TOXICOLOGICAL INVESTIGATIONS ON THE ONION MAGGOT _HYLEMYA ANTIQUA_ (MEIG.)

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TOXICOLOGICAL INVESTIGATIONS ON THE ONION MAGGOT

HYLEMYA ANTIQUA (MEIG.)

BY

HUSEIN M. ELMOSA

A THESIS

Submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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AN ABSTRACT

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Approved Ray Hutson

ABSTRACT

The respective toxicities of 15 chlorinated hydrocarbon and phosphate insecticides to the onion maggot adults *Hylemya antiqua* (Meig.), were determined by topical application. The insecticides tested were methyl parathion, dimethoate, Diazinon (O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), parathion, Bayer 21/199 (Co-ral) (O-(3-chloro-4-methylumbelliferone) O,O-diethyl phosphorothioate), American Cyanamid 18133 (O,O-diethyl O-2-pyrazinyl phosphorothioate), trithion, American Cyanamid 18706 (O,O-diethyl-S-(N-ethylcarbamoylmethyl) phosphorodithioate), Thimet (O,O-diethyl S-(ethylthio) methyl phosphorodithioate), ethion, DDT, Guthion (O,O-dimethyl S-(4-oxo-3H-1, 2, 3-benzotriazine-3-methyl) phosphorodithioate), endrin, dieldrin and heptachlor.

The results showed that of the insecticides tested methyl parathion, dimethoate and Diazinon were the most toxic to the onion fly. Heptachlor, dieldrin, endrin and DDT were the least toxic.

A syringe-microburet which was modified for topical application and used to apply the insecticide solutions to the insect is described.

Onion maggot adults from Michigan, suspected of being resistant to chlorinated hydrocarbon insecticides, were

compared with a laboratory strain of onion flies for susceptibility to dieldrin. The data confirmed that the flies obtained from Michigan were resistant to dieldrin.

Experiments were conducted to determine the effect of different pre-treatment and post-treatment temperatures, various post-treatment relative humidities, age, sex, stage of development and site of application on the toxicity of dieldrin and ethion to the onion maggot adults.

Pre-treatment temperature did not affect the toxicity of either dieldrin or ethion to the onion maggot adults. Post-treatment temperatures had a profound effect on the toxicity of both dieldrin and ethion to the fly. In general, the higher the temperature the greater the toxicity. Variations in post-treatment relative humidity did not affect the toxicity of either dieldrin or ethion to the onion maggot adults.

There was no significant difference in the effect of age on the toxicity of dieldrin and ethion to the onion maggot adults. Although greater kills were obtained with the older flies than the young ones, there was more natural mortality with the older flies. The lesser kills obtained with the younger flies were attributed to vigor resistance.

Female flies were significantly more resistant to the action of dieldrin and ethion than the males. There was no significant difference in weight between female and male flies. It was suggested that the difference in resistance between female and male flies may be a case of physiological resistance and worth investigating. The sex ratio of the onion fly was 55 per cent males to 45 per cent females. This was not statistically significant from a 1:1 ratio.

The onion maggot adults were less resistant to the action of both dieldrin and ethion than the second instar larvae, third instar larvae and pupae. Because at the doses used dieldrin was not effective against the second instar larvae, third instar larvae and pupae, no comparison could be made between these stages. The order of resistance in the stages of development of the onion maggot to ethion was pupa > third instar larva > and adult.

Although there was no significant difference in the effect of the site of application on the toxicity of dieldrin and ethion to the onion maggot adults, greater kills were obtained when ethion was applied to the mesonotum of individual insects than when applied to the dorsum of the abdomen.

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INTRODUCTION

The onion maggot, *Hylemya antiqua* (Meig.) is one of the most important pest of onions in the United States. It was introduced to this country in the first half of the nineteenth century. Harris (1852) reported heavy damage caused by the insect in Massachusetts in 1841. At the present time the onion maggot is found in practically every region in the country where onions are grown. In Michigan, Merrill and Hutson (1953) reported that from a total of 52 species of flies attacking onions, *Hylemya antiqua* caused most of the damage to Michigan grown onions.

Several methods have been employed to control the onion maggot since its introduction into this country. Calomel was used with excellent results for several years but was expensive and phytotoxic. After 1944 with the development of the chlorinated hydrocarbon insecticides, the onion maggot was satisfactorily controlled with these materials.

As early as 1957 there were reports by various investigators from different parts of the country that the onion maggot had developed resistance to the chlorinated hydrocarbon insecticides. In Michigan excellent control had been obtained with the chlorinated hydrocarbon insecticides since 1951, but in 1957 and 1958 the same insecticides failed to control the pest (Guyer & Wells, 1959). The failure of these insecticides to control this insect in the field in 1958 initiated the study of various facets of the insect's biology as well as the laboratory evaluation of various materials.

The insecticides tested were topically applied to the insect by a modified syringe-microburet and the results are described herein.

The effect of temperature on the toxicity of a number of insecticides has been reported by previous workers. A brief summary of these reports on the following pages shows two general trends in the effect of temperature on toxicity. One is an increase in toxicity at the lower temperatures, and the other an increase in toxicity at the higher temperatures. The effect of temperature on the mortality of insects may be of considerable practical importance. This was indicated by Hoffman and Lindquist (1949) when they wrote "From a practical standpoint it appears that house flies are controlled with low dosages of DDT in northern or cool climates. For example, the states of Washington and Oregon have recommended a wettable-powder spray containing only 0.5 per cent of DDT for residue spraying in barns. Because only 2 to 6 ml. of fluid can be applied on a vertical wood surface before run-off, the dosages used ranged from 15 to 50 mg. of active ingredient per square foot. According to extension workers these dosages have given good control. In comparison, the southern States recommended a dosage of 200 mg. per square foot for satisfactory control."

Because relative humidity is closely related to temperature, a study of the effect of various relative humidities on the toxicity of both dieldrin and ethion was considered to be important.

In toxicological investigations it is of importance to be able to obtain consistant results. Research on insecticides involves two components, the insect and the insecticide; and in order to obtain repeatable results both of them must be standardised. It is well known that in a given species of insect, large variation in susceptibility may occur. Busvine (1957) listed the sources of variability in insects under two headings, intrinsic differences and extrinsic differences. Under intrinsic factors which affect the susceptibility of insects to insecticides he listed sex, age and the stage of development. Under extrinsic factors temperature and relative humidity were listed. Also the brief review of literature summarized on the following pages revealed that the relative toxicity of insecticides is affected by such factors as age, sex, site of application and the stage of development of the insect. These factors also could be of practical importance in control, because different stages, ages and sexes of the insect may be present at the same time. This is especially true in case of the stage of development of the animal because with any given species large variations in susceptibility may occur throughout the life cycle. It would seem important for the purpose of practical control that some knowledge be available on the change of resistance with the stage of development in order to establish the correct timing of application.

With these points in view investigations on the effect of temperature, relative humidity, sex, age, site of application and the stage of development on the toxicity of insecticides to the onion maggot were undertaken. The insecticides chosen to be tested were dieldrin and ethion because the insect is resistant to the former but not to the latter. This was done with the hope that some ideas might be obtained as to the mechanism of resistance of the onion maggot to the chlorinated hydrocarbon insecticides.

REVIEW OF LITERATURE

I. Relative toxicities of insecticides to onion maggots.

Considerable attention has been devoted to the control of the onion maggot both in North America and Europe. The control measures employed prior to 1938 were classified by Wright (1938) into four groups:

1. The use of deterrents to prevent egg laying.
2. Trapping and killing adults by poison baits.
3. The use of larval poisons.
4. Cultural measures.

The first category included, among other materials, powdered naphthalene, tar oil-sand mixture, and paraffin emulsions. The disadvantages in the use of all deterrents were the necessity for their constant renewal and when applied over a large area, the adult flies appeared to become accustomed to their presence and suffer little inconvenience.

The use of poisonous baits was not attended with much success and could not be relied upon. Among larval poisons used were various mercury salts, corrosive sublimate solution, and calomel. Satisfactory control has been obtained by the use of powdered calomel, which could be applied to the seed coat. Cultural operations were of little value alone in controlling heavy infestations but could be important in combating minor attacks.

After 1944, with the interest in hydrocarbon insecticides, sparked by DDT, control of the onion maggot appeared to be imminent. McLeod (1946) tried a total of twenty-nine different treatments, involving a number of materials and combinations, to control the onion maggot. A calomel treatment gave almost perfect control but was considered impractical because of its high cost and the retarded growth of treated plots. BHC and DDT also gave satisfactory control. Munro (1947) in North Dakota obtained 90 per cent control of the maggot by means of spraying onions with 1 ounce of 25 per cent DDT per gallon of water. Maan (1947) using DDT in the powder form as seed treatment, at the rate of 40 grams per 100 grams of seeds, obtained better control than with calomel. Morrison and Crowell (1952) reported that aldrin and chlordane gave economical control of the onion maggot in Oregon. Tozloski (1954) working in Massachusetts showed that 10 per cent DDT and 1 per cent dieldrin applied to onion seed as dry materials, and agitated to produce an evenly dispersed mixture of seed and insecticides, gave the most effective control of the onion maggot. Shirck (1957) reported that coating the seed with aldrin, dieldrin, heptachlor, or chlordane emulsions gave the best control on seed crops.

Doane & Chapman (1957) reported that populations of onion maggots which were extremely resistant to several chlorinated hydrocarbon insecticides were present in some localities of Wisconsin. Howitt (1958) stated that prior to 1953 the onion fly was readily controlled with aldrin, heptachlor, chlordane, and dieldrin. After 1953 the chlorinated hydrocarbons became less effective. He also reported that cooperative experiments conducted in Washington, British Columbia, Oregon and Idaho demonstrated that strains of onion maggot highly resistant to the chlorinated hydrocarbons were present at least in certain areas of the Pacific Northwest. In Michigan Drew and Guyer (1958) stated that the onion flies emerging from pupae collected from the field were resistant to chlorinated hydrocarbon insecticides.

