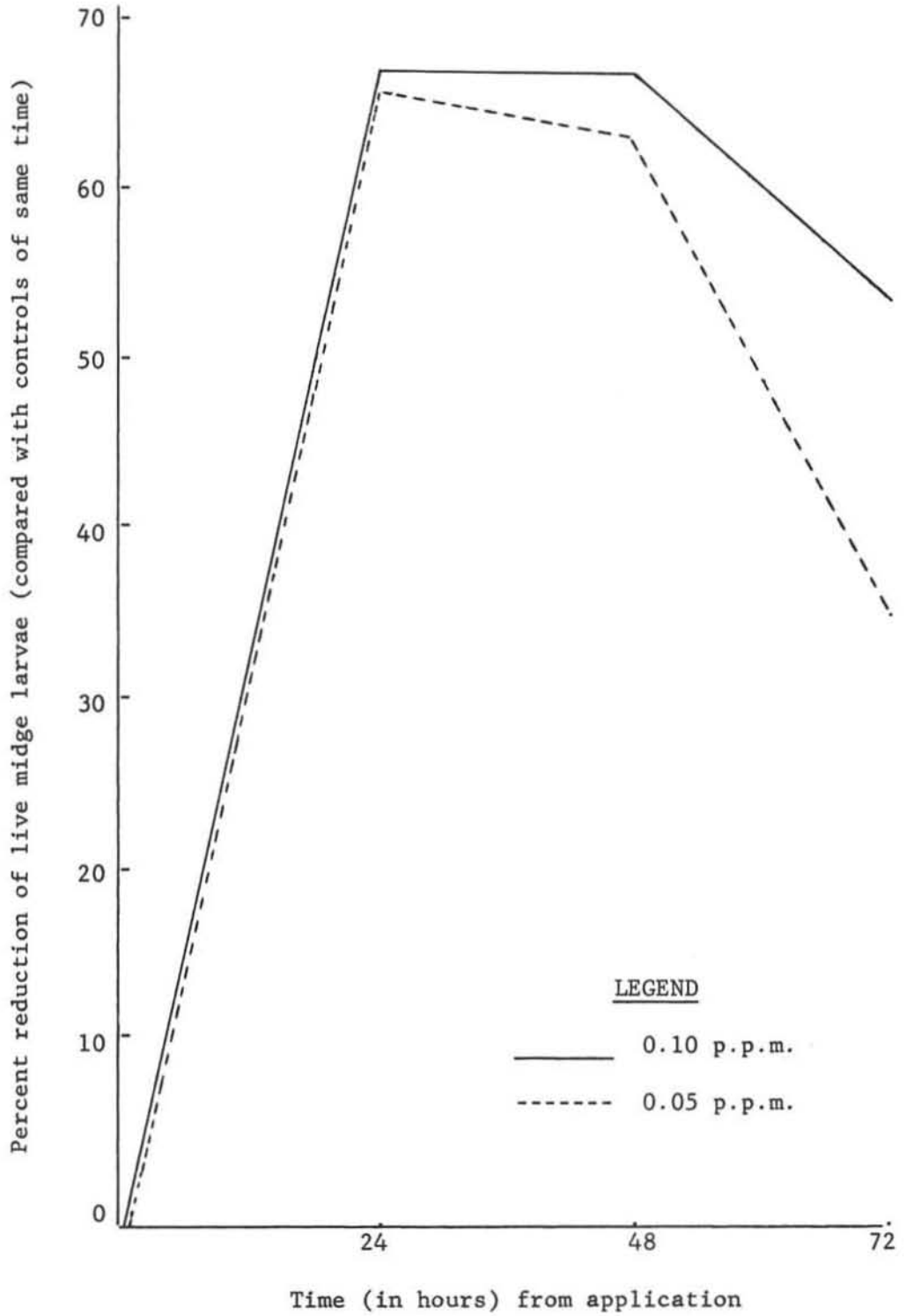

Figure 5. The effect of malathion on Tendipedidae larvae in an area where the bottom was 30 percent covered by vegetation, Bear River Refuge, 1961

The relatively small amount of submersed aquatic plants present in this experiment probably increased the ineffectiveness of toxicity of the insecticide. This sparse cover would allow currents, wind, and sunlight to have greater effect in the dispersal and hydrolysis of the insecticide.

Effects of insecticide on midges in 100 percent vegetative cover. The concentrations of malathion tested in this experiment were 0.05 and 0.10 p.p.m., the same as in the previous malathion experiment. There were, however, different conditions present with respect to the amount of bottom covered by aquatic vegetation. There was complete cover of the bottom soil by submersed aquatic plants in this test compared to 30 percent coverage in the previous malathion experiment.

The reduction of larvae after 24 hours in the range treated with 0.10 p.p.m. malathion averaged 66 percent. This was almost identical with the results obtained in the previous test of malathion for the same treatment. The rings treated with 0.05 p.p.m. of the chemical reduced the midge larvae 41 percent after 24 hours. The difference between this value and the 65 percent reduction of larvae obtained with the same concentration (0.05 p.p.m.) of malathion in the previous test was probably due to the difference in vegetational cover. The greater vegetational cover in this experiment apparently absorbed or rendered unavailable to the invertebrates a portion of the malathion, thus reducing the kill of midge larvae. The reduction of larvae increased slightly in the 0.10 p.p.m. rings to 68 percent after 72 hours. The reduction in the rings with 0.05 p.p.m. malathion decreased to 35 percent after 72 hours (Figure 6).

