THE EFFECT OF TEMPERATURE ON THE TOXICITY OF FIVE INSECTICIDES FOR THE CONTROL OF FIVE SPECIES OF STORED-PRODUCT INSECTS

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ABSTRACT

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Five insecticides, formulated as acetone solutions, were applied on filter papers and their toxicities were evaluated at three temperatures against five species of stored-product insects. The insecticides were DDT, methoxyclor, lindane, malathion and bromophos. The insects were adults of Tribolium castaneum (Herbst), Tribolium confusum (J. du Val), Oryzaephilus surinamensis (L.), Oryzaephilus mercator (Fauvel) and Cryptolestes ferrugineus (Steph.). The three experimental temperatures were 80°, 60° and 50° F and the relative humidity was 60 ± 10 per cent. Tables of LD50 values are presented in terms of deposits of active ingredient of each insecticide (mg/ft^2) obtained for each insect at each test temperature after a 24-hour exposure to the treated filter papers followed by a 72-hour recovery period in flour. The data give a broad view of the effects of temperature on the effectiveness of each insecticide, the susceptibility of each insect species to the different insecticides at the different temperatures and the effective-

ness of each insecticide against the entire range of insect species. Methoxychlor was ineffective against all species, except _T. castaneum_ at 80° F and was, therefore, excluded from further tests.

In general, DDT was more toxic at 60° than at 80° F (negative temperature coefficient) but slightly less toxic at 50° than at 60° F (positive temperature coefficient). Lindane showed variable results at the three temperatures and malathion and bromophos showed a positive temperature coefficient against all insect species. Malathion was very effective against all species at all temperatures. Bromophos was also very effective against all species at 80° F, but was far less effective at 60° and still less effective at 50° F. Lindane was very effective against _C. ferrugineus_ at all temperatures and was also quite effective against the other species. DDT was the least effective at the high temperature. In general, the most resistant species at all temperatures and with all insecticides was _T. confusum_ and the most susceptible was _C. ferrugineus_.

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CHAPTER I

INTRODUCTION

Canada is one of the most important grain producing countries of the world. Although the annual loss in Canada due to storage pests is probably less than 1 per cent of total production, infestations that cause heating, spoilage, grade losses, and customer complaints can have serious consequences for individual farmers, grain companies and food processers.

In the past twenty years, with the introduction of organochlorine and organophosphate insecticides, tremendous advances have been made in the control of stored-product insects. Empty granaries may be treated by spraying insecticides on all inside surfaces to control infestations in cracks and crevices. In addition, grain may be treated directly with certain insecticides as it is binned to protect it from insect infestations during storage.

In Canada, insecticides may be applied over a wide range of climatic conditions which may influence the insecticidal activity of the chemicals.

This is a report of work conducted to determine the effect of different temperatures on the effectiveness of some chemicals in the control of certain stored-product insects.

Problem

The purpose of this study was firstly, to determine the toxicity of the organochlorine insecticides, DDT, lindane and methoxychlor and the organophosphate insecticides, malathion and bromophos against the red flour beetle, *Tribolium castaneum* (Hbst.), the confused flour beetle, *Tribolium confusum* (J. du Val), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L), the merchant grain beetle, *Oryzaephilus mercator* (Fauvel) and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens); and secondly, to determine the effect of three different temperatures on the toxicity of these insecticides.

Economic Importance

It is obvious that the amount of stored foodstuff eaten and, therefore, the amount of weight loss caused by insects is proportional to the size of the population in a granary or warehouse. The main objective, therefore, in the use of insecticides is to reduce or, if possible, to eradicate the insect pest population. Any environmental factor that increases or decreases the effectiveness of chemicals used for insect control or adversely affects the biology of the insect pest must be considered in any insect pest control program.

Optimum conditions for oviposition and development of stored-product insects range from 27.5 to 35° C and 70 to 75 per cent relative humidity. Some chemicals, particularly the organophosphates, are more effective at high temperatures (Lallan et al., 1956). Other contact insecticides, such as, gamma-BHC, aldrin, dieldrin and pyrethrins, are found to be slightly more toxic at high temperatures (Guthrie, 1950; Glynne Jones and Edwards, 1952). On the other hand, organochlorine insecticides, such as, DDT and methoxychlor, are more active at low temperatures (Potter and Gillham, 1946; Guthrie, 1950; Pradhan, 1949; Das and McIntosh, 1961). The amount of chemical needed to cause 50 per cent mortality of an insect pest may thus be reduced significantly with an increase or decrease in temperature, depending on the insecticide used. A knowledge of the most effective chemical or chemicals for the control of a particular pest or group of pests, under different conditions of temperature, would, therefore, assist farmers and others concerned with the protection of stored foods in applying the most efficient and economical type of control.

Information provided by this study, as far as the effectiveness of these chemicals is concerned, may suggest the substitution of one chemical for another for the control of a particular species or group of species of insects. Such a choice may result in more effective, and perhaps less costly, control under certain temperature conditions.

The results of this study will also provide information on the temperature, of a treated granary or warehouse, that favors maximum toxicity of a given insecticide against a certain species. Thus some chemicals may be recommended for use under hot conditions of storage (end of spring or summer) or in countries with year-round high temperatures in storage warehouses. On the other hand, chemicals that show increased biological activity at low temperatures may be recommended for use during the cold months of the year and in countries with prevailing low temperatures throughout most of the year.

CHAPTER II

REVIEW OF THE LITERATURE

Effect of Temperature on the Effectiveness of Insecticides

Temperature, a climatic factor, exerts considerable influence on the physiology of insects. Thus it is not surprising that many facets of the action of insecticides on insects are also radically affected by this factor.

Temperature may also have some indirect effects on test insects. For example, the temperature at which insects are cultured affects their susceptibility to insecticides. Insects reared at high temperatures were found to be smaller in size and more susceptible to insecticides than insects reared at low temperatures (Busvine, 1957). This phenomenon was observed in cockroaches and it was found that when they were kept under warm conditions they were more susceptible to DDT and other contact poisons than those from cooler situations when both were tested at the same temperature (Munson *et al*., 1954). Temperature may also have some direct effects on the susceptibility of insects during and after exposure to an insecticide. In cases where insects are allowed to "dose themselves" by walking over treated surfaces or by flying through an aerosol, the dose acquired depends on the activity of the insects (Armstrong *et al*., 1951; David and Bracey, 1946). It is obvious that temperature exerts a great influence on the activity of insects and, therefore, it also affects the pick-up of the chemical so that the lethal effects are positively correlated with temperature (Pradhan, 1949). The absorption of an insecticide has also been found to be affected by temperature. Solid poisons are more easily absorbed at high temperatures. Thus, 2 to 2.5 times as much DDT can penetrate into a cockroach at 35 than at 15° C (Vinson and Kearns, 1952). Tahori and Hoskins (1953) found, similarly, that 4 times as much DDT may enter the house fly at 32 than at 13.5° C. In both cases, the same dosages were applied to the cuticle of the insects. The internal movement of the poison, assisted by the movement of haemolymph, was found to be more rapid at high temperatures.

It was mentioned above that a rise in temperature favours the penetration and movement of some insecticides like DDT. However, the toxicities of these insecticides are affected in an inverse proportion to increases of temperature. Thus, at 35° C, the lethal dose of DDT to a cockroach is roughly 12 times that at 15° C (Tahori and Hoskins, 1953). This anomaly can be explained by the fact that the solubility of contact poisons, especially DDT, in lipids in the body of the insects rises considerably with increasing temperature. However, lipids are not the active site of poisoning and, therefore, they tend to protect the insect by taking up relatively large amounts of the insecticide. By cooling insects that have been lightly poisoned with DDT, signs of intoxication occur, presumably because of reduced absorption of the insecticide by the fatty tissues (Ferguson, 1939). Lindquist et al., (1944) found that adult house flies that were exposed to films of DDT and then divided into batches after treatment, each of which was kept at a different temperature, showed greater recovery at the higher temperature.

The effectiveness of an insecticide at different temperatures is a characteristic of the insecticide. Thus Potter and Gillham (1946), using DDT spray solutions against adults of the red flour beetle, Tribolium castaneum (Hbst.), obtained higher mortality at low than at high temperature.

Richards and Cutkomp (1946) demonstrated, in the laboratory, that low concentrations of DDT were more effective against mosquito larvae exposed to low temperatures and they named this relationship "negative temperature coefficient".

Hoffman and Lindquist (1949) also found that DDT, TDE and methoxychlor films on glass gave more rapid knockdown and kill of Musca domestica L. at 21 than at 32° C, but the opposite was true with heptachlor, parathion, chlordane, dieldrin and toxaphene.

Vinson and Kearns (1952) studied the effect of two temperatures (15 and 35° C) on the toxicity of DDT, for both topical and injected doses, to the American cockroach, *Periplaneta americana* (L). They found that DDT exhibited a negative temperature coefficient, being more lethal at 15 than at 35° C, and that cockroaches that showed marked DDT symptoms at the lower temperature lost these when brought to the higher one, provided the insects had not been held too long at the lower temperature.

American cockroaches injected with DDT and exposed to post-treatment temperatures of 15 and 30° C, showed a higher percentage mortality at the lower than at the higher post-treatment temperature (Lofgren and Cutkomp, 1956).

Menn *et al.* (1957) studied the effect of three temperatures (15, 25 and 35° C) on the control of resistant and susceptible house flies exposed to DDT in oil. They studied the effect of "time of exposure" at the different temperatures on susceptible and resistant flies. They found that when susceptible flies were exposed to DDT at 15° C they were quickly affected; the same mortality occurred whether the flies were held at 15° C for 24 hours or for only 6 hours and then changed to 35° C. The resistant flies were affected slowly when held at 15° C for 6 hours and at 25° C for 18 hours. The final conclusion drawn from the "time of exposure" factor at high and low temperatures, was that susceptible flies were more sensitive than resistant flies to exposure to DDT during short periods of low temperatures.

The effect of time and temperature on the toxicity of DDT to larvae of *Aedes aegypti* L., was studied by Das and Needham (1961). An increase in temperature during exposure to DDT for one hour, increased the toxic action of this chemical. The mortality of larvae kept at low temperatures was greater than that of larvae kept at high temperature after treatment. The toxic action was reversible; a change from high to low temperature increased paralysis, and larvae that were paralysed after exposure to the chemical at low temperatures recovered when the temperature was raised.

The increased pick-up of chemical resulting from increased activity of the insects at the higher temperature was demonstrated by Pradhan (1949) who showed that when *T. castaneum* adults or larvae of *Plutella maculipennis* (Curtis) were exposed to residues of DDT on filter papers at 14, 21, 27, and 32° C, there was a positive correlation between mortality and temperature. When the insects were treated at a given temperature or were exposed to DDT residues at the four above mentioned temperatures for only a limited period of time and then transferred to clean Petri dishes and again held at the different temperatures, a negative correlation between temperature and mortality occurred. This interesting experiment showed quite clearly the negative temperature coefficient of DDT and explained the positive temperature coefficient observed when the insects were exposed to high temperatures for a longer exposure period. At high temperatures there was increased pick-up of toxicant due to increased activity of the insects.

Das (1961) found a negative temperature coefficient for DDT for _Tenebrio molitor_ L. when post-treatment temperatures were below 10° C. The DDT solutions were applied topically or injected with a micrometer syringe into 2-week old adults, at room temperature. The toxic action of DDT did not increase indefinitely with the lowering of the post-treatment temperature from 10 to -1° C. The temperature coefficient between -1 and 10° C was positive for both injection and topical application although it was always negative at temperatures above 10° C. Das and Needham (1961) called this phenomenon "inversion of temperature coefficient" of DDT. When insects were exposed to a given dose of DDT at -1° C they showed no symptoms of DDT poisoning after three days, but became paralysed when transferred to 10° C.

Whitcomb (1934) studied the effectiveness of 34 materials used against _Tetranychus telarius_ L., at 60 and

80° F. Heavy lubricating oil emulsions were more effective at 80° than at 60° F, whereas light oil emulsions were more effective at 60° F. Soluble sulphur and lime sulphur were better at 80°, but suspended sulphurs (colloidal or wettable sulphur) were better at 60° F. Pyrethrum extracts were less effective at the high temperature in contrast with derris extracts which were more effective at the high temperature.

Guthrie (1950) tested various chemicals applied topically on the German cockroach, Blatella germanica (L.), at 14.5, 22 and 32° C. He found that DDT, pyrethrum and lindane were more toxic at the lower temperatures, while the reverse was true with aldrin, and dieldrin. The results with lindane were quite variable. The toxicity of DDT was approximately 20 times more effective at 14.5 than at 32° C. The effect of temperature on the effectiveness of the other chemicals was considerably less.

The effects of different temperatures on the toxicity of some chemicals injected into the milkweed bug, Oncopeltus fasciatus (Dall), were studied by Woodruff (1950). Nicotine was found to be more toxic at 10 than at 29° C. Rotenone was more toxic at high than at low temperatures.

Harries (1956) studied the effect of temperature and rainfall on the effectiveness of derris and rotenone against Macrosiphum pisi (Harris), a pest of peas. Both chemicals were found to be more effective at high than at low temperature.

Blum and Kearns (1956) found a negative temperature coefficient for pyrethrum when used against P. americana. Two temperatures were used: 15 and 35° C. Prostrated cockroaches at 15° C could recover if transferred to 35° C and this process could be repeated for several hours. Cockroaches from 35° C transferred to 15° C became prostrated more rapidly than those held continuously at 15° C.

Ellisor and Blair (1940) studied the effect of temperature on the toxicity of the stomach poisons, synthetic cryolite, acid lead arsenate, calcium arsenate and basic copper arsenate for the control of 5th instar larvae of Prodenia eridania Cram., and Anticarsia gemmatilis Hbn. They used two temperatures (60 and 80° F) and found that in all but one instance the toxicity was greater at the lower temperature.

Gaines et al. (1949) studied the effect of temperature and humidity on the toxicity of several insecticides used for the control of the boll weevil, Anthonomus grandis Boh. They found that calcium arsenate was not very much affected by high or low temperature, but the toxicity of this compound was reduced by a combination of high temperature and high relative humidity. Toxaphene, like chlordane, lost its toxicity at high temperatures.

Das and McIntosh (1961) studied the effect of time and temperature on the toxicity of several insecticides against the three stored product insects, O. surinamensis, T. castaneum and T. molitor, and on M. domestica. They used the contact poisons rotenone, Dimetan, Valone, x-chlordane, toxaphene and DDT. The two post-treatment temperatures were 10 and 28° C. The aim of their work was to determine whether different post-treatment temperatures affected the speed of action of the insecticides used. Rotenone and Dimetan showed a greater speed of action at 28 than at 10° C. The speed of action of Valone, x-chlordane and toxaphene was also greater at the higher temperature. DDT showed higher toxicity and speed of action at the lower temperature. The general pattern as far as "time" was concerned was that LD_{50} values decreased steadily as time passed.

Strong and Sbur (1960) studied the influence of moisture and temperature on the residual effectiveness of malathion used as grain protectant. The residual effectiveness of malathion was decreased with increase in storage temperature and grain humidity. Experiments concerned with the interrelation of moisture content, temperature and dosages applied, indicated that malathion applied at the rate of 10 ppm could remain effective for at least 12 months when storage temperatures were not higher than

60° F and the grain moisture did not exceed 14 per cent. Similar results of reduced residual activity of malathion under high grain moisture were obtained by Watters (1959).

Lallan <u>et al</u>. (1956) studied the effect of four temperatures (63, 70, 75 and 82° F) on malathion and malathion-piperonyl butoxide acetone solutions, applied topically on a DDT-resistant and a DDT-susceptible strain of house flies. Both chemicals were found to be more toxic at the higher than at the lower temperatures, showing, therefore, a positive temperature coefficient.

Elmosa and King (1964) studied the effect of pre- and post-treatment temperature on the effectiveness of dieldrin and ethion, used for the control of <u>Hylemyia antiqua</u> (Meigen). Pre-treatment temperature did not affect the toxicity of either dieldrin or ethion, but an increase in post-treatment temperature increased, in general, the toxicity of both.

Evans and Gordon (1965), working on the effects of temperature on toxicity of synergized carbamate insecticides (Carbaryl, Pyrolan, T.P.C.) on house flies, found that in the absence of synergists these insecticides were slightly more toxic at 30 than at 20° C. When large amounts of synergists, such as, sesamine, were added, the toxicity of all insecticides used greatly increased and the temperature had no effect on their toxicity.

Temperature and moisture were also found to influence the effectiveness of fumigants used for the control of stored-product insects in food storage. Thus Lindgren and Vincent (1960) found that in stored wheat the concentration of carbon tetrachloride, hydrocyanic acid and methyl bromide needed was greater at 15 per cent than at 10 per cent moisture content. *T. confusum* and *Sitophilus oryza* (L.) were found to be more susceptible at 90 than at 50° F.

The Insecticides Used in the Experiment

Insecticides used to protect stored foods from insects must meet special requirements of safety, toxicity and ease of application. They are usually applied as oil solutions, emulsions or dispersible powders to structural surfaces of food warehouses to control established infestations and to prevent insect outbreaks. Special formulations may also be applied directly to stored grain to protect it from infestation during the storage period.

The insecticides included in the present experiments were DDT, lindane, methoxychlor, malathion and bromophos. The first three are organochlorine insecticides that have been used in stored-product control programs for more than 20 years. Of the two organophosphate insecticides, malathion has been in use as a stored-product insecticide for more than 10 years, bromophos is a candidate stored-product insecticide whose efficacy under various environmental conditions has yet to be proven.

DDT. Late in 1942 when the preliminary work to assess the value of DDT in the control of lice, flies and mosquitoes was well advanced, attention was turned to determine the value of this new insecticide against stored-product insects. From the beginning it was realized that there were difficulties in using DDT freely in food-storage premises because of the danger of food contamination and the resultant toxicological hazards to man and animals (Parkin, 1950).

Parkin (1953) studied the susceptibility of the most common stored-product insects to DDT dust in kaolin. A known amount of dust was applied over the bottom of a 9 cm Petri dish and the LT_{50} and LT_{95} values were evaluated. All 16 species of stored-product insects used in the experiment were found to be susceptible to DDT, although to varying degrees.

Pradhan (1949) showed that residues of DDT on filter papers were effective against *T. castaneum* adults and *P. maculipennis* larvae. Watters and Sellen (1956) reported that a 5 per cent DDT emulsion applied at 1 gallon/ 1000 square feet gave good control of the hairy spider beetle, *Ptinus villiger* (Reit) in warehouses.

Vincent and Lindgren (1957), using topical applications and residual films, tested several contact insecticides on several species of stored-product insects and found DDT to be very effective against almost all the species tested. Similar results were also reported by Kumar and Morrison (1963). *T. confusum* and *C. ferrugineus* adults were found to be susceptible to DDT residues on filter papers. Parkin (1966) used 12 insecticides, formulated as water dispersible powders and applied to strips of filter papers, against eight species of stored-product insects. DDT was found to be effective against all the species used.

