

Apparatus and techniques have been developed to assess the effect of the pyrethrins and mixtures of the pyrethrins and piperonyl butoxide on a number of species of insects. The synergistic action of the piperonyl butoxide has been studied on these species and some comparisons made of the differences between species.

A method of microbioassay using brine shrimps (Artemia salina) has been elaborated and used to study the metabolism of the pyrethrins after application to different species of insects.

A discussion is given on ^{how} piperonyl butoxide synergises the pyrethrins based on the data obtained and on the literature.

The work appears to have been carefully executed whilst the discussion shows evidence of critical appreciation of previous work and of the bearing on it of his own results.

THE ACTION OF SYNERGISED PYRETHRINS ON A NUMBER OF INSECT SPECIES.

by

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ABSTRACT

The toxic action of mixtures of pyrethrins and the synergist piperonyl butoxide on a number of insect species has been examined. The degree to which the addition of the synergist enhanced the toxicity of the pyrethrins has been recorded as the factor of synergism. Synergistic activity of piperonyl butoxide varied between species and could not be demonstrated to occur at all against the moths Plodia interpunctella and Ephestia cautella.

To carry out these experiments, a number of techniques have been adapted and devised, including an electrified barrier to prevent crawling insects leaving a treated surface during exposure to insecticides, and an apparatus for delivering measured volumes of insecticide solutions for topical application to insects. Some sub-lethal responses of test insects to pyrethrins have been briefly described.

The use of the brine shrimp Artemia salina for the biological assay of pyrethrins residues in insects has been investigated. The nauplius larva of the shrimp was considered a more suitable test organism than the adult. Extracts from insects poisoned with pyrethrins exhibited the same order of toxicity to A. salina whether or not piperonyl butoxide was present in the pyrethrins' formulation, although solutions of piperonyl butoxide are quite toxic to the shrimp. When treated insects were left for a period before extraction, loss of toxicity of the extract compared with that of an

extract made immediately after treatment was not demonstrated.

The blood of Schistocerca gregaria nymphs injected with pyrethrins was toxic to nauplii if taken immediately after injection but not if taken 80 minutes after injection. The presence of piperonyl butoxide did not affect these results which are not entirely compatible with the theory that pyrethrins is detoxified by the insect and that synergists prevent this detoxification. The validity of this theory is considered in the discussion.

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N.B.

ON ALL GRAPHS, UNLESS OTHERWISE
STATED:

O - TREATMENT WITH PYRETHRINS

X - TREATMENT WITH PYRETHRINS PLUS
PIPERONYL BUTOXIDE.

INTRODUCTION

Great advances have been made since the last war in the production and use of insecticides, particularly many new synthetic compounds of high efficacy. In spite of these advances, the older insecticides of vegetable origin, especially pyrethrum, still have a place in insect control programmes.

The rapid knockdown action of pyrethrum has led to its use in domestic fly sprays, often in combination with synthetic insecticides. The low mammalian toxicity of pyrethrum recommends it for use against pests of food and those endangering public health.

It is this low mammalian toxicity and to a lesser extent the rapid knockdown action which accounts for the continued use of pyrethrins, although it is more expensive than many other insecticides and residual films are more quickly inactivated by breakdown of the unstable molecule particularly when exposed to light.

The high cost of pyrethrum has stimulated the search for synergists, substances which, although not themselves insecticidal so enhance the toxicity of pyrethrum insecticides that a proportion of the pyrethrum in a formulation may be replaced by the cheaper synergist.

Research into the biological assay of the pyrethrins and of synergised pyrethrins has generally been carried out on a fairly small selection of insects. These include some of the beetles associated with stored products, and a few mosquitoes and other flies, notably Aedes aegypti and Musca domestica. When other species of insect have been used the objective has been to elucidate specific problems regarding their control.

