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COMPARISON OF CONTACT INSECTICIDES RECOMMENDED AGAINST THE MEDITERRANEAN FRUIT FLY *Ceratitis capitata* Wied.

by

Nasri S. Kawar

A Thesis Submitted to the Graduate Faculty
of the School of Agriculture in Partial Fulfillment of
The Requirements for the Degree of

MASTER OF SCIENCE IN AGRICULTURE

Split Major: Entomology-Plant Pathology

Approved:

A. S. Tamouk
In Charge of Major Work

R. H. Porter

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Kawar

ABSTRACT

The present work is an attempt to evaluate, under laboratory conditions, three organic phosphorus and two chlorinated hydrocarbon insecticides against the Mediterranean fruit fly, Ceratitis capitata Wied. Flies were confined for 30 minutes under glass funnels with inside walls surrounded by treated filter papers, after which the flies were moved to clean funnels. Mortalities were recorded at regular intervals. The results were analyzed statistically by the probit analysis and the LD50 of each insecticide was determined. Malathion had the lowest LD50. It was followed in ascending order by Rogor, Dipterex, Toxaphene and DDT respectively.

Laboratory breeding of the flies was also carried out to supply, for the tests, a large number of adult flies bred under uniform conditions. Breeding was most successful at an average temperature of 24-26°C, and the average duration of one life cycle was 14-16 days.

The anatomy and histology of the nervous system were studied, with emphasis on the nerves in the legs, since the insecticidal pick-up is through tarsal contact with treated surfaces. Four stains were tried; the Ranvier-Loewit gold chloride method gave the best results in staining the leg nerves.

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INTRODUCTION

The Mediterranean fruit fly - Ceratitis capitata Wied. is a destructive insect pest belonging to the family Trypetidae, order Diptera. It is well established in all the countries bordering the Mediterranean Sea and on the islands of Cyprus, Malta and Sicily. In Central Europe, it is found in Germany, Austria, Hungary and Switzerland; in South America, in Argentina, Brazil and Uruguay; in Africa, in Eritrea, Gold Coast, Kenya, Tanganyika, Uganda, Union of South Africa and Canary Islands; in Australia, in the western section (C.I.E. 1951). It was observed for the first time in Belgium in 1952 (Van den Brande, 1953), in Costa Rica in March 1955 (Morales, 1955) and in Holland in 1955 (FAO, 1956). In North America this insect was discovered in Florida, U.S.A. in April 1929. Immediately a campaign was organized to eradicate it, and in July 1930, this important pest was completely eliminated from the U.S.A. The cost of the operation amounted to 7,000,000 dollars (Metcalf et al, 1951). However a new infestation was discovered in Florida in April 1956 (U.S. Dep't of Agric. 1956) and once again immediate measures were taken to eradicate it. Shepherd (1957) reported that an extensive program to locate and eradicate this insect was carried out, and by the end of 1956 almost complete success had been achieved.

The wide distribution of C. capitata in the world makes it an insect of great importance in the field of plant protection. Of equal importance is the large number of host

plants that are attacked by it. Bodenheimer (1951) discusses in detail its host relations. He states that "Citrus fruits are rarely a suitable host of Ceratitis, yet the damage caused to them is nevertheless sometimes considerable." This unsuitability depends on three factors which prevent the majority of eggs laid in the rind of citrus fruits from developing into adults. The first factor is the presence of oil glands that secrete etheric oils which, in turn, kill the eggs and young maggots. The maggots that survive and try to bore into the pulp of the fruit are confronted by the second factor which is the thickness and elasticity of the rind. The third factor is the gum secretions in the rind which kill both eggs and maggots. From these factors it is obvious that fruits with a thin rind provide more chances for a successful attack than those with a thick rind. Late-maturing varieties are also more susceptible to attack than early-maturing ones. Sour lemons and citron are immune to the attack by Ceratitis due to their thick and rough rinds. The infestation of oranges and grapefruits depends on the variety, those with a thin rind being more susceptible. Clementines are heavily infested while the tangerines are usually less so.

Bodenheimer (1951) states also that peaches and apricots are the most favored hosts of C. capitata, while plums are relatively resistant. The damage is so severe in some localities that it is not economical to grow such fruits. Pears are also a suitable host while apples and quinces are much less susceptible.

There are many other plants that serve as hosts for this insect, examples of which are - figs, loquats, pomegranates, mangos, guavas, avocados, persimon, kaki, soursop, and coffee. Fruits that are rarely attacked are papayas, dates and grapes (Bodenheimer, 1951).

According to Bodenheimer (1951) green bananas are never attacked by C. capitata because of the presence of tannins in the sap of the fruits. However, Jenkins (1948) recorded a case in Western Australia where green bananas were heavily infested by this fly.

Among the vegetables, tomatoes, eggplants and green peppers are liable to attack by the fly especially in years of heavy infestations. Some hedge plants and shrubs are also attacked by the Mediterranean fruit fly, examples of which are - prickly pear (Opuntia ficus indica), Aberia caffra, and Solanum coagulans. (Bodenheimer, 1951).

The economic losses resulting from the attack of C. capitata can be very high especially in years where the climatic conditions are favorable for the rapid development of this fly. An example of this is cited by Grunberg (1938) in which he states that the damage by C. capitata to the citrus plantations in the Jordan Valley during the growing season 1935-36 was in some cases about 50 per cent of the total cull, and by the end of the season 50-70 per cent of the crop remaining on the trees was damaged.

The damage starts when the rind of the fruit is

pierced by the female fly to lay its eggs. This piercing opens the way for the development of fungi and bacteria which cause the rotting of the fruits. However, rotting does not always occur, because in many cases the fruits will be completely destroyed from the inside by the larvae before the symptoms of damage appear on the outside. The oviposition puncture usually causes a discolored spot on the skin of the fruit which varies with different kinds of fruits. In citrus fruits the puncture becomes surrounded with a yellow circle while the rest of the fruit is still green. In loquats the puncture is surrounded by a green patch even when the fruits turn yellow. In peaches and pears the puncture forms a sunken area which turns brown in color (Bodenheimer, 1951).

According to Bodenheimer (1951) the indirect influence of *C. capitata* is of equal importance to its actual damage. Its presence in some areas in the world has greatly limited the choice of crops to be planted in such areas even though the climatic conditions are favorable. Examples that prove Bodenheimer's statement are numerous. In Lebanon and Syria, the once famous peach variety called "Khitmaly" is no more existing because of the very severe attack by this fly. All trees of this variety were pulled out and burned. Another fruit variety that suffered the same fate is the large French apricot variety called "Pêche de Nancy". In Syria, the pear varieties "Sukkari" and "Uthmani" planted around Damascus and in the Zabadani Plain are threatened in the same way by

this fly[+]. In West Pakistan too, the majority of the peach trees were pulled out because of very severe attack by Ceratitis.[++]

The control measures applied in this country are still unsatisfactory and the economic losses caused by this fly are tremendous. At present there are several insecticides recommended for the control of fruit flies but none has been applied on a large scale in Lebanon.

The present work is an attempt to evaluate some of these insecticides under laboratory conditions. In order to have a large number of homogenous adult flies for the tests, laboratory breeding was found necessary. Since all the poisons employed fall, more or less, in the category of contact poisons, and since the insecticidal pick-up is through tarsal contact with treated surfaces, anatomical and histological investigation of the nervous system and especially in the legs was considered desirable.

[+] In an oral communication by Prof. A. S. Talhouk.

[++] In an oral communication by Dr. Afzal Hussein, Entomologist and former Vice-Chancellor of the Punjab University.

REVIEW OF LITERATURE

A. Binomics of Ceratitis capitata

A good description of the stages of development of Ceratitis capitata has been given by Efflatoun (1925 and 1927). The egg is smooth, shining white in color, long and slightly convex on the dorsal side. It measures 0.9-1.1 mm in length and 0.2-0.25 mm in width.

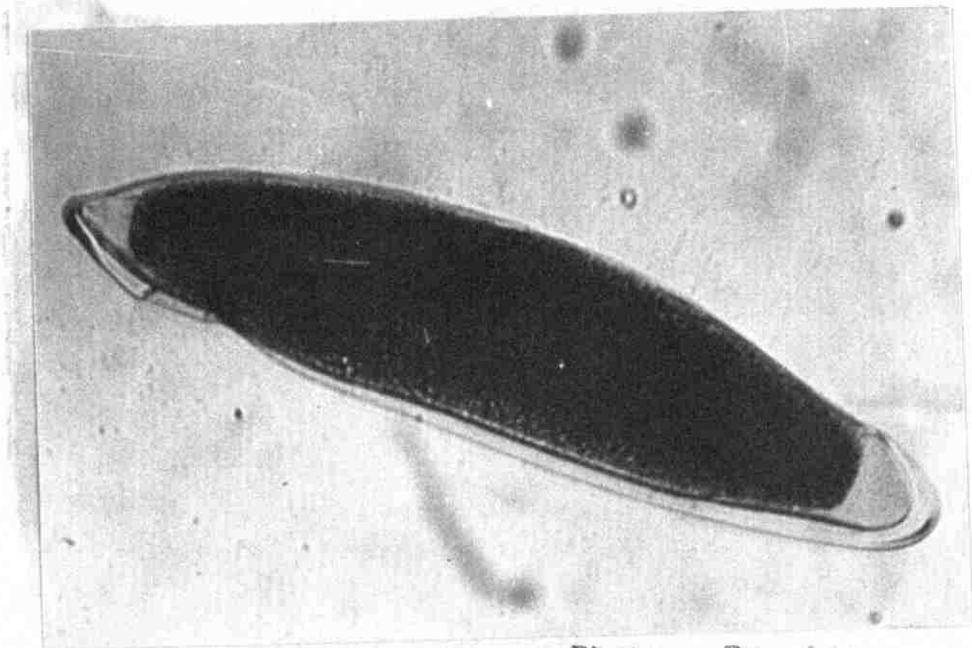


Photo - Dr. Asmar

Fig. 1 - Egg of C. capitata. 125 x

The larva or maggot is creamy-white in color, elongate in shape with a conical head and a sub-cylindric posterior end. It has 2-jointed antennae, small maxillary palpi and oral hooks possessing strong and well curved teeth. When

full-grown, it measures 6.8-8.2 mm in length and 1.5-2.0 mm in width.

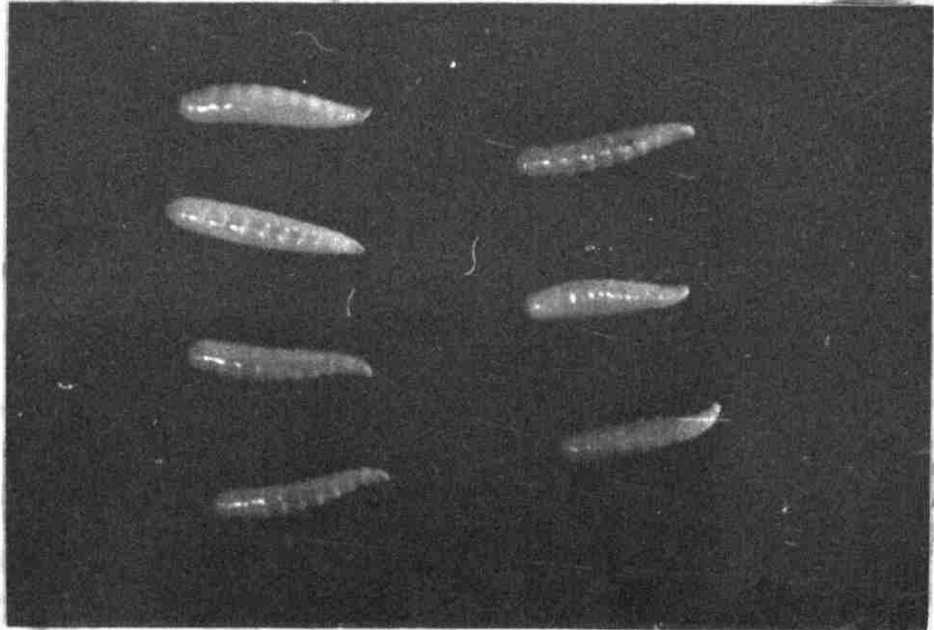


Photo - Dr. Asmar

Fig. - Larvae of C. capitata. 3.5 x

The pupa varies in color from light to dark brown. It is elongate and elliptical in shape and measures 4-4.3 mm in length and 2.1-2.4 mm in width.

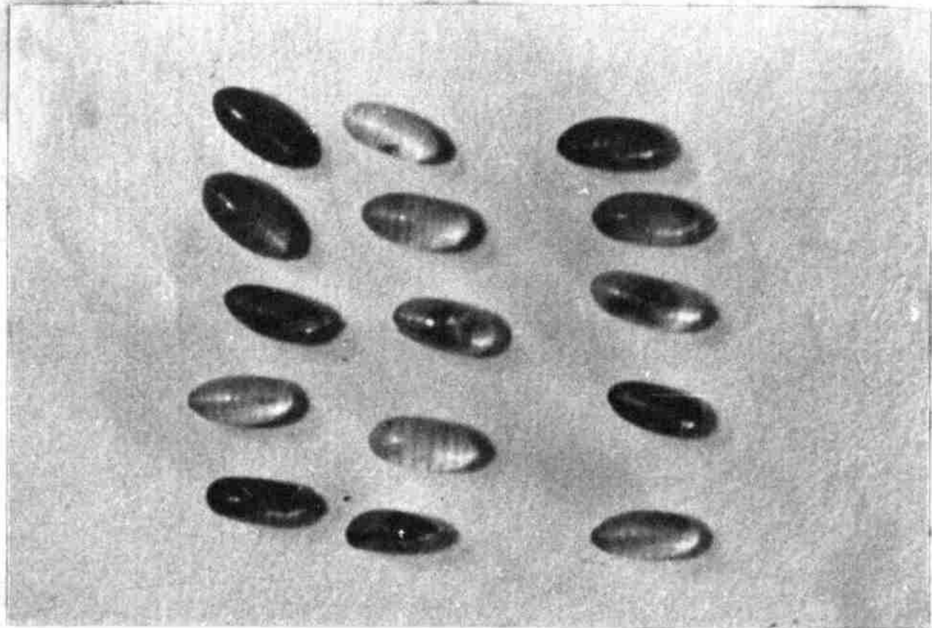


Photo - Dr. Asmar

Fig. 3 - Pupae of C. capitata. 4 x

The color of the adult fly is striking and readily noticeable. The head is yellowish in color except at the base of the antennae where it is dark brown and hairy. The eyes have a combination of green, blue and reddish brown colors. The proboscis and antennae are also yellow. The male possesses two spatulae-shaped appendages at the end of two chaetae which are located at the head posterior to the antennae. The thorax has very characteristic light grey markings on its dorsal side and possesses many strong and erect hairs. The abdomen is orange-yellow in color with two reddish-brown bands and is fairly pubescent. The legs are reddish-yellow and bear rather long bristles and fine hairs. The wings are broad and hyaline with characteristic brown transverse bands.