The reduction of red and white colored larvae was about the same in this experiment in contrast to the results obtained in the previous test

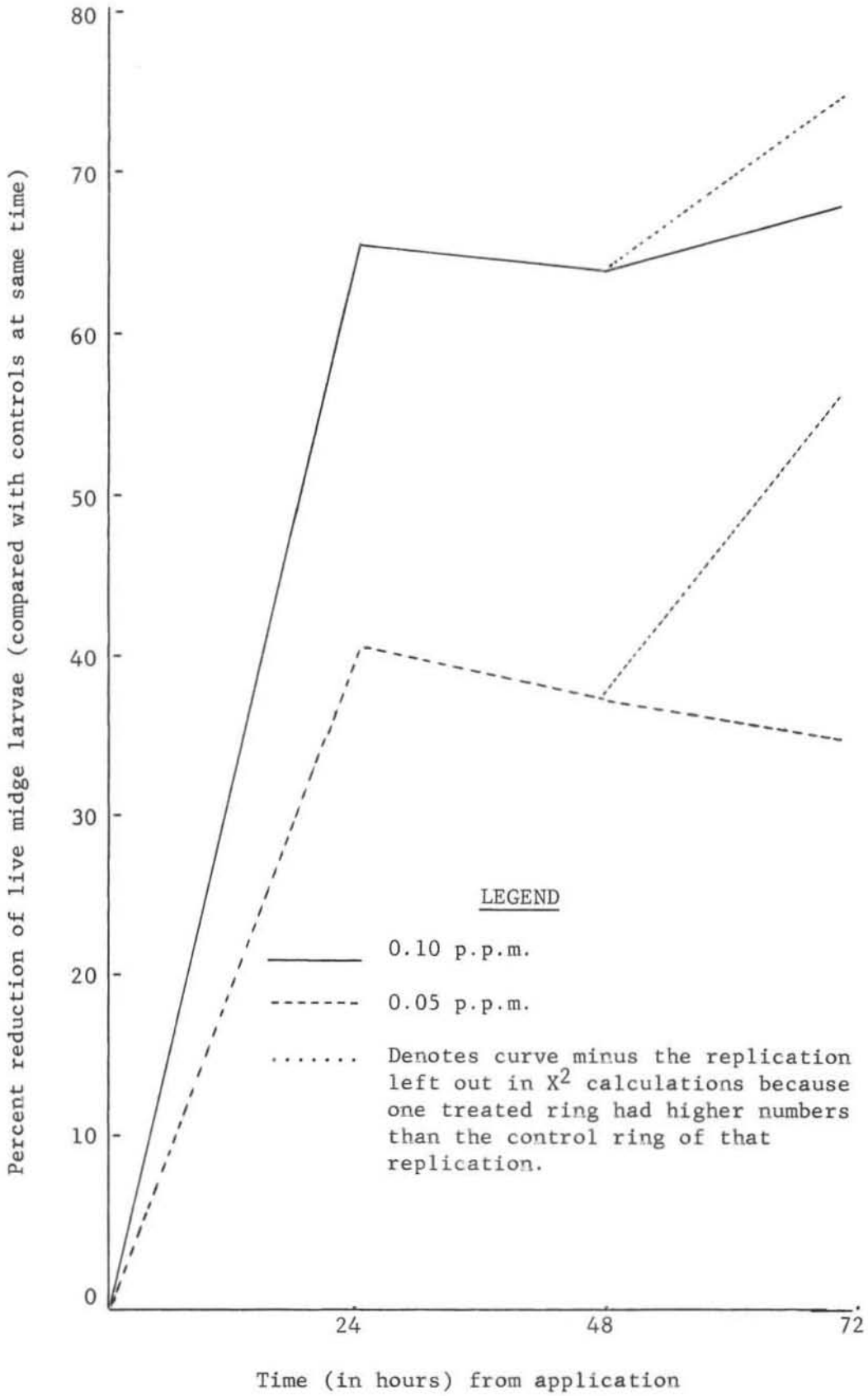

Figure 6. The effect of malathion on Tendipedidae larvae in an area where the bottom was completely covered by vegetation, Bear River Refuge, 1961

of malathion in 30 percent vegetational cover where there was no reduction in the counts of the white larvae but where almost all red larvae were eliminated.

The statistical analysis showed that there was a significant difference between the treatments of 0.05 and 0.10 p.p.m. malathion throughout this experiment (Appendix Table 7). The differences between plots treated alike were significant in all three sampling periods (Appendix Table 10) which shows that the conditions existing at different locations do affect the results of these tests.

DDT experiments

Effects of insecticide on midges in 30 percent vegetative cover. The levels of DDT used in this test (0.1 and 0.5 p.p.m.) were determined from the results of the preliminary field tests. These levels gave a greater kill of midge larvae than was thought desirable. This was partially due to the inability of the preliminary field tests to determine the lasting effects of this chemical. Other undetermined reasons must have allowed the DDT to have a greater effect on the midge larvae at this location as compared to the effect it had at the site of the 1960 tests. The reduction of larvae was over 80 percent for both concentrations after the 24 hour sampling period. This reduction compared to the control samples taken for the same period increased through the 48 and 72 hour sampling periods. The reduction of larvae with the 0.5 p.p.m. level of DDT was almost 98 percent at the 72 hour period (Figure 7).

Statistical analysis showed that the difference between the treatments was not significant at the 24 hour sampling period, but it was significant at the 48 and 72 hour sampling periods after treatment (Appendix Table 8). The differences between plots treated alike were