The increased failure of the chlorinated hydrocarbon insecticides to control this pest prompted the laboratory evaluations reported in the present paper.

II. Effect of pre-treatment temperature on the toxicity of insecticides to insects.

Relatively little work has been done on the effect of pre-treatment temperature on the toxicity of insecticides to insects. Cotton (1932) reported that confused flour beetles preconditioned at lower temperatures for several days required a higher concentration of ethylene oxide to give 100 per cent mortality. Quayle (1934) revealed that in fumigation of scale insects higher kills were obtained when the insects had been conditioned at a lower temperature. Lindgren & Dickson (1941) working on the fumigation of the purple scale with hydrocyanic acid found that insects pre-conditioned at 50°F. were more easily killed than those pre-conditioned at 75°F.; while the latter were killed more readily than those pre-conditioned at 90°F. Munson (1953) reported that American cockroaches were less resistant to the action of DDT when pre-conditioned at 34°C. than when they were pre-conditioned at 17°C. Munson et. al. (1954) found that the American cockroach, *Periplaneta americana* (L.); the vinegar fly, *Drosophila melanogaster* Meig.; and the saw-toothed grain beetle were less susceptible to DDT when kept at low temperature before treatment than when kept at high temperature. Crauford-Benson (1938) showed that temperature before treatment has a definite effect on the toxicity of derris to the foreign grain beetle, *Ahasverus advena* Waltl. The beetles were less susceptible to derris when reared at 20°C. than when kept at 25°C.

III. Effect of post-treatment temperature on the toxicity of insecticides to insects.

Lindquist et. al. (1944) studied the effect of after-treatment temperature on the toxicity of DDT to house flies. Flies were exposed at 65°F. in containers treated with DDT and when all were down they were divided and transferred to clean cages and kept at different temperatures. The results showed 100, 92, and 86 per cent kill when flies were kept at 70°, 90°, and 100°F. respectively. House flies were exposed by Lindquist et. al. (1945) to pyrethrum films and kept in recovery cages at 70°, 80°, 90° and 100°F. The authors showed that mortality was higher at higher temperatures. Härliger (1949) determined the effect of post-treatment temperature on the toxicity of DDT, calcium arsenate, BHC and parathion to the honey bee. When the insects were fed the poison from a micropipette, then placed in containers at 20°, 28°, and 36°C. DDT was much more toxic at lower temperatures, calcium arsenate more toxic at higher temperatures. The post-treatment temperature had no influence on the toxicity of BHC or parathion to the honey bee. Weaver (1949) in an experiment to determine the relative toxicity of certain organic insecticides to the honey bees found that after-treatment temperature has a profound effect on the toxicity of chlordane to the honey bees. At temperatures below 76°C. chlordane was only slightly toxic but at 86°F. its toxicity increased greatly. Pradhan (1949) reported that when adult Tribolium castaneum (Hbst.) were exposed to a film of DDT for about 24 hours at the same temperature and then kept away from it at different temperatures there was a higher kill at the lower temperature. Hoffman & Lindquist (1949) reported that DDT and methoxychlor gave faster knockdown of house flies at 70°F. than at 90°F. These compounds also caused greater mortality at low temperature than at high temperature. The reverse was true with heptachlor, parathion, chlordane, and toxaphene. Guthrie (1950) established the effect of temperature on toxicity of certain organic insecticides applied topically to the German cockroach. The temperature was regulated at 14.5°, 23°, and 32°C. and the treated roaches were subjected to one of the three temperatures for five days. The results showed that DDT, pyrethrum, and lindane were more toxic at the lower temperatures while the reverse was true with aldrin and dieldrin. Vinson et. al. (1952) tested the effect of post-treatment temperature on the toxicity of topically applied and injected DDT to the American cockroach females. Both topical and injected doses of DDT gave less kill at the higher temperature. The LD50 values were 5 to 10 micrograms at 15°C. and 75 to 100 micrograms at 35°C. for topically applied DDT and 2 to 3 micrograms at 15° C. and 20 to 25 micrograms at 35° C. for injected DDT. Cressman *et. al*. (1953) working in California on some factors influencing the effectiveness of parathion against California red scale, found that after treatment temperature had no effect on the toxicity of parathion to the scale. Roth *et. al*. (1953) conducted an experiment to determine the amounts of DDT actually absorbed by the house flies at different temperatures. He found that the flies absorbed 64 per cent more of DDT with a lower mortality when kept at 90° F. than when held at 70° F.

IV. The effect of post-treatment relative humidity on the toxicity of insecticides to insects.

Pradhan (1949) studied the effect of relative humidity on the toxicity of insecticides to insects. He reviewed the literature up to 1949 and concluded "a perusal of literature does not help to deduce any general principal regarding the effect of humidity on the toxicity of insecticides. In some cases the rise in relative humidity has been found to increase the percentage kill and in others to decrease it. There are also cases in which changes in relative humidity have been found to have no appreciable effect on toxicity."

Gains and Dean (1949) investigated the effect of temperature and humidity on the toxicity of certain insecticides to the boll weevil. They reported that high humidity reduced the toxicity of calcium arsenate, toxaphene, and chlordane. Collins and King (1953) studied the effect of humidity on the toxicity of residual films of DDT to the adult of Tribolium confusum Duv. Their results indicated that with the temperature kept constant at 80° F. there was no significant difference in mortality at 33 per cent and 59 per cent relative humidity. Glynne-Jones and Edwards (1952) tested the effect of after-treatment humidity on toxicity of suspensions of acid DNOC and a solution of the sodium salt of DNOC. They found that no difference occurred with the acid DNOC when humidity was changed from 45 - 50 per cent relative humidity to 85 - 90 per cent, but the sodium salt was much more toxic at the higher humidity.

V. Effect of age on the susceptibility of insects to insecticides.

Simanton & Miller (1937) investigated house fly age as a factor in susceptibility to pyrethrum sprays. They tested 1.5 hour, 6 hour, 21 hour, 45 hour, 3 day, 4 day, 7 day and 10 day age groups. The results showed that very young house flies were more easily paralyzed but less easily killed than were older flies. Craufurd-Benson (1938) reported on the resistance of adult Ahasverus advena

Waltl. at different ages to derris. He showed that the insects were less resistant to the insecticides at both very young and increasing ages. McLeod (1944) experimented on the effect of age of the fruit flies on resistance to nicotine sulphate solution. He found that 5-day old flies were more susceptible than 3-day old; and the latter were more susceptible than 1-day old flies. David & Bracey (1946) investigated the change of resistance of Aedes aegypti (Linn.) with age to pyrethrum and DDT. The data indicated that there was a progressive decrease in resistance with age. One-day old insects were more resistant than 3 to 4-day old insects; and the latter were less susceptible than 4 to 5-day old insects. Collins and King (1953) tested the resistance of 7-day old and 54 to 58-day old Tribolium confusum Duv. to residual films of DDT. Their results showed that there was no significant difference in resistance between the two ages with an exposure of 3 hours, but there was highly significant difference with the 6-hour exposure. The mortality was higher with the older beetles. Kerr (1954) studied the variation with age of adult D. melanogaster Meig. in the susceptibility to DDT. He showed that susceptibility was high in young flies, but rapidly decreased with age, to a minimum at about 5 days, thereafter increasing rapidly in males and not significantly in females. He concluded that "the need for sexing and standardizing age in flies used for toxicological investigations was thus demonstrated."

VI. Effect of sex on the susceptibility of insects to insecticides.

Simanton & Miller (1937) in a study of house fly age as a factor in susceptibility to pyrethrum sprays showed that the female house fly was more resistant than the male. A dose of pyrethrum which gave 100 per cent male kill resulted in 55.6 per cent female mortality. Murray (1937) reported that the female house fly was more resistant to the action of pyrethrins than the male. Gough (1940) working on the toxicity of sulfur dioxide to the bed-bug reported that the males and the females were equal in susceptibility. David & Bracey (1946) investigated the relative resistance of male and female _Aedes aegypti_ (Linn.) to insecticides. They found that the females were more resistant than the males to pyrethrins, and to a mixture of pyrethrins and DDT. Barber & Schmitt (1948) working on house fly resistance to DDT residue sprays showed that the females were more resistant than the males. Menusan (1948) experimented on the toxicity of insecticides administered in various ways to several species of insects. He reported that the difference in susceptibility to insecticides between male and female cockroaches varied greatly depending on the insecticide and the method of testing. There was less sex difference in resistance to an insecticide when the material was injected into the blood stream than when the same material was fed or tested for contact toxicity. All materials tested showed the male to be less resistant than the female, with the exception of dichloro ethyl ether where the reverse was true. MacCuaige (1936) determined the resistance of the desert locust and the African migratory locust to DNC in relation to weight, age, and sex. He found no difference in susceptibility of the sexes to DNC.

VII. Effect of stage of development on resistance of insects to insecticides.

Campbell (1926) compared the susceptibility of the last four instars of silkworm larvae to arsenic. His results showed that second instar were 3.5 times as susceptible to arsenic as the fifth instar larvae. He concluded that the susceptibility of the silkworm to arsenic decreased during its larval development. Cotton (1932) working on the relation of the respiratory metabolism of insects to their susceptibility to fumigants showed that when test lots of 50 adults, 25 larvae and 25 pupae of *T. confusum* Duv. were placed in 6-liter glass flasks and fumigated for 5 hours with carbon disulphide, the adults were killed with a dose of 125 mg. per liter, larvae 163 mg. per liter and pupae 314 mg. per liter. Lindgren (1935) investigated the order of resistance of different stages of Tribolium confusum Duv. to various fumigants and reported that with carbon disulphide the resistance of the egg $>$ pupa $>$ adult $>$ larva (low humidity), the pupa $>$ egg $>$ adult $>$ larva (high humidity); with chloropicrin, the resistance of the egg $>$ pupa $>$ adult $>$ larva; with ethylene oxide, the resistance of the pupa $>$ adult $>$ larva $>$ egg. Gunderson and Strand (1939) studied the toxicity of hydrogen cyanide, chloropicrin and ethylene oxide to eggs, nymphs, and adults of the bed-bug, Cimex lectularius L. They showed that the eggs of the bed-bug were less resistant to hydrogen cyanide and ethylene oxide than the nymphs and adults. The eggs were much more resistant to chloropicrin. DuChanois (1947) studied the toxicity of gamma BHC to the pre-imaginal stages of the house fly. The stages used were the eggs, second instar larva, third instar larva, and pupa. He reported that gamma BHC was not toxic to ova. Second instar larvae were the most susceptible and the pupal stage was considerably more resistant than the larval stages. Cochran (1955) compared last instar male and female nymphs with adult females as to their susceptibility to DDT. His findings indicated that there was no difference between adult female, male nymphs, or female nymphs of the American cockroach with respect to DDT susceptibility. Benschoter (1960) investigated the susceptibility of life stages of the Mexican fruit fly to fumigation with ethylene chlorobromide. He found that the order of susceptibility was larva > adult > egg > pupa.