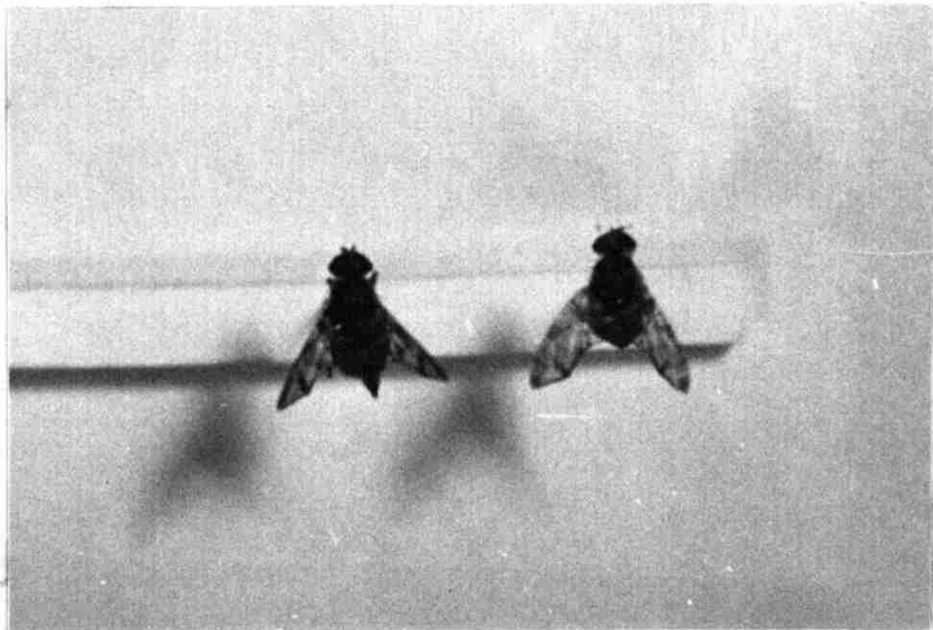


Photo - Dr. Asmar

Fig. 4 - Adult female (left) and male (right) of C. capitata. 4 x

The life cycle of the Mediterranean fruit fly has been investigated in many parts of the world. According to Bodenheimer (1951) the flies are sexually immature when they first hatch. The duration of the preoviposition period depends mainly on the temperature. He found that the preoviposition period in days at different temperatures in degrees centigrade was 18 (2-36 being the extremes) at 25-28°, 27 (3-39) at 29-30° and 121 (71-163) at 16-17°. No oviposition was observed by him at a temperature below 15-16°C. Similar results were obtained by Klein and Paker (1942) from their observations in the Jordan Valley. However Martin (1950) in his field observations on the same insect in Algeria found that the preoviposition period lasted only 3-6 days in summer.

The time for sexual maturation of the male flies is slightly shorter than that of the females. Copulation follows sexual maturity. The male tries to attract the female by giving out a stale mucous smell which stimulates the female (Back and Pemberton, 1918). From laboratory observations Hanna (1947) reports that copulation usually takes place between 9 a.m. and 1 p.m. The male follows the female for sometime until she accepts him. She, then, stops and extends her ovipositor. The male holds her at the anterior part of her abdomen by his forelegs and copulation proceeds. The duration of copulation varies from a few minutes to two or three hours. Copulation takes place repeatedly by both sexes.

The female, when ready to oviposit, wanders on the fruit and looks for a soft place to lay its eggs. It inserts

its ovipositor by rythmic movements and forms a chamber in the fruit to a depth of 1-2 mm in which it lays its eggs. Oviposition continues for about 20 minutes. The average number of eggs per chamber is 22 while the average number of eggs laid by a female is 280 (Hanna, 1947). Higher figures are recorded by Back and Pemberton, (1918) namely 30-300 eggs are found in one chamber. The high figure is due to several ovipositions in the same chamber by different females. They also give the figure 800 as the maximum number of eggs laid by one female. Bodenheimer (1951) states that the average number laid by one female is 266 eggs.

This insect undergoes a complete metamorphosis. The full-grown larva exhibits a strange habit of jumping when removed from its host, and especially if placed on hard surfaces (Hanna, 1947). This habit seems to be associated with an attempt to find a suitable place for pupation (Poutiers, 1930). When full-grown the larva leaves the fruit and drops to the soil where it pupates at a depth of about three centimeters (Hanna, 1947). Experiments performed in France on the depth of pupation showed that adult flies had difficulty in emerging from a depth of more than 3-4 cm of soil especially if the soil was wet. Five pupae were buried at a depth of 5 cm in dry soil, three adults emerged but only two reached the surface. In wet soil and at the same depth, three adults emerged but none reached the surface. When 10 pupae were buried at a depth of 10 cm in dry soil, five adults emerged

but one only reached the surface, and in wet soil four adults emerged and none reached the surface. Of 23 pupae that were buried at a depth of 0.5 cm only 13 adults emerged and all reached the surface (Poutiers, 1938). Delmas and Thermes (1953) also experimented on the depth of pupation. Pupae were buried at various depths in sandy soil in wide glass tubes and the results showed that 85 per cent of the flies emerged from a depth of less than 0.5 inch, 65 per cent at 10 inches, 6.5 per cent at 20 inches and none from a depth of 28 inches.

The longevity of the adult flies is affected chiefly by the availability of food. Hanna (1947) reared 130 couples in the laboratory and fed them sugar-cane solution and egg protein. The average longevity was 32 days, and eleven flies survived for 96 days. Back and Pemberton (1918) kept 482 flies without food and water and all died after the fourth day.

Bodenheimer (1951) found the constants for the development of _C. capitata_ from egg to adult to be:

Threshold of development = 9.3-9.9°C

Thermal constant = 321 day-degrees

The number of generations per year of the Mediterranean fruit fly has been recorded by workers in many countries. Abou Nasser (1954) states that this fly has 7-8 generations per year on the coast of Lebanon and 5-6 in the mountain regions. On the coast the adults appear in early May in the South and late May in the North. The optimum temperature for development ranges between 13 and 24°C. Klein and Paker (1942) report that there are probably not more than five generations

per year of Ceratitis in the Jordan Valley although laboratory breeding results in 6-7 generations. Adults of the first generation appear in early May. These give rise to the second generation flies in August while the third generation adults appear in early October. These flies survive the winter season to initiate again the first generation adults. The length of development of one generation ranges from 57 days at about 16°C to 17 days at about 30°C. Grunberg (1938) working also on Ceratitis in the Jordan Valley reports that at an average temperature of 13.5°C the development of one generation lasts for 100 days while at an average temperature of 24.3°C it takes only 30 days. Hanna (1947) states that the average duration of one generation in Egypt is 66 days at 16°C and 19 days at 27°C.

Under favorable conditions in Italy, C. capitata can have 6-7 generations per year (Constantino, 1929). In Argentina, one generation is usually completed in about 30 days in summer and 60 days in winter (Bergani, 1952), while in Western Australia, about 30 days in summer and 115 days in winter (Jenkins, 1944) are required to complete one generation.

B. Methods of Breeding in the Laboratory

Several methods and media for the rearing of the Mediterranean fruit fly in the laboratory have been developed. Feron and Sacantanis (1955) found that bananas were suitable for the feeding of the flies if the skin of the fruits was

punctured. Two types of cages were developed for the breeding work, one was made of wood, glass and wire-mesh and measured 19 x 18 x 13 inches while the other was cylindrical, about 10 inches high and 8 inches in diameter and made of transparent plastic and muslin. The cages were lighted by two fluorescent tubes with reflectors. The first type had a capacity of 300-500 pairs of adults when used for obtaining eggs but could accommodate 3000 flies, when used as a stock. The second type had a maximum capacity of 100 pairs of adult flies. Bananas were hung in the cages and replaced with fresh ones every 24 hours. Water was provided by means of moist blotting paper. The bananas that contained eggs were kept over dry sand at 25°C and the pupae collected after 16 days.

Ryan (1949) found that when infested fruits were spread on trays of sand, the mold that developed and the juice from the spoiled fruits caused the cementing of the sand and resulted in the death of the majority of the maggots. A new technique was developed by him which employed shallow trays with wire-mesh bottoms on which the infested fruits were placed. The trays were then stacked over each other. The larvae and juice fell into sloping "dip trays" which led to a "splash tray" with a drain for the juice on one side and a tray of sand on the other. The larvae could pupate in the dry sand.

Shaw and Starr (1946) developed a method for collecting _Ceratitis_ eggs. The flies were allowed to lay their eggs

in pieces of fruit skin with little pulp adhering to it and fixed on glass plates with paraffin wax. The eggs were then removed with a camel's-hair brush and kept on filter paper moistened with 0.1 per cent cupric chloride to prevent the growth of fungi. When the eggs hatched, the larvae were fed on pieces of fruit and left to pupate in sand.

Many food media have been developed for the rearing of the larvae and adults of Ceratitis capitata. Marlowe (1934) used an artificial medium composed of a mixture of extracted honey, crushed fruits, brown sugar, agar-agar and water for the feeding of the maggots and adult flies. This medium provided satisfactory food for 6 to 16 weeks and in comparison with fresh fruits greatly decreased the time required for the care of the flies.

A fortified carrot medium, developed by Finney (1956) for the rearing of the Oriental fruit fly, Dacus dorsalis Hendel, proved to be somewhat useful for the rearing of Ceratitis capitata. The medium consisted of crushed carrots to which was added "Butoben" (n-butyl p-hydroxybenzoate) to prevent the growth of molds, brewers' yeast to compensate for nutritional deficiencies and 2 N hydrochloric acid to inhibit the development of bacteria.

Christenson et al (1956) substituted dehydrated for fresh carrots used in the medium developed by Finney and found that it resulted in larvae of D. dorsalis that were more uniform in size. It also improved the rearing of C. capitata.

C. Anatomy and histology of the nervous system.

The nervous system of insects is divided into the central, visceral and peripheral sensory nervous systems.

In insects the generalized type of central nervous system is formed of a series of double ganglia joined longitudinally by nerve cords known as connectives, and transversely by commissures. It is divided into three major parts, namely the supraoesophageal ganglion or "brain", the suboesophageal ganglion and the ventral nerve cord which is composed of a number of thoracic and abdominal ganglia. However, in the higher forms of insects, for example in the order Diptera, sub-order Cyclorrhapha, to which Ceratitis capitata belongs, the thoracic and abdominal ganglia are fused into one big thoracic ganglion. Nerve cords are distributed from this ganglion to the legs, the dorsal part of the thorax, and the abdomen. The brain is located directly above the oesophagus, and is mainly an association center. It controls the reflex responses in the rest of the body in accordance with the stimuli received from the great sense organs of the head. It is responsible for orientation and for all the more complex forms of behavior" (Wigglesworth, 1950).

The suboesophageal ganglion lies below the oesophagus and is formed by the fusion of the mandibular, maxillary and labial ganglia (Imms, 1951). It contains the motor centers for the mouth parts and innervates them. It influences also the motor activity of the entire insect (Wigglesworth, 1950).

The visceral or sympathetic nervous system is divided into the oesophageal sympathetic and the ventral sympathetic. The first is connected with the brain and innervates the fore and middle intestines, and heart. The second consists of a pair of transverse nerves system. The transverse nerves supply the spiracles in the body segments. Nerves also arise from the last abdominal ganglion and innervate the reproductive system and the hind-intestine (Imms, 1951).

The peripheral sensory nervous system consists of delicate nerve fibers and cells located in the integument below the hypodermis. These nerves include both motor and sensory fibers which lead to various sense organs. The sense organs of insects and especially those that are located on the legs are of importance in toxicological tests.

The simplest type of sense organs is the sensillum which is a hair innervated by a sensory cell. The external shape of the sensillum varies considerably from a spine to a scale to a minute peg. It may be located superficially on the integument, or may arise from a deep cavity. There are also two groups of sense organs which are related to the sensillae but lack the external processes. These are the sense pores which are marked externally by pits, and the plate organs which are covered externally by oval or elliptical plates surrounded by membranes (Snodgrass, 1935).

A typical sensillum consists of the cuticular or external part of the organ which is connected to its tricho-

genous or formative cell. A sense cell, which lies in the hypodermis, is connected to the trichogenous cell by its distal process. At the end of the sensory process, there is a minute sense rod which is attached to the cuticle of the organ. The sensory cell is connected to a ganglion of the central nervous system by a nerve fiber (Imms, 1951).