The reason for this lack of variety in the choice of test insects is to some extent historical. As has already been pointed out, the properties of the pyrethrins, in particular their relatively high cost and instability when exposed to strong light, largely confined their use to the spheres of public health and stored products. The insects mentioned can be easily maintained in culture whilst the use of a large number of species involves much extra work and space in insectaries, especially as some of the cultures may be more difficult to maintain than those of the test insects commonly reared.

Lloyd and Hewlett (1958) classified the susceptibility of about thirty Coleoptera and Lepidoptera species associated with stored products, to formulations containing 0.3% pyrethrins plus 3.0% piperonyl butoxide, which they applied by exposing insects to films and to a spray. They also compared the toxicity of the mixture of pyrethrins plus piperonyl butoxide mixture with the toxicities of a formulation containing 1.3% pyrethrins. To some three-quarters of the insect species tested the two formulations

were equally toxic, thus showing that piperonyl butoxide by acting as a synergist enhanced the toxicity of the weaker 0.3% solution of pyrethrins and gave it a toxicity equivalent to that of the stronger solution containing 1.3% pyrethrins. The mixture was conspicuously more toxic than the plain pyrethrum formulation to Calandra oryzae and Mezium affine when applied as a film and to C. oryzae when applied as a spray, indicating that the synergistic action of the piperonyl butoxide was greater against these two insects than against the majority of those tested. On the other hand, to Tenebrio obscurus and T. molitor, Gibbium psyllodes, Tribolium castaneum, Ephestia cautella and Anagasta kuhniella, the pyrethrins were more toxic in the 1.3% solution than in the 0.3% solution mixed with piperonyl butoxide, in one or both methods of application. This result indicates that piperonyl butoxide is less effective in enhancing the toxicity of pyrethrins against these insects than against the majority of those tested.

McGouran and Fales (1944) showed that the synergistic action of sesame oil and of n isobutylundecyleneamide with pyrethrins was less marked against Aedes aegypti than against Musca domestica.

Although few direct comparisons have been made of the synergistic effect of any one synergist in enhancing the toxicity of pyrethrum to different insect species, the results quoted above show that the degree of enhancement does vary with the species. In view of the importance and widespread use of pyrethrum synergists, it is surprising that their action has not been investigated more

thoroughly. In this work the degree to which piperonyl butoxide enhances the toxicity of pyrethrins to a number of insect species has been investigated.

Variation in susceptibility of insects to insecticides may occur within a species as well as between species. Bruce (1957) compared the actions of a number of pyrethrins synergists on nine strains of housefly. His findings show that, while there is considerable variation in the susceptibilities of the strains to pyrethrins, the enhancement of toxicity brought about by the synergists was similar with each strain. It is likely, therefore, that the differences in synergistic activity of piperonyl butoxide between insect species recorded here, are greater than the differences between strains of the same species.

In an attempt to elucidate the mode of action of piperonyl butoxide and pyrethrins, residues extracted from insects poisoned with pyrethrins have been assayed biologically, using the brine shrimp Artemia salina as the test organism.

Synergism.

Metcalf (1955) defines synergistic action as occurring when two chemicals are applied as a mixture to a population of insects and their toxicity produces a response greater than the sum of their independent effects. The term, activation, is used to describe the special case of synergism in which a substance, with no toxicity at the dosage employed, increases the effect of a

toxicant. Chemicals which function as activators have proved of great commercial importance in the use of pyrethrins and are commonly called synergists.

It has been found convenient in this work to refer to piperonyl butoxide as a synergist and to its joint action with pyrethrins as synergism, although this might equally well be called activation.

To obtain some quantitative idea of synergistic action the term "factor of synergism" is employed. This term is in wide use and has been defined by Goodwin-Bailey (1956) as follows:-

Let the concentration of pyrethrins, in a formulation containing only pyrethrins, that is required to produce a given response (often 50%) in an insect population be x .

Let the concentration of pyrethrins, in a formulation containing pyrethrins and synergist, that is required to produce the same response in insects from the same population be y .

Then factor of synergism = $\frac{x}{y}$.