VIII. Effect of loci of application on susceptibility of insects to insecticides.

O'Kane et. al. (1933) studied the effect of nicotine, pyrethrum, strychinne sulphate, cocoanut oil, fatty acid, white petrolium oil and kerosene applied to different spots of the American cockroach, Oriental cockroach, German cockroach and the larvae of the mealworm. They found that the reaction rate to a given dose depended on the location of application. Wilson (1949) investigated the effect of pyrethrum and two of its synergists, piperonyl butoxide and piperonyl cyclonene on house flies and blow flies when applied to different parts of the body. He found that when placed on the top of the thorax, 0.1 microgram of pyrethrins knocked down only 40 per cent of house flies and 85 per cent of blow flies. The same dose knocked down 100 per cent of the flies when applied to the mouth parts. Bull and Beck (1951) working on the role of the circulatory and nervous systems in the toxic action of parathion to the American cockroach reported that the site of application was important. They found that the closer the site of application to the central nervous system the shorter the knockdown time. Fisher (1952) reported on the importance of the locus of application on the effectiveness of DDT for the house fly. His results showed that lower mortalities were produced as the locus of application moved distally along the legs, and posteriorly from head to abdomen. Hoskins et. al. (1952) reported on some factors influencing the contact of chemical and test insect. He found that application of lindane to the tarsi of the house fly was more effective than to the ventral surface of the thorax. MacCuaig (1956) studied the resistance of the desert locust and the African migratory locust to DNC. His findings showed that the site of application had relatively little effect on the kills obtained, except when the dose was applied to the femur or wings.

MATERIALS AND METHODS

Method of Obtaining Flies

The flies used in these experiments, with the exception of experiment 1, part II, were obtained from pupae which had been collected from infested Michigan onion fields. The pupae were stored at 33° F. When flies were required for testing, pupae were transferred to a temperature cabinet at 77° F. and adults allowed to emerge. After emergence the flies were held in a small insect cage until they were taken to the laboratory to be tested. The method of obtaining the flies used in experiment I, part II, will be described under rearing methods. The food for the flies consisted of honey and brewers yeast prepared on a moist slice of bread. The petri dish containing the food was placed in the cage. Also small droplets of a mixture of honey and brewers yeast were placed on top of the cage. Water was supplied by cotton wicks inserted through a cork in a small Erlenmeyer flask.

Rearing Methods

The few references in the literature concerning successful rearing techniques for the onion fly were summarized by Workman (1958).

In the present research, attempts were made to rear insecticide-susceptible onion flies obtained from Cornell University, and chlorinated hydrocarbon-resistant onion flies from Michigan, in a basement room at Michigan State University. An air conditioner kept the temperature in the rearing room between 65° F. and 75° F. at all times. Cages 20 inches long, 16 inches wide, and 16 inches high were used for rearing the onion fly. The cages were covered with 16 mesh screen with cloth sleeve openings at the front.

Light for the rearing cages was supplied by four, forty watt, white fluorescent lamps. The four foot lamps were suspended 18 inches above the rearing cages. The lights were controlled by a time switch and were in operation from 8 a.m. until midnight, giving 16 hours light each day.

At first the food for the adult flies consisted of honey and brewers yeast prepared on a moist slice of bread in a petri dish and placed in the rearing cages. The flies from Cornell University produced eggs satisfactorily with this diet, but the flies from Michigan produced very few eggs. The diet was then changed to milk (with formaldehyde, two parts per thousand, to retard spoilage) and honey. To 25 parts of honey was mixed one part of a liquid vitamin concentrate containing vitamins A, D, B_1, B_2, B_6, C, E, niacinamide, pantothenic acid, choline chloride, and inositol. Milk was supplied to the onion flies in small jars inverted on the screen on the top of the cage with cheese cloth placed over the jar opening to prevent spillage. The honey was placed on top of the cage in small droplets. Satisfactory egg production resulted from this diet with the insecticide-susceptible flies from Cornell University. Chlorinated hydrocarbon-resistant onion flies from Michigan still failed to produce many eggs. This diet was the same one used by Workman (1958) in rearing the onion fly successfully in the greenhouse in Oregon.

Small onion bulbs were planted in 16 x 10 inch enamel pans containing muck soil and placed in the rearing cages. Females oviposited on the onion plants or on the muck. Also halves of large onion bulbs were placed cut side down on the soil for the emerging larvae to feed on.

It is important to note that the onion flies obtained from Michigan and reared simultaneously by the same methods as the flies from Cornell University, failed to produce eggs satisfactorily. There is no complete explanation for this; however, Hoskins and Gordon (1956) stated that the fertility of resistant insects was often so low that propagation was impossible. This may have been the case with the chlorinated hydrocarbon-resistant onion flies.

Topical Application Apparatus

In topical application of insecticides to individual insects accuracy and ease of operation are most important. Topical application is usually accomplished by treating individual insects with small volumes of insecticide solutions. Trevan (1922) suggested that small droplets of fluid could be produced by using the pitched thread of a machinist's micrometer to drive the plunger of a small syringe. After Trevan developed the micrometer-syringe, topical application was widely employed in insect toxicology, and most of the topical application apparatus in use today embodies Trevan's principle (Metcalf, 1952). The apparatus used for topical application in this research was a syringe-microburet, made by the Micro-Metric Instrument Company of Cleveland, Ohio. The syringe-microburet (Fig. 1) employs a thumbscrew-operated shaft which advances the stem of the direct-dial micrometer gauge. This stem, which is carried through the gauge, advances the plunger of the syringe and delivers the insecticide solution through the syringe. The syringe used in the work reported here delivers a volume of 0.2 microliter of solution for each unit of displacement on the dial. Syringes of other sizes especially calibrated for use with this instrument are available from the manufacturer.

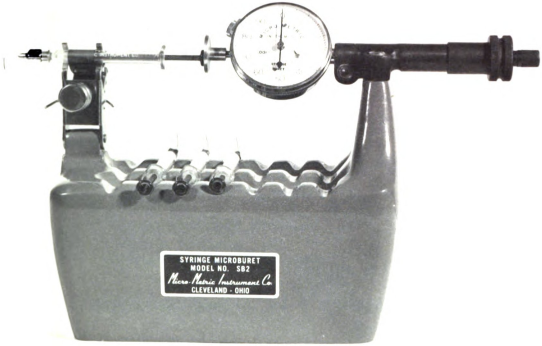


Figure 1. The modified syringe-microburet

Because this apparatus is intended primarily for microtitration, the manufacturer supplies glass buret tips which can be attached to the syringes. With these glass buret tips attached it was impossible to deliver the small volumes of insecticide solutions necessary for topical application. In order to use this syringe-microburet for topical application, it was necessary to replace the buret tips by 26-gauge hypodermic needles polished at the tips with a fine abrasive stone and bent at 90° angle.

The advantage of the modified syringe-microburet is threefold:

1. Most important, volume deliveries are made quickly and accurately, and they are read on a direct-reading dial gauge.
2. The syringes fit interchangeably into a V-block at the delivery end of the buret stand.
3. Storage facilities for the filled syringes are provided on the buret stand.

Temperature Control

Four electric incubators with thermostatic controls were employed. In order to produce temperatures below room temperature it was necessary to place two of the electric incubators in a cold room. One of these was regulated at 60° F. and the other at 75° F. One of the two remaining cabinets was regulated at 77° F. and the other at 90° F.

Humidity Control

Constant humidities were obtained by supersaturated* salt solutions. Chemically pure salts appropriate for each relative humidity at different temperatures were used (Hodgman, 1949). They were placed at the bottom of desiccators and enough distilled water was added to cover the surface of the salt.

Method of Treating Onion Flies with the Insecticide Solutions

In the following experiments, individual insects were treated with the respective insecticide solutions after they had been anesthetized with carbon dioxide. The carbon dioxide was obtained as a liquified gas in cylinders under a pressure of about 800 pounds per square inch. The gas was allowed to escape through a pressure reducing valve at a slow rate through a 4 inch Büchner funnel in which the test insects were confined. The modified syringe-microburet was used to apply the insecticide solutions to the individual insects (Fig. 2).

After treatment the flies were held in pint-size

*Should read saturated, with an excess of salt present, here and wherever mentioned in this context later.

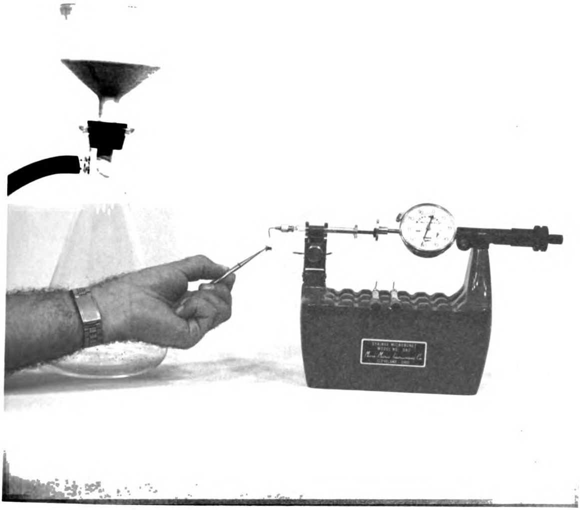


Figure 2. The method by which the toxicant was topically applied to the insects.

ice cream cartons which were covered with cheese cloth to prevent the escape of treated flies. The food for the treated insects consisted of honey and brewers yeast placed on top of the cheese cloth. Water was supplied in small droplets added to the ice cream cartons.