The tactile sensillae which are the simplest of the sense organs are distributed all over the insect's integument and especially on the antennae, palpi, legs and cerci; they are usually of the trichoid type - slender and pointed. The olfactory (smell) and gustatory (taste) sensillae are more developed than the tactile sensillae. The cuticular processes of the smell and taste sensillae vary greatly in appearance. All types of sensillae however, are characterized by their thin cuticle (Imms, 1951). The organs of vision and hearing bear no direct relation to the subject of this thesis, hence they are not described.

D. Penetration of contact insecticides

A complete review of literature on the penetration of contact insecticides is not feasible because of the large amount of work that has been done in connection with the subject, especially after the discovery of DDT. A number of workers have discussed the mode of penetration of various insecticides through the cuticle of insects. Savit _et al_ (1946) found that gamma-BHC was equally effective against _Periplaneta americana_ when applied either to the body surface

or as intra-abdominal injections. There was no significant difference between the two median lethal doses. DDT exhibited similar effects. They concluded that "this finding emphasizes the importance of the absorptive capacity of the insect body surface for contact poisons in contributing to the effectiveness of insecticides." Dresden and Krijgsman (1948) found that the specific insecticidal properties of DDT, BHC and rotenone were based on the efficient absorption through the insect cuticle rather than on the high toxicity of the respective insecticides. Similar results were obtained when the insecticides were tested both on the skin of the insects and by injections. This demonstrated that the cuticle did not act as a barrier but rather permitted a quick and complete penetration of insecticides. Wiesmann (1946) tested a solution of DDT in oil on adults of *Calliphora vomitoria* and *Blattela germanica*, and found that its penetration was dependent on the structure and chemical composition of the cuticle and the lipophile properties of DDT. Penetration was possible through areas which contained lipoids, and where the exo- and endo- cuticles were very thin, unpigmented and flexible. Such areas were the sense organs and the intersegmental skin. Areas that were not permeable contained sclerotin in their exo- and endocuticles.

Fisher (1952) tested DDT activity on *Musca domestica*. He found that the cuticle played an important part in DDT toxicity, since a certain dose of DDT applied to the mesonotum

of the fly produced 16 times more toxicity than a dose three times higher when applied internally into the tissues of the mesonotum. He also found that the location and size of the cuticular area was important. The effectiveness of DDT was increased as the loci of application approached the body or head. Treatment of the labella gave the highest effectiveness.

Armstrong _et al_ (1951) studied the mode of penetration of four benzene hexachloride isomers through the cuticle of grain weevils. They found that the amount of each isomer taken up by the insects was related to its solubility in hydrocarbon solvents. The gamma isomer was able to penetrate through the outer layers of the insect cuticle much more rapidly than the other three isomers tested, namely alpha, beta and delta. From these results they concluded that the pick-up of the insecticides by the insects was a simple solution of the insecticides in the outer waxy covering of the epicuticle, and that the structure of the insecticide seemed to play an important part in the ability of penetration through the insect cuticle as well as in its toxicity at the site of action.

The penetration of contact insecticides through the tarsi of insects was found to be of importance also. Potts and Vanderplank (1945) tested DDT and pyrethrum against the tse-tse fly (_Glossina_ spp.). The flies were killed after touching the insecticides with their tarsi for only two seconds. They concluded that the susceptibility of insects

to these insecticides is correlated with the development of the pulvilli. Hickin (1945) questioned the preceding conclusion by experimenting with two species of lice that have very poorly developed pulvilli. When their tarsi came in contact only with DDT, they died in 120 minutes, while n-carbitol thiocyanate, under the same conditions, killed them in only six minutes. He concluded that the relative size of the pulvillus is unlikely to have any special significance in the entry of insecticides, and that different insecticides show a wide variation in their speed of action on the same species. Burtt (1945) experimented with a number of insecticides on the sheep tick, _Ixodes ricinus_. The insecticides were applied both on the backs of the ticks and on their feet. DDT and rotenone required an average of four days to produce intoxication when applied on the dorsal side of the ticks, yet they acted in an average of eight hours when applied to the legs. The feet of the ticks proved to be the most vulnerable part of the body to all the insecticides tested. Hayes and Liu (1950) compared the histology of the tarsi of _Musca domestica_, _Blattella germanica_ and _Epilachna varivestis_. They found that in the house fly, which is susceptible to DDT poisoning, the tarsi possessed many chemoreceptive sensillae and the cuticle is thin, 12.5-25 micra in thickness. No chemoreceptors were found on the tarsi of the other two insects which are resistant to DDT poisoning, and their cuticles were found to be thicker than that of the house fly. Fisher (1952)

disagrees with these findings since he found that penetration of DDT was more effective through the first tarsomere of the fly's leg, which bears no sensillae, than through the third, fourth and fifth tarsomeres which have large numbers of sensillae. He adds "the claim that sensillae offer better entry points is based on the thinness of the articular membranes, but there is no evidence that the cuticle thickness in house flies plays any part in preventing or retarding DDT penetration." Srivastava (1957) working on the mode of entry of aldrin into the body of the grasshopper Hieroglyphus nigrorepletus, found that the pretarsus region was the most susceptible part. Entry of the insecticide through the other parts of the leg was slower than through the pretarsus. He concluded that when aldrin was applied to the pretarsus region, concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 per cent of the insecticide were able to effect the mortality of H. nigrorepletus to the extent of 6, 20, 60, 90 and 98 per cent respectively by the end of 72 hours after application.

The mode of action of organic phosphorus insecticides has been studied also by a number of workers. Metcalf and March (1949) studied the mode of action of parathion and its derivations on cockroaches, house flies and worker bees. They found that parathion and para-oxon were readily absorbed through the cuticle of the American cockroach, and thus the median lethal doses for topical application and for injection were almost identical. Ball and Beck (1951) studied the

effect of parathion on the American cockroach. They found that the central nervous system and the blood of the cockroach were active in translocating parathion in the body. However the nervous system was more active in this translocation. They also found that knockdown of the cockroaches was directly related to the proximity of the site of insecticidal application to the central nervous system. Hartley and Brown (1955) tested the effect of 32 insecticides on the cholinesterase of the American cockroach. They found that the 13 chlorinated hydrocarbons tested, including DDT, lindane and the chlordane compounds, had no significant effect on the cholinesterase from the head of the cockroach. Of the organic phosphorus insecticides tested, schradan and EPN had no effect, while Diazinon, TEPP, parathion and malathion had an anticholinesterase property.

MATERIALS AND METHODS

A. Breeding in the laboratory

The attempt to rear the Mediterranean fruit fly in the laboratory at the Faculty of Agricultural Sciences, American University of Beirut, was started in April, 1957. A search for infested oranges and mandarins was begun in the citrus growing districts of Antelias and Sin-el-Fil, north of Beirut. However this search was not successful since, as was stated by Bodenheimer (1951) the mortality of the eggs and larvae of *Ceratitis capitata* in citrus fruits was found to be very high.

In July, 1957 the attempt to collect *Ceratitis* larvae was resumed. A large number of peaches suspected to be infested were collected and placed on top of sand in aluminum trays. Sand was used because the larvae can move easily through it and pupate. This work was carried on until October, 1957 but the flies were not obtained in numbers sufficient to start breeding. The reasons for this failure, although peaches are known to be a favored host of *Ceratitis* in Lebanon is thought to be caused by the high rate of mortality among the larvae as a result of the quick rotting and molding of the peaches and the excess juice coming out which could easily drown the larvae. Another possible reason for the failure to get adult flies could be attributed to the extremely large numbers of *Drosophila* maggots that developed in the peaches. They seemed to have spoiled the medium for *Ceratitis* larvae.

When this method to obtain adult flies proved to be unsuccessful, the writer asked the Geigy Company in Basel, Switzerland to send him some pupae to start the breeding work. In October, 1957 he received around 100 pupae from which some adults appeared on the day of arrival.

The method of rearing the Mediterranean fruit fly in the laboratory was essentially similar to that which the writer has seen used by the Geigy Company in Basel, Switzerland and the Bayer Company in Leverkeusen, Germany. Rearing cages, 85 x 55 x 55 centimeters with a glass door and window, a wooden bottom and fine wire-mesh sides, were used. To stop the entrance of Drosophila, which proved to be a nuisance in breeding Ceratitis, the sides of the cages were covered with cheese cloth which had smaller holes than those of the wire-mesh. The glass window was replaced with a piece of cloth in the shape of a sleeve through which the hand could be inserted into the cage without allowing the escape of any flies.

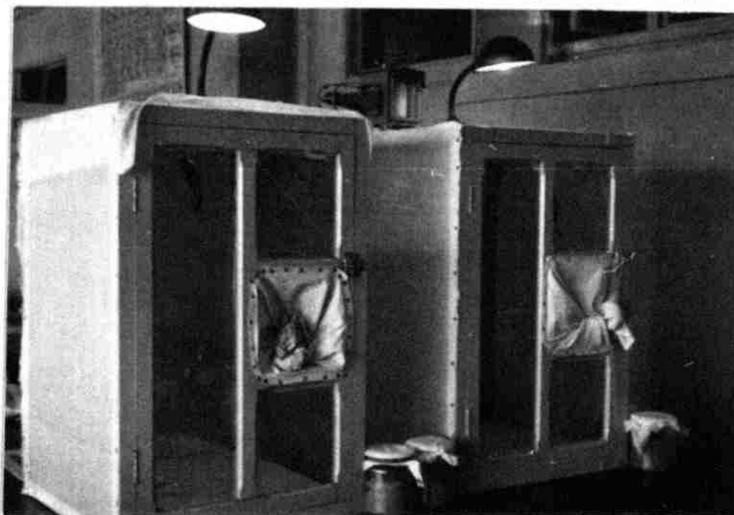


Photo - N.S.Kawar

Fig. 5 - Rearing cages for C. capitata. Note jars of sterilized sand for pupation.

Light was provided at night from two 100-watt Tungsten desk lamps, placed on top of the cages.

Banana fruits were used for the feeding and oviposition of the flies. A number of triangular holes about one centimeter on each side were cut in the skin of the fruits. The purpose of these holes was to facilitate the feeding and the oviposition by the flies in the fruits themselves which were hung from the tops of the cages. A peeled banana was also placed on the floor of the cage to supply additional food. The bananas were replaced every two or three days with fresh fruits as considered necessary.

Supplementary feeding consisted of a 20 per cent protein hydrolyzate solution, an 80 per cent sugar solution and tap water. It was found that the protein hydrolyzate is very effective in shortening the preoviposition period and in increasing the fecundity of the female flies, while the sugar solution is needed as a source of carbohydrates (Hagen and Finney, 1950). Filter papers soaked with these solutions were replenished daily.

In order to control the fungus that developed on the bananas in the cages and the jars, the fruits were brushed with a 10 per cent Hydrogen Peroxide solution before they were hung in the cages.

Banana fruits that were known to contain Ceratitis eggs were transferred from the cages to glass jars, each containing a layer of sterilized sand about 10 centimeters

thick. In these jars the eggs hatched into larvae, fed on the bananas, pupated in the sand and finally emerged as adult flies. The length in days of each stage in the life cycle was observed and recorded in Tables I, II and III. Other characteristics such as the site of oviposition, number of eggs in a puncture, habits of larvae and duration of mating were also observed.

To study the effect of temperature on the rate of development, the jars were kept under three different conditions: 1) a temperature of 30°C and a relative humidity of 75 per cent, 2) a temperature of 35°C and a relative humidity of 75 per cent, and 3) room temperature.

In the first two cases a relative humidity of 75 per cent was obtained by providing a saturated sodium chloride solution in an open beaker placed on the middle shelf of the incubator.

In the third case, the temperature in the room was recorded by two thermographs, one placed on top of the cage and the other on the table next to the cage.

B. Techniques applied in the anatomical and histological study.

The anatomy of the nervous system of the Mediterranean fruit fly was studied by dissecting a number of adult flies and tracing the central nervous system and its branches to the various parts of the insect's body. This study was found necessary to facilitate the histological work which followed later. The gross anatomy of the legs of the fly was also

studied and the various types of setae were observed.

The histological study of the nervous system followed this preliminary work. Particular emphasis was placed on nerve endings in the legs since they are the part of the body where contact insecticides are picked up as the fly walks on treated surfaces. The tissues were either stained before or after they were embedded in paraffin and sectioned. In the former case, i.e. *in toto* stain, two methods were used - 1) Ranson's method, (Carleton, 1926) which is a modification of Cajal's technique for neurofibrils; it gave good results in staining the supraoesophageal ganglion (brain) and the compound eyes but failed to give satisfactory results in staining the nerves in the leg. 2) Ranvier-Loewit gold chloride method (Carleton, 1926) which gave good results in staining the nerves in the legs. After staining, the tissues were embedded in paraffin, sectioned and fixed on slides.