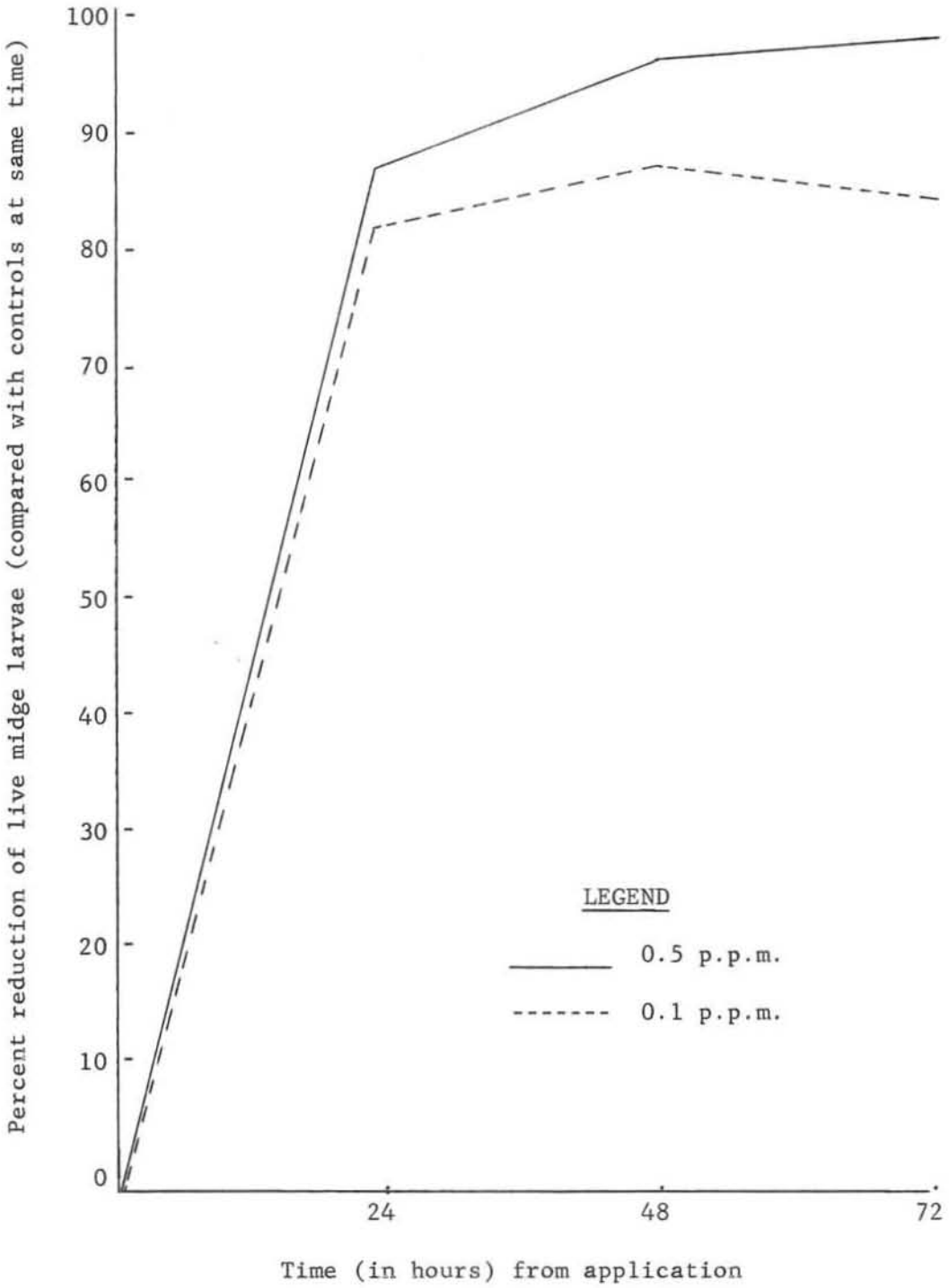

Figure 7. The effect of DDT on Tendipedidae larvae in an area where the bottom was 30 percent covered by vegetation, Bear River Refuge, 1961

not significant after 24 hours, but became so after the 48 and 72 hour periods (Appendix Table 11).

Effects of insecticide on midges in area with no vegetative cover. The concentrations of DDT used in this experiment were 0.025 and 0.05 p.p.m. The levels were reduced from those used in the DDT experiment in 30 percent vegetative cover which were found to be too high. A kill of midge larvae was obtained within the desired range. The 0.05 p.p.m. DDT concentration reduced the midge larvae about 52 percent in 24 hours and continued to kill them until a reduction of about 68 percent was obtained by the time of the 72 hour sampling period. The 0.025 p.p.m. level of DDT reduced the numbers of tendipedids about 22 percent in 24 hours and this reduction increased to almost 36 percent after 72 hours (Figure 8).

Statistical analysis showed a significant difference between the treatments in all three sampling periods of this experiment (Appendix Table 8). The differences between plots treated alike were significant through all three time periods that were tested (Appendix Table 12).

It was found that the insecticide DDT affects the red and white colored larvae about the same. White, or colorless, larvae were reduced in numbers slightly more than the red ones, but this difference was not significant.

Lasting effects of DDT on midge larvae. Two experiments were set up to determine how long DDT would affect the numbers of midge larvae present in an area after treating with the insecticide. Experiment A, which was conducted within the polyethylene rings, was designed to show the lasting effects of DDT in an enclosed area or when an entire unit of water was treated at one time. A concentration of 0.1 p.p.m. DDT was placed in each of three rings and samples were taken periodically to