Insecticides

The insecticides used, their purity, and the sources from which they were obtained are listed below. The names used here are those approved as of April, 1959 by the Committee on Insecticide Terminology of the Entomological Society of America.

Insecticide	Per Cent Purity	Source
Methyl parathion	80	Nutritional Biochemical Corp., Cleveland, Ohio
Dimethoate	Technical	American Cyanamid Co. Stamford, Connecticut
Diazinon (O,O-diethyl O-(2-isoprpyl-4-methyl-6-pyrimidinyl) phosphorothioate	Technical	Geigy Chemical Corp. Mcintosh, Alabama
Parathion	Technical	Nutritional Biochemical Corp., Cleveland, Ohio
Bayer 21/199 (Co-ral) (O-(3-chloro-4-methylumbellifer-one) O,O-diethyl phos-phorothioate)	Technical	Chemagro Corp. New York, New York

Insecticide	Per Cent Purity	Source
American Cyanamid 18133 (O,O-diethyl O-2-pyrazinyl phosphorothioate)	Technical	American Cyanamid Co. Stamford, Connecticut
Trithion (S-(chlorophenylthio) methyl O,O-diethyl phosphorothioate)	Technical	Stauffer Chemical Corp. Richmond, California
American Cyanamid 18706 (O,O-dimethyl-S-(N-ethyl-carbamoylmethyl) phosphorothioate	Technical	American Cyanamid Co. Stamford, Connecticut
Thimet (O,O-diethyl S-(ethylio) methyl phosphorothioate)	Technical	American Cyanamid Co. Stamford, Connecticut
Ethion	Technical	Niagara Chemical Div. Food Machinery and Chemical Corp., Middleport, New York
DDT	Technical	Nutritional Biochemical Corp., Cleveland, Ohio
Guthion (O,O-dimethyl S-(4-oxo-1,2,3-benzotriazine-3-methyl) phosphorodithioate)	16.7	Chemagro Corp. New York, New York
Endrin	Technical	Velsicol Chemical Corp. Chicago, Illinois
Dieldrin	85	Nutritional Biochemical Corp., Cleveland, Ohio
Heptachlor	72	Nutritional Ciochemical Corp., Cleveland, Ohio

EXPERIMENTAL PROCEDURES AND RESULTS

Experiment I

Part I

This experiment was conducted in 1958 to determine the respective toxicities of 15 chlorinated hydrocarbon and organic phosphate insecticides to the onion maggot adults. All insecticides tested were of technical grade with the exception of methyl parathion, heptachlor, dieldrin, and Guthion (O,O-dimethyl S-(4-oxo-3H-1,2,3-benzotriazine-e-methyl) phosphorothioate). These were 80 per cent, 72 per cent, 85 per cent, and 16.7 per cent pure respectively.

The LD50 in terms of micrograms of insecticides per gram of fly was determined by the probit analysis method (Finney 1952). An example of the mathematical procedure in determining the LD50 for Thimet by the probit analysis method is shown in the appendix.

Flies 2 to 5-days old, obtained as previously described were used for testing. Each toxicant was dissolved in acetone and applied in minute droplets to the mesonota of individual insects by the syringe-microburet. One microliter of solution was applied to each fly as described previously. At least four dose levels of each insecticide were used to determine the LD50. Four to five replicates of 10 flies each were used at each dose level. Also a control treatment of 10 flies was conducted with each replicate. These controls received acetone only. Mortality counts were taken 24 hours after treatment. The criterion for mortality was the inability of the flies to show active locomotion.

The LD50 in micrograms of toxicant per gram of fly, 95 per cent confidence limits, and the slopes of the log. dose-probit lines for the 15 insecticides tested are presented in Table 1. The most effective insecticides against the onion maggot were methyl parathion, dimethoate, and Diazinon (O,O-diethyl O-(2-isopropyl-4 methyl-6-pyrimidinyl) phosphorothioate). The least effective were the four chlorinated hydrocarbon insecticides tested. It is to be noted that the sample of Guthion used was only 16.7 per cent pure and was therefore not comparable to the samples of other materials. By calculation, it may be estimated that a sample containing 83.5 per cent Guthion (5 times as pure as used in the laboratory) would have an LD50 of 4.59 micrograms per gram of fly, and would be comparable to Trithion (S-(p-chlorophenylthio) methyl O,O-diethyl phosphorodithioate) in toxicity.

Table 1 -- Toxicities of 15 insecticides as determined by topical application to the onion fly.

Insecticide	LD50 in microgram per gram fly	95% confidence limits	Slope of Log. dose probit line
Methyl parathion	1.30	0.95-1.80	3.45
Dimethoate	1.32	0.71-2.44	2.79
Diazinon	1.35	0-67-2.71	4.94
Parathion	2.09	1.50-2.90	5.73
Bayer 21/199 (Coral)	2.24	1.74-2.88	3.82
Am. Cyanamid 18133	2.24	1.24-4.05	4.76
Trithion	5.72	4.75-6.90	6.05
Am. Cyanamid 18706	6.92	5.70-8.40	5.95
Thimet	7.06	4.38-11.40	3.30
Ethion	7.41	6.05-9.08	6.01
DDT	11.17	7.18-17.38	4.58
Guthion	21.93	16.14-29.93	6.41
Endrin	37.95	24.43-58.88	2.98
Dieldrin	542.00	328-895	2.23
Heptachlor	2,979.00	2,518-3,524	6.42

In a review of arthropod resistance to chemicals Hoskins and Gordon (1956) stated that the LD50 is an index of the mean tolerance or the mean resistance of the insects tested. The LD50, if obtained with wild unexposed insects gives the tolerance of the species, or if obtained with individuals from an area treated with an insecticide it gives a measure of the resistance that has been developed. Determination of the LD50 from time to time will show how the average resistance varies and how it is affected by any chosen procedure, such as continued use of insecticide, substitution of another, or use of none at all. Therefore, the LD50 is a measure of what already happened and gives little indication of what may be expected. For the latter purpose the slope of the log.dose-probit line is much more informative.

The slope of the log.dose-probit line is a measure of the diversity of response or the heterogeneity of the insects toward the toxicant used. In the normal susceptible condition of insects, the slope is great. When resistance begins to develop the slope decreases. This decrease in slope will continue until resistance tends to reach a plateau, and subsequently the slope increases.

This phenomenon seems to be true with the slopes recorded in table 1 for the insecticides tested. Table 1 shows large LD50 and small slopes for endrin and dieldrin. This indicates that the flies are resistant to the two insecticides, and resistance did not reach its limit. It is expected that the onion fly will develop still greater resistance to endrin and dieldrin. On the other hand, both the LD50 and the slope for heptachlor are large. This indicates that the onion fly resistance to heptachlor has reached the limit and no further resistance is expected to develop. Table 1 also shows that both the LD50 and the slope for dimethoate are small. One may speculate that there is a great heterogeneity in the onion fly with respect to resistance to this insecticide and resistance is apt to develop.

It would be interesting to determine the LD50 for the insecticides tested from time to time to show how resistance varies. Besides the LD50 the slope of the log. dose-probit line should be reported, because it is more informative.

Part II

The purpose of this experiment was to determine the LD50 of dieldrin to an insecticide susceptible strain of onion maggot adult. This LD50 would be compared to the LD50 for dieldrin obtained in part I of this experiment with samples of resistant onion flies from Michigan. This experiment was conducted in May, 1960.

The onion flies tested were reared from a culture of flies obtained from Cornell University. As far as is known this culture of flies had had no previous contact with insecticides. The flies since 1958 have been reared in the laboratory at Michigan State University by the previously described method.

The LD50 of dieldrin was determined by the method described in Part I of this experiment. The LD50 and its 95 per cent confidence limits were 1.38 and 0.80-2.39 micrograms per gram of fly. The slope of the log.dose-probit line is 2.009. Table 1 shows that the LD50 with the onion flies obtained from Michigan is 542 micrograms per gram of fly. From this it can be concluded that the samples of onion flies from Michigan are highly resistant to dieldrin.

It is important to note that the difference in weight between the resistant onion flies from Michigan and the susceptible flies obtained from Cornell University was not significant. The individual weights of 20 flies from each of the two localities are recorded in the appendix.

Experiment II

The following experiment was conducted in 1959 to determine the effect of different pre-treatment temperatures on the toxicity of both ethion and dieldrin to chlorinated hydrocarbon resistant onion maggot adults.

Three temperature incubators were employed. One incubator was kept at 60° F., another incubator at 75° F., and the third cabinet was regulated at 90° F. The relative humidity in the incubators was maintained at 53 per cent. One thousand micrograms of dieldrin or 8 micrograms of ethion per gram of fly were applied to 2 to 5-day old flies obtained by the method described previously. Four replicates of 5 males and 5 females of flies were used at each dose level and temperature. Also a control treatment was conducted in conjunction with each replicate. These control flies received acetone only. The flies were conditioned at the specified temperatures for three days. After conditioning the flies were removed from the temperature cabinets and treated with the toxicant at room temperature. The toxicant was dissolved in acetone and applied to the mesonota of individual insects in one microliter amounts by the method described previously. After treatment the flies were held in pint-size ice cream cartons and transferred to a constant temperature cabinet at 77° F. Food and water were supplied to the treated insects. Mortality counts were made 24 hours after treatment.

The results obtained are shown in tables 2 and 3. Analysis of variance of the data indicated that there was no significant difference in the effect of different pretreatment temperatures on the toxicity of dieldrin or ethion to the onion maggot adults.

Table 2 -- Number of adult onion maggots killed with dieldrin after conditioning for three days at different temperatures. Each lot consisted of 10 flies.