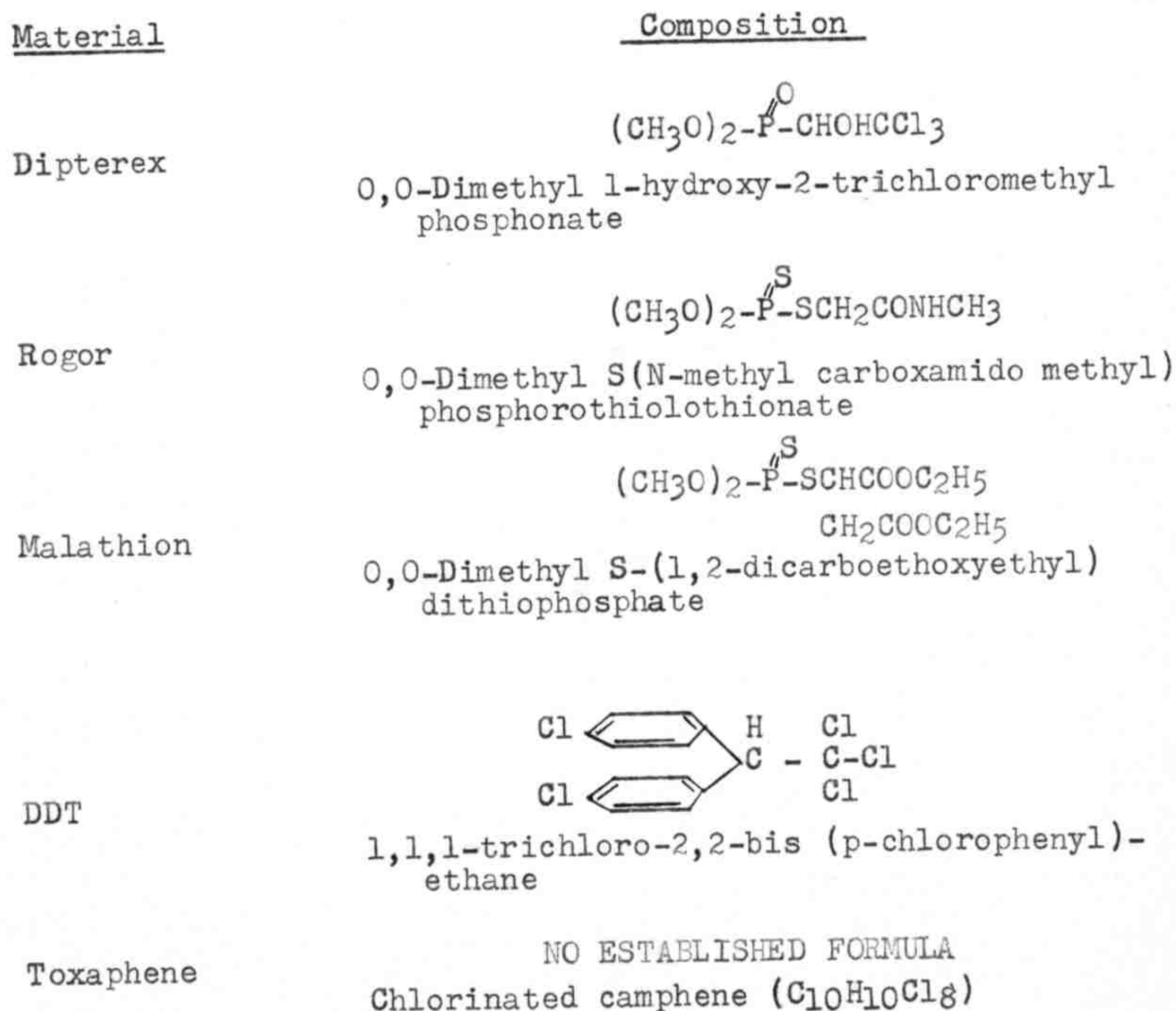
Tissues that were sectioned before staining were first fixed in 10 per cent formaldehyde for 24 hours, washed in running tap water for several hours and then embedded in paraffin and sectioned by the usual technique (Guyer, 1953). Two stains were used on the sections, i.e. Luxol Fast Blue (Kluver, 1944) and the Silver-diamino hydroxide method (Nassar and Shanklin, 1955). Both stains gave satisfactory results on the head but were unsatisfactory in staining the nerves in the leg.

Sections of the head were cut 7 micra thick while those of the legs were cut 10 micra in thickness.

C. Testing contact insecticides

The effectiveness of five insecticides against *C. capitata* was tested in the laboratory by finding their median lethal doses (LD_{50}).

The insecticides used in these tests were the following:



Material	Composition
Dipterex	$(CH_3O)_2-\overset{O}{\overset{\|}{P}}-CHOHCCl_3$ 0,0-Dimethyl 1-hydroxy-2-trichloromethyl phosphonate
Rogor	$(CH_3O)_2-\overset{S}{\overset{\|}{P}}-SCH_2CONHCH_3$ 0,0-Dimethyl S(N-methyl carboxamido methyl) phosphorothiolothionate
Malathion	$(CH_3O)_2-\overset{S}{\overset{\|}{P}}-SCHCOOC_2H_5$ $CH_2COOC_2H_5$ 0,0-Dimethyl S-(1,2-dicarboethoxyethyl) dithiophosphate
DDT	$(Cl-C_6H_4)_2CH-CCl_3$ 1,1,1-trichloro-2,2-bis (p-chlorophenyl)-ethane
Toxaphene	NO ESTABLISHED FORMULA Chlorinated camphene ($C_{10}H_{10}Cl_8$)

Two of these insecticides, namely DDT and Toxaphene belong to the chlorinated hydrocarbons while the other three, i.e. Dipterex, Malathion and Rogor are organic phosphates.

Each insecticide was tested at three different concentrations which were prepared by diluting the insecticide in paraffin oil and petroleum spirit in the ratio of 1:4. This combination ensured a rapid and uniform spreading of the insecticidal film on the filter paper (Blackith, 1950). Petroleum spirit, being quite expensive, was replaced by ordinary automobile gasoline. Only the gasoline fraction, boiling between 45 and 80°C, was used. This gasoline fraction proved to be satisfactory and was much cheaper than petroleum spirit. All the insecticides used were in the form of emulsifiable concentrates. The concentrations of the insecticides were calculated on the basis of percentage by volume rather than on the percentage by weight which give more accurate results. The concentrations used in the final tests were:

Material	Concentration %
Dipterex	0.16
	0.08
	0.04
Rogor	0.1
	0.05
	0.025
Malathion	0.04
	0.02
	0.01

Material	Concentration %
DDT	2
	1
	0.5
Toxaphene	1
	0.5
	0.25

In order to insure continuous contact with the insecticidal film, the flies were kept under glass funnels resting on Whatman No. 1 treated filter papers 11 cm in diameter. The inside walls of the funnels were fitted with semi-circular pieces of the same kind of filter paper. The funnels were then connected to vaccum suction tubes to prevent the accumulation of fumes. This method was adapted from Pradhan (1949) who used several devices for confining insects on treated surfaces.

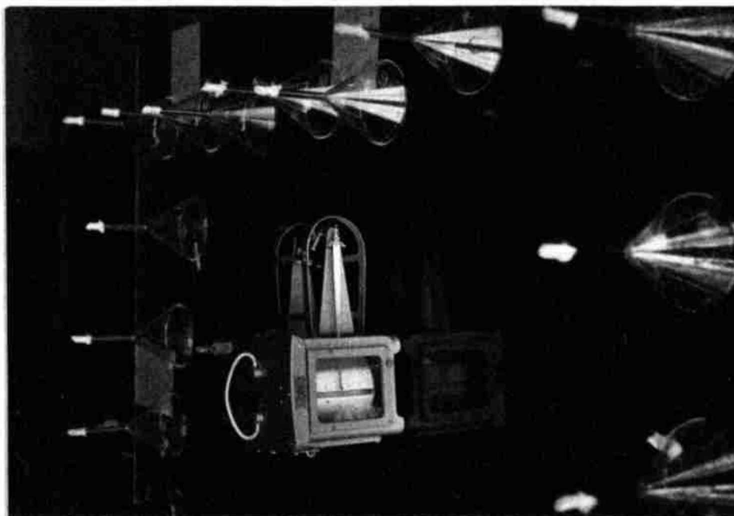


Photo - N.S.Kawar

Fig. 6 - A battery of funnels, with treated filter papers, attached to suction pump to exclude action of gases

The circular filter papers were placed on glass sheets and treated with 1.5 cc and the semicircular ones with 2.5 cc of the insecticide. The insecticidal solutions were applied by means of syringe pipettes. This procedure was adapted from Busvine and Barnes (1948) and Stringer (1949). The filter papers were allowed to dry for 14-15 hours before being used.

When the whole set-up was ready the flies were collected by an aspirator, stupefied by carbon dioxide, and placed on the treated surfaces. They were confined for 30 minutes inside the funnels after they recovered from the anaesthetic. They were then restupefied, and removed to clean funnels placed on a glass-topped table and provided with bananas and pieces of filter papers soaked with tap water.

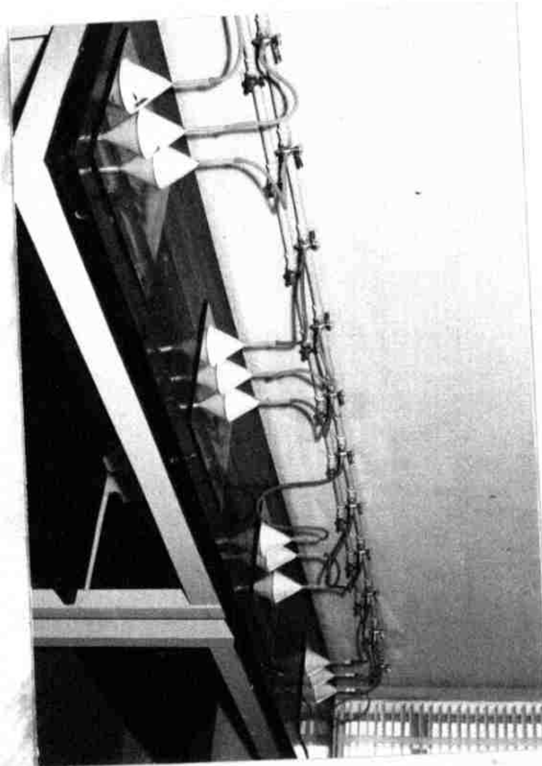


Photo - N.S.Kawar

Fig. 7 - A thermograph and a set of funnels under which treated flies were kept for the duration of the experiment

Two other methods, besides carbon dioxide, were tried for immobilizing the flies. The first was an attempt to chill the flies in a refrigerator to a temperature below their threshold of activity. This method was not successful since the flies recovered rather rapidly. The other method was the use of ether. This was discontinued since the vapors of ether possess a fat-solvent ability and thus rendered the results inaccurate (Busvine, 1957). Carbon dioxide was found to be the safest material to use for immobilizing the flies (Sherman, 1953).

The flies were observed occasionally for the first 24 hours and then once every 24 hours for three days. Mortalities were recorded after every observation.

Several pilot tests were conducted to determine the age of the flies and the concentrations of the insecticides to be used in the final tests. In the pilot tests, 15 flies were used for every concentration giving a total of 270 flies, including the controls, for the whole test. In the final tests, however, 30 flies were used giving a total of 540 flies. The tests were repeated three times. The control solution consisted of the paraffin oil and the petroleum spirit only.

The results of these tests were analyzed statistically by the Probit analysis (Finney, 1947. Busvine, 1957).

Calculation of the regression equation proceeds in the following manner:

1) Results of the tests with the insecticide at different concentrations are set down in the first three columns.

2) The mortality rates are corrected for control deaths, by Abbott's formula: $P = \frac{P'-C}{100-C}$ where P = corrected mortality, P' = observed mortality C = control mortality.

3) The concentrations are converted to logarithms. Negative values are avoided by adding one or more units.

4) The per cent mortalities are converted into probits and the results are tabulated in column (vi).

5) The emperical probits (vi) are plotted against log doses (v) on ordinary graph paper. A provisional line is drawn by the sum of squares method.

6) Expected probits (Y) for the log.-dose values are determined from this provisional line.

7) Working probits (y) are then calculated from the formula $y = Yo + kp$, where p = corrected mortality (Busvine, 1957. Table VII).

8) Weighting coefficients for each point are also read from the same table. Each coefficient is multiplied by the number of flies used and the products form the weights (column x).

9) Columns (xi) and (xii) are formed by multiplying w by x and w by y.

10) Adding up columns (x), (xi) and (xii) gives the sums of w, wx and wy. The means are also calculated, $\bar{x} = \frac{Swx}{Sw}$ and $\bar{y} = \frac{Swy}{Sw}$.

11) Swx^2 is obtained by multiplying wx by x for each line and adding up the products.

12) Similarily, Swy^2 and Swxy are obtained.

13) The value b for the regression equation is calculated by the formula: $b = \frac{Swxy - \bar{x}\, Swy}{Swx^2 - \bar{x}\, Swx}$

14) The regression equation is $y = \bar{y} + bx - b\bar{x}$

15) The appropriate values of $\bar{y}$, b and $\bar{x}$ are substituted to give the final regression equation for the insecticide.

16) Values of x corresponding to y = 5.0 and some other values, (y = 7.0 and y = 3.0) are calculated so as to draw the regression line which gives the exact LD50.

17) The variance is calculated by the formula

$$V = \frac{1}{b^2}\left(\frac{1}{Sw} + \frac{(m-\bar{x})^2}{Swx^2 - \frac{(Swx)^2}{Sw}}\right)$$

where m is the log. LD50 calculated above, and the other quantities have been calculated.

18) The 95% confidence limits (m_1 and m_2) for the log. LD50 (m) are calculated by the following formulae:

$$m_1 = m - 1.96 \times \text{Standard deviation}$$

$$m_2 = m + 1.96 \times \text{Standard deviation}$$

where the standard deviation is equal to the square root of the variance.

RESULTS AND DISCUSSION

Breeding of *C. capitata* was performed in the laboratory using jars kept under three different conditions: 1) in an incubator with a temperature of 30°C and 75 per cent relative humidity, 2) in an incubator with a temperature of 35°C and 75 per cent relative humidity, and 3) on the table at room temperature. A number of observations were recorded during the time of breeding. Temperature plays a major role in the rate of development of the stages of this fly. In breeding at 30°C and a relative humidity of 75 per cent, 14 replicates were performed. The results are shown in Table I.

Table I. Duration in Days of the Different Stages of *C. capitata* at 30°C and 75 per cent Relative Humidity

Replicate	Egg stage in days	Larval stage in days	Pupal stage in days	Total in days
1	4	8	5	17
2	4	8	5	17
3	4	8	5	17
4	3	8	5	16
5	3	7	6	16
6	5	8	6	19
7	3	7	7	17
8	4	7	6	17
9	4	8	6	18
10	4	8	6	18
11	5	8	6	19
12	5	8	6	19
13	3	7	7	17
14	3	7	6	16
Average	3.9	7.6	5.9	17.4

Apparently the above mentioned conditions are not the most favorable for the development of Ceratitis since, as seen from Table III, a faster rate of development resulted at lower temperatures.