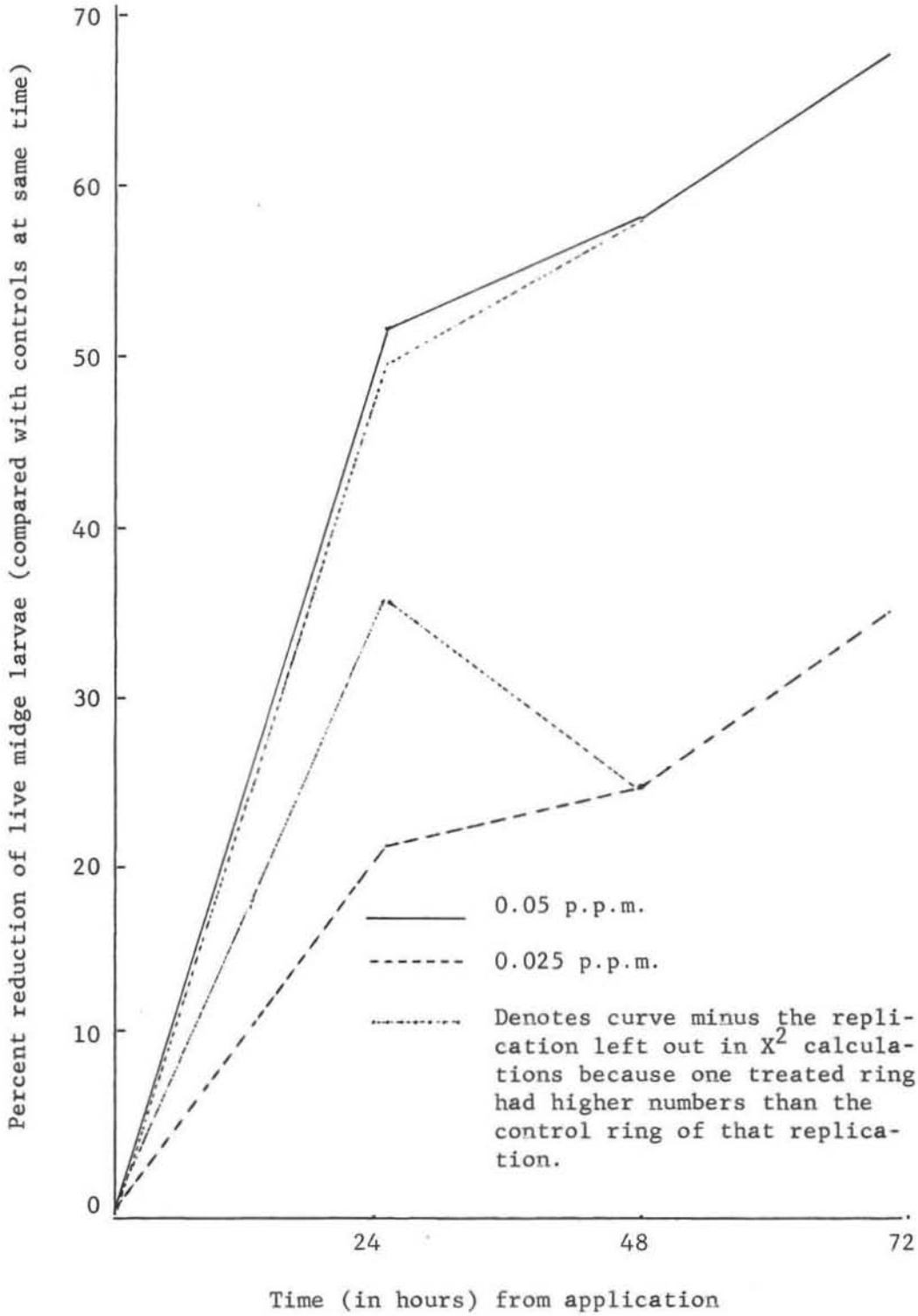

Figure 8. The effect of DDT on Tendipedidae larvae in an area where the bottom had no vegetation (originally the bottom was 30 percent covered, but it was clipped for this experiment), Bear River Refuge, 1961

determine how long the numbers of larval midges would be held in check by this treatment.

There was a sharp reduction in larvae numbers during the first few days after treating. After five days only about 2 percent of the original population remained alive. From the fifth day on there was little additional reduction of the midge larvae population within the rings. The numbers did continue to go down slightly without any rise back toward normal levels until the ninth day after treatment (Figure 9). This was as long as the experiment was continued. The treatment level of insecticide was, perhaps, excessive, as the reduction in numbers far exceeded that thought to be sufficient for control purposes. That may be one reason for the numbers remaining low for so long (Table 3).

Experiment B was set up in open plots exposed to the effects of winds and currents. The purpose of this test was to determine the lasting effects of DDT under different conditions of vegetation and currents when only a portion of the area was treated with the insecticide. Numbers of living midge larvae were used as indicators. Unmeasured amounts of insecticide were applied to the three plots tested.

Apparently the plot located in a calm water area received the heaviest dose of DDT. It was reduced the most in larvae numbers and took the longest time (9 days) to return to the previous population level. This was expected as the chemical would not be diluted nor rapidly carried away by the water currents. The plot located in a shallow channel received a substantial dose of insecticide which kept the midge larvae population below normal numbers for approximately five days. The plot located in an area completely covered by plants (Potamogeton sp.) apparently did not get as much chemical to the larvae on the bottom as