DDT (50 per cent wettable powder or 25 per cent emulsifiable concentrate) is recommended today against insect pests of warehouses where packaged food does not come in contact with treated surfaces (Gray and Watters, 1954).

Lindane. Lindane is widely used throughout the world for the control of stored-product insects in food warehouses, flour mills and empty granaries. In Canada, this insecticide has been recommended for the control of stored-product insects in bins (Watters, 1959, 1961) and for the control of spider beetles in flour warehouses (Watters, 1961).

Lindane has been recommended ahead of DDT for use in warehouses, bins, etc., because it shows higher toxicity than DDT to insects and mites, is more rapid in action and has highly toxic vapors (Parkin, 1950, 1952, 1955, 1958, 1960).

Bouchet (1952) found that lindane, at a concentration of 0.08 grams per cubic meter, was non-corrosive, non-toxic to mammals and effectively protected stored materials against a great number of stored-product insects without the necessity of airtight conditions during the experiment.

Methoxychlor. Methoxychlor is one of the safest insecticides available today (Negherborn, 1959). It is used in Canada and the United States of America as a granary and warehouse chemical against stored-product insects. Methoxychlor is much less toxic than DDT or lindane (Strong *et al.*, 1961; King *et al.*, 1962; Walker, 1960), but Ashrafi and Aijas (1965) reported that methoxychlor was effective against *T. castaneum* up to 10 days after treatment. According to Parkin (1966) and Rowland (1967) methoxychlor has little value as a residual insecticide. However, their assessments were based on laboratory experiments. In Canada, Watters (1961) has shown that methoxychlor in the form of wettable powder was effective for the control of spider beetles in flour warehouses.

Malathion. Because of its low toxicity to humans and other vertebrates and its high toxicity to insects, malathion is one of the most important insecticides in stored-products entomology. It can be applied as a prophylactic spray on the inside surfaces of empty granaries and food storage warehouses. It can also be used as a grain protectant mixed with grain (Watters, 1959).

Parkin (1966) reported that malathion was effective against several species of stored-product insects. Lemon (1966) also reported that malathion was effective against *T. castaneum* and *T. confusum*. Heavy infestations of *O. surinamensis* were readily controlled by an application of 10 ppm malathion which also gave good protection to stored barley for as long as eight months (Green and Tyler, 1966).

Malathion was found to break down very rapidly following application to stored grain. In general, this breakdown is favored by high storage temperature and damp grain conditions (Strong *et al.*, 1961; La Hue, 1966; Papworth, 1961).

Watters (1956) found that malathion, applied as a residual spray on wooden panels, remained effective for two months against *C. ferrugineus*. According to Parkin (1966) malathion was persistent on wood for three to four months. Despite rapid degradation of malathion after storage, biological effectiveness appears to remain at a high level for about one year provided that the grain is not damp (Watters, 1967). Watters (1957, 1959) and Strong and Sbur (1960) reported that malathion was less persistent and less effective in damp grain than in dry grain. However, malathion was found to break down very rapidly on some structural surfaces, particularly on concrete. Thus, Lemon (1966) found that malathion applied as a water-dispersible powder on concrete blocks lost its biological activity very rapidly. The very rapid loss of toxicity of malathion on concrete was in agreement with the results of Parkin (1966) who also found that malathion lost its toxicity almost immediately on cement and very rapidly on white-wash and tile.

Bromophos. Bromophos is a new experimental organophosphorus insecticide and acaricide which is very effective against a great variety of agricultural and stored-product insects. One of the important properties of this chemical is its low mammalian toxicity and its persistence on alkaline surfaces (Immel and Geisthardt, 1964; Kinkel *et al*., 1966). It is stable in alkaline media up to pH 9, according to the technical information provided by CELA (Germany) "Experimental insecticide bromophos-1965-10". Bromophos is recommended for treatment of empty storages.

Lemon (1966) tested the relative susceptibilities of *T. castaneum* and *T. confusum* to 16 organophosphorus insecticides applied topically and found that bromophos was only slightly less effective than malathion against both species.

Lemon (1966), realizing the value of bromophos as an insecticide suitable for sprays on alkaline surfaces and as a grain protectant, continued his experiments on the effectiveness of this chemical against 10 species of stored-product insects. He determined the effectiveness of the chemical applied topically, its residual toxicity on a concrete surface, and finally, its persistence on wheat. During these experiments bromophos was found to be quite effective against all species used. High mortalities were obtained with bromophos 16 weeks after application on concrete surfaces. Forty weeks later, bromophos (1.5 g/m^2) gave 91.8 per cent mortality of *T. confusum* on concrete, whereas the toxicity of malathion was zero. From the experiments with dust formulations on wheat, bromophos was found to be less effective than malathion.

CHAPTER III

MATERIALS AND METHODS

I. Materials

Experimental Insects

The following five species of stored-product insects of the Order Coleoptera, were selected for the insecticide tests.

The red flour beetle, *Tribolium castaneum* (Hbst.), Tenebrionidae.

The confused flour beetle, *Tribolium confusum* (J. du Val), Tenebrionidae.

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), Silvanidae.

The merchant grain beetle, *Oryzaephilus mercator* (Fauvel), Silvanidae.

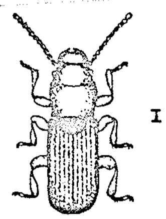
The rusty grain beetle, *Cryptolestes ferrugineus* (Steph.), Cucujidae.

Biology of Test Insects

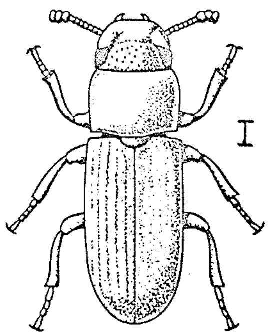
T. castaneum and *T. confusum*. *T. castaneum* and *T. confusum* are reddish-brown and about 1/8 inch long. They are distinguishable by the shape of their antennae (Fig. 1, b, d). In hot dry climates (over 71.5° F and less than

FIGURE 1

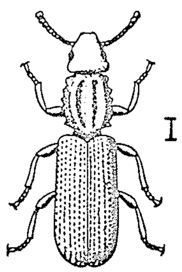
INSECTS USED IN INSECTICIDE EXPERIMENTS



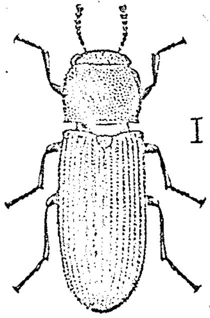
a
RUSTY GRAIN BEETLE
Cryptolestes ferrugineus (Stephens)



b
RED FLOUR BEETLE
Tribolium castaneum (Herbst)



c
SAW-TOOTHED GRAIN BEETLE
Oryzaephilus surinamensis (Linnaeus)



d
CONFUSED FLOUR BEETLE
Tribolium confusum (Jacquelin du Val)

60 per cent R.H.) or in hot and damp climates (over 71.5° F and 70 per cent R.H.) both species occur together. In moderate climates (less than 71.5° F and over 55 per cent R.H.), like the climatic conditions of Canada, _T. confusum_ is the principle species (Freeman, 1962). The adults of both species are general feeders of farinaceous foods and are serious pests of prepared cereal foods. Both are common in warehouses, granaries, mills, milling machinery and grain shipments. The females lay up to 500 eggs in flour, grain or other foodstuffs. The larvae are yellowish and feed extensively on flour or on the germ of wheat kernels. The development period from egg to adult may be as short as four weeks under favorable conditions (Cotton, 1963; Watters, 1967).

A survey in Manitoba, Saskatchewan and Alberta in 1958, showed that _T. castaneum_ and _T. confusum_ were present in 25 per cent and 4 per cent, respectively, of granaries examined (Liscombe and Watters, 1962).

O. surinamensis and _O. mercator_. Both have similar habits, occur in the same places and resemble each other. However, they are distinguishable on the basis of the size of the region directly behind the eye (Hinton and Corbet, 1963). The adults are dark brown and about 1/8 inch long (Fig. 1, c). They may be readily recognized by the six tooth-like projections on the prothorax. The females lay their eggs loosely among foodstuff or on kernels; each female may lay 45 to 280 eggs. The eggs hatch in three to five days. The larvae feed for two weeks during the summer and then construct a delicate cocoon-like covering by joining together small grains or fragments of foodstuffs with a sticky secretion. The pupal stage lasts about one week. Development from egg to adult may take place in three to four weeks in summer (Cotton, 1963; Watters, 1967).

During the 1958 survey in Manitoba, Saskatchewan and Alberta, 4 per cent of the granaries were found to be infested by _O. surinamensis_ (Liscombe and Watters, 1962). No data were presented for _O. mercator_.

C. ferrugineus. _C. ferrugineus_ is flat, reddish-brown and about 1/16 inch long (Fig. 1 a). It is cosmopolitan in distribution and a very common pest of stored grain in hot dry climates [over 71.5° F and less than 60 per cent R.H. (Freeman, 1962)]. The larvae feed on the germ of wheat. At maturity they form small cocoons of a gelatinous substance and later emerge as adults. Under favorable conditions, this species may complete its development from egg to adult in five to nine weeks. This species is resistant to cold weather and is commonly found in stored grain in Canada; 36 per cent of granaries were found to be infested during the 1958 survey in Manitoba, Saskatchewan and Alberta (Liscombe and Watters, 1962).

Source and Breeding of Test Insects

The parent cultures of the five species of insects used, were obtained from the standard stocks of the Crop Protection Section, Research Station, Canada Department of Agriculture, Winnipeg, Manitoba.

All the species, except C. ferrugineus, were reared in a constant temperature cabinet at 78±2° F and 60±5 per cent R.H. C. ferrugineus was cultured in another constant temperature cabinet at 78±2° F and 70±5 per cent R.H.

The food media on which the test insects were cultured are listed in Table I.

The cultures were kept in 64 fl oz jars covered with filter paper. The filter paper was sealed to the top of the jar with wax to avoid infestation by mites.

As far as possible, the insects used during the experiment were two to four weeks old. In order to obtain adults of known age, 200 to 300 adults of each species were added to fresh food media in glass jars. After a three to four day egg-laying period, the adults were removed from the food media and a new generation was obtained from the hatched eggs in approximately four weeks.

The rusty grain beetle, which is slow in its development at 78° F, required from six to eight weeks to complete

TABLE I

FOOD MEDIA OF TEST INSECTS

Species	Culture medium
T. castaneum	Sifted whole wheat flour and 5% Brewer's yeast
T. confusum	Sifted whole wheat flour and 5% Brewer's yeast
O. surinamensis	Rolled oats and 5% Brewer's yeast
O. mercator	Rolled oats and 5% Brewer's yeast
C. ferrugineus	Whole wheat kernels and 5% wheat germ

a generation. For this reason adults of unknown age were used.

Insecticides

Names and sources of insecticides. The following five synthetic organic insecticides were used.

DDT - p,p' - 1:1:1-trichloro-2:2-bis(p-chlorophenyl) ethane. 99+%, $C_{14}H_9Cl_5$, m.p. 108.5° C; City Chemical Corporation, New York.

Lindane - 100% -isomer of Benzene Hexachloride. 1:2:3:4:5:6-hexachlorocyclohexane. $C_6H_6Cl_6$, m.p 112.9° C; City Chemical Corporation, New York.

Methoxychlor - 1:1:1-trichloro-2:2-bis(p-methoxyphenyl) ethane. $C_6H_{15}Cl_3O_2$, m.p. 86-88° C. Pure chemical obtained by crystallization of 50% technical methoxychlor wettable powder (Marlate 50% W.P); Du Pont de Nemours & Co. (Inc.).

Malathion - 0,0-dimethyl S-(1,2-dicarb=ethoxyethyl). $C_{10}H_{19}O_6PS_2$, 96% liquid, b.p 156-157° C; City Chemical Corporation, New York.

Bromophos - 0,0-dimethyl-0-2,5-dichloro-4-bromophenyl phosphorothioate. 40% emulsifiable concentrate; Green Cross Products, Montreal.

A 5 per cent acetone solution was made from each chemical. Various concentrations of each chemical were then prepared by diluting a given volume of the 5 per cent solution with a solvent consisting of 3 parts petroleum ether (reagent grade), 1 part acetone (reagent grade) and 1 part Shell Risella Oil (117) (Champ and Cribb, 1965). The solutions were kept in a refrigerator at approximately 40° F.

Technique for obtaining pure methoxychlor. Pure crystals of methoxychlor were obtained by repeated recrystallization of alcoholic extracts from a 50% W.P (Marlate 50% W.P). The crystals were extracted with hot alcohol. The liquor was concentrated to obtain crystals. The crystals were then washed with cold alcohol and then redissolved in hot alcohol. The solution was reconcentrated and a second batch of crystals were obtained. The process of washing, dissolving and precipitating new crystals was repeated for a third time. The purity of the final crystals was assessed by determination of melting point which was found to be 86 to 88° C (Robertson and Jacobs, 1962).

Temperature

The experiments were conducted in cabinets with controlled temperature and humidity. The three temperatures selected for the tests were 80, 60 and 50° F. The relative humidity at each temperature was maintained at 60±10 per cent.

II. Methods

The method used was similar to that of Busvine and Nash (1953). Briefly, it consisted of impregnating filter papers with known concentrations of insecticide and exposing the test insects to the papers for a predetermined period of time. After exposure, the insects were placed in food media for another predetermined period of time, at the end of which mortality was recorded.

Application procedure

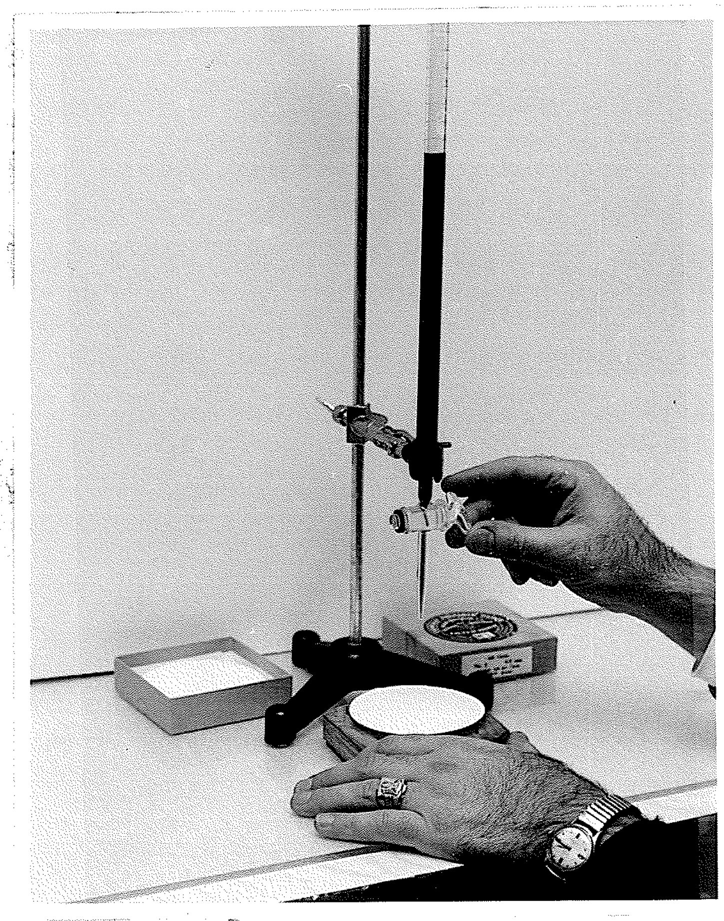
The various concentrations of each chemical were applied with a 100-ml burette on Whatmann No. 1 filter paper, 9 cm in diameter (63.64 cm^2). The volume of solution required to give a uniform distribution of chemical on a filter paper was found, after several tests, to be 1.5 ml per filter paper. The size and type of filter papers used, as well as the volume of solution per filter paper, were kept constant for all the insecticides used throughout the experiment.

During application, the filter paper was balanced on a bed of nails fixed on a rectangular piece of wood, 10 x 10 cm. The tip of the burette was kept approximately 4 cm above the filter paper and a constant volume of solution was applied spirally, as uniformly as possible, on the filter paper (Fig. 2); the solution was not allowed to over-

FIGURE 2

APPARATUS FOR APPLYING INSECTICIDE

ON FILTER PAPER



flow from the filter paper. The burette was washed with acetone after applying each concentration of each chemical. Filter papers used for the checks were treated with solvent alone. After application of each insecticide, the filter papers were marked, placed in a horizontal position on small nails fixed on pieces of cardboard, and allowed to dry for 24 hours at room temperature (Fig. 3). The amount of active ingredient for each dose was calculated in terms of mg/square foot.

Twenty-four hours after application, three filter papers (three replications), each with the same dosage and the same chemical, were placed on rectangular glass plates (40 x 10 cm). A barrier glass ring (8.5 cm in diameter and 4 cm high) was placed on top of each filter paper to confine the test insects (Fig. 4). When O. surinamensis and O. mercator were to be tested, the glass rings were dipped in "Fluon" (Polytetrafluoroethylene dispersion. I.C.I.) to a depth of 3 cm, to prevent the insects from climbing and escaping from the barrier ring (Fig. 5).

Four replications were used at the beginning of the experiment. Later it was found that the variation in mortalities between replications was so small that only three replications were necessary.