At 35°C and a relative humidity of 75 per cent, only four replicates were performed. The results are tabulated below.

Table II. Duration in Days of the Different Stages of *C. capitata* at 35°C and 75 per cent Relative Humidity

Replicate	Egg stage in days	Larval stage in days	Pupal stage in days	Total in days
1	2	5	All died	-
2	3	3	All died	-
3	2	4	All died	-
4	2	4	All died	-
Average	2.3	4	-	-

All the larvae turned charcoal black in color when they died which may indicate that the high temperature was responsible for the death of these larvae.

Table III gives the average duration of the different stages in the laboratory during the year starting November 1, 1957 and ending October 31, 1958. The monthly average of the temperature was calculated by the method of Bodenheimer which follows. The average daily temperature (a.d.t.) is calculated by recording the temperatures at 7 a.m., 2 p.m. and 9 p.m., multiplying the evening temperature by 2, adding the three readings and dividing by 4. The monthly average is calculated by adding the a.d.t.'s for the month and dividing by the number of days in that month.

Table III. Average Duration in Days of the Different Stages of C. capitata in the Laboratory During Different Months of the Year

Month	Egg stage in days	Larval stage in days	Pupal stage in days	Total in days	Average Room Temperature in °C
November 1957	4	12	8	24	21.7
December 1957	5	12	9	26	21.2
January 1958	5	14	10	29	20.3
February 1958	5	14	11	30	20.1
March 1958	3	8	5	16	24.5
April 1958	4	8	6	18	23.6
May 1958	4	8	6	18	22.7
June 1958	3	7	4	14	26.5
July 1958	3	7	4	14	27.2
August 1958	1	6	5	12	29.3
September 1958	2	6	5	13	27.8
October 1958	3	7	5	15	24.1

It must be remembered that the laboratory in which the flies were reared was heated during the period starting in late November and ending in the middle of May. However, the average temperatures varied from one month to another in a way sufficient to give an idea of the effect of temperature on the rate

of development. The shortest life cycle was obtained during the month of August when the average temperature was 29.3°C. However, a higher rate of mortality of pre-adult stages seems to have taken place. Similar results were obtained during the months of July and September. The most successful breeding of flies was obtained at an average temperature of 24-26°C.

Some observations on the habits of the Mediterranean fruit fly were also recorded. Copulation was observed to take place usually from 9 a.m. to 3 p.m. The average duration of a single copulation was found to be two hours. Oviposition was observed to take place shortly after copulation. The female would wander around searching for a suitable place to lay the eggs. The majority of the egg chambers were found in the flesh of the banana fruit which was exposed (see p. 25). However more egg chambers were found in the peeled banana placed on the floor of the cage. The number of eggs in a chamber was found to vary considerably (5-22). A few females oviposited by piercing the peel of the fruit, but among those that did so, a number apparently were unable to withdraw the ovipositor and death resulted.

The larvae, that were kept in the jars, exhibited a positive phototropic behavior, since they always clustered on the lighted side of the jar. The jumping movement of the larvae was also observed and especially when they were placed on hard surfaces. One larva was placed on a table, and the average distance of jumping was measured and found to be 10.5

cm. In jumping, the larva sticks its head and tail vertically on the table until it is arched, and then it releases itself quickly. This sudden release makes it jump.

The depth of pupation was found to vary considerably. Some larvae pupated in the cheese cloth which covered the jar, while others pupated on the surface of the sand, but the majority pupated in the sand at a depth ranging from 1-6 cm.

In certain cases where the food supply in the jar was not sufficient for all the larvae, some of them pupated before becoming full-grown. This early pupation gave rise to smaller flies than the normal ones.

The anatomy of the central nervous system of C. capitata was studied by dissecting a number of flies. Dissecting the head to locate the brain and the suboesophageal ganglion, was practically impossible because of its small size. However, the thoracic ganglion could be located with more ease. It is whitish in color and heart-shaped. It is connected to the legs and the abdominal region.

A study of the external anatomy of the legs was next undertaken. The coxa and trochanter have no striking exoskeletal features. The femur of the fore leg is shorter and broader than those of the middle and hind legs, and bears longer and more numerous spines and bristles. These give it a darker color than the other parts of the legs. The tibia in the three legs are long and slender and do not differ much from one leg to another. However, that of the second

leg bears a prominent spine at its posterior end while those of the first and third do not.

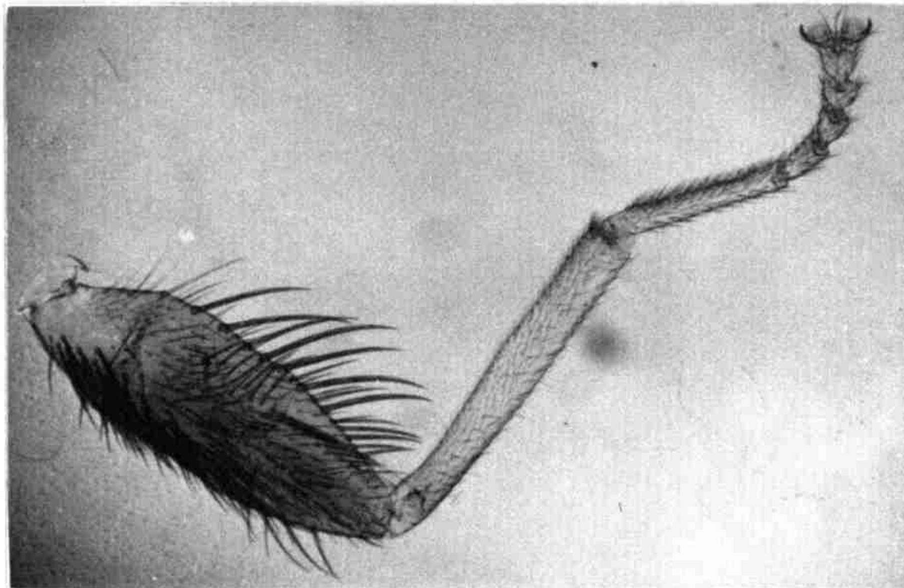


Photo - Dr. Asmar

Fig. 8 - Fore leg of C. *capitata*
60 x

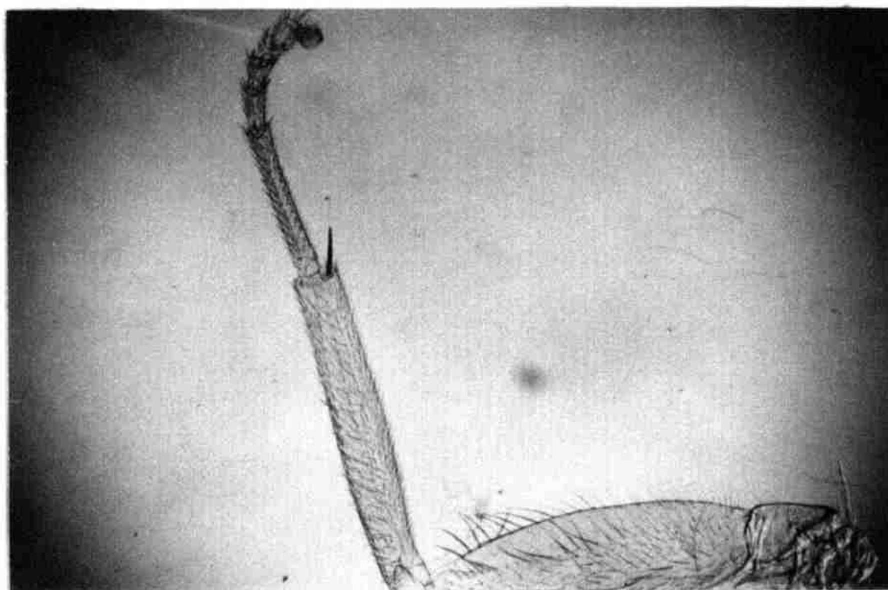


Photo - Dr. Asmar

Fig. 9 - Middle leg of C. *capitata*.
Note spine at tip of tibia. 60x

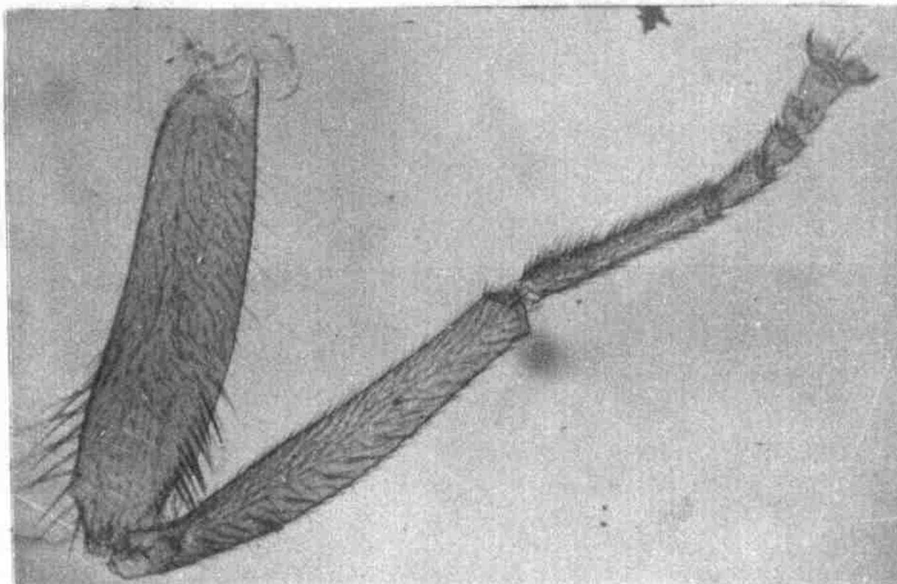


Photo - Dr. Asmar

Fig. 10 - Hind leg of C. capitata. 60x

The tarsus is five-segmented, the first segment is longer than the other four. At its distal end is located the pretarsus which is composed of two pad-like pulvilli which help the fly in holding on smooth surfaces, two strong claws and a long bristle-like process located between them. Imms (1951) states that this process is the flexor plate which has elongated to form the bristle-shaped structure called the empodium. The tendon of the flexor muscle of the claws is attached to the empodium. The photographs of the legs show the high number of spines, bristles, tactile and probable chemoreceptors which cover the legs.

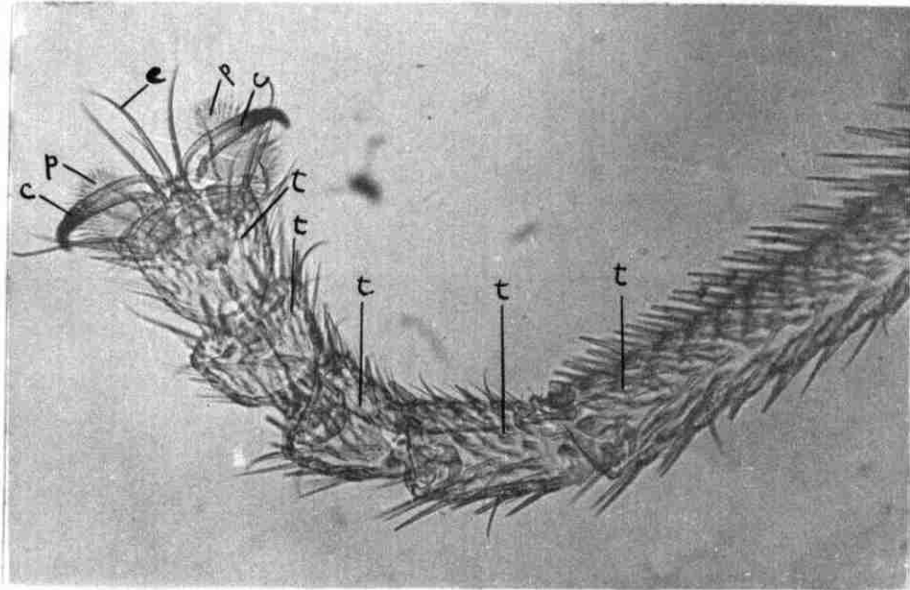


Photo - Dr. Asmar

Fig. 11 - Tarsal segments (t) and pretarsus of the leg of C. capitata showing the claws (c), pulvilli (p) and empodium (e) 95x

The histological study of the nervous system of the flies followed the anatomical study. A number of stains were tried first on the brain to find their suitability in staining the nerve tissues. This was thought to be helpful in spotting finer nerves in other parts of the body. The gold-chloride method gave good results in staining the nerves in the legs. The following photograph shows the comparatively thick nerves passing through the femur to the tibia and tarsi. This is an indication of the important part played by the proximal end of the leg in sense perception, whether it is physical or chemical. It is mainly through contact with the tarsi that the fly picks up the insecticide from dry films, and it is through the nerves that the insecticide acts. Therefore the importance of innervation of the leg cannot be over-emphasized.