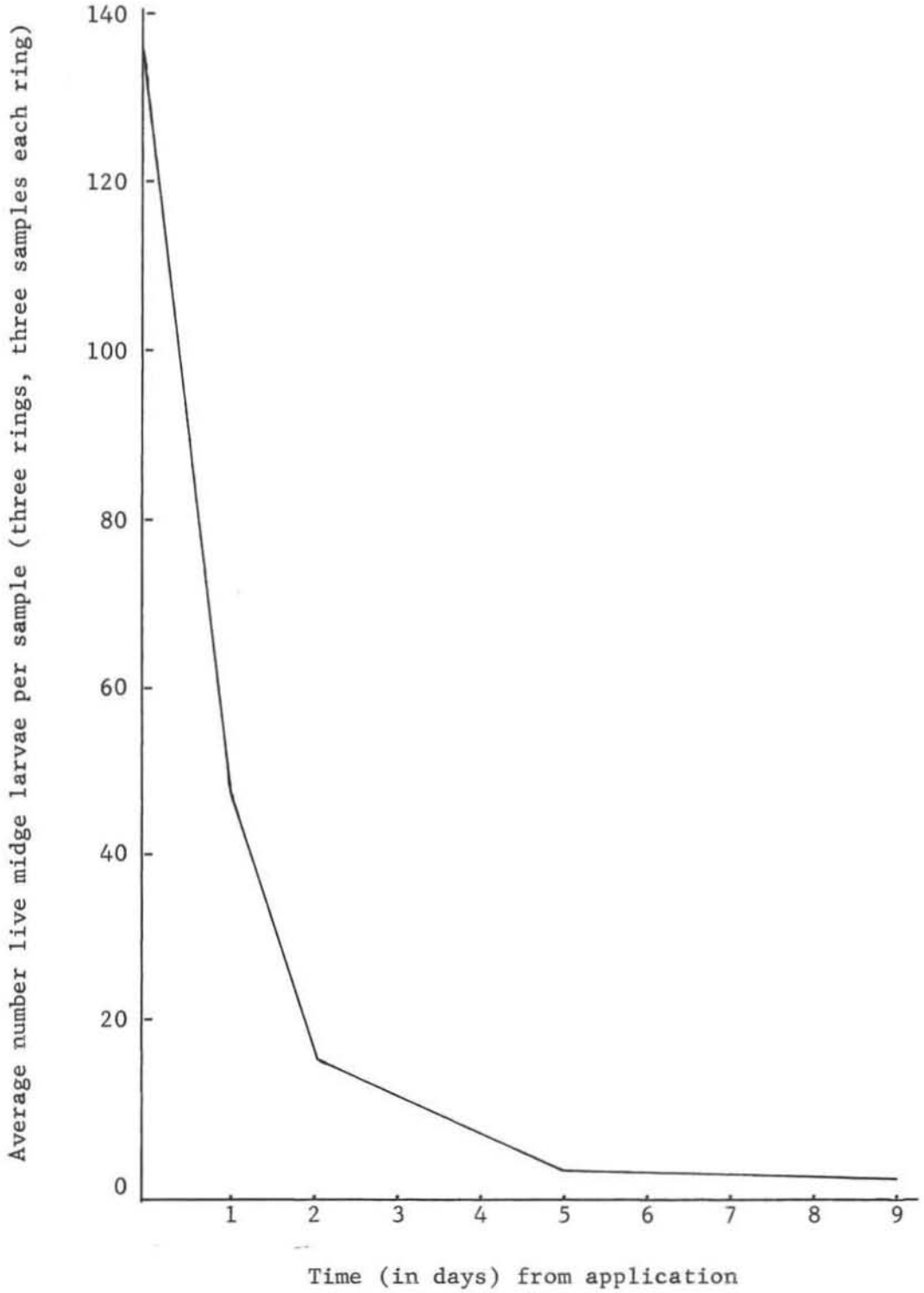

Figure 9. The lasting effects of 0.1 p.p.m. DDT on Tendipedidae larvae in polyethylene rings, Bear River Refuge, 1961

did the other two plots. The population was reduced about 50 percent after two days, but had returned to near normal numbers about four days after treating (Figure 10).

Table 3. The lasting effects of 0.10 p.p.m. DDT in maintaining low levels of midge larvae populations in polyethylene rings, Bear River Refuge, 1961[a]

Days after treatment	0.1 p.p.m.		
	Ring 1	Ring 2	Ring 3
Controls	153.0	146.0	106.0
1	60.0	59.0	24.3
2	24.3	14.3	8.7
3			
4			
5	1.7	3.0	1.7
6			
7	3.0	1.3	1.0
8			
9	2.3	1.3	0.3

[a]Figures given are averages of three samples. Samples were not taken on days left blank.

Using such small plots (5' x 5') it would be possible for the larvae from outside the treated areas to migrate into them quite rapidly as soon as the toxicity was reduced or the poison carried away by wind action or water currents. A natural reduction in larvae numbers was observed toward the end of this experiment. Such a reduction usually occurs in the fall of the year as the water temperature decreases. Since it was September when this experiment was taking place, this reduction was not unexpected (Table 4).