The term "R D 50" is used to indicate the dose necessary to produce a 50% response.

Outline of Experimental Work.

It was decided that an examination of the action of synergised pyrethrins on a wider range of insect species than has at present been investigated would be of interest. To this end biological assays of pyrethrins, with and without the addition of the synergist

piperonyl butoxide, have been undertaken using eleven species of insect. The enhancement in the toxicity of the pyrethrins brought about by the addition of piperonyl butoxide has been evaluated.

The most widely held theory to explain the action of pyrethrins synergists is that they reduce in some way an insect's ability to detoxify the pyrethrins' molecules thus increasing the effect of the pyrethrins on the insects.

The experiments described in the first part of this work showed that there is considerable variation in the reaction of different insects to synergised pyrethrins. This variation may have been due to differences in the efficiency of the detoxification mechanism in different species.

To investigate the physiological action of synergised pyrethrins, it is desirable to be able to assay residues of pyrethrins in the tissues of poisoned insects. Winteringham (1955) has investigated pyrethroid residues in the housefly using radiotracer techniques. In the present work an attempt has been made to obtain further information by estimating the quantities of residual pyrethrins in insect tissue using biological assay. A biological assay technique using nauplii of the brine shrimp, Artemia salina, was devised for this purpose and is described in the second part of this work.

The possibilities of such a technique have been demonstrated. Rapid detoxification of pyrethrins could not be detected and these

results are discussed further below.

The description of experimental work has been divided into two parts.

1. The biological assay of pyrethrins together with the synergist piperonyl butoxide using a selection of test insects.

2. The biological assay of pyrethrins residues using the brine shrimp Artemia salina.

STATISTICAL.

Where a statistical analysis of the results is considered necessary, the methods described by Finney (1952a, 1952b) have been employed.

The responses of insects to a series of doses of insecticides have been expressed as regression lines drawn by plotting the logarithm of the dose on the x axis and the transformed percentage response on the y axis.

Probit and angular transformations of the response rates were used. For the majority of the experiments the angular transformation was used because the ensuing calculation of the regression parameters was thought to be simpler than with probits. The weighting coefficient associated with the angular transform is constant ($\frac{1}{820.7}$) but if the probit transformation is used, the weighting coefficient must be calculated with the help of tables for each dosage.

In interpreting the significance of differences of slope or position of regression lines, the doses required to give very high or low responses may be deduced only with low precision. The toxicities of various preparations are therefore quoted in terms of the concentration of pyrethrins required for a 50% response. Whilst the angular transformation appears to afford equal precision over the entire range of responses, this gain is bought at the expense of a mathematical model which is increasingly unreal at

the extremes of the range of response. It is at least possible to maintain that the probit transformation, which is based on the normal transformation, is a closer representation than the angular of the effective distribution of response amongst the insects.

In the biological assay of pyrethrins the insects treated at each dosage were generally divided into from two to five replicates.

KNOCKDOWN AND MORTALITY.

The use of mortality as a response to treatment with an insecticide in biological assay is often unreliable because of the difficulty involved in defining mortality. Where mortality has been used as a response in these experiments, an insect is considered to have responded if it did not respond in any way to stimulation and was completely motionless. This definition excludes insects badly affected by the insecticide but which may still be twitching or making other occasional movements.

Carefully defined sub-lethal responses, making use of the paralysing effects of pyrethrins, have been extensively used. These sub-lethal responses are described as "knockdown" and they are further defined in the accounts of individual experiments which follow.

RESPONSES IN THE CONTROLS.

"Controls" must be included in all biological assay experiments to ensure that responses of the treated insects are due to the

insecticide and not to handling, carrier oil or other causes. Control treatments should be identical with those of other insects in the assay except for the absence of the toxicant.

When small control responses occur, they do not invalidate an experiment, though they do lower the sensitivity a little. They may be ignored on the assumption that a proportion of those responding in the control treatment would have responded to the toxicant. Alternatively, the number responding in the control treatment may be subtracted from the responses obtained for each insecticidal treatment on the grounds that they are not part of the assay.