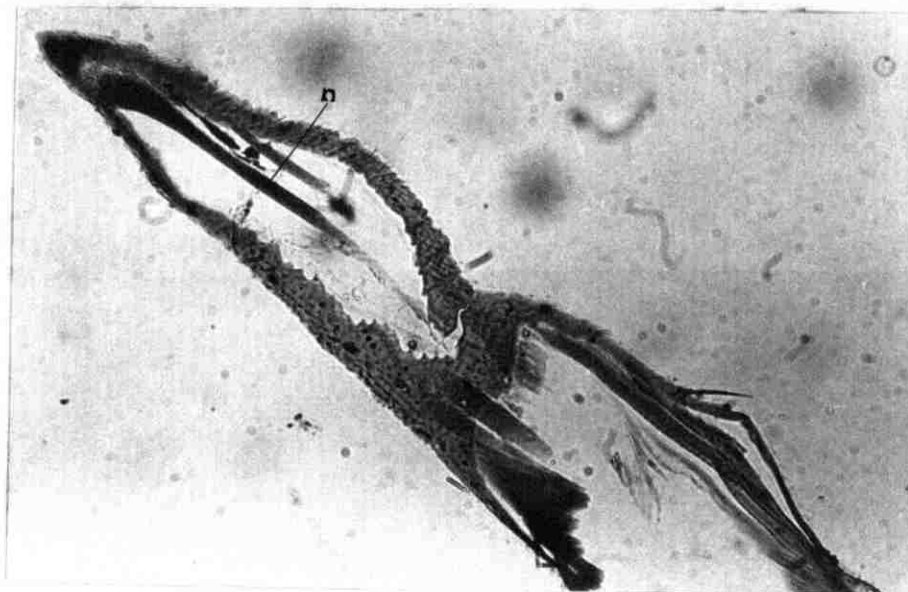


Photo - Dr. Asmar

Fig. 12 - Longitudinal section through the leg of C. capitata showing the nerve (n) passing from the femur to the tibia. 85x

Tests with chemicals were carried in three steps. The first was performed to find whether there was any relation between the age of flies and their susceptibility to the action of insecticides. The flies that were used were 12 days old and had already copulated and oviposited. Tests were carried with Dipterex, Malathion and DDT. The results are shown in Table IV.

Table IV. Effect of Insecticides on Flies 12 Days of Age

Material	Conc.%	No. of flies used	No. of flies dead after 24 hrs.	% Mortality
Dipterex	0.04	13	11	84.6
	0.02	15	6	40.0
	0.01	15	6	40.0
Malathion	0.02	12	8	66.7
	0.01	13	7	53.9
	0.005	12	4	33.3
DDT	0.032	15	10	66.7
	0.016	19	8	42.1
	0.008	15	3	20.0
Control	-	42	15	35.7

The above table shows that the flies were highly susceptible to low concentrations of insecticides, and that the "controls" had a high rate of mortality+. Therefore, it was decided to use young flies, three to four days old, before they had a chance of copulating and ovipositing.

Two pilot tests were then conducted, one comprised five treatments, and the other six. Forty five flies were used in each treatment, and the concentration of the insecticides in each treatment was taken at three levels. Low mortality rates were obtained in the first test as seen from Table V. As a result, the concentrations were raised, and approximate LD50's were determined. The results of the second pilot test are given in Table VI.

+ This is more striking especially when compared to Tables V, VI and VII.

Table V. Per cent Mortality of Flies at Different Concentrations of Dipterex, Malation, DDT and Toxaphene+

Material	Conc.%	No. of flies used	No. of flies dead after 24 hrs.	% Mortality
Dipterex	0.04	15	1	6.67
	0.02	15	0	0
	0.01	15	0	0
Malathion	0.02	15	8	53.33
	0.01	15	1	6.67
	0.005	15	0	0
DDT	0.5	15	3	20.00
	0.25	15	1	6.67
	0.125	15	1	6.67
Toxaphene	0.4	15	9	60.00
	0.2	15	3	20.00
	0.1	15	1	6.67
Control	-	45	2	4.44

Table VI. Per cent Mortality of Flies at Different Concentrations of Dipterex, Rogor, Malathion, DDT and Toxaphene

Material	Conc.%	No. of flies used	No. of flies dead after 24 hrs.	Mortality
Dipterex	0.16	15	15	100.00
	0.08	15	9	60.00
	0.04	15	1	6.67
Rogor	0.1	15	14	93.33
	0.05	15	3	20.00
	0.025	15	0	0
Malathion	0.04	15	14	93.33
	0.02	15	11	73.33
	0.01	15	0	0
DDT	2	15	15	100.00
	1	15	11	73.33
	0.5	15	5	33.33
Toxaphene	1	15	14	93.33
	0.5	15	9	60.00
	0.25	15	5	33.33
Control	-	45	1	2.22

+Rogor was not yet received when this test was performed.

The results obtained in the second pilot test were found to be satisfactory, and therefore the final tests were conducted using the same concentrations as shown in Table VI. The number of flies per treatment was doubled to give more accurate results. The experiment was replicated three times on different dates, and each replicate contained the six treatments.

Table VII. Per cent Mortality of Flies in Three Replications at Different Concentrations of Dipterex, Rogor, Malathion, DDT and Toxaphene

Material	Conc. %	No. of flies used	No. of flies dead after 24 hrs. 1st test	2nd test	3rd test	Average	% Mortality
Dipterex	0.16	30	30	30	29	29.57	98.90
	0.08	30	18	16	15	16.33	54.43
	0.04	30	2	1	0	1	3.33
Rogor	0.1	30	28	27	27	27.33	91.10
	0.05	30	6	6	6	6	20.00
	0.025	30	0	1	1	0.67	2.23
Malathion	0.04	30	30	29	27	28.67	95.56
	0.02	30	22	21	21	21.33	71.10
	0.01	30	1	1	0	0.67	2.23
DDT	2	30	30	30	29	29.67	98.90
	1	30	22	21	19	20.67	68.90
	0.5	30	8	6	5	6.33	21.10
Toxaphene	1	30	30	29	27	28.67	95.56
	0.5	30	18	16	15	16.33	54.43
	0.25	30	8	6	6	6.67	22.23
Control	-	90	2	2	2	2	2.22

The results shown in Table VII were analyzed statistically by the Probit analysis. In this analysis the concentration of the insecticide is expressed by its logarithm, while the per cent mortality is transformed into a probit (Busvine, 1957. Table VI). The log.- concentration/probit line is plotted on ordinary graph paper and probit 5, equivalent to the LD50 is determined. The results are satisfactory in the majority of cases, however a regression equation which gives the exact LD50 can be calculated. Tables VIII to XII summarize the results of calculation of the regression equations for the different insecticides. The method of calculating the regression equation is outlined on pages 32-34.

Table VIII. Calculation of Log.-Dose/Probit Regression Equation for Dipterex

i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
Conc. of Dipterex %	No. of flies used	% dead	Conc. % kill	Log (+2) dose	Emperical probit	Expected probit	Working probit	Weighting coefficient	Weight		
				x		Y	y		w	wx	wy
0.16	90	98.90	98.88	1.204	7.30	7.5	7.21	0.050	4.5	5.418	32.445
0.08	90	54.43	53.40	0.903	5.09	5.0	5.11	0.637	57.3	51.742	292.803
0.04	90	3.33	1.14	0.602	2.71	2.6	2.74	0.062	5.6	3.371	15.344
0	90	2.22	-	-	-	-	-	-	-	-	-

$Sw = 67.4$; $Swx = 60.531$; $\bar{x} = \frac{Swx}{Sw} = 0.8981$; $Swy = 340.592$;

$\bar{y} = \frac{Swy}{Sw} = 5.0533$; $Swx^2 = 55.276$; $Swy^2 = 1772.194$; $Swxy = 312.702$

$b = 7.465$, Regression Equation: $y = 7.465x - 1.651$ if $y = 5, x = 0.8910$

LD50 (m) = Log. 0.8910 = 0.078%

Variance = 0.000267. Standard deviation = 0.0163

95% Confidence Limits = $0.860 < m < 0.922$

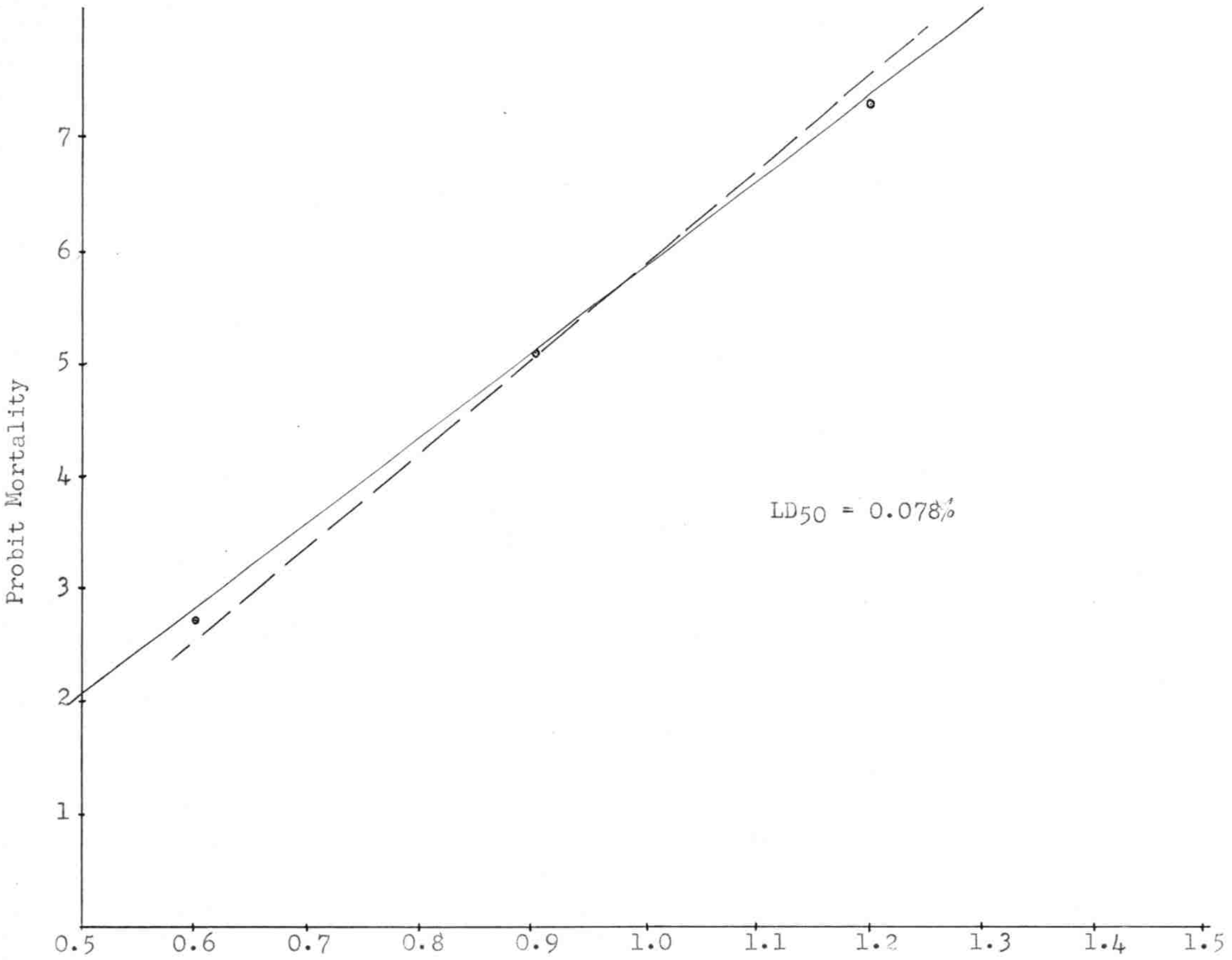


Fig. 13 - Log.-dose/probit regression line for Dipterex

Table IX. Calculation of Log.-Dose/Probit Regression Equation for Rogor

i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
Conc. of Rogor %	No. of flies used	% dead	Conc. % kill	Log (+2) dose	Emperical probit	Expected probit	Working probit	Weighting coefficient	Weight		
				x		Y	y		w	wx	wy
0.1	90	91.10	90.90	1.000	6.33	6.2	6.32	0.370	33.3	33.300	210.456
0.05	90	20.00	18.18	0.699	4.09	4.4	4.13	0.558	50.2	35.090	207.326
0.025	90	2.23	1.02	0.398	2.67	2.5	2.73	0.050	4.5	1.791	12.285
0	90	2.22	-	-	-	-	-	-	-	-	-

$Sw = 88.0$; $Swx = 70.181$; $\bar{x} = \frac{Swx}{Sw} = 0.7975$; $Swy = 430.067$;

$\bar{y} = \frac{Swy}{Sw} = 4.8871$; $Swx^2 = 58.541$; $Swy^2 = 2219.876$; $Swxy = 360.267$.