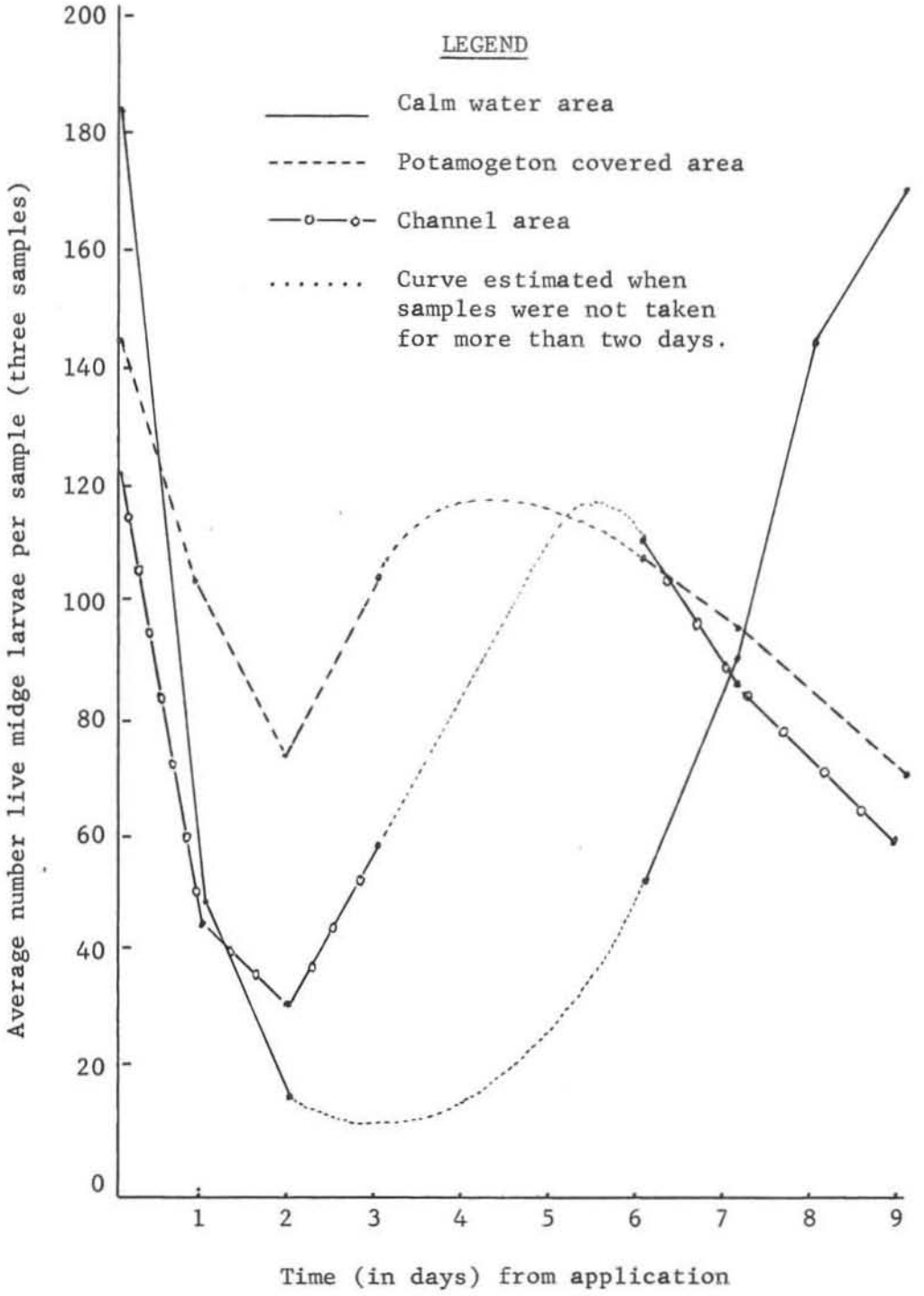

Figure 10. The lasting effects of variable amounts of DDT on Tendipedidae larvae in exposed plots having different conditions, Bear River Refuge, 1961

Table 4. The lasting effects of variable amounts of DDT in maintaining low levels of midge larvae populations in exposed plots, Bear River Refuge, 1961[a]

Days after treatment	Plot locations		
	Channel area	Potamogeton covered area	Calm water area
Controls	121.0	148.0	183.0
1	45.3	103.7	48.0
2	29.7	75.0	15.0
3	58.7	106.3	
4			
5			
6	108.3	104.7	55.0
7	81.7	92.0	85.7
8			144.7
9	61.0	69.3	170.3

[a]Figures given are average of three samples. Samples were not taken on days left blank.

DISCUSSION

One of the first questions encountered during the course of this investigation was why more insecticide was required in distilled or river water alone to obtain a kill comparable to that obtained in more natural media. One of the main reasons for this occurrence was probably the lack of organic matter particles in the water media. The insecticide would not be carried into the bodies of the larvae along with ingested food particles as there was no food for them to eat. Ordinarily the poison settles on the bottom and is ingested with food eaten by the larvae or carried into their tubes by the water currents they set up to provide food and oxygen.

Another interesting phenomenon was observed when both corixids and red tendipedids were present in the same pan of insecticide being tested in the laboratory. It was observed that the corixids would seize and hold fast to a larva that was available (either by being in a water-only medium or by being affected by the chemical enough to leave its tube). After seizing a larva, the corixids were observed to "suck" out all the red hemoglobin-like substance leaving the larvae still alive but colorless. Why they did this was not determined in this study. Possibly it is a nutritive source of food.

Little definite correlation of temperature with percent kill of midge larvae was found. The indication that DDT did a little better when temperatures were cooler agreed with the findings of Metcalf (1955).

The data obtained for malathion in the 1960 and 1961 field tests were almost identical. DDT, though, gave better results in the 1961

tests than in those conducted in 1960. Part of this was due to the lasting effects of the toxicity of the poison for more than the 24 hours studied in 1960. Possibly the difference in locations due to soil, water depth, organic matter, currents or vegetation may have had some effect as well.

The larval populations in the study area averaged 10,400 larvae per square yard and were as high as, or higher than, those reported by Brues (1946), which he says may attain densities as great as 7,000 larvae per square yard. He also reported that great variations occur among midges with reference to the presence of sand, mud or clay on the bottoms of the areas where they develop. Large variations were found in the densities of larvae between points of close proximity.

The differences between concentrations of insecticides found effective for control of midge larvae in this study and those of other studies is attributed to a difference of conditions and objectives. Other studies have attempted eradication. In this study only a partial control was sought.

MANAGEMENT RECOMMENDATIONS

Since the areas are relatively small where the birds afflicted with botulism are found and the amount of insecticide required for control purposes is also slight, it is economically feasible to use chemicals as a means of control of midge larvae possibly associated with avian botulism outbreaks. How and when to use chemicals are other questions that must be answered.

Fellton (1941, p. 194) stated:

> The one most important treatment, if one can be selected, in the control of aquatic midges is that made in the spring when the overwintering larvae have become active but before pupation begins, since there are no eggs or adults of the insects present at that time to cause immediate reinfestation of the breeding place. If this treatment results in a kill which approaches extermination of the larvae present, the control problem during the remainder of the season will be made much easier.

Fellton found that when the water temperature reached 50° F in the spring, the midge larvae became quite active and soon after the adults began to emerge and lay eggs.

Since midge larvae occur in the mud at the bottom of the impoundments, most of the common insecticidal formulations are unsatisfactory because they are lighter than water. A formulation with specific gravity greater than that of water is required. Oil solutions without an emulsifier are not considered suitable for aircraft application since the small droplets do not penetrate the surface film, and large droplets result in poor dispersion, both in the air and under water. A good material for aircraft application would be a granular formulation in which the individual insecticide-carrying particles are large enough and heavy enough to settle through the air without excessive drift, to penetrate the

surface film of the water on contact, and to sink rapidly to the bottom. Emulsions and/or emulsion concentrates might be used provided they were quick-breaking and heavy enough to settle after breaking. Emulsions would probably be preferable in underwater dispersion from a boat.

A stomach or contact poison would be best to use in control of midge larvae because of the breathing and feeding habits of these invertebrates. Their breathing and feeding habits serve to bring suspended particles of undissolved poisons which have been applied to the water into the tubes of the larvae.

Fellton (1941) found that the "bloodworms" tended to migrate from areas that had just been sprayed if the insecticide was slow in its toxic effect. They moved toward the shore and necessitated a careful spraying of the shoreline after the body of the lake had been treated.