FIGURE 3

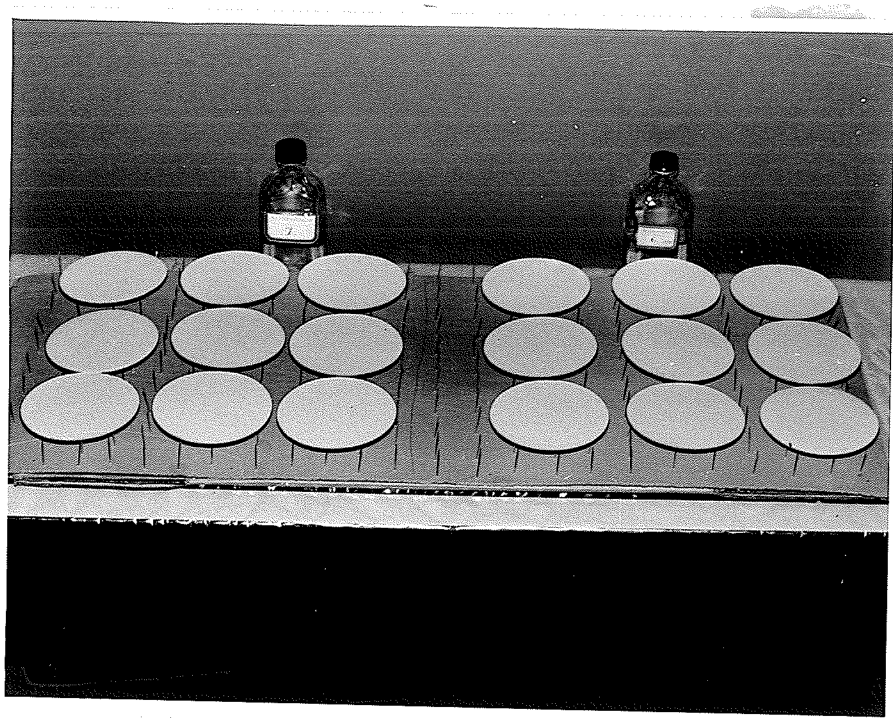
BED OF NAILS FOR SUPPORTING TREATED

FILTER PAPERS DURING DRYING

FIGURE 4

GLASS PLATES AND TREATED FILTER PAPER WITH

BARRIER RINGS CONFINING TEST INSECTS



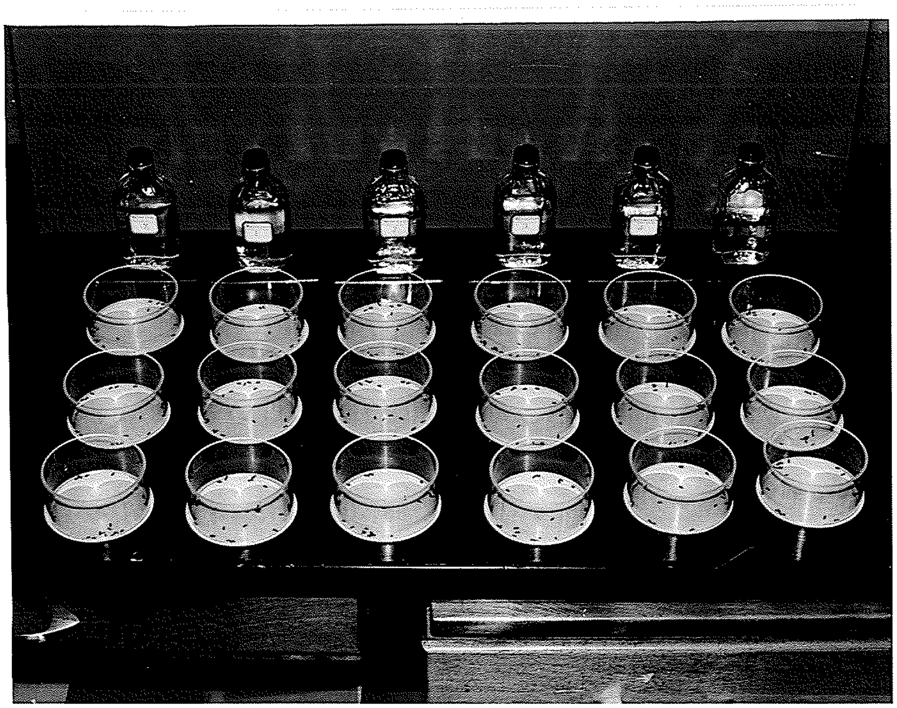


FIGURE 5

GLASS RINGS TREATED WITH FLUON TO PREVENT ESCAPE OF CERTAIN SPECIES OF TEST INSECTS

Exposure of Test Insects to Residual Films

To test the effect of starvation on the susceptibility of test insects to insecticides, a preliminary experiment was carried out to compare insects starved for 24 hours prior to exposure to a chemical, with insects that were not starved. The difference in mortality after exposure to the same dosage of the same chemical under the same environmental conditions was less than one per cent. Thus, in subsequent experiments, the test insects were not starved prior to exposure.

Two- to four-week old adult insects from the culture media were collected at random, without sexing, into batches of 20. A batch was exposed, within the barrier ring, to each treated filter paper on the glass plate as previously described. This unit was then transferred to a cabinet maintained at the appropriate experimental temperature.

Conditions During Exposure to Residual Films

Temperature was maintained within 0.5° C, and relative humidity within 5 per cent, in the temperature cabinets. The exposure period was 24 hours in darkness, to avoid increased activity of the test insects which could influence the degree of poisoning.

Conditions after Exposure to Residual Films

After 24 hours of exposure, the glass plates with the filter papers and insects were removed from the cabinet. The insects were collected from the filter papers with an aspirator and placed in clean glass jars (5 cm in diameter and 8 cm high) containing approximately 6 grams of flour. The jars and flour had been stored in the cabinet for three days prior to introduction of the insects. The open jars were returned to the cabinet and maintained at the same temperature at which the insects were exposed to the treated filter papers. This period is known as "recovery period" and indicates the time that the insects were allowed to recover in the food media before mortality was assessed. A preliminary experiment showed that a recovery period of 72 hours after exposure was adequate for comparison of mortalities.

Assessment of Mortality

In order to obtain repeatable results and data which could be used to calculate the LD_{50} and LD_{95} values from regression lines, the experimental insects were classified as either alive or dead. To assess mortality, the insects were placed in a white enamel tray and examined under a strong light. Insects that showed any sign of life, such as movement of legs or antennae, were recorded as being alive; insects which showed no movement at all, even after gentle probing, were considered dead. Immobile beetles were stimulated by heat from either a 100-watt bulb or a lighted cigarette to determine whether or not they were feigning death. Insects showing movement in response to either heat source were classified as alive. Some individuals of all species feigned death.

The assessment was made in the laboratory at approximately 78° F. After assessing mortality the data were corrected by Abbott's formula (Abbott, 1925). The concentrations, in terms of mg/ft^2, were plotted on a logarithmic scale and mortality on a Probit scale. The efficiency of the Probit regression line was tested by Probit analysis (Finney, 1952).

CHAPTER IV

RESULTS

The LD50 values were obtained from Probit analysis of the data. Wherever the Probit analysis indicated, after a x^2 test, that the regression coefficient was significant, or in other words that the heterogeneity of the Probit log. concentration-mortality values were not significant, the Fiducial or 95% confidence limits for the LD50 and LD95 values are given. When the regression coefficient was not significant, no confidence limits are given.

A regression line was obtained for each insect species for each insecticide at each temperature. These lines were analyzed by joint Probit analysis for a test of parallelism. When the data (observed and expected frequencies of mortalities) did not contradict the hypothesis of parallelism, after a x^2 test, the three regression lines were drawn in separate figures for a test of homogeneity in the population of the insect concerned, based on the slopes of the lines. The regression equation of each line was also calculated. The new LD50 and LD95 values of the parallel lines and the 95% confidence limits were also calculated. The 95% confidence limits of the LD50 values were used to test whether there were significant differences between any two lines. When the 95% confidence limits of the LD50 values of each regression line did not overlap with the 95% confidence limits of another line of the same insect and insecticide, there was a significant difference between the LD50 values obtained at the two temperatures.

From the beginning of the experiment it was realized that methoxychlor, under the standard conditions of preparation, was an ineffective insecticide. Of the five insect species tested, only *T. castaneum* showed some susceptibility to this insecticide. The LD50 value obtained for this species at 80° F was 18.2 mg/ft^2. For the remaining species of insects, doses up to 7,225 mg/ft^2 were used without any toxic effects. For this reason methoxychlor was excluded from further tests.

Toxicity of the Insecticides at the Three Experimental Temperatures

Table II summarizes the LD50 values obtained with DDT, lindane, malathion and bromophos for the five species of test insects at 80°, 60° and 50° F. The numerical values in each row indicate the effectiveness of any of the insecticides against any of the insect species at the same temperature and the values in each column indicate the tolerance of each insect species to each of the insecticides. The response of each insecticide at the three temperatures is also shown by the three LD50 values, given at the three temperatures at each intersection of species and insecticide. The LD50 and

TABLE II

THE LD50 VALUES (MG/FT^2) OF FOUR INSECTICIDES APPLIED AGAINST FIVE SPECIES OF STORED-PRODUCT INSECTS AT THREE TEMPERATURES

Insecticide	Temp. °F	*T. castaneum*	*T. confusum*	*O. surinamensis*	*O. mercator*	*C. ferrugineus*
D D T	80	36.62	60.07	120.10	109.30	11.29
	60	30.55	37.33	16.85	12.95	7.48
	50	36.32	51.55	23.22	13.98	9.48
Lindane	80	8.68	11.20	25.24	22.25	0.03
	60	36.09	43.96	31.21	11.03	0.38
	50	13.38	36.30	19.93	22.51	1.41
Malathion	80	.90	2.78	.44	.80	.75
	60	6.00	13.19	4.62	3.16	4.00
	50	10.86	20.50	6.99	7.18	6.04
Bromophos	80	2.39	6.72	3.32	3.50	0.69
	60	42.54	124.60	39.52	9.38	6.29
	50	185.40	280.20	139.60	30.57	12.90

LD95 values, together with their 95% confidence limits, the x^2 values, the regression equation and the relative potencies of DDT, lindane, malathion and bromophos are given in Tables III, IV, V and VI, respectively.

The LD50 values (Table II and Figure 6) show that the effectiveness of each insecticide, under the controlled conditions of the experiment, was affected by high or low temperatures. Thus, DDT showed a negative temperature coefficient from 80° to 60° F, being more effective at 60° than at 80° F against all insect species, and a slight positive temperature coefficient from 60° to 50° F, being more effective at 60° than at 50° F. This trend was consistent for each of the five species. The relative toxicity of DDT at 80° and 60° F was found to vary among the insect species. Thus, the 95% confidence limits of the LD50 values at 80° and 60° F for the various insect species show significant differences for T. confusum, O. surinamensis and O. mercator; for T. castaneum and C. ferrugineus, the 95% confidence limits overlap, indicating that the differences in the LD50 values do not differ significantly (Table III). Comparing the differences in effectiveness of DDT for each insect species, it will be noted that approximately 1.6 times as much DDT is needed for T. confusum at 80° for the same level of mortality as at 60° F. Similarly, for O. surinamensis and O. mercator 7 times and 8.4 times as much DDT is needed,

TABLE III

COMPARATIVE TOXICITY OF DDT TO FIVE SPECIES OF STORED-PRODUCT INSECTS AT THREE DIFFERENT TEMPERATURES

Species	Temp. °F	Heterogeneity at p=0.05	Regression equation Y = A + Bx	LD50 mg/sq ft	Fiducial limits		LD95	Fiducial limits		Relative potency*
					Lower	Upper		Lower	Upper	
T. castaneum	80	x^2 = .649	Y = -.247 + 3.355x	36.62	31.72	42.16	113.20	91.62	150.70	0.025
T. confusum	80	x^2 = 1.851	Y = -.731 + 3.222x	60.07	51.47	70.56	194.60	146.10	309.50	0.046
O. surinamensis	80	x^2 = 1.854	Y = -.431 + 2.611x	120.10	102.60	140.70	512.50	390.50	749.20	0.003
O. mercator	80	x^2 = 5.154	Y = .359 + 2.276x	109.30	91.77	120.90	577.10	423.30	894.90	0.007
C. ferrugineus	80	x^2 = 5.388	Y = 3.950 + .996x	11.29	6.65	33.08	505.90	107.60	22.34	0.066
T. castaneum	60	x^2 =11.280	Y = 2.991 + 1.353x	30.55	9.35	192.80	502.10	114.70	10.0^9	0.196
T. confusum	60	x^2 = .339	Y = -.151 + 3.276x	37.33	31.86	45.28	118.60	86.00	201.20	0.353
O. surinamensis	60	x^2 = .650	Y = 1.620 + 2.755x	16.85	14.31	19.92	66.61	49.76	102.50	0.274
O. mercator	60	x^2 = 1.029	Y = 2.575 + 2.179x	12.95	10.44	15.76	73.68	50.80	134.10	0.244
C. ferrugineus	60	x^2 = 9.750	Y = 3.546 + 1.664x	7.48	Not significant		72.87	Not significant		0.534
T. castaneum	50	x^2 =12.870	Y = 2.565 + 1.560x	36.32	14.38	213.40	411.40	109.30	10.0^7	0.299
T. confusum	50	x^2 = 2.106	Y = 2.913 + 1.218x	51.55	38.07	77.46	1154.00	490.30	5149.00	0.397
O. surinamensis	50	x^2 = 1.618	Y = 2.067 + 2.147x	23.22	19.19	28.98	135.50	90.32	248.00	0.301
O. mercator	50	x^2 = .834	Y = 3.004 + 1.742x	13.98	10.99	19.20	123.00	68.62	315.30	0.513
C. ferrugineus	50	x^2 = 6.892	Y = 3.273 + 1.766x	9.48	Not significant		80.93	Not significant		0.637

*The figures of relative potency have been worked out using LD50 as a criterion and taking that of malathion as one at the same temperature.

TABLE IV

COMPARATIVE TOXICITY OF LINDANE TO FIVE SPECIES OF STORED-PRODUCT INSECTS AT THREE DIFFERENT TEMPERATURES

Species	Temp. °F	Heterogeneity at p=0.05	Regression equation Y = A + Bx	LD50 mg/sq ft	Fiducial limits Lower	Fiducial limits Upper	LD95	Fiducial limits Lower	Fiducial limits Upper	Relative potency*
T. castaneum	80	$x^2 = 4.099$	Y = 1.526 + 3.700x	8.68	Not significant		24.17	Not significant		0.104
T. confusum	80	$x^2 = 4.658$	Y = .140 + 4.632x	11.20	Not significant		25.37	Not significant		0.248
O. surinamensis	80	$x^2 = 4.021$	Y = .347 + 3.318x	25.24	Not significant		79.04	Not significant		0.017
O. mercator	80	$x^2 = 5.398$	Y = 1.597 + 2.525x	22.25	18.80	26.87	99.71	71.47	162.00	0.035
C. ferrugineus	80	$x^2 = 3.416$	Y = 6.342 + .903x	.03	.02	.05	2.16	1.01	6.58	22.730
T. castaneum	60	$x^2 = 4.435$	Y = .645 + 2.796x	36.09	30.73	42.44	139.90	106.40	207.20	0.166
T. confusum	60	$x^2 = 1.421$	Y = .257 + 2.886x	43.96	37.58	51.73	163.30	123.90	243.20	0.300
O. surinamensis	60	$x^2 = 1.658$	Y = -.021 + 3.360x	31.21	26.71	37.09	96.34	72.07	153.30	0.148
O. mercator	60	$x^2 = 5.494$	Y = 2.135 + 2.748x	11.03	8.86	13.29	43.75	32.50	70.18	0.286
C. ferrugineus	60	$x^2 = 8.448$	Y = 5.939 + 2.223x	.38	Not significant		2.07	Not significant		10.520
T. castaneum	50	$x^2 = .913$	Y = 1.328 + 3.259x	13.38	11.56	15.80	42.79	31.45	72.25	0.812
T. confusum	50	$x^2 = 2.614$	Y = -.027 + 3.222x	36.30	31.33	42.08	117.60	92.90	163.60	0.565
O. surinamensis	50	$x^2 = 2.511$	Y = 1.826 + 2.442x	19.93	16.82	24.04	94.01	67.35	152.30	0.351
O. mercator	50	$x^2 = 4.129$	Y = -.521 + 4.082x	22.51	18.22	26.90	56.94	40.91	152.50	0.419
C. ferrugineus	50	$x^2 = 8.053$	Y = 4.690 + 2.053x	1.41	10.0^{-4}	8.84	8.96	3.07	10.0^{31}	4.284

*The figures of relative potency have been worked out using LD50 as a criterion and taking that of malathion as one at the same temperature.

TABLE V

COMPARATIVE TOXICITY OF MALATHION TO FIVE SPECIES OF STORED-PRODUCT INSECTS AT THREE DIFFERENT TEMPERATURES

Species	Temp. °F	Hetero-geneity at p=0.05	Regression equation Y = A + Bx	LD50 mg/sq ft	Fiducial limits		LD95	Fiducial limits		Relative potency*
					Lower	Upper		Lower	Upper	
T. castaneum	80	x^2 = 2.673	Y = 5.167 + 3.642x	.90	.79	1.02	2.54	2.05	3.47	1.000
T. confusum	80	x^2 = 2.329	Y = 3.033 + 4.426x	2.78	2.49	3.08	6.55	5.54	8.32	1.000
O. surinamensis	80	x^2 = 2.124	Y = 7.148 + 5.982x	.44	.39	.48	.82	.73	.96	1.000
O. mercator	80	x^2 = 3.007	Y = 5.505 + 5.200x	.80	.73	.86	1.65	1.46	1.98	1.000
C. ferrugineus	80	x^2 = 4.186	Y = 5.477 + 3.913x	.75			1.98			1.000
T. castaneum	60	x^2 = .739	Y = 1.222 + 4.852x	6.00	5.48	6.68	13.11	10.78	17.94	1.000
T. confusum	60	x^2 = 2.679	Y = 2.611 + 6.793x	13.19	12.28	14.38	23.04	19.92	28.96	1.000
O. surinamensis	60	x^2 = 3.992	Y = 1.620 + 5.081x	4.62			9.74			1.000
O. mercator	60	x^2 = 7.219	Y = 2.213 + 5.574x	3.16			6.24			1.000
C. ferrugineus	60	x^2 = 5.005	Y = 2.865 + 3.546x	4.00	3.38	4.69	11.64	9.15	16.72	1.000
T. castaneum	50	x^2 = 1.083	Y = -.329 + 5.145x	10.86	10.04	11.71	22.67	19.85	27.31	1.000
T. confusum	50	x^2 =12.280	Y =-4.094 + 6.933x	20.50			35.40			1.000
O. surinamensis	50	x^2 = 4.670	Y = .982 + 4.755x	6.99	6.41	7.66	15.52	13.03	20.36	1.000
O. mercator	50	x^2 = 9.950	Y = 2.955 + 2.388x	7.18	6.08	8.67	35.06	22.94	76.02	1.000
C. ferrugineus	50	x^2 = 1.216	Y = 2.815 + 2.798x	6.04	5.05	7.39	23.37	16.62	39.63	1.000

*The figures of relative potency have been worked out using LD50 as a criterion and taking that of malathion as one at the same temperature.