Insecticide	Rep.	Pre-treatment temperature		
		60° F.	75° F.	90° F.
Dieldrin	a	7	6	6
	b	6	6	4
	c	6	5	6
	d	5	5	5
	Mean	6.00	5.50	5.25
Control	a	1	1	0
	b	1	0	0
	c	0	1	0
	d	0	0	0
	Mean	.5	.5	0

Analysis of Variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Temperature	2	1.6	.8	0.19	3.55
Dose	1	165.4	165.4		
Interaction	2	.3	.15		
Within cells	18	7.7	.43		

Conclusion: The difference between 60°, 75° and 90°F. pre-treatment temperatures on the toxicity of dieldrin to the onion maggot adults is not significant.

Table 3 -- Number of adult onion maggots killed with ethion after conditioning for three days at different temperatures. Each lot consisted of 10 flies.

Insecticide	Rep.	Pre-treatment temperature 60° F.	75° F.	90° F.
Ethion	a	8	9	7
	b	5	5	5
	c	7	7	5
	d	8	6	5
	Mean	7.00	6.75	5.50
Control	a	0	0	1
	b	0	0	0
	c	0	0	0
	d	0	0	0
	Mean	0	0	.25

Analysis of Variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Temperature	2	1.8	0.9	0.87	19.43
Dose	1	240.6	240.6		
Interaction	2	3.6	1.8		
Within cells	18	18.5	1.03		

Conclusion: The difference between 60°, 75°, and 90°F. pre-treatment temperatures on the toxicity of ethion to the onion maggot adults is not significant.

Experiment III

This experiment was conducted in 1959 to determine the effect of different post-treatment temperatures on the toxicity of both dieldrin and ethion to the chlorinated hydrocarbon resistant onion fly.

A dose of 1,000 micrograms of dieldrin or 8 micrograms of ethion per gram of flies was used. Three electric incubators were used in this experiment. One cabinet was regulated at 60° F., another at 75° F. and the third incubator was kept at 90° F. Two to 5-day old flies were obtained as described previously. Four replicates of 5 females and 5 males of flies were used at each dose level and temperature. A control treatment was conducted along with each replicate. The toxicants were dissolved in acetone and topically applied to the mesonota of individual insects. The flies were treated at room temperature and held in pint-size ice cream cartons. Immediately after treatment the flies were transferred to the specified temperature. Mortality counts were made 24 hours after treatment.

The number of adult onion maggots killed with dieldrin and ethion at the different post-treatment temperatures were recorded in tables 4 and 5. Analysis of variance of the data in tables 4 and 5 showed that there were highly significant differences in the effects of post-treatment temperature on the toxicity of ethion and dieldrin to the onion fly.

Table 4 -- Number of adult onion maggots killed with ethion when held at different temperatures after treatment. Each lot consisted of 10 flies.

Insecticide	Rep.	Post-treatment temperature		
		60° F.	75° F.	90° F.
Ethion	a	0	5	10
	b	1	3	6
	c	0	2	10
	d	0	5	9
	Mean	.25	3.75	8.75
Control	a	0	0	1
	b	1	0	0
	c	0	0	0
	d	1	0	0
	Mean	.5	0	.25

Analysis of variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.99}$
Temperature	2	69.8	34.9	31.7^{xx}	6.01
Dose	1	96.0	96.0		
Interaction	2	76.7	38.3		
Within cells	18	20.0	1.1		

Conclusion: The effect of post-treatment temperature on the toxicity of ethion to the onion fly is highly significant.

Further statistical analysis revealed that the three means differ significantly from one another. The means were compared by the modified Tukey method (Snedecor 1956, p. 251).

Table 5 -- Number of adult onion maggots killed with dieldrin when held at different temperatures after treatment. Each lot consisted of 10 flies.

Insecticide	Rep.	Post-treatment temperature		
		60° F.	75° F.	90° F.
Dieldrin	a	2	1	10
	b	1	3	9
	c	0	2	8
	d	1	3	8
	Mean	1	2.25	8.75
Control	a	0	0	0
	b	0	0	0
	c	0	0	1
	d	0	0	2
	Mean	0	0	.75

Analysis of variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.99}$
Temperature	2	84.2	41.1	74.7 xx	6.01
Dose	1	84.4	84.4		
Interaction	2	55.8	27.9		
Within cells	18	10.2	.57		

Conclusion: The effect of post-treatment temperature on the toxicity of dieldrin to the onion fly is highly significant.

Further statistical analysis revealed that the three means differ significantly from one another. The means were compared by the modified Tukey method (Snedecor 1956, p. 251).

Experiment IV

The purpose of this experiment was to determine the effect of various after-treatment relative humidities on the toxicity of ethion or dieldrin to the resistant onion maggot adults. This experiment was performed in 1959.

Three desiccators containing supersaturated salt solutions were placed in a temperature cabinet at 77° F. The relative humidities in these desiccators were 35, 55, and 75 per cent. The doses of ethion and dieldrin applied were 8 or 100 micrograms per gram of fly respectively. Four replicates of 5 male and 5 female flies were used at each dose level and humidity. A control treatment was conducted along with each replicate. The modified syringe-microburet previously described was used to apply the insecticide solutions to the mesonota of individual insects. The flies were treated at room temperature and held in pint-size ice cream cartons. After treatment the flies were transferred to the specified humidity. Mortality counts were made 24 hours after treatment.

The number of adult onion maggots killed when held at different humidities were recorded in tables 6 and 7. Analysis of variance of the data in the forementioned tables showed that humidity after treatment had no significant effect on the toxicity of dieldrin or ethion to the onion maggot adults.

Table 6 -- Number of adult onion maggots killed with ethion when held at different humidities after treatment. Each lot consisted of 10 flies.

Insecticide	Rep.	Post-treatment relative humidity		
		35%	55%	75%
Ethion	a	6	7	9
	b	3	5	6
	c	3	6	4
	d	5	5	8
	Mean	4.25	5.75	6.75
Control	a	0	0	1
	b	0	0	0
	c	0	0	0
	d	0	0	0
	Mean	0	0	.25

Analysis of variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Humidity	2	7.59	3.80	2.73	3.55
Dose	1	181.51	181.51		
Interaction	2	5.24	2.62		
Within cells	18	25	1.39		

Conclusion: The effect of post-treatment humidity on the toxicity of ethion to the onion maggot is not significant.

Table 7 -- Number of adult onion maggots killed with dieldrin when held at different humidities after treatment. Each lot consisted of 10 flies.

Insecticide	Rep.	Post-treatment relative humidity		
		35%	55%	75%
Dieldrin	a	6	6	6
	b	6	3	8
	c	8	8	9
	d	6	8	7
	Mean	6.50	6.25	7.50
Control	a	0	0	0
	b	0	0	0
	c	1	0	0
	d	2	0	0
	Mean	.75	0	0

Analysis of variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Humidity	2	0.58	0.29	0.17	19.43
Dose	1	240.66	240.66		
Interaction	2	4.09	2.05		
Within cells	18	30.50	1.69		

Conclusion: The effect of post-treatment humidity on the toxicity of dieldrin to the onion maggot is not significant.

Experiment V

To show the extent to which the age of the onion fly affects mortality, an experiment was conducted in 1959 in which given doses of ethion or dieldrin were tested against flies from the same batch of pupae at specified intervals after emergence.

The age groups tested were 2 - 5 day, 9 - 12 day, and 16 - 19 day old flies. Flies were obtained as described previously. The insecticides were dissolved in acetone and applied to the mesonota of individual insects by the topical application apparatus. Four replicates of 10 males and 10 females each were tested at each dose and age group. Also, a control treatment with acetone was performed. A dose of 1,000 micrograms of dieldrin or 8 micrograms of ethion was applied per gram of fly. After treatment the flies were held in pint-size ice cream cartons and placed in a temperature cabinet at 77°F. Mortality counts were made 24 hours after treatment. The number of flies killed for each age group are recorded in tables 8 and 9. Also, the data are recorded in tables 10 and 11 after correction for natural mortality by Abbott's formula (Finney 1952, p.88).

Analysis of variance of data in the preceding tables before and after correction for natural mortality showed no significant difference in susceptibility of the various age groups to ethion or dieldrin. Nevertheless, inspection of tables 8 and 9 revealed that more kills were obtained with the older flies. Also, there was more natural mortality with the older insects. The high natural mortality in the older group was expected because the onion maggot adult male flies reach their peak in natural mortality at 25 days of age (Workman, 1958). Also, the writer observed an increase in natural male mortality in the rearing cages of 16 - 19 day old group.

Table 8 -- Number of adult onion maggots of various age groups killed with dieldrin. Each lot consisted of 20 flies.

Micrograms of Dieldrin per gram of fly	Age Group		
	2-5 day	9-12 day	16-19 day
1000	11	11	17
1000	12	14	12
1000	11	9	13
1000	9	10	15
Mean	10.75	11	14.25
Control	1	3	4

Analysis of variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Treatment	2	30.5	15.25	3.69	5.14
Replicate	3	8.7			
Error	6	24.8	4.13		

Conclusion: The difference in susceptibility of the various age groups tested with dieldrin is not significant.

Table 9 -- Number of adult onion maggots of various age groups killed with ethion. Each lot consisted of 20 flies.

Micrograms of ethion per gram fly	Age Group		
	2-5day	9-12 day	16-19 day
8	11	17	10
8	13	12	14
8	13	16	12
8	8	--	15
Mean	11.25	15	12.75
Control	0	2	6

Analysis of variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Treatment	2	24.10	12.05	2.12	4.46
Error	8	45.50	5.69		

Conclusion: The difference in susceptibility of the various age groups tested with ethion is not significant.

Table 10 -- Per cent mortality[1] in various age groups of the onion maggot adults treated with dieldrin.

Micrograms of dieldrin per gram of fly	Age Group		
	2-5 day	9-12 day	16-19 day
1000	53	47	81
1000	58	65	50
1000	53	35	56
1000	42	41	69
Mean	51.5	47	64
Control	5	15	20

Analysis of Variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Treatment	2	6.10	3.05	1.98	5.14
Replicate	3	3.00			
Error	6	9.24	1.54		

Conclusion: The difference in susceptibility of the various age groups tested with dieldrin is not significant.