$b = 6.722$. Regression Equation: $y = 6.722x - 0.477$ if $y = 5$, $x = 0.8143$

LD50 (m) = Log. 0.8143 = 0.065%

Variance = .000254 Standard deviation = 0.0159

95% Confidence Limits = $0.7827 < m < 0.8459$

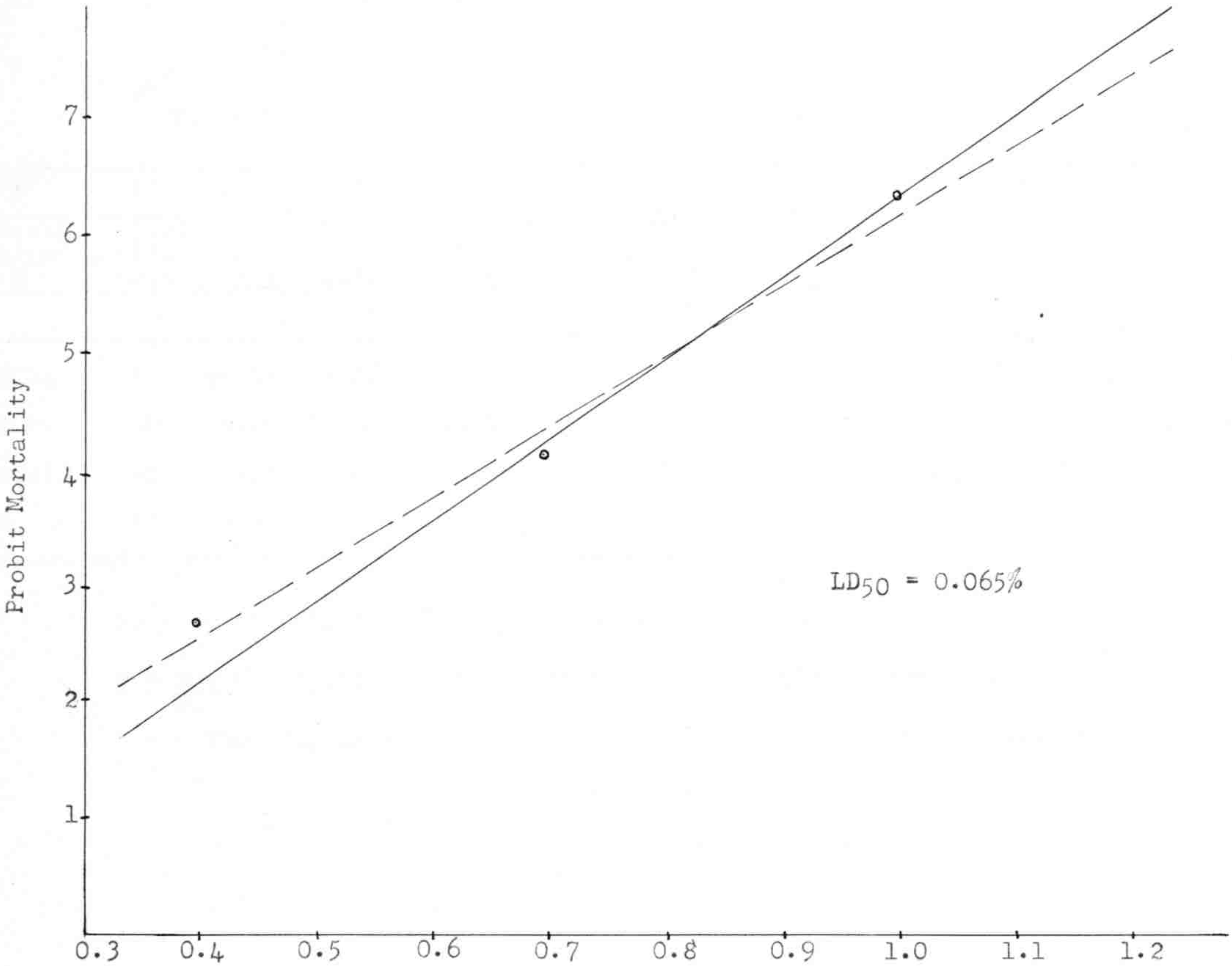


Fig. 14 - Log.-dose/probit regression line for Rogor

Table X. Calculation of Log.-Dose/Probit Regression Equation for Malathion

i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
Conc. of Malathion %	No. of flies used	% dead	Conc. % kill	Log (+2) dose	Emperi-cal probit	Expect-ed probit	Work-ing probit	Weighting coef-ficient	Weight		
				x		Y	y		w	wx	wy
0.04	90	95.56	95.46	0.602	6.69	7.0	6.58	0.131	11.8	7.104	77.644
0.02	90	71.10	70.44	0.301	5.53	5.0	5.52	0.637	57.3	17.247	316.296
0.01	90	2.23	1.02	0.000	2.67	3.0	2.77	0.131	11.8	0.000	32.686
0	90	2.22	-	-	-	-	-	-	-	-	-

$Sw = 80.9$; $Swx = 24.351$; $\bar{x} = \frac{Swx}{Sw} = 0.3010$; $Swy = 426.626$;

$\bar{y} = \frac{Swy}{Sw} = 5.2735$; $Swx^2 = 9.468$; $Swy^2 = 2347.392$; $Swxy = 141.948$.

$b = 6.330$. Regression Equation: $y = 6.330x + 3.368$. if $y = 5$, $x = 0.2578$

LD50 (m) = Log. 0.2578 = 0.018%

Variance = 0.000330. Standard deviation = 0.0182

95% Confidence Limits = $0.240 < m < 0.276$

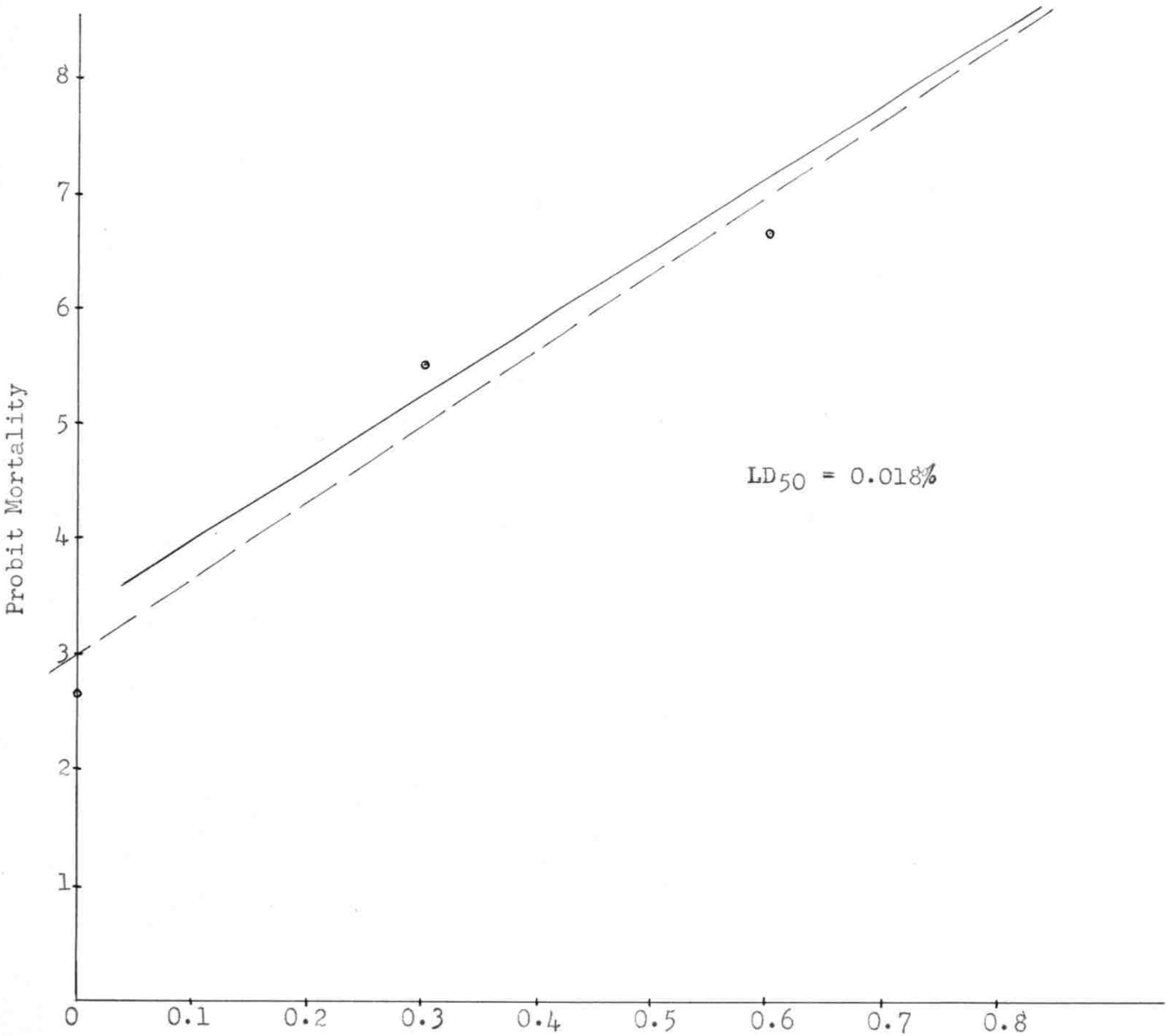


Fig. 15 - Log.-dose/probit regression line for Malathion

Table XI. Calculation of Log.Dose/Probit Regression Equation for DDT

i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
Conc. of DDT %	No.of flies used	% dead	Conc. % kill	Log (+2) dose	Emperical probit	Expected probit	Working probit	Weighting coefficient	Weight		
				x		Y	y		w	wx	wy
2	90	98.90	98.87	2.301	7.29	7.2	7.27	0.092	8.28	19.052	60.196
1	90	68.90	68.19	2.000	5.48	5.6	5.47	0.558	50.22	100.440	274.703
0.5	90	21.10	19.30	1.699	4.13	4.0	4.13	0.439	39.51	67.125	163.176
0	90	2.22	-	-	-	-	-	-	-	-	-

$Sw = 98.01$; $Swx = 186.617$; $\bar{x} = \frac{Swx}{Sw} = 1.9040$; $Swy = 498.075$

$\bar{y} = \frac{Swy}{Sw} = 5.0818$; $Swx^2 = 358.769$; $Swy^2 = 2614.167$; $Swxy = 965.154$.

$b = 4.884$. Regression Equation: $y = 4.884x - 4.127$. if $y = 5$, $x = 1.8888$

LD50 (m) = Log. 1.8888 = 0.774%

Variance = 0.000432. Standard deviation = 0.0207

95% Confidence Limits = $1.848 < m < 1.929$

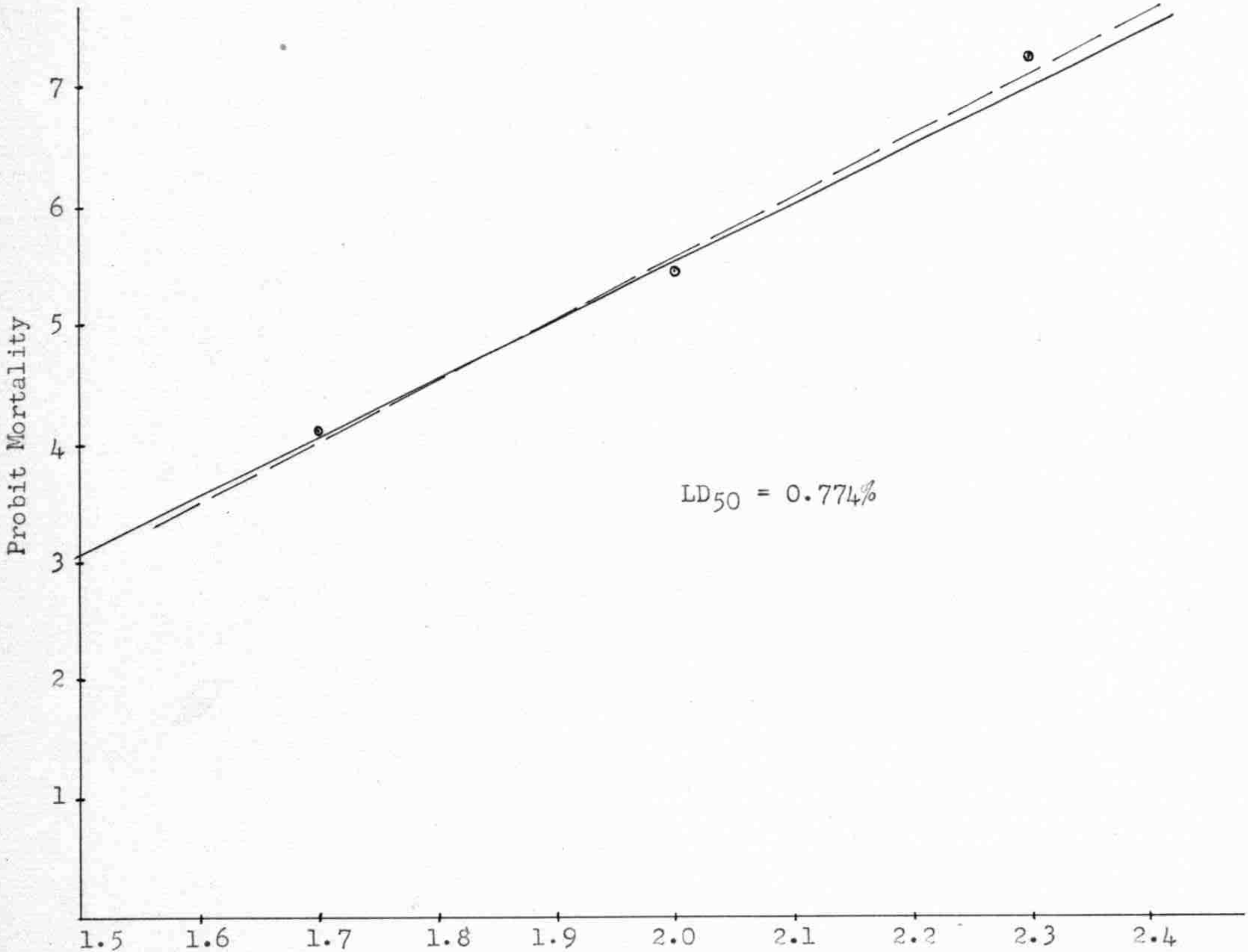


Fig. 16 - Log.-dose/probit regression line for DDT

Table XII. Calculation of Log.-Dose/Probit Regression Equation for Toxaphene

i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
Conc. of Toxaphene %	No. of flies used	% dead	Conc. % kill	Log (+2) dose	Emperical probit	Expected probit	Working probit	Weighting coefficient	Weight		
				x		Y	y		w	wx	wy
1	90	95.56	95.46	2.000	6.69	6.6	7.69	0.238	21.4	42.800	164.566
0.5	90	54.43	53.40	1.699	5.09	5.3	5.11	0.616	55.4	94.125	283.094
0.25	90	22.23	20.46	1.398	4.17	4.0	4.19	0.439	39.5	55.221	165.505
0	90	2.22	-	-	-	-	-	-	-	-	-

$Sw = 116.3$; $Swx = 192.146$; $\bar{x} = \frac{Swx}{Sw} = 1.6521$; $Swy = 613.165$;

$\bar{y} = \frac{Swy}{Sw} = 5.2723$; $Swx^2 = 322.717$; $Swy^2 = 3405.589$; $Swxy = 1041.487$.