TABLE VI

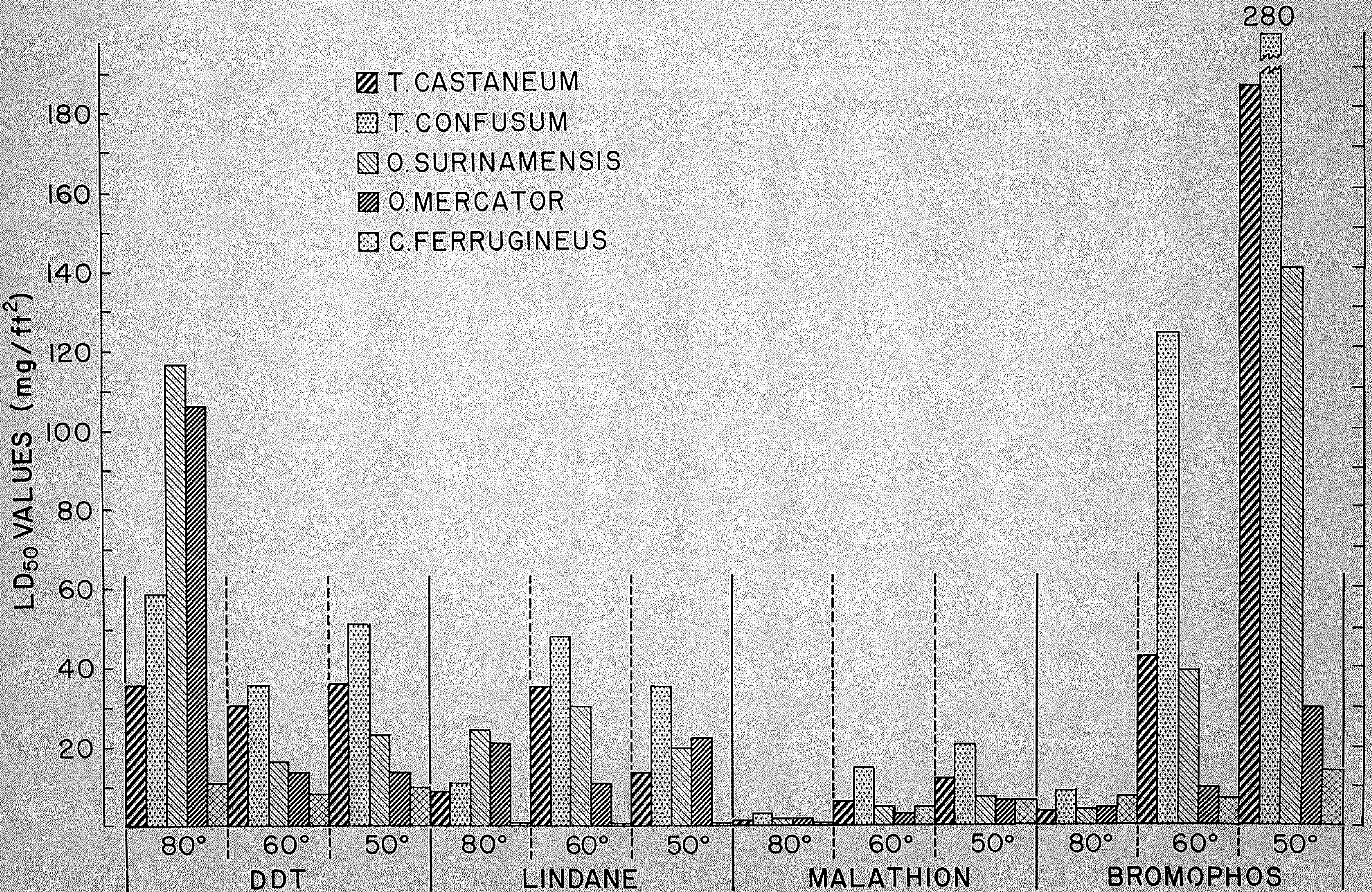
COMPARATIVE TOXICITY OF BROMOPHOS TO FIVE SPECIES OF STORED-PRODUCT INSECTS AT THREE DIFFERENT TEMPERATURES

Species	Temp. °F	Hetero-geneity at p=0.05	Regression equation Y = A + Bx	LD50 mg/sq ft	Fiducial limits		LD95	Fiducial limits		Relative potency*
					Lower	Upper		Lower	Upper	
T. castaneum	80	x^2 = .532	Y = 3.080 + 5.075x	2.39	2.19	2.60	5.04	4.38	6.10	.376
T. confusum	80	x^2 = 2.844	Y = 1.476 + 4.257x	6.72	6.10	7.41	16.37	13.42	22.54	.414
O. surinamensis	80	x^2 = .266	Y = 2.574 + 4.653x	3.32	3.03	3.63	7.49	6.48	9.13	.132
O. mercator	80	x^2 = 1.725	Y = 2.916 + 3.828x	3.50	3.16	3.88	9.42	7.85	12.17	.228
C. ferrugineus	80	x^2 =10.850	Y = 5.575 + 3.679x	.69			1.95			1.080
T. castaneum	60	x^2 = .419	Y = .632 + 2.681x	42.54	34.66	49.62	174.70	138.10	250.90	.141
T. confusum	60	x^2 = .352	Y = .598 + 2.100x	124.60	88.22	152.30	756.70	522.60	1592.00	.106
O. surinamensis	60	x^2 = .219	Y = 1.338 + 2.293x	39.52	30.43	47.43	206.20	154.70	330.90	.117
O. mercator	60	x^2 = 2.699	Y = 1.543 + 3.555x	9.38	6.60	11.31	27.23	22.82	37.65	.337
C. ferrugineus	60	x^2 = 6.195	Y = 2.833 + 2.711x	6.29			25.46			.636
T. castaneum	50	x^2 =10.730	Y =-1.362 + 2.804x	185.40	63.66	294.60	715.70	388.60	10.0^6	0.058
T. confusum	50	x^2 = 5.881	Y =-3.120 + 3.317x	280.20	249.30	311.60	877.70	695.00	1279.00	.073
O. surinamensis	50	x^2 = 3.108	Y =-3.032 + 3.745x	139.60	126.50	156.10	383.80	306.10	542.40	.050
O. mercator	50	x^2 = .162	Y = .855 + 2.790x	30.57	24.95	35.72	118.80	92.95	174.80	.235
C. ferrugineus	50	x^2 = 1.881	Y = 2.265 + 2.420x	12.90	10.64	16.31	60.09	39.87	116.60	.478

*The figures of relative potency have been worked out using LD50 as a criterion and taking that of malathion as one at the same temperature.

FIGURE 6

COMPARATIVE EFFECTIVNESS OF FOUR INSECTICIDES AGAINST
FIVE SPECIES OF INSECTS AT THREE DIFFERENT TEMPERATURES


T. CASTANEUM
T. CONFUSUM
O. SURINAMENSIS
O. MERCATOR
C. FERRUGINEUS
LD50 VALUES (mg/ft2)
280
80° 60° 50°
DDT
LINDANE
MALATHION
BROMOPHOS

respectively, at 80° as at 60° F. The differences in the LD50 values between 60° and 50° F were not as large as those between 80° and 60° F. Thus, the only insect species that showed significant differences in the LD50 values at 60° and 50° F was O. surinamensis. Comparing the LD50 values of DDT obtained at 80° and 50° F for the different insect species it is apparent that for O. surinamensis and O. mercator, the LD50 values differ significantly. Joint Probit analysis for the test of parallelism showed that the three lines obtained for each species at the three temperatures were not all parallel. The species whose regression lines were found not to contradict the hypothesis of parallelism were O. surinamensis (Table VII and Figure 7), O. mercator (Table VIII and Figure 8) and C. ferrugineus (Table IX and Figure 9). For O. surinamensis and O. mercator the regression lines at 80° differ significantly from the regression lines at 60° and 50° F, but the regression lines at 60° and 50° F did not differ significantly from each other (Tables VII and VIII). The slopes of the regression lines for both species were approximately the same (2.45 for O. surinamensis and 2.04 for O. mercator). Given that a low slope is a reflection of different variances in susceptibility of individuals in the population, it is apparent that the populations of both species were quite homogeneous. For C. ferrugineus the regression lines for the three temperatures were parallel,

TABLE VII

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR O. surinamensis EXPOSED TO DDT AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	120.133	a	101.758	141.819
	LD95	561.658		447.504	736.890
60	LD50	16.935	b	14.126	20.331
	LD95	79.177		62.176	105.549
50	LD50	22.274	c	18.884	140.954
	LD95	104.139		81.103	140.954

a and b are significantly different ($P<0.05$)

a and c are significantly different ($P<0.05$)

b and c are not significantly different ($P>0.05$)

FIGURE 7

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

O. surinamensis TO DDT AT THREE TEMPERATURES

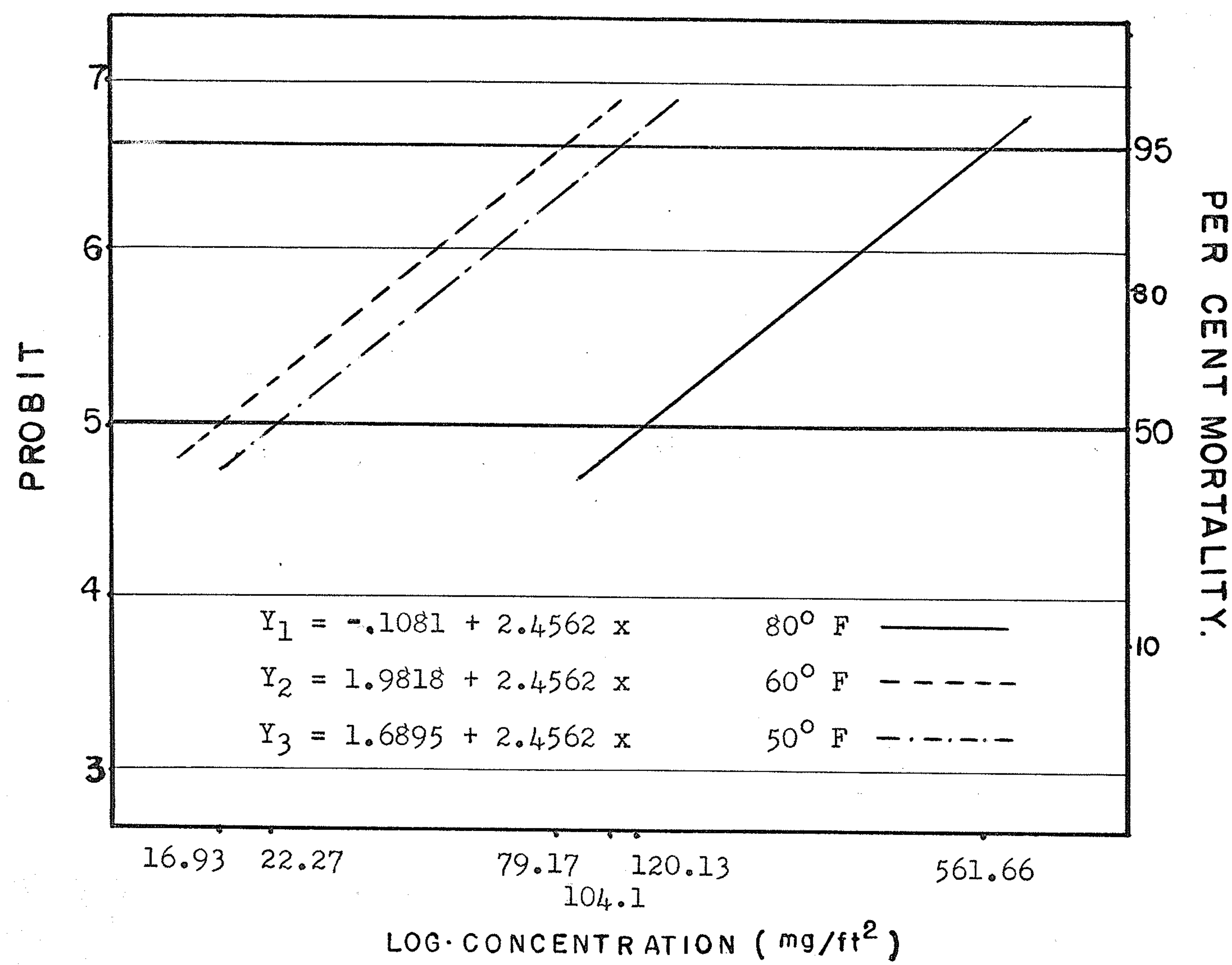

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = -.1081 + 2.4562 x
Y2 = 1.9818 + 2.4562 x
Y3 = 1.6895 + 2.4562 x
80° F
60° F
50° F
16.93
22.27
79.17
104.1
120.13
561.66
LOG·CONCENTRATION (mg/ft2)

TABLE VIII

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *O. mercator* EXPOSED TO DDT AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	108.998	a	89.963	131.948
	LD95	696.414		526.632	982.925
60	LD50	12.835	b	10.362	15.816
	LD95	82.008		61.903	115.447
50	LD50	12.809	c	10.530	15.864
	LD95	81.842		58.852	123.783

a and b are significantly different ($P<0.05$)

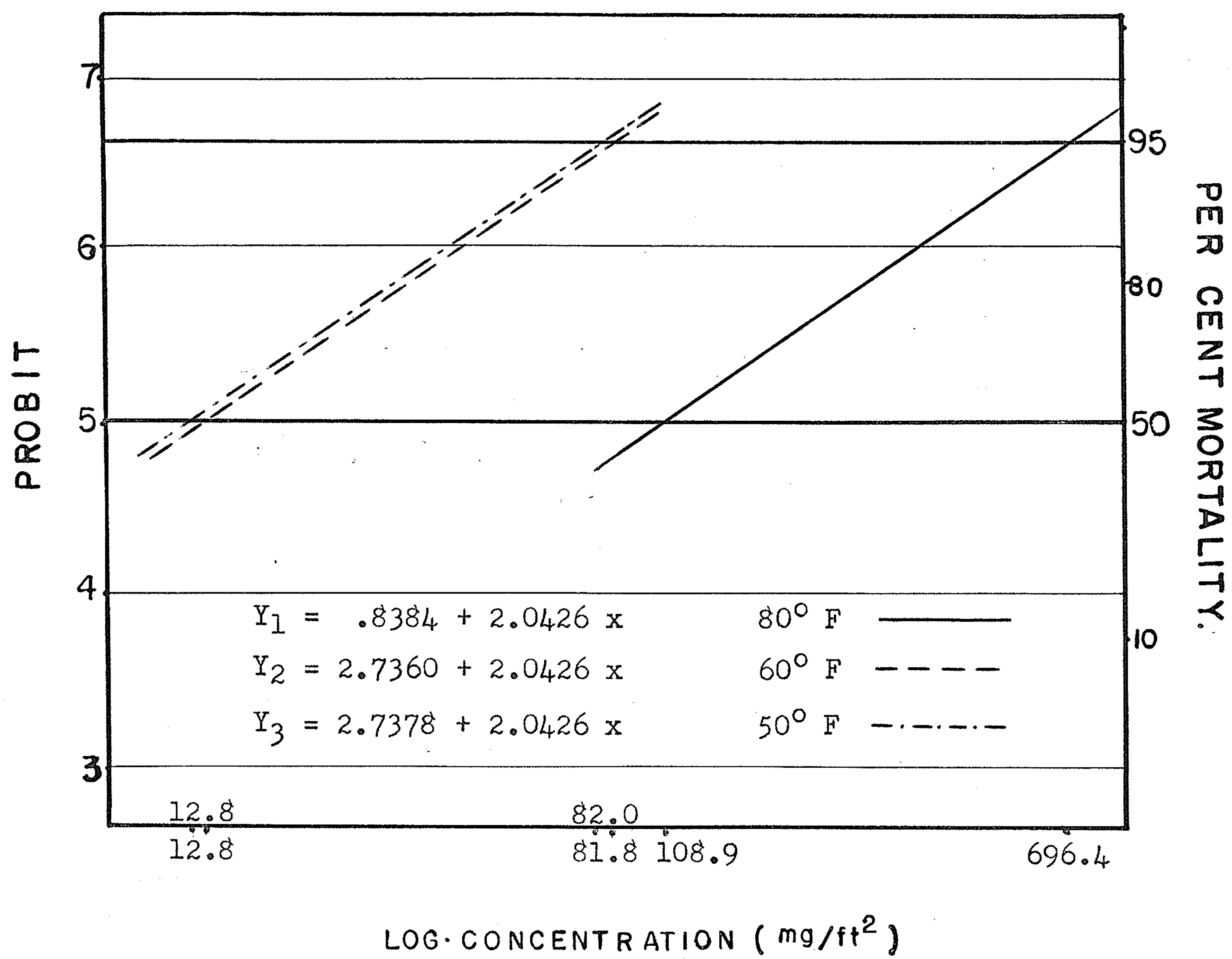
a and c are significantly different ($P<0.05$)

b and c are not significantly different ($P>0.05$)

FIGURE 8

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

O. mercator TO DDT AT THREE TEMPERATURES



PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = .8384 + 2.0426 x
Y2 = 2.7360 + 2.0426 x
Y3 = 2.7378 + 2.0426 x
80° F
60° F
50° F
12.8
12.8
82.0
81.8
108.9
696.4
LOG·CONCENTRATION (mg/ft2)

TABLE IX

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *C. ferrugineus* EXPOSED TO DDT AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	7.775	a	3.750	24.472
	LD95	109.593		31.770	2092.630
60	LD50	8.376	b	4.054	25.748
	LD95	118.066		34.637	2183.440
50	LD50	11.429	c	5.272	40.067
	LD95	161.099		44.337	3452.130

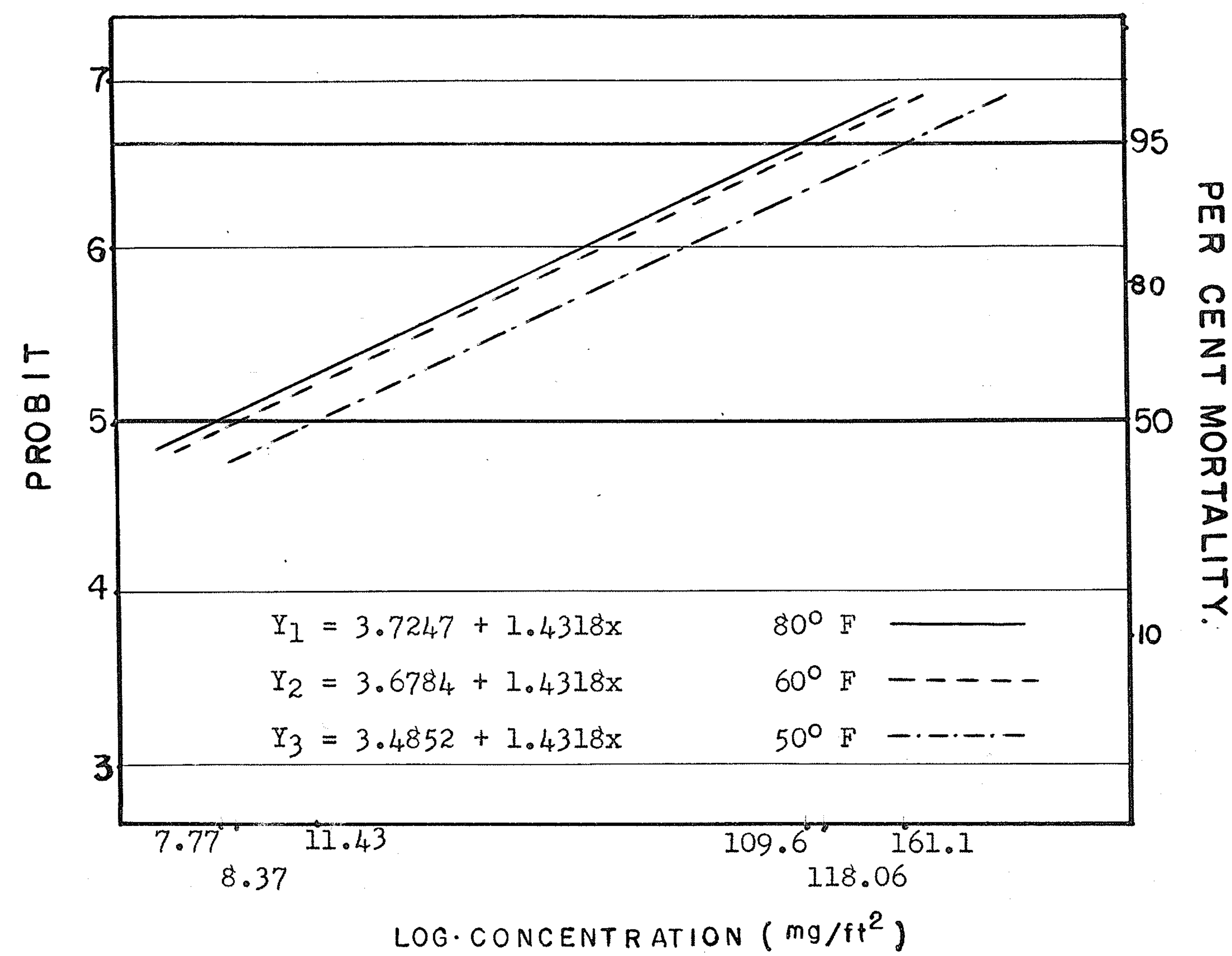
a and b are not significantly different $(P > 0.05)$

a and c are not significantly different $(P > 0.05)$

b and c are not significantly different $(P > 0.05)$

FIGURE 9

REGRESSION LINES OBTAINED AFTER EXPOSURE OF *C. ferrugineus* TO DDT AT THREE TEMPERATURES


PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 3.7247 + 1.4318x
80° F
Y2 = 3.6784 + 1.4318x
60° F
Y3 = 3.4852 + 1.4318x
50° F
7.77
8.37
11.43
109.6
118.06
161.1
LOG·CONCENTRATION (mg/ft2)

but they did not differ significantly from one another. The common slope of the regression lines for C. ferrugineus was smaller (1.43), that is, the regression lines were flatter than those for O. surinamensis and O. mercator, indicating a more heterogeneous population of this species or, perhaps, individuals in the population resistant to DDT.