[1]After correction for natural mortality.

Table 11 -- Per cent mortality[1] in various age groups of the onion maggot adults treated with ethion.

Micrograms of ethion per gram of fly	Age Group		
	2-5 day	9-12 day	16-19 day
8	55	83	29
8	65	56	57
8	65	78	43
8	40	--	64
Mean	56.25	72.33	48.25
Control	0	10	30

Analysis of Variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Treatment	2	10.00	5.00	2.56	4.46
Error	8	15.62	1.95		

Conclusion: The difference in susceptibility of the various age groups tested with ethion is not significant.

[1]After correction for natural mortality.

Experiment VI

Purpose: To determine the difference in susceptibility of the sexes of onion maggot adults to ethion or dieldrin.

The flies tested in this experiment in 1959 were obtained as previously described. The insecticide solutions were topically applied to the mesonota of 2 to 5 day old insects. The LD50 of both ethion and dieldrin were determined as described in experiment I. At least four dose levels of each insecticide and four replicates of 10 flies of the same sex were treated to determine the LD50. After treatment at room temperature, the flies were held in pint-size ice cream cartons and transferred to a constant temperature cabinet at 77° F. Mortality counts were made 24 hours after treatment. The LD50 for the male and female of the onion fly in micrograms of toxicant per gram of fly, 95 per cent confidence limits, and the slopes of the log. dose-probit lines for ethion and dieldrin are shown in tables 12 and 13.

A comparison of data in tables 12 and 13 revealed that the difference between the sexes in the LD50 when exposed to dieldrin or ethion was significant. The females were less susceptible to the action of the chemicals than the males.

It is well known that the lethality of poison is influenced by the weight of the test animal. This suggested the possibility of significant difference in weight between the male and female insects. To find whether or not there was significant difference in weight, 40 females and 40 males were weighed individually. These figures are given in table 23 in the appendix. The average weight of the female insect was 10.92 mg. and that of the male was 10.50 mg. Analysis of variance of the weights disclosed that the difference in weight between male and female insects was not significant.

After the difference in susceptibility of the sexes of the onion fly to ethion and dieldrin was found to be significant, it was decided to determine the sex ratio of the fly. At regular intervals 100 pupae were taken from the stock of field-collected pupae stored at 38° F. These pupae were transferred to a temperature cabinet at 77° F. and allowed to develop. The flies which emerged from each 100 pupae were sexed and counted. In a total of 712 flies counted, there were 323 females and 389 males. This represented a sex ratio of 55 per cent males to 45 per cent females. This is not statistically significant from a 1:1 ratio.

Table 12 -- Toxicities of dieldrin and ethion as determined by topical application to the onion fly females.

Insecticide	LD50 in micro-grams per gram of fly	95% confidence limits	Slope of log. dose-probit line
Dieldrin	894	586-1364	2.95
Ethion	11.5	9.2-14.5	6.20

Table 13 -- Toxicities of dieldrin and ethion as determined by topical application to the onion fly males.

Insecticide	LD50 in micro-grams per gram of fly	95% confidence limits	Slope of log. dose-probit line
Dieldrin	329.2	165.8-653.8	3.15
Ethion	5.90	4.40-7.90	3.90

Experiment VII

Purpose: To determine the relationship between the stage of development of the onion fly and susceptibility to both dieldrin and ethion.

The following stages of development of the onion fly were compared as to their susceptibility to dieldrin: Second instar larvae, third instar larvae, pupae and adults. Third instar larvae, pupae, and adults were compared as to their susceptibility to ethion. The insecticides were dissolved in acetone and applied topically to the insects. Larvae were obtained from infested onion bulbs which were brought to the laboratory from an onion field in Michigan.

Larval treatment: Second and third instar larvae were taken from infested onion bulbs and treated with the following doses of dieldrin: 1000, 2000, and 4000 micrograms per gram of larvae. Also, third instar larvae were treated with 15 and 100 micrograms of ethion per gram of larvae. The individual weights of 20 second instar larvae and 40 third instar larvae are recorded in table 23 in the appendix. Two to 4 replicates of 20 larvae were tested at each dose level. Also, a control treatment was conducted with each dose level. After treatment the larvae were held in pint-size ice cream cartons and transferred to a constant temperature cabinet at 77° F. The larvae were provided with slices of onions for food. Mortality counts were taken 24 hours after treatment. The number of larvae pupated, larvae survived and larvae killed are recorded in tables 14, 15 and 18.

Pupal treatment: Pupae were obtained by allowing some of the larvae which were brought from the field to pupate. The concentrations of insecticides used were 1000, 2000, and 4000 micrograms of dieldrin and 100 micrograms of ethion per gram of pupae. The individual weights of 20 pupae are shown in table 23 in the appendix. Insecticide solutions were dissolved in acetone and applied topically to the pupae. Four replicates of 20 pupae were treated at each dose level. Also, a control treatment was conducted along with each replicate. After treatment the pupae were held in pint-size ice cream cartons and placed in a temperature cabinet at 77° F. The pupae were allowed to develop, and the emerged flies were counted. The number of adults that emerged and pupae which failed to develop are shown in tables 16 and 19.

Adult treatment: Adults were obtained by allowing some pupae which were obtained for pupal treatment to develop. Doses of 1000 and 2000 micrograms of dieldrin per gram of fly were applied to 2 to 5 day old adults. Fifteen and 100 micrograms of ethion per gram of fly were applied. The insecticides were dissolved in acetone and applied to the mesonota of individual insects. After treatment the flies were held in pint-size ice cream cartons and transferred to a temperature cabinet at 77° F. Mortality data obtained from treating the onion maggot adults with different doses of both dieldrin and ethion is recorded in tables 17 and 20.

Table 14 -- Number of survived and dead larvae after treatment of the second instar larvae of the onion fly with different doses of dieldrin. Each lot consisted of 20 larvae.

Micrograms of dieldrin per gram of larvae	Larvae survived	Larvae dead
1000	20	0
1000	20	0
1000	20	0
Control	20	0
2000	18	2
2000	18	2
2000	20	0
2000	17	3
Control	19	1
4000	19	1
4000	17	3
4000	20	0
4000	18	2
Control	20	0

Table 15 -- Number of larvae pupated, larvae survived, and larvae dead after treating the third instar larvae with different concentrations of dieldrin. Each lot consisted of 20 larvae.

Micrograms of dieldrin per gram of larvae	Larvae pupated	Larvae survived	Larvae dead
1000	7	13	0
1000	8	12	0
1000	4	16	0
1000	3	17	0
Control	5	15	0
2000	5	15	0
2000	0	20	0
2000	3	14	3
Control	2	18	0
4000	2	17	1
4000	4	16	0
4000	1	19	0
Control	2	18	0

Table 16 -- Number of adults emerged and undeveloped pupae after treatment with different doses of dieldrin. Each lot consisted of 20 pupae.

Micrograms of dieldrin per gram of pupa	Adults emerged	Undeveloped pupae
2000	5	15
Control	9	11
2000	6	14
Control	7	13
2000	10	10
Control	6	14
2000	7	13
Control	5	15
4000	5	15
Control	5	15
4000	10	10
Control	16	4
4000	14	6
Control	6	14
4000	14	6
Control	9	11

Table 17 -- Number of adult onion maggots killed when exposed to different concentrations of dieldrin. Each lot consisted of 20 flies.

Micrograms of dieldrin per gram of fly	Dead	Alive
1000	12	8
1000	14	6
1000	11	9
Control	1	19
2000	15	5
2000	13	7
2000	14	6
Control	0	20

Table 18 -- Number of pupae, larvae dead, and larvae survived after treating the third instar larvae with different concentration of ethion. Each lot consisted of 20 larvae.

Micrograms of ethion per gram of larvae	Pupae	Larvae survived	Larvae dead
15	5	14	1
15	3	16	1
Control	6	12	2
100	2	0	18
100	1	0	19
100	2	0	18
100	0	0	20
Control	16	0	4

Table 19 -- Number of adults that emerged and pupae which failed to develop after treatment with ethion. Each lot consisted of 20 pupae.

Micrograms of ethion per gram of pupa	Adults emerged	Pupae failed to develop
100	9	11
Control	9	11
100	8	12
Control	9	11
100	10	10
Control	8	12
100	6	14
Control	8	12

Table 20 -- Number of adult onion maggots killed when exposed to different concentrations of ethion. Each lot consisted of 20 flies.

Micrograms of ethion per gram of fly	Dead	Alive
15	13	7
15	16	4
15	14	6
Control	0	20
100	20	0
100	20	0
100	20	0
Control	2	18

An inspection of tables 14 and 15 indicates that the second and third instar larvae of the onion maggot were extremely resistant to the action of dieldrin. A dose of dieldrin as large as 4000 micrograms per gram of larvae was ineffective against the second and third instar larvae. Also, table 16 shows that a dose of 4000 micrograms of dieldrin per gram of pupae was ineffective, because more treated pupae developed than in the control group. This would not be the case if dieldrin was effective against the pupa. On the other hand, table 17 reveals that dieldrin is effective at 1000 and 2000 micrograms per gram of onion maggot adults. This indicates that the adult stage of the onion maggot is less resistant to dieldrin than the other stages tested.

Tables 18, 19 and 20 demonstrate that a dose of 100 micrograms of ethion per gram of third instar larva, pupa or adult was very effective against the third instar larvae and adults, but was ineffective against the pupae. Also, tables 18 and 20 show that a dose of 15 micrograms of ethion per gram of third instar larva or adult was ineffective against third instar larvae but effective against the adults. Therefore, it was concluded that the order of resistance of the life stages of onion maggot tested with ethion was pupa $>$ third instar larva $>$ and adult.

Experiment VIII

This experiment was carried out in 1959 to determine whether or not the site of application has any effect on the toxicity of both ethion and dieldrin to the onion maggot adults.