The latter alternative is thought to be preferable. When all responses are expressed as percentages, the corrected response to a treatment (p_c) is calculated as

$$p_c = \frac{p - c}{100 - c} \times 100$$

where

p = recorded response

c = control response.

This correction has been tabulated by Healy (1952) and is known as Abbot's correction.

THE INSECTS.

During the course of this work the following species of insect have been used.

Orthoptera,

Acrididae

Schistocerca gregaria Forsk.

Hemiptera,

Aphididae,

Acyrtosiphon pisum (Harris).

Pyrrhocoridae,

Dysdercus fasciatus Signoret.

Lepidoptera,

Phycitidae,

Plodia interpunctella (Huebner).

Ephestia cautella (Walker)

Galleriidae,

Galleria mellonella (L.).

Achroia grisella (F.).

Pieridae,

Pieris brassicae (L.).

Coleoptera,

Curculionidae,

Calandra (Sitophilus) granaria (L.).

Nitidulidae,

Carpophilus dimidiatus (F.).

Crysomelidae,

Phaedon cochleariae (F.).

Hymenoptera,

Formicidae,

Lasius (Acanthomyops) fuliginosus (Latreille).

In the biological assay of insecticide residues, the brine shrimp Artemia salina (L). (Crustacea, Anostraca) was used, and a few experiments were carried out using larvae of Aedes aegypti (L) (Diptera, Culicidae).

The culture of the above-mentioned insects will now be briefly described. The methods used are those commonly adopted at Imperial College Field Station.

X. Acyrtosiphon pisum.

Bean plants were grown in sand in the greenhouse at an average temperature of 68°F (range 55-80°F.). Twenty-five days after sowing the plants had generally reached a height of 8-12 inches with four pairs of fully developed leaflets. Gravid female aphids were placed on the plants for 24 hours only. During this time they deposited nymphal aphids (about 2-3 per adult) which developed to adults in about 10 days under the conditions obtaining in the greenhouse. By leaving the adults on the plants for only 24 hours it was possible to record the age of the next generation of insects. The number of instars in the life history of this insect is reported to vary from 5 to 11 and was generally seven in these cultures. The aphids were used in biological assay when they were 7-8 days old and were probably in their second last instar.

When the first experiments were carried out with A. pisum, this system of breeding was not in use. The age of the insects was not precisely known but they were probably all in the two instars preceding the adult stage.

Dysdercus fasciatus.

D. fasciatus were kept at a temperature of 25°C in 7 lb. jars on a substrate of dampened bulb fibre. They were fed on cotton seeds which had been previously soaked in water for 24 hours and

which were presented to the insects in small open petri dishes. The supply of seed was renewed three times per week.

Adult D. fasciatus were allowed to lay eggs in the bulb fibre. The eggs were often placed near the side of the culture jar and could therefore be observed. During development a large proportion of the nymphs died.

Plodia interpunctella.

P. interpunctella, the "Indian meal-moth", was cultured in 7 lb. jars containing about 120-150 g. of heat-sterilized middlings and about 5 cc. "Bemax stabilized wheat germ" which was moistened with glycerol. 25-40 young adult moths were put in each jar which was kept at a temperature of 25°C and a relative humidity of 65% $\pm$ 10%. Under these conditions the life cycle was completed in approximately 4 weeks.

Ephestia cautella.

E. cautella, the "dried currant moth" was kept under similar conditions to P. interpunctella. Its life cycle occupied about five weeks and it proved to be rather more difficult than Plodia to breed in large numbers.

Galleria melonella

Achroia grisella

G. melonella, the "greater wax moth" or "bee-moth" and A. grisella the "lesser wax moth" were both cultured on old honey-comb which had been sterilized by fumigation with methyl bromide or on a

mixture of old honeycomb and an artificial diet medium containing cornmeal, whole wheat flour, milk powder, middlings, dried yeast, honey and glycerine.