Lindane showed variable results at the three temperatures (Table II, page 40, and Table IV, page 43). For T. castaneum, T. confusum, O. surinamensis and C. ferrugineus, lindane showed a positive temperature coefficient, being more effective at 80° than at 60° F. For O. mercator, lindane showed a negative temperature coefficient, being more effective at 60° than at 80° F. Lindane showed a pronounced fumigant action, especially at 80° F, characterized by high mortalities in the checks. For this reason a large constant temperature room, instead of the constant temperature cabinets, was used for exposing insects to lindane. At 50°, lindane was significantly more effective than at 60° F against T. castaneum, O. surinamensis and T. confusum; but it was significantly less effective against O. mercator and C. ferrugineus. The action of lindane against C. ferrugineus was positively correlated with temperature. The joint Probit analysis for the test of parallelism indicated that the three regression lines obtained from the LD50 and LD95 values, at each temperature, were parallel for

T. castaneum (Figure 10), *O. surinamensis* (Figure 11), *O. mercator* (Figure 12) and *C. ferrugineus* (Figure 13). The regression lines at the three temperatures for *T. castaneum* differ significantly from one another (Table X). For *O. surinamensis* there was a significant difference between the regression lines at 60° and 50° F (Table XI). For *O. mercator* the regression lines at 80° and 60° F differ significantly (Table XII). For *C. ferrugineus* the regression lines at 80° and 50° F differ significantly but not at 80° and 60° or at 60° and 50° F (Table XIII). The common slope of the regression lines of *C. ferrugineus* is very low (1.16), in contrast with *T. castaneum*, *O. surinamensis* and *O. mercator* which ranged from 3.1 to 2.8, indicating a heterogeneous population of this species or probably individuals in the population, resistant to lindane.

Malathion showed a positive temperature coefficient for all insect species, being most effective at 80°, intermediate at 60° and least toxic at 50° F (Table II, page 40, and Table V, page 44). Thus, the three LD50 values obtained for *T. castaneum* at the three temperatures, differ significantly amongst each other. The LD50 values for *T. confusum* differ significantly between 80° and 60° F. For *O. surinamensis* and *O. mercator* the LD50 values differ significantly between 80° and 50° F. The joint Probit analysis for the test of parallelism showed that in all insect species the

FIGURE 10

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

T. castaneum TO LINDANE AT THREE TEMPERATURES

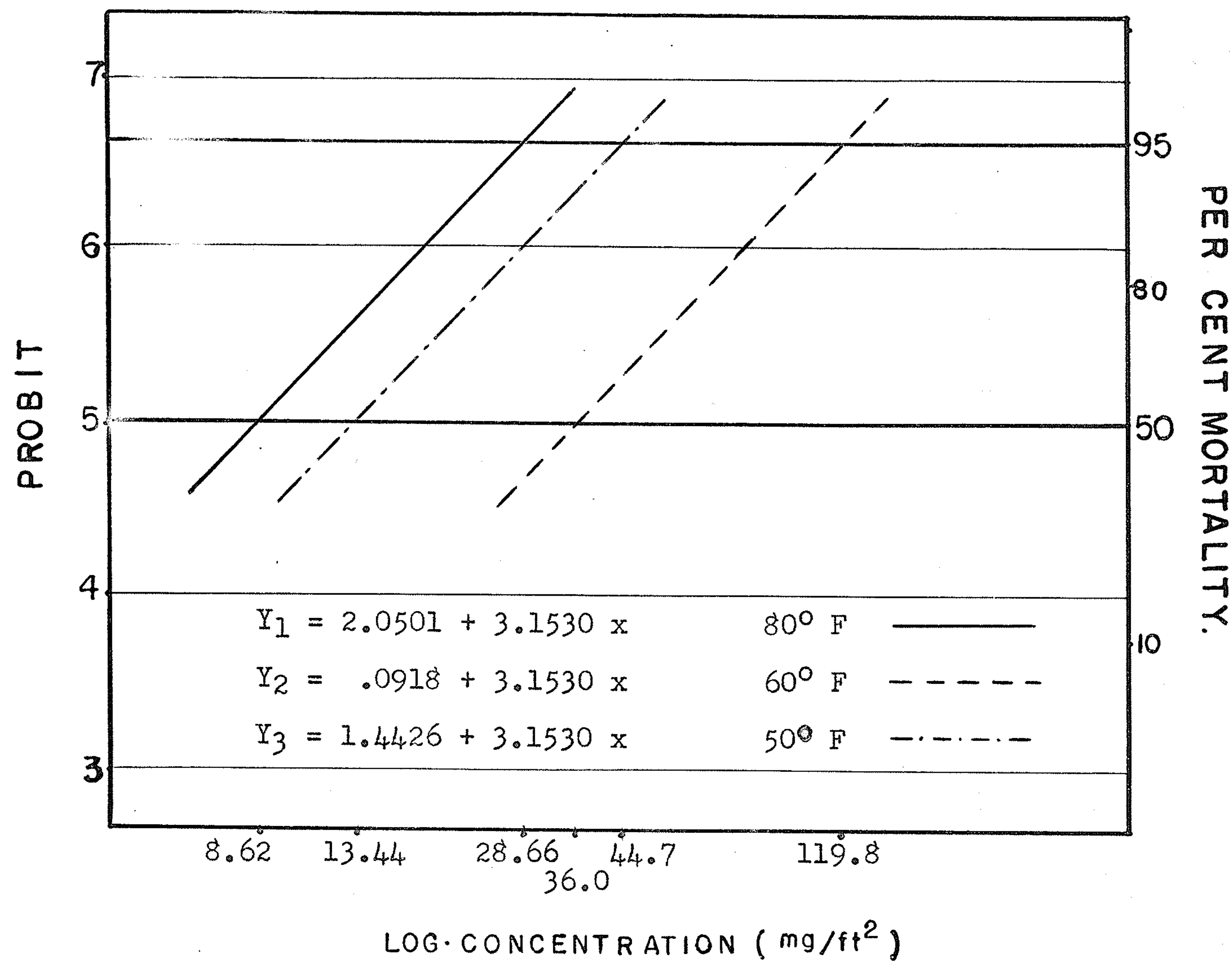

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 2.0501 + 3.1530 x 80° F
Y2 = .0918 + 3.1530 x 60° F
Y3 = 1.4426 + 3.1530 x 50° F
8.62
13.44
28.66
36.0
44.7
119.8
LOG·CONCENTRATION (mg/ft2)

FIGURE 11

REGRESSION LINES OBTAINED AFTER EXPOSURE OF *O. surinamensis* TO LINDANE AT THREE TEMPERATURES

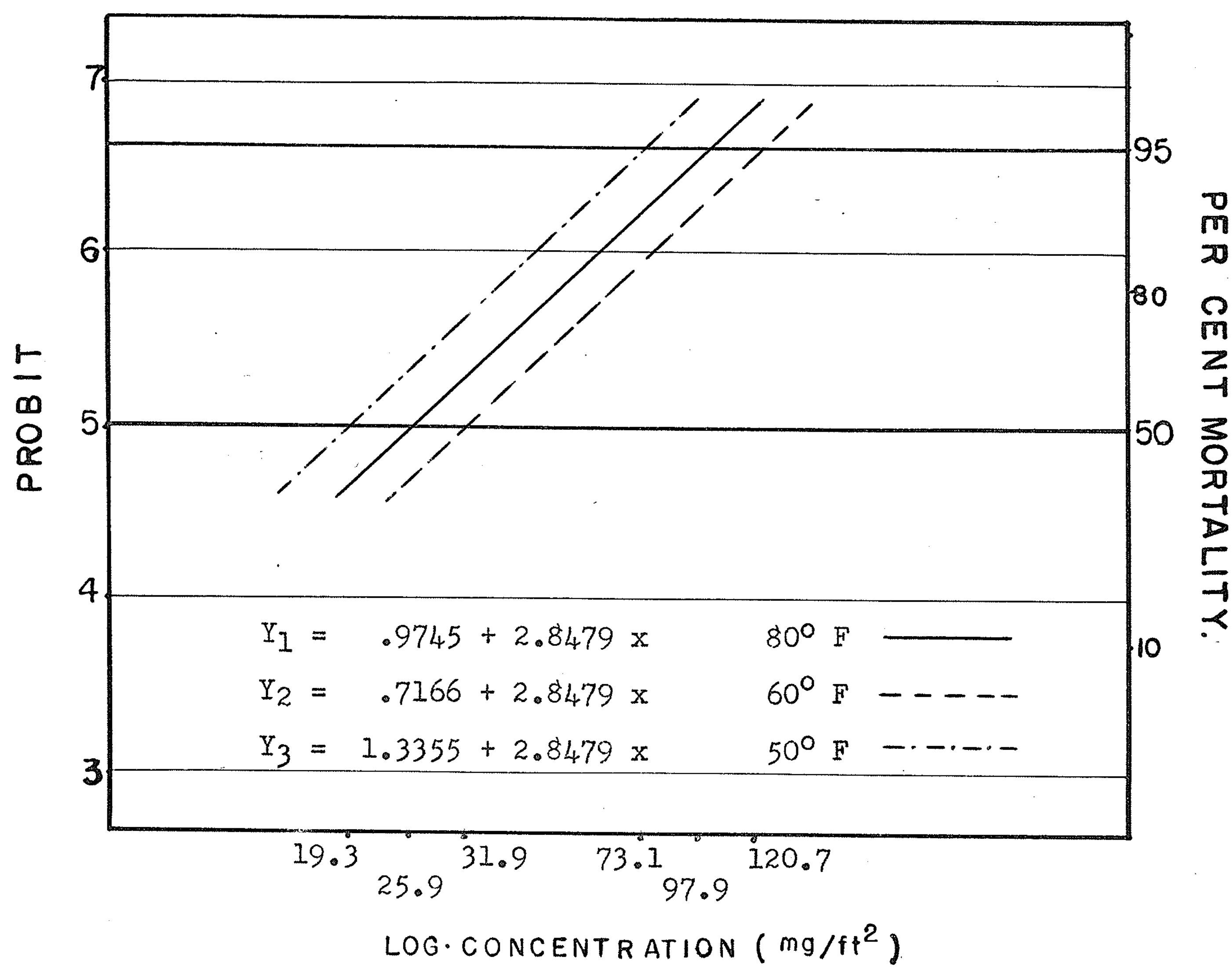

PROBIT
7
6
5
4
3
95
80
50
10
PER CENT MORTALITY.
Y1 = .9745 + 2.8479 x 80° F
Y2 = .7166 + 2.8479 x 60° F
Y3 = 1.3355 + 2.8479 x 50° F
19.3
25.9
31.9
73.1
97.9
120.7
LOG·CONCENTRATION (mg/ft2)

FIGURE 12

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

O. mercator TO LINDANE AT THREE TEMPERATURES

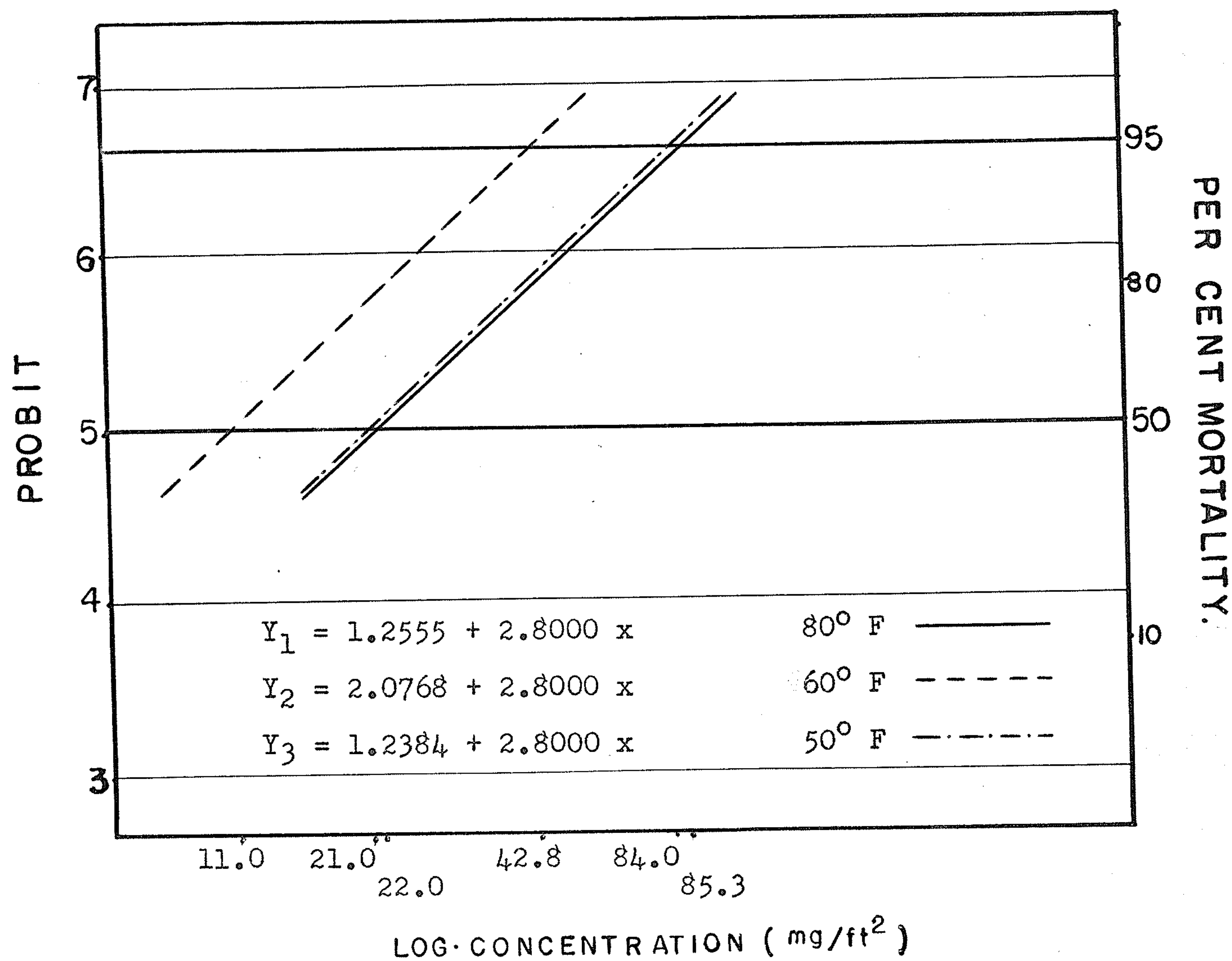

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 1.2555 + 2.8000 x
Y2 = 2.0768 + 2.8000 x
Y3 = 1.2384 + 2.8000 x
80° F
60° F
50° F
11.0
21.0
22.0
42.8
84.0
85.3
LOG·CONCENTRATION (mg/ft2)