Doses of 12 micrograms of ethion or 300 micrograms of dieldrin per gram of fly were applied. Four replicates of 10 females and 10 males at each dose level and locus of application were used. The insecticides were dissolved in acetone and applied to the mesonota or to the middle of the abdominal dorsum of individual flies. The insecticide solutions were topically applied by the modified microburet described previously. After treatment the flies were held in pint-size ice cream cartons and transferred to a constant temperature cabinet at 77° F. Food and water were supplied to the treated insects. Mortality counts were made 24 hours after treatment. The number of dead insects obtained from applying ethion or dieldrin to different locations of the onion maggot adults are recorded in tables 21 and 22.

Table 21 -- Number of adult onion maggots killed by applying dieldrin to the thoracic mesonotum or dorsum of abdomen of the insect. Each lot consisted of 20 flies.

Micrograms of dieldrin per gram of fly	Site of application	
	Dorsum of abdomen	Mesonotum of thorax
800	7	10
800	9	9
800	8	8
800	6	3
Mean	7.5	7.5

Analysis of Variance

Source of variation	D.F.	S.S.	Mean S.	F.
Site of application	1	0	0	0
Replicate	3	25	8.3	
Error	3	9	3	

Conclusion: The difference in toxicity of dieldrin topically applied to the thorax or abdomen of the onion fly is not significant.

Table 22 -- Number of adult onion maggots killed by applying ethion to the thoracic mesonotum or dorsum of abdomen of the insect. Each lot consisted of 20 flies.

Micrograms of ethion per gram of fly	Site of application	
	Dorsum of abdomen	Mesonotum of thorax
12	4	9
12	1	10
12	2	6
12	5	6
Mean	3	7.75

Analysis of Variance

Source of variation	D.F.	S.S.	Mean S.	F.	$F_{.95}$
Site of application	1	45.13	45.13	8.27	10.13
Replicate	3	6.38			
Error	3	16.37	5.46		

Conclusion: The difference in toxicity of ethion applied topically to the thorax or the dorsum of abdomen of the onion fly is not significant

DISCUSSION

In measuring the relative toxicity of a number of insecticides to a particular pest, it is important to test such insecticides under field conditions where they will ultimately be used. This was recognized by Busvine (1957) when he wrote "Logically, of course the best criterion is a full scale field trial; but such trials are expensive, slow and owing to the difficulty of providing adequate replications to make up for great variability, do not always produce unambiguous conclusions. Therefore they should be reserved till laboratory tests have narrowed the choice down to two or three substances." Laboratory tests to evaluate several insecticides against the onion maggot were carried out in 1958 in Michigan. Table 1 indicates that the organic phosphate insecticides were much more effective against the onion maggot than the chlorinated hydrocarbon insecticides used. Also table 1 strongly indicates that the pest was resistant to the chlorinated hydrocarbon insecticides.

Increased resistance in an insect population is first suspected because of the failure of a standard treatment to give control equal to that obtained previously. This, however, is not a positive proof that the insect is actually resistant to a certain insecticide. In order to ascertain if a particular insect is resistant to a certain insecticide, laboratory tests are usually carried out to give a direct comparison between a known normal strain and the one under suspicion. Although there was a strong indication that the onion fly in certain areas in Michigan was resistant to the chlorinated hydrocarbon insecticides it was not experimentally confirmed. To confirm if the onion maggot was resistant to dieldrin, samples of the suspected resistant and non-resistant flies from two localities were tested in the laboratory, and the LD50 for the two populations was established. Table 1 shows that the LD50 of dieldrin for the onion maggot adults obtained from Michigan was 542 micrograms per gram of fly. The LD50 of dieldrin for the Cornell strain of flies was 1.38 micrograms per gram of fly. This confirmed that the samples of Michigan onion maggots tested were positively resistant to dieldrin.

From the scanty reports on the effect of pre-treatment temperature on the toxicity of insecticides, no conclusions can be reached. However, Cotton (1932), Munson (1953, 1954) and Craufurd-Benson (1938) reported that insects were less susceptible to the action of chemicals when pre-conditioned at lower temperatures. On the other hand, Quayle (1934) and Lindgren (1941) revealed that insects were less resistant to insecticides when pre-conditioned at lower temperatures. Tables 2 and 3 of this work shows that the pre-treatment temperature had no significant effect on the toxicity of either ethion or dieldrin to the onion maggot adults.

It seems that temperature is the most important factor affecting the insect during insecticidal tests. Also, the temperature affects the killing efficiency of insecticides. A brief review of literature indicated three general trends of the effect of post-treatment temperatures on the toxicity of insecticides to insects. First, is an increase in toxicity at lower temperatures, and second an increase in toxicity at the higher temperatures. Also, there were cases in which the post-treatment temperatures have no effect on toxicity. Experiment III of the present research showed that the post-treatment temperature has a profound effect on toxicity of both dieldrin and ethion to onion maggot adults. Dieldrin and ethion were much more toxic to the insect at the higher temperature. Careful inspection of tables 4 and 5 revealed that at 60° F. dieldrin and ethion were ineffective against the onion fly. The toxicity of the materials increased as the temperature increased. Guthrie (1950) reported on the effect of post-treatment temperature. He indicated that dieldrin was more toxic at higher temperatures when applied topically to the German cockroach. This is the only report found on the effect of temperature on the toxicity of dieldrin to insects. No reports are available in the literature on ethion.

According to the few reports on the effect of relative humidity on the action of insecticides, it seemed that increasing humidity increased or decreased toxicity depending on the poison and the test insect. Also, there were cases in which relative humidity had no significant effect on toxicity of insecticides. Tables 6 and 7 in experiment IV shows that relative humidity after treatment had no significant effect on the toxicity of either dieldrin or ethion to the onion maggot adults. Potter and Way (1958) reviewed the literature on the effect of relative humidity before and during treatment on the toxicity of insecticides. They concluded that the relative humidity before and during treatment does not affect the subsequent toxicity of a poison.

It has previously been shown that the susceptibility of insects to insecticides increased as they became older (Simanton 1937, Craufurd-Benson 1938, McLeod 1944, David 1946, Collins 1953, and Kerr 1954). All of the literature available and known to the writer on the effect of age of insects on the toxicity of insecticides indicated that older insects were more susceptible than their younger counterparts. Although there was no significant difference in the effect of age on susceptibility of the onion fly to either dieldrin or ethion, greater kills were obtained with the older flies. Also, there were more deaths due to natural mortality. This is shown in tables 8 and 9. It appears that the slightly lower kills obtained with the younger flies was due to vigor resistance.

The majority of reports in the review of literature on the effect of sex on the toxicity of insecticides to insects indicated that the female insects were more resistant than the male. This is in agreement with the present research. Tables 12 and 13 show that the LD50 of dieldrin for female and male onion flies was 894 and 529.2 micrograms per gram of fly respectively. Also, the LD50 of ethion for female and male onion flies was 11.5 and 5.9 micrograms of ethion per gram of fly respectively. It is important to note that the difference in weight between the female and male insects was not significant. This eliminated any suggestion that the difference in resistance may be due to significant difference in weight between male and female insects. The significant difference in resistance of the onion fly to both dieldrin and ethion appears to be a case of physiological resistance, and it would seem worthwhile to investigate. After the difference in resistance between males and females of the onion fly was found to be significant it was decided that a determination of the sex ratio would be important. An investigation to determine the sex ratio of the onion fly revealed that the sex ratio is 55 per cent males and 45 per cent females. This is not significant from a 1:1 ratio. There are no reports available in the literature on this subject.

When it is stated that an insecticide is effective against a certain insect species, it usually means that the insecticide has been proved toxic to a certain stage in the life cycle of the insect. Little is known of the extent of variation in susceptibility of the different stages in the life cycle. There are no reports dealing with this subject specifically on the onion maggot. The literature review on the effect of the stages of development on resistance of insects to insecticides revealed that large variation in susceptibility may occur throughout the life cycle. The present research found this to be true with the onion maggot. The adult stage of the onion maggot was less resistant to the action of dieldrin than the second instar larva, third instar larva and pupa. Also, the order of resistance in the life stages of the onion maggot to ethion were pupa $>$ third instar larva $>$ and adult. This should be taken into consideration in devising control measures.

Aside from biological factors, there are physical factors which may affect the results of insecticide tests.

One of these factors is the site of application. The majority of reports in the preceding review of literature on the effect of location of dose on toxicity of insecticides to insects indicated that the closer the site of application to the central nervous system, the higher the mortality. Although the present investigations showed no significant difference in the effect of site of application on the toxicity of either dieldrin or ethion, inspection of table 22 reveals that more kills were obtained when ethion was applied to the mesonotum than when applied to the dorsum of the abdomen. It is to be mentioned that the possibility of a slight spreading of the insecticide solutions to the neighboring areas may have contributed to the insignificant difference in the effect of site of application on the toxicity of both dieldrin and ethion to onion maggot adults.

SUMMARY AND CONCLUSIONS

The respective toxicities of 15 insecticides to the onion maggot adults Hylemya antiqua (Meig.), were determined by topical application. A modified syringe-microburet was used to apply the insecticide solutions to the insects. Also, a laboratory strain of onion maggot adults was compared with samples of the suspected dieldrin-resistant onion fly in Michigan.

Experiments were conducted to determine the effect of different pre-treatment and post-treatment temperatures, various after-treatment relative humidities, age, sex, stage of development, and site of application on the individual toxicity of dieldrin and ethion to the onion maggot. The results indicated:

1. Of the insecticides tested, methyl parathion, dimethoate, and Diazinon (0,0-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) were the most toxic to the onion maggot adults. Heptachlor, dieldrin, endrin, and DDT were the least toxic.
2. The samples of flies obtained from Michigan were found to be much more resistant to dieldrin

than a normal strain of flies reared in the laboratory.