The insects were kept in 7 lb. jars at a temperature of 25°C. The length of the life-cycle varied.

When disturbed both species of adult moth became very active and difficult to catch, they were trapped in 2 in. x 1 in. diameter specimen tubes, three or four at a time. The newly hatched larvae were also very active and small enough to get through all but the finest muslin gauze. The fully grown larvae are voracious eaters and were quite capable of chewing through muslin, silk, nylon cloth, polythene gauze and cork; they could only be confined completely with rubber bungs in which holes were bored to allow ventilation and covered with metal gauze.

Pieris brassicae.

P. brassicae, the "cabbage white butterfly" was reared by the method described by Champ (1958). All stages of the insect were bred in a room with a diurnal temperature variation of about 20°C to 30°C. P. brassicae adults were kept in a large cage illuminated by natural and artificial light to give a day length of 16 hours and so prevent the onset of diapause. The food provided for the adults was a 5% solution of honey in water, which was presented in artificial flowers. Two ten-inch potted cabbage plants were provided daily on which the females oviposited. After hatching the larvae were reared on field-grown cabbage leaves in 2 ft. cube muslin cages. The fully fed

larvae migrated and pupated on the walls of the cages.

By keeping the relative humidity of the breeding room at about 40% and maintaining good air circulation, it was possible to reduce fermentation of the food material and disease outbreaks to a minimum.

Calandra (Sitophilus) granaria.

C. granaria, the "granary weevil", was reared in 2 lb. jam jars half-filled with heat-sterilized wheat and kept at a temperature of 25°C and relative humidity of $65\% \pm 10\%$.

Under these conditions the life cycle was completed in about 5-6 weeks, and large numbers of insects could be collected from a culture jar in which 1200 to 1500 C. granaria adults had been allowed to lay for 2 days.

Carpophilus dimidiatus.

C. dimidiatus was reared in 2 lb. jar containing about 50-75 g. of rolled oats and a little "Bemax stabilized wheat germ". They were kept at a temperature of 25°C and relative humidity of $65\% \pm 10\%$.

Forty adult beetles were put in each culture and the life cycle was completed in about six weeks. Provided they can be kept free from mites, these beetles are very easy to rear.

Phaedon cochleareae.

P. cochleareae, the "mustard beetle", was reared in cultures on young turnip plants transplanted to 12 litre cylindrical breeding cages consisting of a metal base to contain soil and plants, perspex

sides and a perforated metal lid. About 50 adult P. cochleareae were allowed to lay eggs on the turnip plants for 48 hours and were then removed. The cages were kept illuminated in a constant temperature room at 25°C or in a heated greenhouse. The feeding larvae crawl fairly actively over the plants which were renewed as required. At 25°C adult P. cochleareae emerged after about 28 days.

In cultures reared by Mr. M. Tanton of Imperial College Field Station, up to 800 adults were obtained under these conditions; however, these numbers were not obtained by the author and time did not permit an examination of the reasons for this difficulty.

Lasius (Acanthomyops) fuliginosus.

L. fuliginosus was not cultured but the workers were collected as required from a nest in a tree near the laboratory. The method of collection is described below.

Schistocerca gregaria was kept in culture at the insectaries of Imperial College Field Station and collected as required. No culturing of this insect was undertaken by the author.

The culture of Artemia salina is described in Part II of this thesis.

PYRETHRINS AND SYNERGISTS.

Extracts of Pyrethrum flowers contain a number of insecticidal esters: For the sake of brevity the insecticidal constituents of the extract of pyrethrum flowers are referred to in this thesis as pyrethrins.

Pyrethrins used in this work were obtained from Messrs. Stafford Allen and Sons Ltd. as an extract containing approximately 20% weight/weight pyrethrins. All the experiments described here are comparative and there was no reason to determine the exact concentration of this approximately 20% solution. For convenience all dilutions of pyrethrins used have been worked out assuming a concentration of 20% in the original extract. Also, for convenience, all dilutions of pyrethrins and of synergists have been made on a volume/volume basis.