FIGURE 13

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

C. ferrugineus to LINDANE AT THREE TEMPERATURES

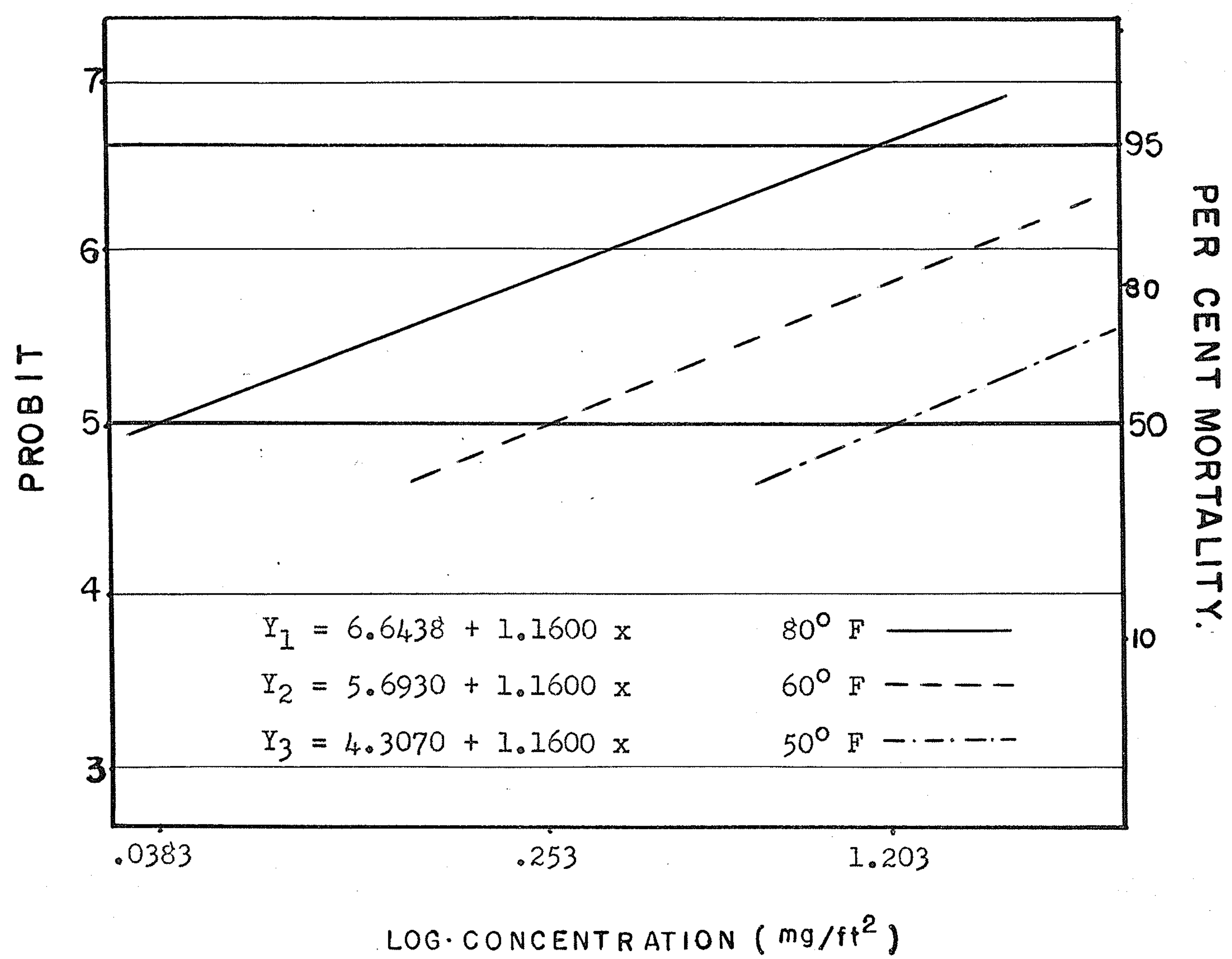

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 6.6438 + 1.1600 x
Y2 = 5.6930 + 1.1600 x
Y3 = 4.3070 + 1.1600 x
80° F
60° F
50° F
.0383
.253
1.203
LOG·CONCENTRATION (mg/ft2)

TABLE X

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *T. castaneum* EXPOSED TO LINDANE AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	8.621	a	7.400	10.028
	LD95	28.667		23.589	36.225
60	LD50	36.033	b	31.292	41.510
	LD95	119.813		98.853	121.305
50	LD50	13.436	c	11.553	15.690
	LD95	44.676		36.273	57.541

a and b are significantly different ($P < 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are significantly different ($P < 0.05$)

TABLE XI

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *O. surinamensis* EXPOSED TO LINDANE AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	25.911	a	21.910	30.831
	LD95	97.988		77.521	130.244
60	LD50	31.918	b	26.574	38.525
	LD95	120.705		94.777	161.445
50	LD50	19.352	c	16.731	22.516
	LD95	73.184		58.756	95.827

a and b are not significantly different ($P > 0.05$)

a and c are not significantly different ($P > 0.05$)

b and c are significantly different ($P < 0.05$)

TABLE XII

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *O. mercator* EXPOSED TO LINDANE AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits	
				Lower	Upper
80	LD50	21.743	a	16.104	30.527
	LD95	84.120		54.012	175.288
60	LD50	11.067	b	7.396	15.948
	LD95	42.816		28.119	80.790
50	LD50	22.051	c	13.370	35.922
	LD95	85.310		50.601	182.796

a and b are significantly different ($P < 0.05$)

a and c are not significantly different ($P > 0.05$)

b and c are not significantly different ($P > 0.05$)

TABLE XIII

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *C. ferrugineus* EXPOSED TO LINDANE AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	.038	a	.014	.089
	LD95	1.003		.360	5.793
60	LD50	.253	b	.078	.653
	LD95	6.619		2.296	36.762
50	LD50	1.203	c	.463	2.825
	LD95	31.507		11.013	196.941

a and b are not significantly different ($P > 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are not significantly different ($P > 0.05$)

three regression lines obtained for the three temperatures were parallel. The regression lines differ significantly for *T. castaneum* (Table XIV and Figure 14), *O. surinamensis* (Table XVI and Figure 16), *O. mercator* (Table XVII and Figure 17) and *C. ferrugineus* (Table XVIII and Figure 18). For *T. confusum*, the regression lines at 80° and 60°, and 80° and 50° F, showed significant differences, but not at 60° and 50° F (Table XV and Figure 15). The slopes of the regression lines for all the insect species were similar, ranging from 3.2 to 5.2, indicating homogeneous populations with no individuals resistant to malathion.

Bromophos, like malathion, showed a positive temperature coefficient (Table II, page 40, and Table VI, page 46). Comparing the LD50 values of malathion, obtained at the three different temperatures for each species, it will be noted that the LD50 values do not differ greatly. With bromophos, on the other hand, the differences are, in some cases, spectacular. Thus, approximately 18 times as much bromophos is needed at 60° and 77.5 times as much at 50° to cause the same level of mortality as that at 80° F against *T. castaneum*. It is obvious that bromophos is far less toxic at low, than at high, temperatures. In all insect species at all temperatures the LD50 values were significantly different. The test of parallelism showed

TABLE XIV

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *T. castaneum* EXPOSED TO MALATHION AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	.895	a	.804	.998
	LD95	2.149		1.864	2.548
60	LD50	6.092	b	5.499	6.777
	LD95	14.627		12.624	17.482
50	LD50	10.801	c	9.862	11.818
	LD95	25.931		22.864	30.185

a and b are significantly different ($P < 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are significantly different ($P < 0.05$)

FIGURE 14

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

T. castaneum TO MALATHION AT THREE TEMPERATURES

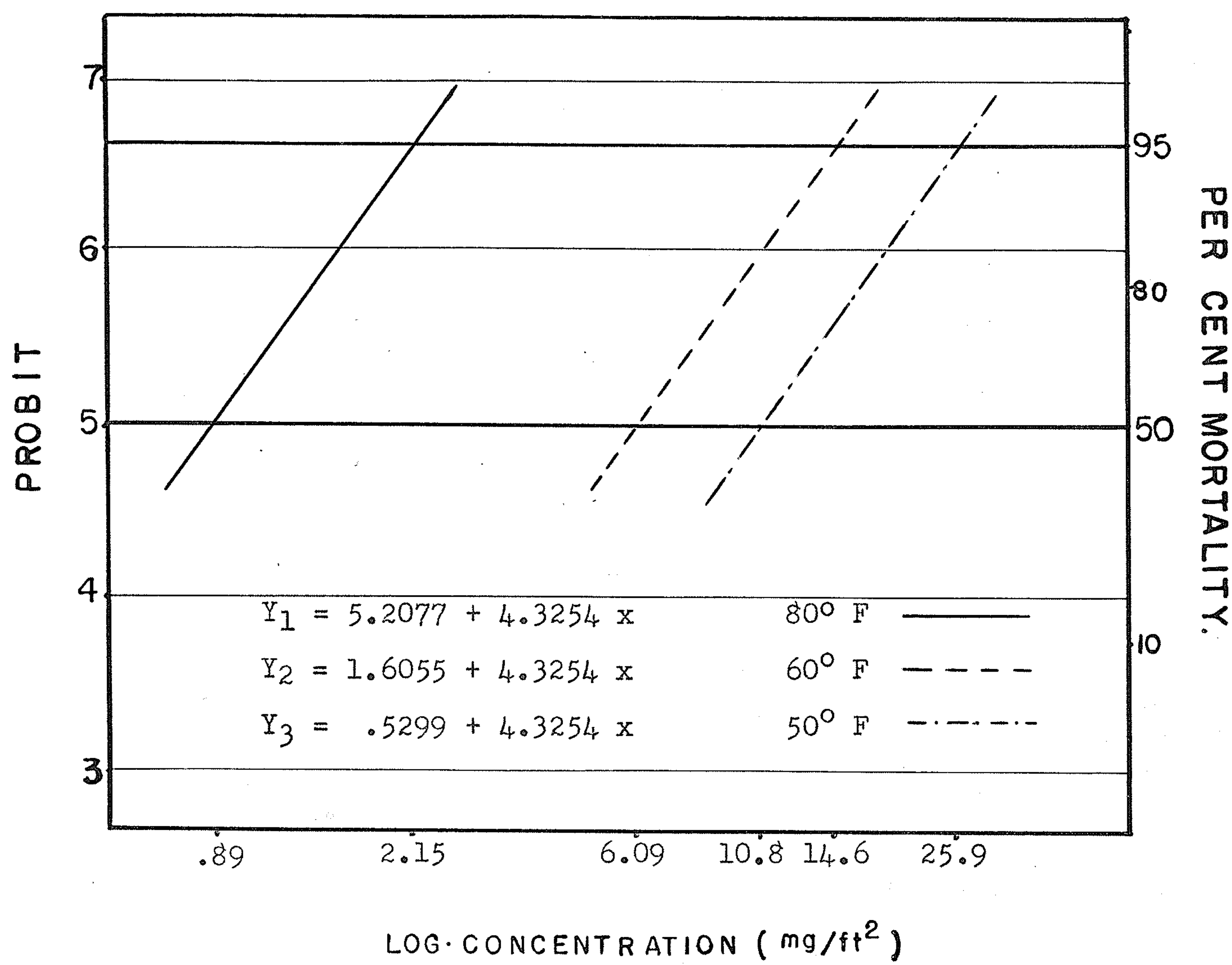

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 5.2077 + 4.3254 x
Y2 = 1.6055 + 4.3254 x
Y3 = .5299 + 4.3254 x
80° F
60° F
50° F
.89
2.15
6.09
10.8
14.6
25.9
LOG·CONCENTRATION (mg/ft2)

TABLE XV

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *T. confusum* EXPOSED TO MALATHION AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits	
				Lower	Upper
80	LD50	2.806	a	2.095	3.681
	LD95	5.789		4.287	10.540
60	LD50	13.650	b	10.345	19.220
	LD95	28.163		19.835	58.737
50	LD50	20.454	c	16.055	25.955
	LD95	42.203		31.760	76.883

a and b are significantly different ($P < 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are not significantly different ($P > 0.05$)

FIGURE 15

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

T. confusum TO MALATHION AT THREE TEMPERATURES

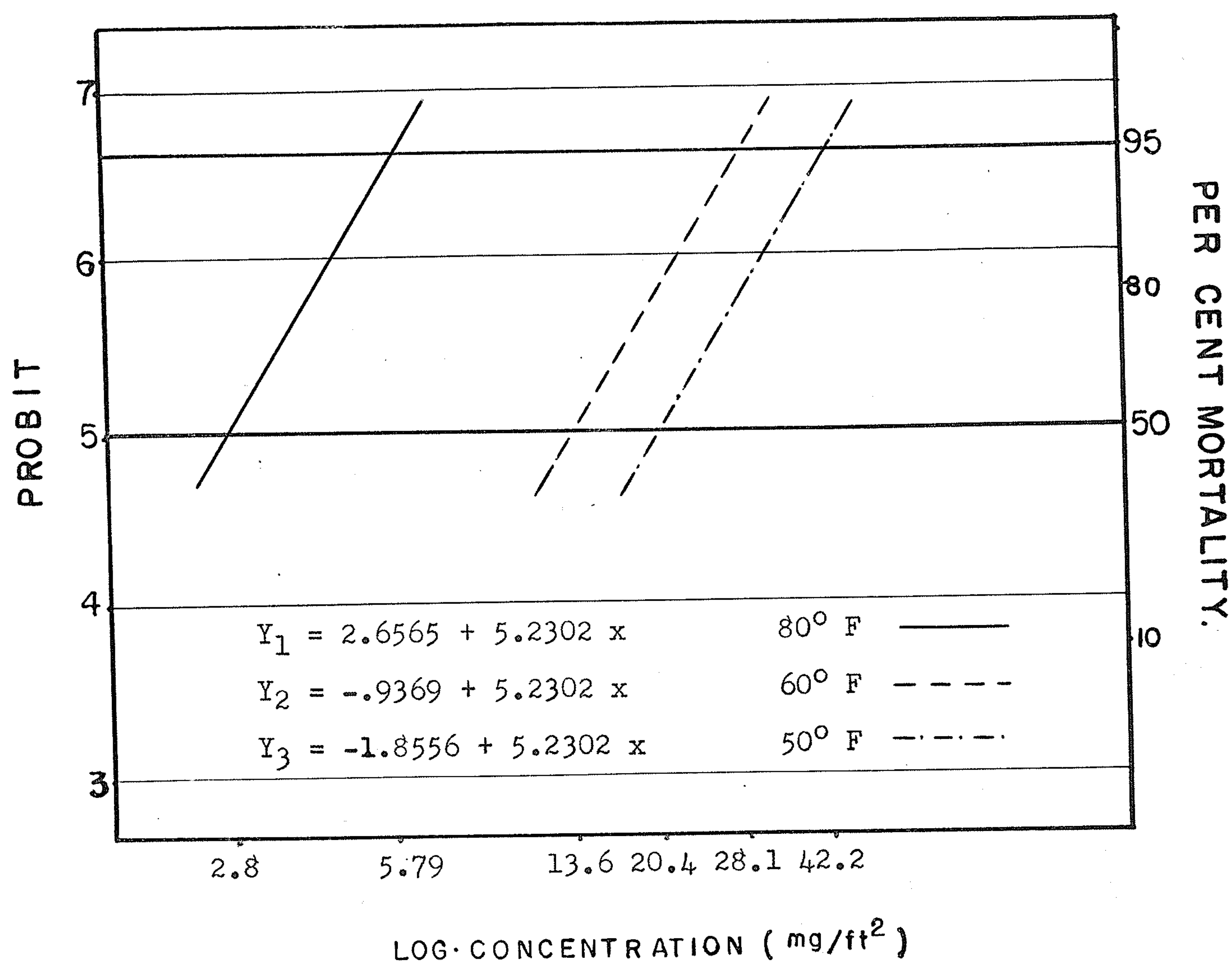

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 2.6565 + 5.2302 x
Y2 = -.9369 + 5.2302 x
Y3 = -1.8556 + 5.2302 x
80° F
60° F
50° F
2.8
5.79
13.6
20.4
28.1
42.2
LOG·CONCENTRATION (mg/ft2)

TABLE XVI

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR O. surinamensis EXPOSED TO MALATHION AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	.429	a	.386	.475
	LD95	.886		.790	1.011
60	LD50	4.620	b	4.256	5.020
	LD95	9.539		8.524	10.913
50	LD50	6.981	c	6.451	7.559
	LD95	14.413		12.915	16.439

a and b are significantly different ($P < 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are significantly different ($P < 0.05$)

FIGURE 16

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

O. surinamensis TO MALATHION AT THREE TEMPERATURES

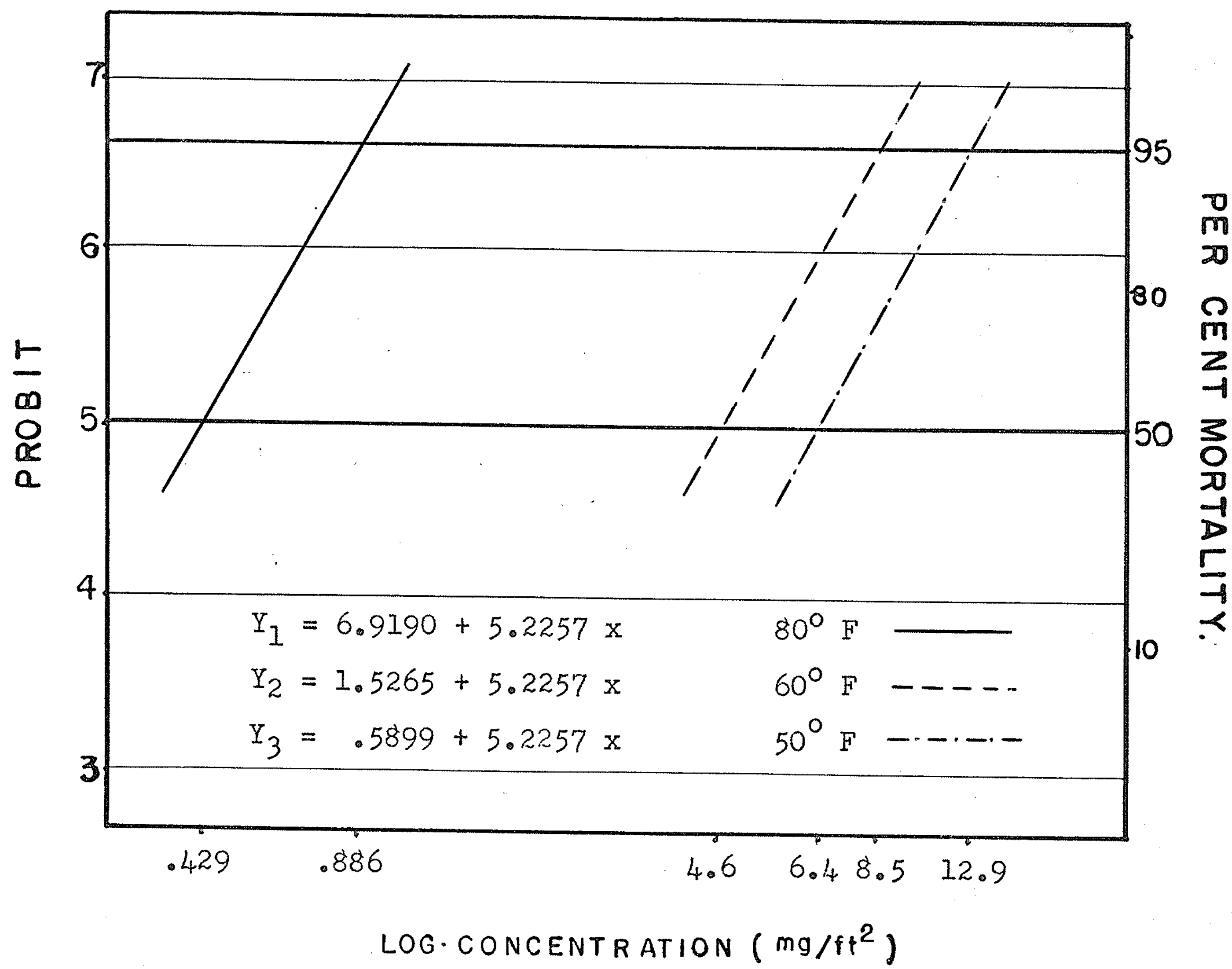

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 6.9190 + 5.2257 x
Y2 = 1.5265 + 5.2257 x
Y3 = .5899 + 5.2257 x
80° F
60° F
50° F
.429
.886
4.6
6.4
8.5
12.9
LOG·CONCENTRATION (mg/ft2)