3. Different pre-treatment temperatures did not effect the toxicity of either dieldrin or ethion to the onion maggot adults.
4. Post-treatment temperature had a profound effect on the toxicity of dieldrin and ethion. The higher the temperature the higher the toxicity.
5. Various after-treatment relative humidities did not effect the toxicity of dieldrin and ethion to the onion maggot adults.
6. There was no significant difference in the effect of age on the toxicity of either dieldrin or ethion to the onion maggot adults. More kills were obtained with the older flies than young ones. Also, there was more natural mortality with the older flies. The lesser kills obtained with the younger insects was attributed to vigor resistance.
7. The female flies were significantly more resistant to the action of both dieldrin and ethion than the males. There was no significant difference in weight between female and male flies. It was suggested that the difference

in resistance between females and males may be a case of physiological resistance and worth investigating.

8. The onion maggot adults were less resistant to the action of both dieldrin and ethion than the second instar larvae, third instar larvae, and pupae. Because at the doses used, dieldrin was not effective against the second instar larvae, third instar larvae, and pupae no comparison could be made between these stages. The order of resistance in the stages of development of the onion maggot to ethion was pupa > third instar larva > and adult. On a weight basis it was concluded that adults were the least resistant stage tested.
9. Although there was no significant difference in the effect of the site of application on the toxicity of either dieldrin or ethion to the onion fly, more kills were obtained when ethion was applied to the mesonotum of individual insects than when applied to the dorsum of the abdomen.

In toxicological investigations on the onion maggot, temperature after treatment, age, sex, and site of application should be standardized.

APPENDIX

The Mathematical Procedure in Determining the LD50 for Thimet by the Probit Analysis Method

Dose	Per cent[1] dead	Log.dose X	X^2	Provisional Probit	Expected Probit	Working Probit Y	Y^2
20	98	1.301	1.693	7.954	6.600	6.914	47.803
15	80	1.176	1.383	5.842	6.180	5.770	33.293
12	82	1.079	1.164	5.915	5.915	5.915	34.987
8	51	0.903	0.815	5.025	5.400	5.005	25.050
2	9	0.301	0.091	3.659	3.450	3.700	13.690
Control 15							

NW	NWX	NWX^2	NWY	NWY^2	NWXY
2.002	2.605	3.389	13.842	95.702	18.011
3.142	3.695	4.345	18.129	104.607	21.380
3.839	4.142	4.469	22.703	134.315	24.500
4.731	4.272	3.356	23.679	118.512	21.381
0.651	0.196	0.059	2.409	8.912	0.725
14.365	14.910	16.118	80.767	462.048	85.937

[1] After correction for natural mortality by Abbott's formula.

$$\bar{X} = \frac{NWX}{NW} = \frac{14.910}{14.365} = 1.038$$

$$\bar{Y} = \frac{NWY}{NW} = \frac{80.767}{14.365} = 5.622$$

$$Wx^2 = NWX^2 - \bar{X}(NWX)$$

$$= 16.113 - 1.038(14.910) = .641$$

$$Wxy = NWXY - \bar{Y}(NWX)$$

$$= 85.937 - 5.622(14.910) = 2.113$$

$$b = \frac{Wxy}{Wx^2} = \frac{2.113}{.641} = 3.296$$

The equation for the probit regression line is:

$$Y = \bar{Y} + b(X - \bar{x})$$

$$= 5.622 + 3.296(1.301 - 1.038) = 6.489$$

$$= 5.622 + 3.296(1.176 - 1.038) = 6.077$$

$$= 5.622 + 3.296(1.079 - 1.038) = 5.757$$

$$= 5.622 + 3.296(.903 - 1.038) = 5.177$$

$$= 5.622 + 3.296(.301 - 1.038) = 3.193$$

The above values of Y for 5 values of X are plotted on the following graph to draw the log.dose-probit line.

Find the logarithm LD50 as the value of X which gives Y = 5:

$$m = \frac{5 + b\bar{X} - \bar{Y}}{b}$$

$$= \frac{5 + 3.296 \times 1.038 - 5.832}{3.296} = .849$$

$$\text{Variance of } (m) = \frac{1}{b^2} \left\{ \frac{1}{NW} + \frac{(m - \bar{X})^2}{NW(X - \bar{X})^2} \right\}$$

$$= \frac{1}{10.864} \left\{ \frac{1}{14.910} + \frac{(.849 - 1.038)^2}{.841} \right\} = .0112983$$

The standard error of m is $\sqrt{.0112983} = .1063$

The 95 per cent confidence limits are:

$$m \pm ts_{\bar{X}}$$

$$= .894 \pm 1.96 \times .1063$$

$$= .894 + .208 = 1.057$$

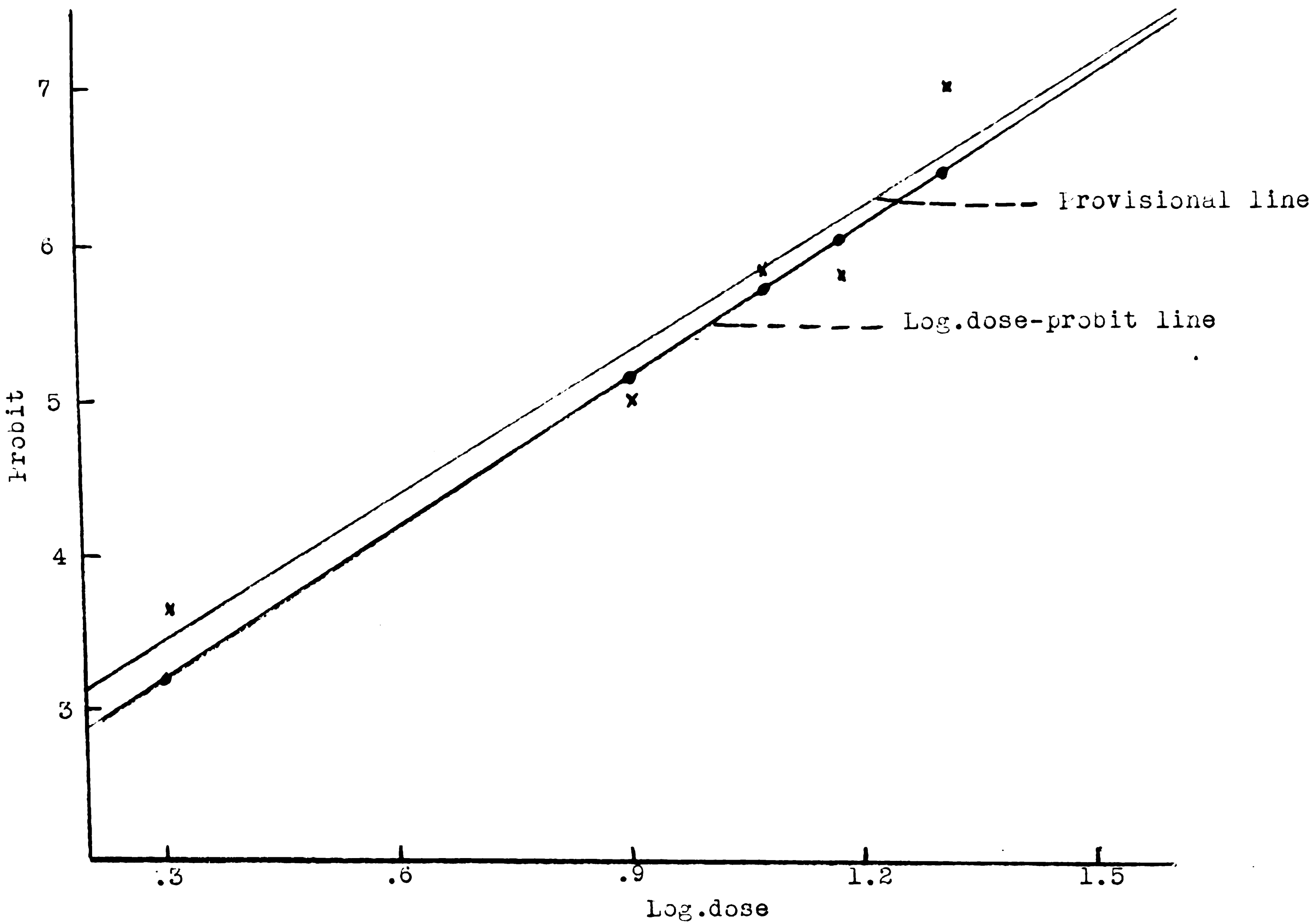
$$= .894 - .208 = .641$$

Antilogarithm of .849 = 7.06

Antilogarithm of 1.057 = 11.40

Antilogarithm of .641 = 4.38

Whencethe LD50 for Thimet is 7.06 micrograms per gram of fly and the 95 per cent confidence limits are 11.40 and 4.38.



7
6
5
4
3
Probit
Provisional line
Log.dose-probit line
.3
.6
.9
1.2
1.5
Log.dose

Table 23 -- The individual weights in milligrams of onion flies obtained from Michigan, onion flies from Cornell University, male and female Michigan onion flies, second instar larvae, third instar larvae, and pupae.

Flies from Michigan	Flies from Cornell U.	Male Flies	Female Flies	Second Instar Larvae	Third Instar Larvae	Pupae
11	11	10	12	5	17	14
11	9	12	11	5	18	14
13	12	11	11	5	19	15
10	9	11	11	5	18	12
11	8	11	9	4	14	12
10	9	kk	10	5	19	12
12	10	11	13	4	18	12
12	10	10	13	5	17	13
13	10	11	12	6	20	11
9	9	9	12	5	18	12
11	10	10	14	6	17	12
12	9	10	9	6	17	11
11	13	10	9	5	18	11
12	11	10	10	5	16	11
10	9	11	10	4	17	13
12	9	12	12	5	18	13
11	10	11	12	4	18	12
13	10	11	10	5	18	12
12	10	10	11	5	16	15
13	11	10	10	4	15	11
		10	10		15	
		11	10		16	
		9	14		19	
		10	10		16	
		13	11		17	
		10	13		18	
		10	9		16	
		12	11		17	
		12	12		14	
		10	12		17	
		9	14		17	
		9	10		18	
		11	13		12	
		10	9		17	
		11	9		16	
		12	11		17	
		9	9		14	
		9	9		14	
		10	11		13	
		11	9		13	
Mean 11.45	9.95	10.50	10.92	4.9	16.35	12.40

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