A 10% stock solution of pyrethrins was prepared with equal quantities of the 20% pyrethrins concentrate and of absolute alcohol. To precipitate and remove oleoresins (which tend to make apparatus difficult to clean after use) this mixture was left standing in a refrigerator for 24 hours and then filtered through cotton-wool.

The 10% stock solution was kept in a refrigerator and further diluted when required with the appropriate solvent as described in the experimental section below.

Because of the breakdown and loss of toxicity of pyrethrins caused by light and warmth during storage, fresh solutions were

made up from the 10% stock solution before each experiment.

The synergist piperonyl butoxide (3, 4-methylenedioxy-6-propylbenzyl butyldiethylene glycol ether) was obtained from John Powell Inc. of New York. It was soluble in all the solvents used and was diluted as required.

The synergist referred to as van Dyke 264 (N-Octyl bicycloheptene dicarboximide) ceased to be known generally by this trade name some years ago but it has been retained here for ease of reference.

In one experiment the synergist sesoxane was also used.

TEMPERATURE.

As far as was practicable all the biological assays and insect rearing were carried out at 25°C. It was of course not possible to breed insects such as P. brassicae, A. pisum or P. cochleareae or to collect L. fuliginosus under these conditions. In some cases insects were brought into the laboratory for topical application but they were at once returned to the constant temperature room.

SECTION 1.

THE BIOLOGICAL ASSAY OF PYRETHRINS TOGETHER WITH THE SYNERGIST PIPERONYL BUTOXIDE USING A SELECTION OF TEST INSECTS.

TECHNIQUES.

The methods of obtaining and rearing the insects used in this work have already been described.

Methods used in biological assay must be modified to suit the size, behaviour and stage in the life history of the particular insect under investigation. In these experiments it has been found necessary to develop a number of different techniques. They fall into three categories:

- A. Contact over a period of time with a surface impregnated with the insecticide.
- B. Topical application of the insecticide.
- C. Spraying of the insects with the insecticide.

It is thought to be more convenient to describe categories A and C in detail in the course of the descriptions of the treatment of each species of insect which follow. Therefore only a general consideration is included here.

The primary consideration in designing a biological assay is to ensure, as near as possible, that all the insects under test do in fact receive the same dose of insecticide. For this reason, biological assays in which measured quantities of solution are placed directly on the insect or injected into it are in many ways ideal; they are, however, subject to the criticism that these methods of application are far removed from everyday insect control with

insecticides. A crawling-insect type of assay involving contact over a period of time with a poisoned surface is therefore desirable for confirmation of results obtained by the other methods. Also, there are many cases, for example if the insect is small, when topical application or injection is not practicable.

A. Contact over a period with a surface impregnated with the insecticide.

In an assay in which the test insects crawl over surfaces impregnated with the insecticide, it is generally advisable that the insect remains in continuous contact with the treated surface during the period of exposure. Various means of achieving this have been tried and in the detailed description of experiments which follows the more successful of these means are given.

However, it has been shown in these experiments that insects which move about continuously during the period of exposure (e.g. adult moths), and do not settle in one place for long periods, may be successfully assayed if confined in a small space over an insecticide-treated surface. The results obtained seem to indicate that all the insects spend a similar time on the surface and, therefore, have a reasonable chance of picking up a similar dose of insecticide.

Contact assays used in this work are all based on the method of crawling insect assay of Blackith (1950) and have been modified to a greater or lesser extent to suit the requirements of each insect. It is convenient to describe these methods in the course of detailed accounts of the experimental work rather than here. The basic method used by Blackith is described in part 1 dealing with Calandra granaria,

and the various modifications are mentioned in the appropriate context, including the electrified barrier which is described in part 3.

B. Topical application of insecticide.

The two methods of topical application used, required little or no modification to dose different insects; they are therefore described here.

In earlier experiments the topical application method of Kerr (1954) was used.