TABLE XVII

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *O. mercator* EXPOSED TO MALATHION AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	.766	a	.565	.999
	LD95	2.023		1.485	3.330
60	LD50	3.138	b	2.321	4.217
	LD95	8.279		5.872	14.598
50	LD50	6.856	c	5.310	9.004
	LD95	18.088		12.900	32.464

a and b are significantly different ($P < 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are significantly different ($P < 0.05$)

FIGURE 17

REGRESSION LINES OBTAINED AFTER EXPOSURE OF *O. mercator* TO MALATHION AT THREE TEMPERATURES

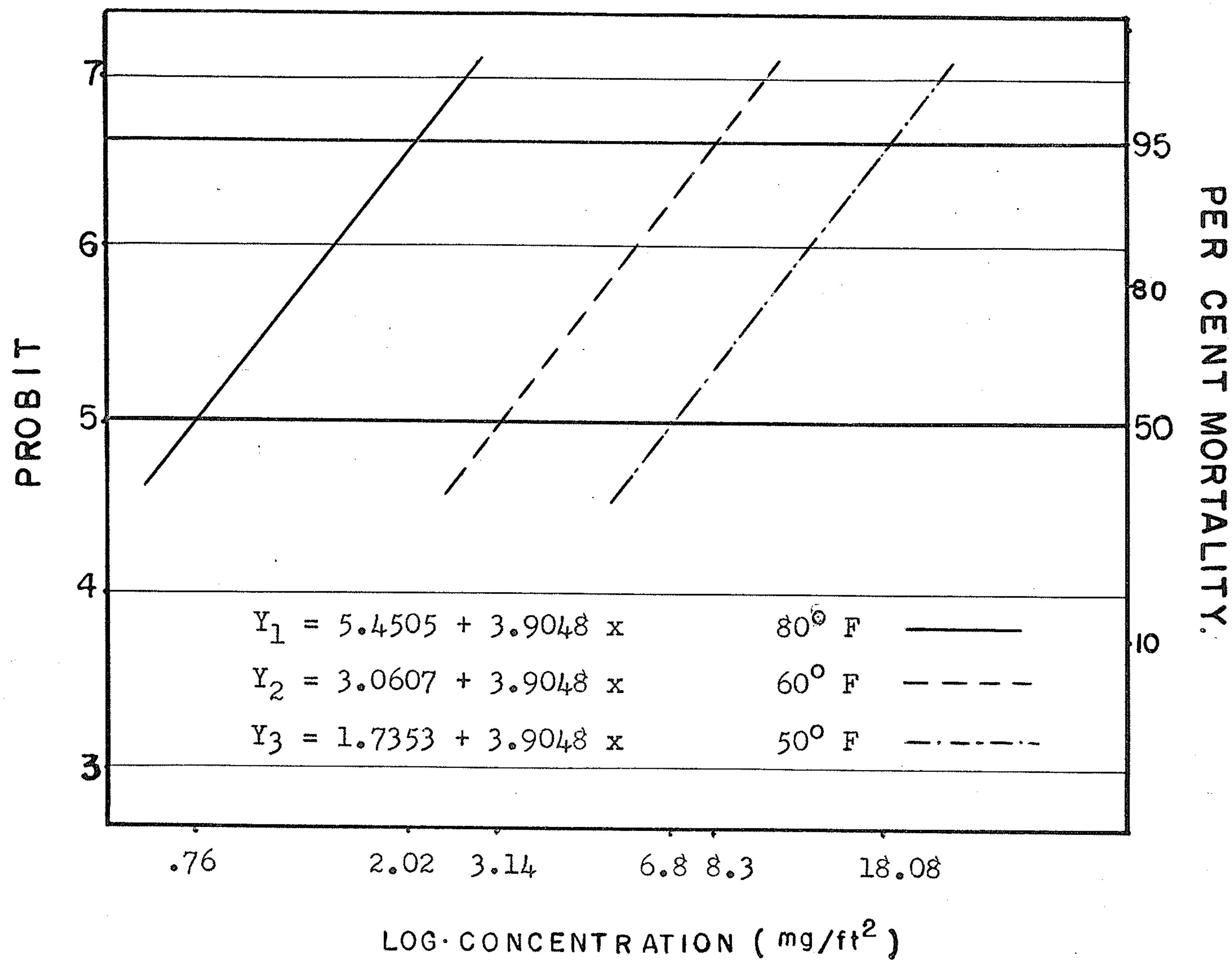

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
Y1 = 5.4505 + 3.9048 x
80° F
Y2 = 3.0607 + 3.9048 x
60° F
Y3 = 1.7353 + 3.9048 x
50° F
.76
2.02
3.14
6.8
8.3
18.08
LOG·CONCENTRATION (mg/ft2)

TABLE XVIII

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *C. ferrugineus* EXPOSED TO MALATHION AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	.728	a	.592	.886
	LD95	2.364		1.885	3.098
60	LD50	3.973	b	3.325	4.732
	LD95	12.906		10.374	16.882
50	LD50	5.870	c	5.029	6.910
	LD95	19.067		15.164	25.506

a and b are significantly different ($P < 0.05$)

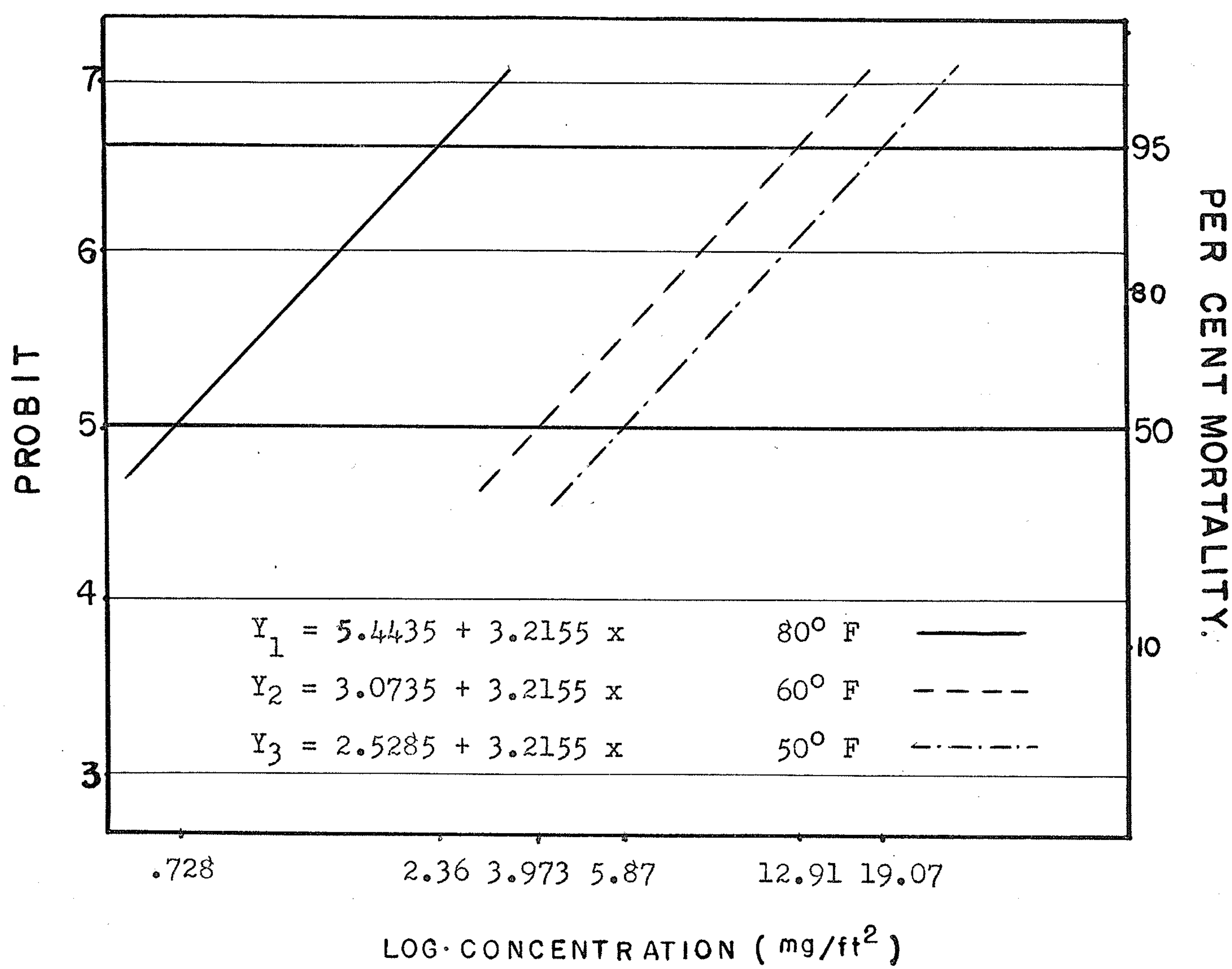
a and c are significantly different ($P < 0.05$)

b and c are significantly different ($P < 0.05$)

FIGURE 18

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

C. ferrugineus TO MALATHION AT THREE TEMPERATURES


PROBIT
7
6
5
4
3
95
80
50
10
PER CENT MORTALITY.
Y1 = 5.4435 + 3.2155 x 80° F
Y2 = 3.0735 + 3.2155 x 60° F
Y3 = 2.5285 + 3.2155 x 50° F
.728
2.36 3.973 5.87
12.91 19.07
LOG·CONCENTRATION (mg/ft2)

that the three regression lines for O. mercator and C. ferrugineus were parallel (Figure 19 and Figure 20). For O. mercator all the regression lines were significantly different (Table XIX) and for C. ferrugineus the regression lines for 80° and 60° and 80° and 50° F differed significantly (Table XX). The slope of the lines for C. ferrugineus is smaller than O. mercator, indicating a heterogeneous population or probably the presence of resistant individuals in the population.

Species Susceptibility to Each Insecticide

In general, the various insect species used in the experiment showed different susceptibility levels to each insecticide (Table III, page 42, Table IV, page 43, Table V, page 44, and Table VI, page 46). The susceptibility levels of the different insect species to each insecticide, and at each temperature, are summarized as follows:

FIGURE 19

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

O. mercator TO BROMOPHOS AT THREE TEMPERATURES

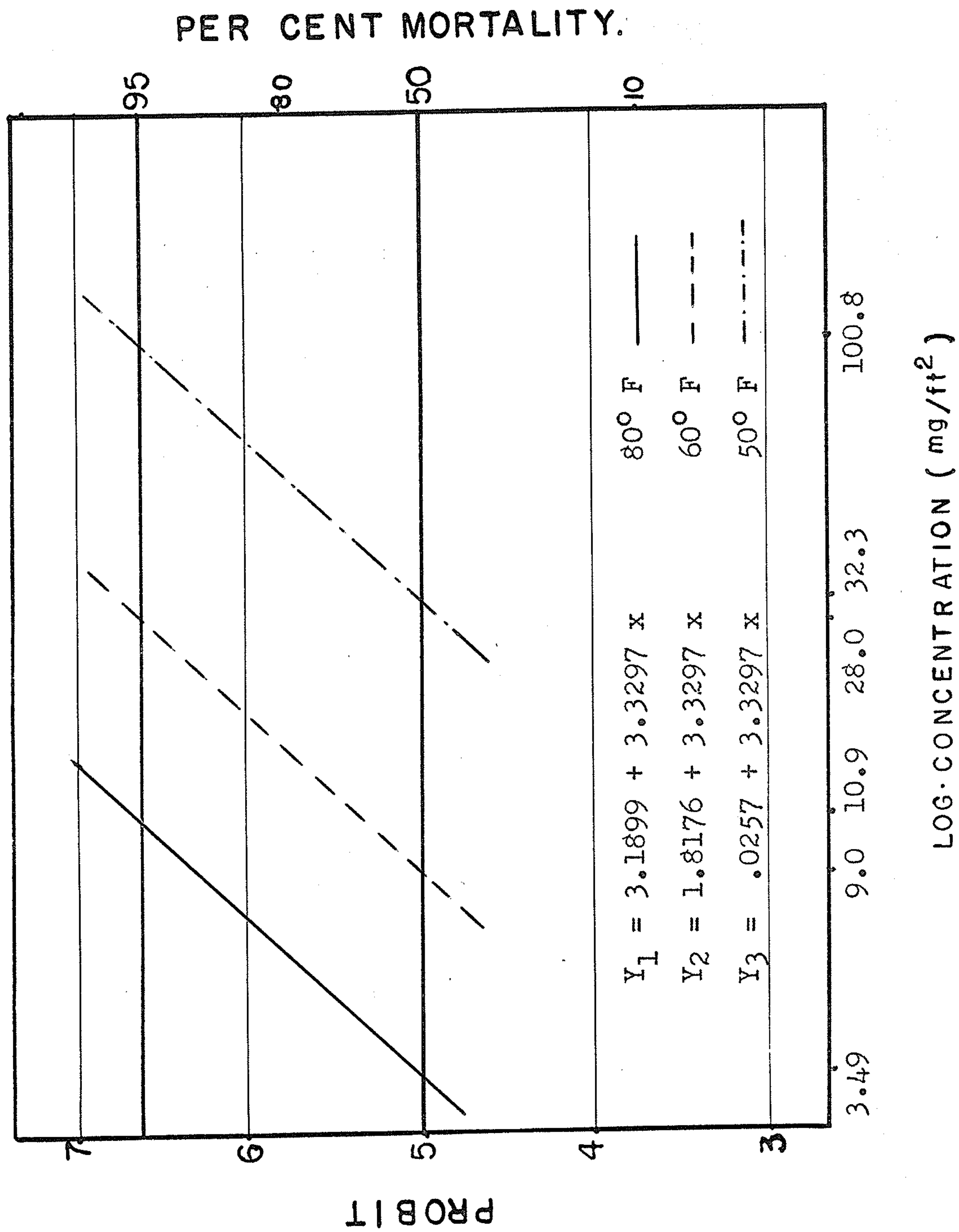

PER CENT MORTALITY.
95
80
50
10
PROBIT
7
6
5
4
3
LOG·CONCENTRATION (mg/ft²)
3.49
9.0
10.9
28.0
32.3
100.8
Y1 = 3.1899 + 3.3297 x 80° F
Y2 = 1.8176 + 3.3297 x 60° F
Y3 = .0257 + 3.3297 x 50° F

FIGURE 20

REGRESSION LINES OBTAINED AFTER EXPOSURE OF

C. ferrugineus TO BROMOPHOS AT THREE TEMPERATURES

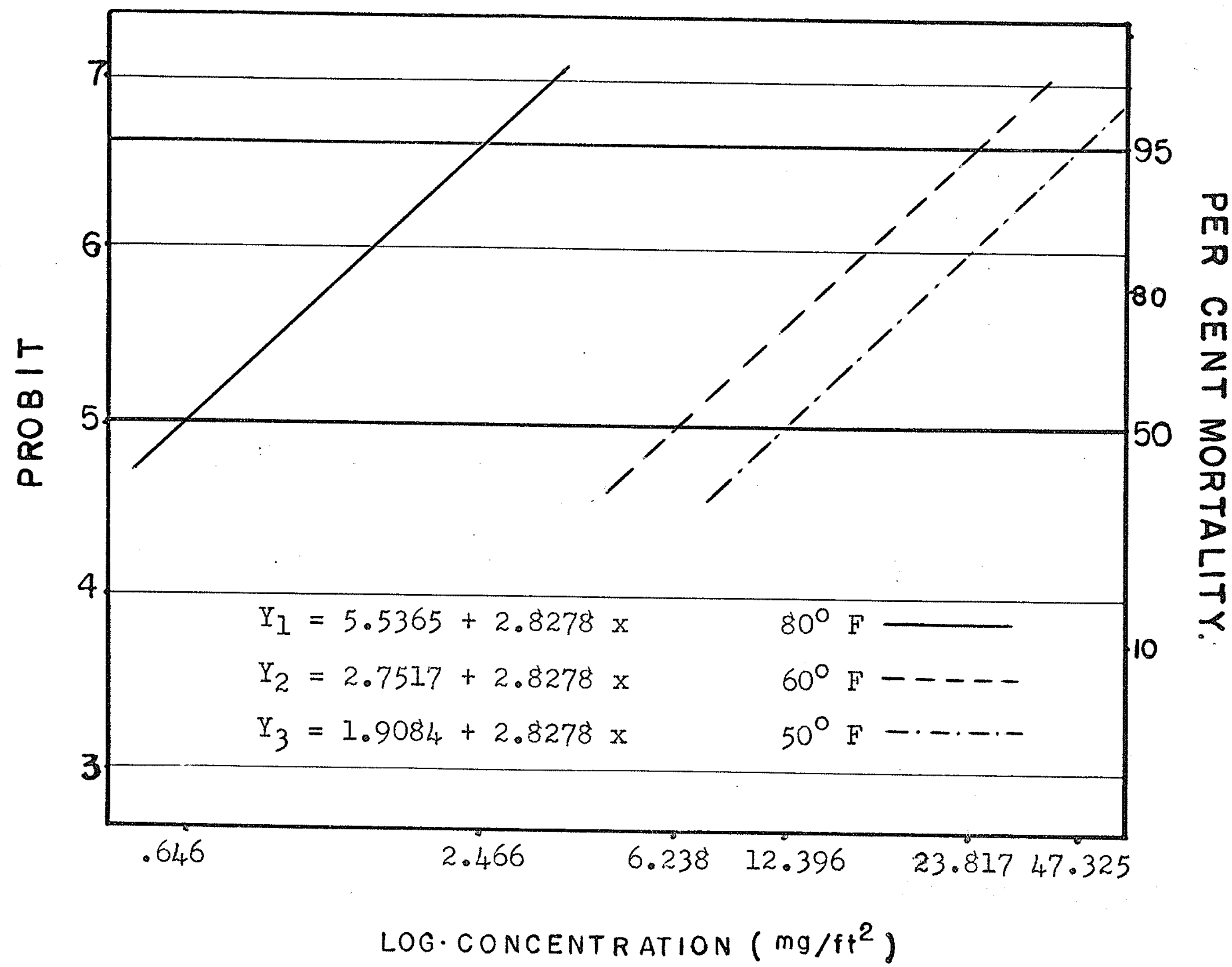

PROBIT
7
6
5
4
3
PER CENT MORTALITY.
95
80
50
10
$Y_1 = 5.5365 + 2.8278\ x$ 80° F
$Y_2 = 2.7517 + 2.8278\ x$ 60° F
$Y_3 = 1.9084 + 2.8278\ x$ 50° F
.646
2.466
6.238
12.396
23.817
47.325
LOG·CONCENTRATION (mg/ft²)

TABLE XIX

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *O. mercator* EXPOSED TO BROMOPHOS AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits	
				Lower	Upper
80	LD50	3.496	a	3.112	3.926
	LD95	10.907		9.139	13.631
60	LD50	9.032	b	7.553	10.538
	LD95	28.175		24.222	33.508
50	LD50	32.312	c	28.139	36.668
	LD95	100.801		86.024	122.319

a and b are significantly different ($P < 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are significantly different ($P < 0.05$)

TABLE XX

THE LD50, LD95 (MG/FT^2) VALUES AND 95% CONFIDENCE LIMITS OBTAINED UNDER THE HYPOTHESIS OF PARALLELISM FOR *C. ferrugineus* EXPOSED TO BROMOPHOS AT THREE TEMPERATURES

Temperature °F	LD value			Exact 95% confidence limits Lower	Upper
80	LD50	.646	a	.313	1.132
	LD95	2.466		1.377	7.212
60	LD50	6.238	b	3.552	12.164
	LD95	23.817		12.204	99.029
50	LD50	12.396	c	7.347	23.827
	LD95	47.325		24.446	200.293

a and b are significantly different ($P < 0.05$)

a and c are significantly different ($P < 0.05$)

b and c are not significantly different ($P > 0.05$)

DDT

°F

80 *C. ferrugineus* > *T. castaneum* > *O. surinamensis* > *O. mercator* > *T. confusum*

60 *C. ferrugineus* > *O. mercator* > *O. surinamensis* > *T. castaneum* > *T. confusum*

50 *C. ferrugineus* > *O. mercator* > *O. surinamensis* > *T. castaneum* > *T. confusum*

Lindane

°F

80 *C. ferrugineus* > *T. castaneum* > *T. confusum* > *O. mercator* > *O. surinamensis*

60 *C. ferrugineus* > *O. mercator* > *O. surinamensis* > *T. castaneum* > *T. confusum*

50 *C. ferrugineus* > *T. castaneum* > *O. surinamensis* > *O. mercator* > *T. confusum*

Malathion

°F

80 *O. surinamensis* > *C. ferrugineus* > *O. mercator* > *T. castaneum* > *T. confusum*

60 *O. mercator* > *C. ferrugineus* > *O. surinamensis* > *T. castaneum* > *T. confusum*

50 *C. ferrugineus* > *O. surinamensis* > *O. mercator* > *T. castaneum* > *T. confusum*

Bromophos

°F

80 *C. ferrugineus* > *T. castaneum* > *O. surinamensis* > *O. mercator* > *T. confusum*

60 *C. ferrugineus* > *O. mercator* > *O. surinamensis* > *T. castaneum* > *T. confusum*

50 *C. ferrugineus* > *O. mercator* > *O. surinamensis* > *T. castaneum* > *T. confusum*

In all the insecticides and temperatures, with the exception of malathion at 80° and 60° F, C. ferrugineus was the most susceptible insect species and in all but two instances (DDT and lindane at 80° F) T. confusum was the most resistant insect species. It is interesting to note that C. ferrugineus is the smallest and T. confusum the largest of the insect species used.