Control operations when dealing with midge larvae would probably be best if initiated in early spring before emergence of the adults. One or two applications of DDT or periodic applications of malathion would probably be required to maintain control in an area. If an applicator can be devised for underwater application of an emulsion from an airboat, it would probably be the least expensive and most versatile means of distribution. Airplane application is expensive, subject to weather conditions, and sometimes not too accurate in getting a complete coverage of the required area (without overlapping or hitting areas not to be treated such as shoreline vegetation). Many times, however, it is the best means available, especially when dealing with large expanses of shallow water.

SUMMARY

In 1960 and 1961, an investigation of selected insecticides was made for the purpose of controlling aquatic midge (Tendipedidae) larvae populations which are thought to be a major factor in outbreaks of avian botulism. This study took place at Bear River Migratory Bird Refuge near Brigham City, Utah.

Laboratory experiments were conducted using malathion, DDT, rotenone, pyrethrum, toxaphene and dibrom. DDT emulsion and powder, malathion emulsion, and rotenone powder gave favorable results. Pyrethrum emulsion, granular toxaphene, and dibrom emulsion required a concentration that might be detrimental to wildlife in order to be effective.

Field testing was confined to the use of malathion and DDT emulsions. These insecticides have shown relatively little harm to wildlife in past usage. Preliminary field tests were conducted in 1960 within three galvanized steel rings. These tests, run for 24 hours, showed malathion to be very effective at concentrations of 0.1 and 0.5 p.p.m. in reducing the numbers of midge larvae. DDT was not as effective as malathion. It did give adequate control, however, at 0.1 to 0.5 p.p.m. A combination of the two insecticides gave poor results at 0.1 p.p.m. each and good results at a concentration of 0.5 p.p.m. each.

Field testing in 1961 was conducted in nine polyethylene rings. These were set up to give three replications of each experiment. Two tests, of both malathion and DDT, were conducted. Each test was run under different conditions of vegetative cover. The indications were that the submerged aquatic vegetation (Potamogeton sp.) tended to reduce

the kill when low concentrations of insecticide were used. The vegetation also seemed to prolong the effects of the insecticide, especially malathion.

The midge larvae were found to have great variation in densities among the locations tested. Even the variation within a particular ring was sometimes large.

A difference was noted in the reactions of "bloodworms" and colorless larvae to most of the insecticides tested. The colorless larvae, on the whole, showed more resistance to insecticides such as malathion and rotenone than did the red colored ones. This was apparent in the use of malathion. The reactions of the two groups to DDT were about the same.

Estimates were made by probit transformation of the LD 50's for malathion and DDT. LD 50 for malathion 24 hours after being treated was 0.04 p.p.m. and for DDT the LD 50 was 0.03 p.p.m. after 72 hours.

A difference was noted in the residual effects of the two chemicals. The residual effect of malathion was gone after one to two days, and the larvae began to build up again in numbers almost immediately. DDT, on the other hand, had a much longer residual effect. This effect was still causing tendipedid larvae mortality at the end of nine days within the rings. The residual effect of DDT lasted four to eight days when it was not confined within the rings, but was exposed to the effects of currents, wind and sun.

It is difficult to generalize on the direct and indirect effects of large scale treatments from these few data. It would appear, however, that either malathion or DDT would provide effective control with little or no loss to wildlife if used correctly. Rotenone, if tested more extensively, might also fit into this same category. Since the areas where control of the midge larvae is sought are relatively small, there is little danger of depleting an essential waterfowl food to a dangerous level.

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A P P E N D I X

Table 5. Results of principal field experiments on the effects of malathion on midge larvae, Bear River Refuge, 1961

Experiment 1 - 30 percent vegetative cover

	P_1			P_2			P_3		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
T_1	139.5	170.5	145.0	111.5	188.5	143.0	138.0	166.5	126.0
T_2	54.0	58.0	46.0	41.5	56.0	64.0	118.5	67.0	94.0
T_3	67.5	48.5	35.5	68.0	40.5	38.0	74.0	61.5	66.0

Experiment 2 - 100 percent vegetative cover

	P_1			P_2			P_3		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
T_1	95.0	126.0	102.0	86.5	79.0	61.5	51.0	114.5	76.5
T_2	82.3	60.7	46.3	30.0	75.3	36.0	73.7	31.3	52.0
T_3	39.7	37.0	32.3	31.3	34.3	16.0	28.7	27.7	19.7

P_1 = 24 hour sampling period

P_2 = 48 hour sampling period

P_3 = 72 hour sampling period

R_1, R_2, R_3 = Replications of basic experiment

T_1 = Controls - average of two samples

T_2 = 0.05 p.p.m. malathion - average of two samples in Experiment 1 and three samples in Experiment 2

T_3 = 0.10 p.p.m. malathion - average of two samples in Experiment 1 and three samples in Experiment 2

Table 6. Results of principal field experiments on the effects of DDT on midge larvae, Bear River Refuge, 1961

Experiment 3 - 30 percent vegetative cover

	P_1			P_2			P_3		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
T_1	236.0	132.5	125.5	58.5	79.0	72.0	176.0	108.5	98.0
T_2	46.7	23.0	24.3	8.0	9.3	7.7	44.3	10.0	9.0
T_3	15.7	16.7	22.0	2.7	2.3	1.3	2.3	3.3	2.3

Experiment 4 - no vegetative cover

	P_1			P_2			P_3		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
T_1	109.0	100.0	167.0	139.0	100.5	174.0	193.0	149.5	285.5
T_2	124.7	67.3	102.3	103.7	81.0	126.3	107.7	141.0	154.7
T_3	46.7	70.3	62.7	71.0	51.3	49.0	73.7	80.7	42.7

P_1 = 24 hour sampling period

P_2 = 48 hour sampling period

P_3 = 72 hour sampling period

R_1, R_2, R_3 = Replications of basic experiment

T_1 = Controls - average of two samples

T_2 = Average of three samples in both experiments. Treatment was 0.10 p.p.m. in Experiment 3 and 0.025 p.p.m. in Experiment 4.