$b = 5.401$. Regression Equation: $y = 5.401x - 3.651$. if $y = 5$, $x = 1.6017$

LD50 (m) Log. 1.6017 = 0.400%

Variance = .000132 Standard deviation = .0176

95% Confidence Limits = $1.567 < m < 1.636$

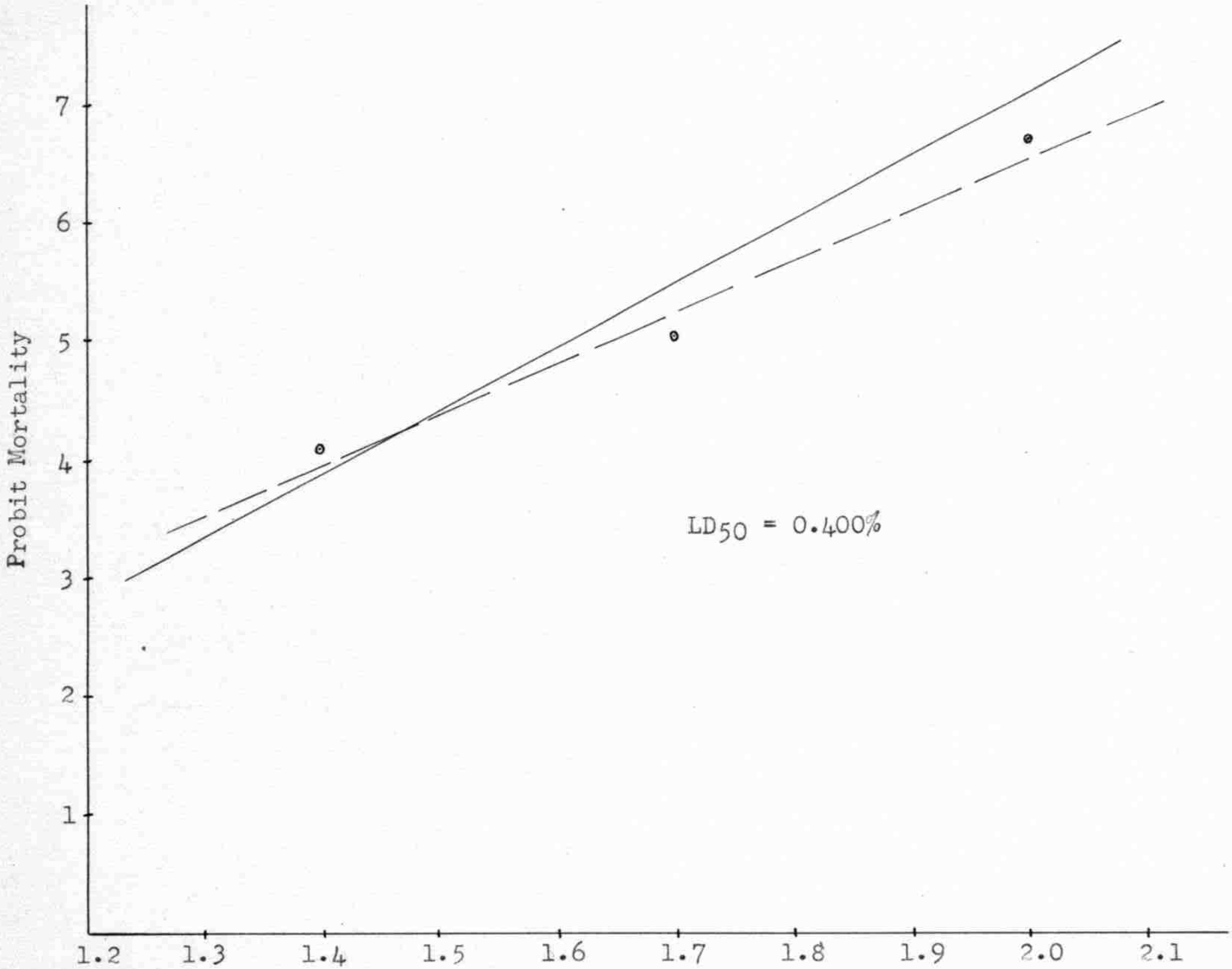


Fig. 17 - Log.-dose/probit regression line for Toxaphene

A comparison of the LD50's of the five insecticides tested can be made by relating them to the LD50 of one insecticide. Sun (1950) suggested a method whereby the LD50's are converted into "Toxicity Indexes" which make comparisons easier. In this method the LD50 of one of the insecticides tested is taken as the standard (=100), and then the toxicity index is calculated by finding the ratio between the LD50 of the standard insecticide and the LD50 of the test sample, multiplied by 100.

It was decided to use DDT as the standard, because it is the classical insecticide and is still widely used against the Mediterranean fruit fly. Table XIII gives the toxicity indexes of the five insecticides and shows that the three organic phosphorus and one chlorinated hydrocarbon insecticides are effective against _Ceratitis_ at lower concentrations than DDT.

Table XIII. Toxicity Indexes of the Insecticides Used Against _C. capitata_

Material	LD50 %	Toxicity Index
DDT	0.774	100
Dipterex	0.078	992
Rogor	0.065	1190
Malathion	0.018	4300
Toxaphene	0.400	194

Malathion has the highest toxicity index, followed

by the other two organic phosphates, Rogor and Dipterex, and finally Toxaphene. As to activity the organic phosphates seem definitely to be more effective than the chlorinated hydrocarbons at the tested concentrations. It was also noticed while conducting the tests that the organic phosphates were very quick acting while the chlorinated hydrocarbons were relatively slow in their action. At the highest tested concentration, Malathion gave around 95 per cent mortality only one hour after the flies were exposed to the residual film. Dipterex, at the highest tested concentration, gave an almost 100 per cent mortality after three hours. Rogor was slower than the first two, while Toxaphene and DDT needed about 12 to 14 hours to produce the percent mortalities given in Table VII.

Ebeling (1953) conducted extensive laboratory tests on the control of fruit flies and he obtained an LD50 of 0.98 per cent for DDT and 0.45 per cent for Toxaphene. The result for Toxaphene is very close to that obtained by the writer while that for DDT differs. An interesting point was brought up by Ebeling in his discussion. He found that the regression line slopes of the insecticides against a given species are remarkably similar. Fig. 18 gives the regression lines for the five insecticides tested in the experiments. It shows that the slopes of these lines are more or less similar, a conclusion in accordance with the results mentioned by Ebeling.

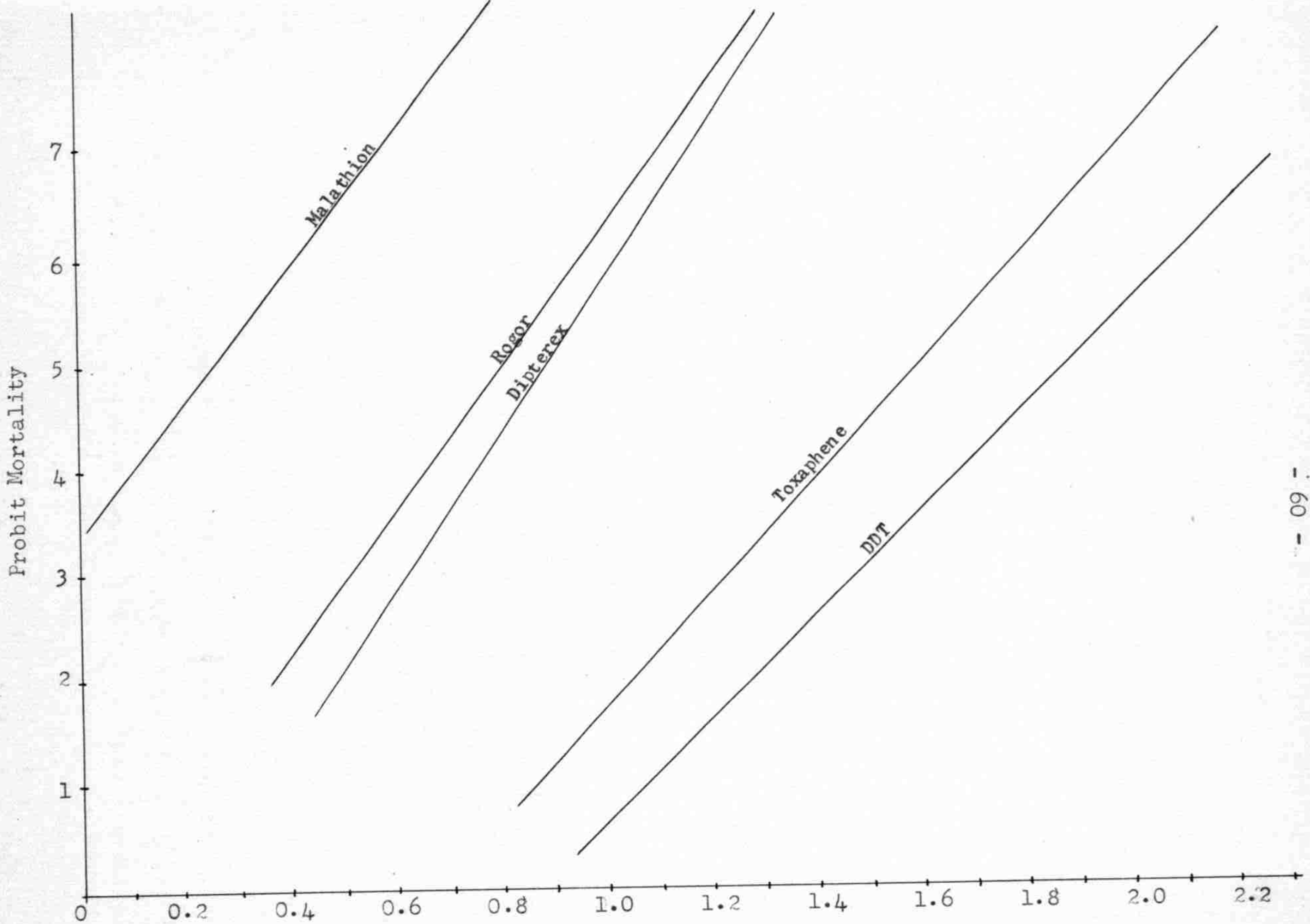


Fig. 18 - Log.-dose/probit regression lines for the five insecticides tested.

SUMMARY AND CONCLUSION

The Mediterranean fruit fly, Ceratitis capitata Wied. is a destructive insect pest which is well established in many countries in the world where it infests a wide variety of plants.

The present work attempted to evaluate five insecticides under laboratory conditions. In order to have a large number of adult flies for the tests, bred under uniform conditions, laboratory breeding was found necessary. Inasmuch as all the poisons employed fall, more or less, in the category of contact poisons, and since the insecticidal pick-up is through tarsal contact with treated surfaces, anatomical and histological investigation of the nervous system and especially in the legs was considered desirable.

The flies were bred in wooden cages with wire-mesh and glass sides. Banana fruits were used for feeding and oviposition. Additional food consisted of sugar and protein hydrolyzate solutions. Banana fruits that were known to contain Ceratitis eggs were transferred from the cages to glass jars containing sterilized sand. In these jars, the eggs developed into larvae which pupated after feeding for some time and finally adult flies emerged.

The effect of temperature on the rate of development of the fly was studied by keeping the jars under three different conditions: 1) a temperature of 30°C and a relative humidity of 75 per cent; 2) a temperature of 35°C and a relative humidity

of 75 per cent; and 3) room temperature. The most successful breeding was observed at an average temperature of 24-26°C and the average duration of one life cycle was 14-16 days.

The anatomy and histology of the nervous system of the fly was also studied. Four stains were tried; the Ranvier-Loewit gold chloride method gave the best results in staining the nerves in the legs.

The effectiveness of five insecticides against *C. capitata* was tested in the laboratory by finding the median lethal dose of each. For the pilot and final tests, three concentrations each of Dipterex, Rogor, Malathion, DDT and Toxaphene were tried, and for each concentration 30 flies were used making a total of 540 for each test. The pilot tests were conducted at first to determine the age of the flies and the concentrations of the insecticides to be used in the final tests. When these were determined the final tests were conducted in three replicates and each replicate contained six treatments. In the tests the flies were confined for 30 minutes under glass funnels resting on treated filter papers to insure constant contact with the insecticide. The flies were then moved to clean funnels and supplied with food and water. Mortalities were recorded at regular intervals and the results were analyzed statistically by the probit analysis.

The results of this work show that Malathion had the lowest LD50 (=0.018%). It was followed by Rogor, Dipterex, Toxaphene and DDT with LD50's of 0.065, 0.078, 0.400 and

0.774% respectively. This can be considered a step towards a practical solution of the problem of the Mediterranean fruit fly.

The next step will be the application of these results in large field tests to see the behavior of the different insecticides against what is generally termed "weathering", which may radically change the over-all picture.

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APPENDIX

While breeding C. capitata in the laboratory five insect and one mite species were observed in the jars in which the bananas were kept. Two of these were easily identified. They were:-

1) Fruit fly - Drosophila melanogaster

2) Beetle - Carpophilus hemipterus

The other four species were sent to the British Museum (Natural History) in London for identification. Mr. G. Owen Evans, Department of Zoology has kindly sent the following identification, none of which was specifically known:

1) Fly: Eumerus sp. undetermined - probably new (R. Coe, Entomology Department).

2) Parasite: Pteromalidae (Chalcidoidea).

3) Fly: Muscidae? Coenosia sp.

4) Mite: Proctolaelaps sp. nr. scolyti Evans, 1958.