Relative Susceptibility of Each Insect Species to Each Insecticide at Each Temperature

Examination of the LD50 values along each column of Table II (page 40) indicates the tolerance of an insect species to each insecticide at each temperature. This is seen more readily in the leader table at the end of this section. Since malathion was the most effective insecticide at all temperatures for all the test insect species except C. ferrugineus, the LD50 values of malathion obtained for each insect species at the same temperature, were used to calculate the relative potency of the other insecticides, i.e. $\frac{\text{LD50 malathion}}{\text{LD50 of each insectide}}$. The relative potency values of DDT, lindane, malathion and bromophos are given in a separate column of Table III, page 42; Table IV, page 43; Table V, page 44 and Table VI, page 45, respectively.

T. castaneum. The most effective insectide against *T. castaneum*, at 80° F, was malathion followed by bromophos, lindane and DDT. With all test insects, except *C. ferrugineus*, the same pattern of insecticide effectiveness was obtained at 80° F. Thus, as compared to malathion at 80° F, approximately 2.6 times as much bromophos, 9.6 times as much lindane and 40.7 times as much DDT was needed for the same level of mortality of this species. At 60° F, malathion was also the most effective followed by DDT, lindane and bromophos. As compared with malathion at 60° F, approximately 5.1 times as much DDT, 6 times as much lindane and 7 times as much bromophos was needed for the same level of mortality. At 50° F, malathion was the most effective followed by lindane, DDT and bromophos. Thus as compared with malathion at 50° F approximately 1.3 times as much lindane, 3.3 times as much DDT and 17.1 times as much bromophos was needed for the same level of mortality.

T. confusum. In general, the same pattern of insecticide effectiveness was obtained at the three temperatures for this species as that of *T. castaneum*. Thus, at 80° F, malathion was the most effective followed by bromophos, lindane and DDT. As compared to malathion at 80° F, 2.4 times as much bromophos, 4 times as much lindane and 21.6 times as much DDT was needed for the same level of mortality. Similarly, as compared to malathion at 60° F, 2.8 times as much DDT, 3.3 times as much lindane and 9.4 times as much bromophos was needed for the same level of mortality. At 50° F, as compared to malathion, 1.8 times as much lindane, 2.5 times as much DDT and 13.6 times as much bromophos was needed for the same level of mortality.

O. surinamensis. The same pattern of insecticide effectiveness obtained for the two previous species at the three temperatures was also obtained for O. surinamensis. Thus, at 80° F, malathion was the most effective insecticide followed by bromophos, lindane and DDT. As compared with malathion at 80° F, approximately 7.5 times as much bromophos, 57.4 times as much lindane and 273.0 times as much DDT was needed for the same level of mortality. At 60° F, as compared to malathion, approximately 3.6 times as much DDT, 6.7 times as much lindane and 8.5 times as much bromophos was needed for the same level of mortality. At 50° F, as compared with malathion, approximately 2.8 times as much lindane, 3.3 times as much DDT and 20.0 times as much bromophos was needed for the same level of mortality.

O. mercator. At 80° F, malathion was the most effective insecticide followed by bromophos, lindane and DDT. As compared to malathion at 80° F, approximately 4.4 times as much bromophos, 27.8 times as much lindane and

136.6 times as much DDT was needed for the same level of mortality. At 60° F, as compared with malathion, approximately 2.9 times as much bromophos, 3.5 times as much lindane and 4.1 times as much DDT was needed for the same level of mortality. At 50° F, as compared with malathion, approximately 1.9 times as much DDT, 3.1 times as much lindane and 4.2 times as much bromophos was needed for the same level of mortality.

C. ferrugineus. The most effective insecticide against C. ferrugineus at 80° F, was lindane followed by bromophos, malathion and DDT. Thus, as compared to lindane at 80° F, approximately 20.9 times as much bromophos, 22.7 times as much malathion and 342.0 times as much DDT was needed for the same level of mortality. At 60° F, lindane was also the most effective insecticide and approximately 10.5 times as much malathion, 16.5 times as much bromophos and 19.7 times as much DDT was needed for the same level of mortality. At 50° F, lindane was the most effective insecticide and approximately 4.2 times as much malathion, 6.7 times as much DDT and 9.2 times as much bromophos was needed for the same level of mortality.

Comparing the LD50 values obtained in all species, except C. ferrugineus, for all insecticides at 80° F it is obvious that the most effective insecticide was malathion followed by bromophos, lindane and DDT. At 60° F, malathion was still the most effective followed by either DDT or lindane and finally by bromophos. At 50° F, malathion was the most effective followed by lindane, DDT and bromophos.

T. castaneum	80° F	Malathion > Bromophos > Lindane > DDT
	60° F	Malathion > DDT > Lindane > Bromophos
	50° F	Malathion > Lindane > DDT > Bromophos
T. confusum	80° F	Malathion > Bromophos > Lindane > DDT
	60° F	Malathion > DDT > Lindane > Bromophos
	50° F	Malathion > Lindane > DDT > Bromophos
O. surinamensis	80° F	Malathion > Bromophos > Lindane > DDT
	60° F	Malathion > DDT > Lindane > Bromophos
	50° F	Malathion > Lindane > DDT > Bromophos
O. mercator	80° F	Malathion > Bromophos > Lindane > DDT
	60° F	Malathion > Bromophos > Lindane > DDT
	50° F	Malathion > DDT > Lindane > Bromophos
C. ferrugineus	80° F	Lindane > Bromophos > Malathion > DDT
	60° F	Lindane > Malathion > Bromophos > DDT
	50° F	Lindane > Malathion > DDT > Bromophos

CHAPTER V

DISCUSSION

The ineffectiveness of methoxychlor, applied in acetone to filter papers, corresponds with the results of Strong and Sbur (1961) who found that methoxychlor was less effective against four stored-product insects when it was applied in an acetone rather than in an oil solution. Parkin (1966) also realized that methoxychlor, applied in the form of water dispersible powder, had little value as a residual insecticide, since it was the least effective of twelve insecticides against eight species of stored-product insects. Similar poor results with methoxychlor were obtained by Vincent and Lindgren (1957), Walker (1960), Strong *et al.* (1961), King *et al.* (1962) and Rowlands (1967). In the results it was mentioned that *T. castaneum* was the only species that showed some susceptibility to methoxychlor. This observation is in agreenment with Ashrafi and Aijas (1965) who also reported that methoxychlor was effective against *T. castaneum* for at least 10 days. On the other hand, Watters (1961) reported that methoxychlor, in the form of wettable powder, although slightly less effective than DDT, effectively controlled spider beetles in flour warehouses. It is possible that the poor toxic results obtained with methoxychlor are due primarily to the presence of acetone in the spray solution which prevented the formation of crystals on the filter paper after evaporation of the solvent. Methoxychlor in the crystalline form is soluble in acetone but no crystals can be formed after evaporation of this solvent. For this reason during the purification technique in the laboratory, alcohol was used as the solvent.

DDT

According to the results obtained by this study, DDT showed, in general, a negative temperature coefficient between 80° and 60° F and a slight positive temperature coefficient from 60° to 50° F. Similar results were obtained by Woodruff (1950) who also found that when DDT was injected into nymphs and adults of Oncopeltus fasciatus (Dall.), a positive temperature coefficient was obtained in the range of temperatures 10° to 22° C (50° to 75° F) and a negative temperature coefficient from 22° to 29° C (72° to 84° F). Pradhan (1949) also obtained a positive temperature coefficient for DDT when the test insects were allowed to dose themselves for long periods of time on DDT residues on filter papers before the assessment of mortality. This phenomenon was explained on the basis of increased activity of the test insects at high temperatures, resulting in increased pick-up of chemical. When the insects were allowed to dose themselves for short periods of time at the different temperatures and then transferred to clean food media for a given period of time before assessment of mortality, a negative temperature coefficient was obtained. The negative temperature coefficient of DDT has been reported by many workers (Lindquist *et al.*, 1945; Potte and Gillham, 1946; Dustan, 1947; Hoffman and Lindquist, 1949; Barker, 1957; Vinson and Kearns, 1952; Das, 1961; and Das and McIntosh, 1961).

The slight positive temperature coefficient of DDT, obtained from 60° to 50° F, may be explained on the basis of reduced activity of the test insects at the lower temperature. If the LD50 values of DDT obtained at 80°, in all insect species, are compared with those obtained at 50° F, DDT still shows a negative temperature coefficient, being more effective at 50° than at 80° F.

With regard to species susceptibility to DDT, Parkin (1953) reported that *C. ferrugineus* was more susceptible than *O. surinamensis* and *O. mercator* to DDT dust at 25° C (77° F) and 70 per cent R. H. and that *O. surinamensis* was more resistant than *O. mercator*. He also mentioned that *T. castaneum* was more susceptible than *T. confusum*. These results are in agreement with those obtained in this study. Parkin (1960) working with DDT applied topically to *T. castaneum* and *T. confusum* at 25° C (77° F), reported that both species showed approximately the same level of susceptibility to DDT. These results are not in agreement with the results obtained in this study which showed that _T. confusum_ was more resistant than _T. castaneum_. Parkin (1953) reported that DDT dust and DDT water dispersible powder (Parkin, 1966) was more effective against _O. surinamensis_ and _O. mercator_ than _T. castaneum_ and _T. confusum_. These results are not in agreement with the results obtained in this study. This disagreement may be explained partly by the fact that in the tests by Parkin insects were exposed for longer periods of time (2 to 3 days) to the DDT residues and _O. surinamensis_, being more active than the other two species, picked up more insecticide, and partly by the fact that Parkin (1966) used a lower temperature. On the other hand, a different method of assessing mortality was used. Parkin (1966) placed living insects on their backs and if they were unable to regain their normal position they were recorded as dead. In this study, insects that showed any sign of life were recorded as alive. The difference in results may be attributed to the fact that _O. surinamensis_ and _O. mercator_ showed more prolonged post treatment toxic symptoms than _T. castaneum_ and _T. confusum_. However, in this study, at 60° F, _T. castaneum_ and _T. confusum_ were found to be more resistant than _O. surinamensis_ and _O. mercator_.

Lindane

The variable results obtained for lindane in this study, at the three experimental temperatures, are in agreement with the results obtained by Hoffman and Lindquist (1949) who also found that lindane showed different action at different temperatures when used against different insect species, sometimes showing a positive and sometimes a negative temperature coefficient. In general, lindane was more effective than DDT against most of the insect species at 80° and 50° F, and less effective at 60° F. Parkin (1960) reported that *C. ferrugineus* was far less resistant than *O. surinamensis* and *T. castaneum*, to lindane, at 25° C. These results are in agreement with those obtained in this study. Parkin (1966) reported that *T. castaneum* was more resistant than *O. surinamensis*. This finding does not agree with the results obtained in this study at 80° F. However, at 60° F, *T. castaneum* was found to be more resistant to lindane than *O. surinamensis*.

Malathion

Malathion showed a positive temperature coefficient, being more effective at the higher than at the lower temperatures for all test insects. It showed extreme toxicity to all insect species, and the reduction of its effectiveness at lower temperatures was slight. These results are in agreement with the results obtained by Lallan *et al.* (1956)

who also reported that malathion showed a positive temperature coefficient. Similar results were obtained by Parkin (1958, 1960, 1966); Strong and Sbur (1960) and Kalkat et al. (1961). Parkin (1966) reported that O. surinamensis was more susceptible than T. castaneum to residues of malathion on filter papers at 25° C. This agrees with the results obtained in this study. Lemon (1966) obtained higher mortalities with T. castaneum than with T. confusum in tests with malathion applied topically at 25° C and 70 per cent R. H. The difference in susceptibility of these two species was explained by the fact that the insects differ in size, T. confusum being 26 per cent heavier than T. castaneum. Similar results were obtained in the present work.

Bromophos

Bromophos showed a positive temperature coefficient at the three experimental temperatures for all test insects. In general, bromophos at 80° F was slightly less effective than malathion against all experimental insects. This finding is in agreement with Lemon (1966) who also reported similar results with this insecticide. At 60° and 50° F bromophos was far less effective against all insect species in this study. Lemon (1966, 1967) reported that T. confusum was more resistant than T. castaneum to bromophos. Similar levels of species susceptibility were obtained in the present work.

Species Susceptibility to Different Insecticides

In general, each test insect showed different levels of susceptibility to the different insecticides at the same temperature. Shi *et al.* (1961), using various insecticides sprayed directly on *T. castaneum*, reported that malathion was more effective than DDT. These results agree with the results obtained in this work. Vincent and Lindgren (1957) evaluated the effectiveness of different insecticides, applied topically, against *T. confusum* and reported that lindane was most effective, followed by DDT and malathion. These findings are not in agreement with the results obtained in this study, presumably because of different methods of applying the insecticide to the test insects. Strong and Sbur (1961), after exposing *T. confusum* for 28 days to wheat treated with various insecticides, reported that lindane was the most effective insecticide followed by malathion and DDT. These results agree partly with the results obtained in this study because although DDT was the least effective insecticide, malathion was more effective than lindane. The greater toxicity of lindane may be attributed, in part, to the fumigant action as well as to the contact exposure which occurred during the 28 days of the experiment. Because different methods were employed, the results are not comparable. Lemon (1966) reported that *T. confusum* was more susceptible to malathion than to bromophos, both applied topically. These results agree with those obtained in this study. Parkin (1960) reported that *O. surinamensis*, which was highly resistant to lindane, was extremely susceptible to malathion and that *T. castaneum* was also much more susceptible to malathion than to lindane. These results are in full agreement with the results obtained here. The greater effectiveness of lindane, compared with malathion to *C. ferrugineus*, may be attributed to the smaller size of this insect in relation to the other species, resulting in a higher surface area to volume ratio. This may result in *C. ferrugineus*, absorbing more lindane, through the fumigant action of the chemical, than large species with smaller surface area to volume ratios. According to Gratwick (1957) small insects tend to pick up more insecticide, in the form of dust, from treated surfaces than do larger species.

The general conclusion from the literature is that most of these results confirm the broad and general picture presented by the LD50 values in Table II, page 40, in spite of different testing techniques, different insect strains and different methods of assessing mortality and expressing results.

CHAPTER VI

CONCLUSIONS

In conclusion, temperature proved to be an important factor affecting the toxicity of some of the insecticides commonly used to control several important stored-product insects. The different insecticides showed different toxicities to each species at the three experimental temperatures. On the other hand, some insecticides were found to be more toxic than others against some stored-product insects under certain temperature conditions.

Under the conditions of these tests, the following conclusions were reached:

1. In general, at the three experimental temperatures, DDT showed a negative temperature coefficient, lindane a variable action and the organophosphorus insecticides, malathion and bromophos, a positive temperature coefficient.
2. Malathion was the most effective insecticide against most of the insect species, except *C. ferrugineus*, at the three temperatures of the experiment. At 80° F, the order of insecticide effectiveness against all insect species, except *C. ferrugineus*, was malathion, bromophos, lindane and DDT. At 60° F, malathion, except for *C. ferrugineus*, was the most effective

followed by either DDT or bromophos. At 50° F, malathion, except for *C. ferrugineus*, was the most effective followed by either lindane or DDT. Bromophos was the least effective at 50° F in all test insects.

3. Bromophos was very effective at 80° F (slightly less effective than malathion) against all the species tested, but was much less toxic at the lower temperatures.
4. Lindane was especially effective against *C. ferrugineus* at all temperatures and was also quite effective against the other species. Temperature had less effect on the toxicity of lindane than on the other chemicals.
5. DDT was the least effective insecticide at the high temperature, but quite effective at the lower temperatures.
6. The most resistant insect species at all temperatures and with all insecticides, except DDT and lindane at 80° F, was *T. confusum*; the most susceptible was *C. ferrugineus*, except in the case of malathion at 80° and 60° F.

From the results of these tests some practical conclusions, which may be expected to hold true in field experiments may also be drawn. Thus, under a wide range of storage temperatures, malathion, which is an insecticide of low acute and chronic mammalian toxicity, may be recommended when long residual life is not required and when many insect species of stored-product insects are present. When storage temperatures are above 80° F and when long residual life is required, bromophos which is also very effective at high temperatures, may be recommended. When storage temperatures vary considerably and when C. ferrugineus alone is present, lindane should be recommended in preference to malathion. At low storage temperatures, lindane and DDT are also expected to be effective against many species of stored-product insects.

In the Tropics, or in countries with prevailing high storage temperatures throughout most of the year, malathion and bromophos should be recommended in preference to the organochlorine insecticides.

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