T_3 = Average of three samples in both experiments. Treatment was 0.50 p.p.m. in Experiment 3 and 0.05 p.p.m. in Experiment 4.

Table 7. Chi-square analysis of goodness-of-fit on survival of midge larvae after treatment with insecticide malathion

Sources of variation	df	Probability of success	X^2	Level of significance
Experiment 1 - 30 percent vegetative cover				
Period 1	1	.340	.207	N.S.
Period 2	1	.348	1.120	N.S.
Period 3	1	.559	28.659	*
Experiment 2 - 100 percent vegetative cover				
Period 1	1	.462	40.161	*
Period 2	1	.491	31.412	*
Period 3	1	.342	14.990	*

*Significant at the .01 level of probability
N.S. Not significant

Table 8. Chi-square analysis of goodness-of-fit on survival of midge larvae after treatment with insecticide DDT

Sources of variation	df	Probability of success	X^2	Level of significance
Experiment 3 - 30 percent vegetative cover				
Period 1	1	.160	6.587	N.S.
Period 2	1	.075	12.074	*
Period 3	1	.093	47.530	*
Experiment 4 - no vegetative cover				
Period 1	1	.566	9.878	*
Period 2	1	.583	97.082	*
Period 3	1	.478	135.801	*

*Significant at the .01 level of probability
N.S. Not significant

Table 9. Chi-square test of homogeneity of replicates within Experiment 1 for insecticide malathion in 30 percent vegetative cover

Sources of variation	df	Probability of success	X^2	Level of significance
Period 1 - 24 hours				
Replicate 1	1	.435	2.657	N.S.
Replicate 2	1	.312	1.232	N.S.
Replicate 3	1	.281	1.881	N.S.
Pooled X^2	3		5.771	N.S.
Sum X^2	1		.207	N.S.
Heterogeneity X^2	2		5.564	N.S.
Period 2 - 48 hours				
Replicate 1	1	.491	12.600	*
Replicate 2	1	.256	3.346	N.S.
Replicate 3	1	.357	10.301	*
Pooled X^2	3		26.247	*
Sum X^2	1		1.120	N.S.
Heterogeneity X^2	2		25.127	*
Period 3 - 72 hours				
Replicate 1	1	.697	34.003	*
Replicate 2	1	.386	.383	N.S.
Replicate 3	1	.635	13.422	*
Pooled X^2	3		47.808	*
Sum X^2	1		28.659	*
Heterogeneity X^2	2		19.149	*

*Significant at the .01 level of probability
N.S. Not significant

Table 10. Chi-square test of homogeneity of replicates within Experiment 2 for insecticide malathion in 100 percent vegetative cover

Sources of variation	df	Probability of success	X^2	Level of significance
Period 1 - 24 hours				
Replicate 1	1	.642	41.563	*
Replicate 2	1	.388	9.389	*
Replicate 3	1	.385	4.057	N.S.
Pooled X^2	3		55.009	*
Sum X^2	1		40.161	*
Heterogeneity X^2	2		14.848	*
Period 2 - 48 hours				
Replicate 1	1	.354	.043	N.S.
Replicate 2	1	.694	50.069	*
Replicate 3	1	.423	13.326	*
Pooled X^2	3		67.665	*
Sum X^2	1		31.412	*
Heterogeneity X^2	2		36.253	*
Period 3 - 72 hours				
Replicate 1				
Replicate 2	1	.258	.296	N.S.
Replicate 3	1	.469	27.383	*
Pooled X^2	3		27.679	*
Sum X^2	1		9.878	*
Heterogeneity X^2	2		17.801	*

*Significant at the .01 level of probability
N.S. Not significant

Table 11. Chi-square of homogeneity of replicates within Experiment 3 for insecticide DDT in 30 percent vegetative cover

Sources of variation	df	Probability of success	X^2	Level of significance
Period 1 - 24 hours				
Replicate 1	1	.132	17.747	*
Replicate 2	1	.188	.357	N.S.
Replicate 3	1	.184	.140	N.S.
Pooled X^2	3		18.244	*
Sum X^2	1		6.587	N.S.
Heterogeneity X^2	2		11.657	*
Period 2 - 48 hours				
Replicate 1	1	.091	2.889	N.S.
Replicate 2	1	.073	4.559	N.S.
Replicate 3	1	.063	4.855	N.S.
Pooled X^2	3		12.303	*
Sum X^2	1		12.074	*
Heterogeneity X^2	2		.229	N.S.
Period 3 - 72 hours				
Replicate 1	1	.132	43.630	*
Replicate 2	1	.061	3.596	N.S.
Replicate 3	1	.058	4.216	N.S.
Pooled X^2	3		51.442	*
Sum X^2	1		47.530	*
Heterogeneity X^2	2		3.912	N.S.

*Significant at the .01 level of probability
N.S. Not significant

Table 12. Chi-square test of homogeneity of replicates within Experiment 4 for insecticide DDT with no vegetative cover

Sources of variation	df	Probability of success	X^2	Level of significance
Period 1 - 24 hours				
Replicate 1				
Replicate 2	1	.688	.210	N.S.
Replicate 3	1	.494	18.783	*
Pooled X^2	3		18.993	*
Sum X^2	1		9.878	*
Heterogeneity X^2	2		9.115	N.S.
Period 2 - 48 hours				
Replicate 1	1	.628	16.472	*
Replicate 2	1	.756	2.699	N.S.
Replicate 3	1	.504	68.685	*
Pooled X^2	3		87.855	*
Sum X^2	1		97.082	*
Heterogeneity X^2	2		9.227	*
Period 3 - 72 hours				
Replicate 1	1	.470	12.023	*
Replicate 2	1	.741	63.440	*
Replicate 3	1	.346	97.122	*
Pooled X^2	3		172.585	*
Sum X^2	1		135.801	*
Heterogeneity X^2	2		36.784	*

*Significant at the .01 level of probability
N.S. Not significant