An 0.2 mm. interval diameter "Vesidia" capillary glass tube was drawn out to a point at one end and mounted in a special device on the mechanical stage of a microscope. The insecticide solution was drawn into the capillary and it was adjusted so that the meniscus was in the field of view. Using a 32 mm. objective and a X 6 eyepiece and strong illumination, the image of the capillary and meniscus was projected through a Sartory "camera lucida," mounted on the microscope with the lens reversed, on to a scale placed beside the microscope. When an insect was touched by the drawn out tip of the capillary tube, the solution flowed on to the insect and the quantity of liquid applied to it could be measured by watching the image of the meniscus moving on the scale which had previously been calibrated.

In order that the solution should flow when the insect touched the capillary and cease when it was taken away, careful adjustment of the internal diameter of the tip of the capillary and/or of the viscosity of the solution was necessary. In these

experiments the pyrethrins were dissolved in odourless kerosene and generally 0.05 μ l of solution was applied to each insect.

This technique necessitated working in reduced light and was not particularly quick. In later topical application experiments, the use of the Kerr apparatus was abandoned in favour of an "Aglia" micrometer syringe with a device attached so that the required dose could be delivered without the operator having to look at the scale, thus leaving him free to concentrate on the insect held to the tip of the needle. This device is shown in diagram in figure 1. The three rings which could be attached to the barrel of the syringe enabled doses of ^{0.4}~~0.04~~ μ l, 1.0 μ l and 2.0 μ l to be administered, or multiples of these figures. The pyrethrins solution forms a drop on the tip of the needle when the barrel was turned and the insect could be gently brought to this drop, which then flowed over the insect. In these experiments the pyrethrins were dissolved in acetone. This solvent is not ideal as it evaporates too easily. In any further work it is suggested that a less volatile solvent could be used with advantage but it did not seem necessary to change the solvent in the midst of a series of experiments.

C. The spraying of insects with the insecticide.

In the biological assays using aphids the pyrethrins were sprayed on to the test insects using a "Spray Tower" as described by Potter (1952). As the spray tower was only used with the aphids, its use is more conveniently described in the section dealing with the assays employing these insects.

Compared with the accuracy of topical application, the actual dose received by an insect when sprayed was uncertain. The spray tower was therefore only used when other methods proved impracticable.

1. Calandra granaria.

The crawling insect assays described in these experiments are all based on the method used by Blackith (1950) and by Goodfidge (1954) in the biological assay of Calandra granaria adults and of other insects. To gain familiarity with the procedure, a series of experiments were carried out using C. granaria. This procedure, in modified forms, has been used in many of these experiments and will now be described.

The insecticide-treated surfaces presented to the test insects in these assays were No. 1 Whatman filter papers, 7 cm. in diameter and impregnated with a solution of pyrethrins or of pyrethrins plus synergist in Risella oil. A stock solution of pyrethrins was prepared as described above. The insecticide was diluted to the required concentration with a mixture of solvents consisting of 1 part "Shell Risella 17" oil and 4 parts petroleum (60-80°F boiling range) and referred to hereafter as the "mixed dilu~~z~~ent".

0.5 ml. of pyrethrins solution of the required dilution was spread over the filter paper (which was placed on a glass plate) from a graduated pipette by drawing the tip of the pipette over the paper in a spiral so as to obtain even distribution. The mixed dilu~~z~~ent has a very low viscosity and, consequently spreads quickly and evenly over and through the filter paper. Within 2-4 minutes of application, the bulk of the petroleum ether evaporated leaving the filter paper impregnated with a solution of the insecticide in

Risella oil. The papers were left for a further period of at least 30-40 minutes to allow the last traces of the petroleum ether to evaporate.

During the period of exposure of the test insects, the filter papers were placed on glass plates; either a number of papers on one large plate or individually on plates 7 cm. square, as was most convenient. The insects were confined on the papers by brass rings 2.5 cm. deep and 5.4 cm. internal diameter, having the internal surface nickel-plated and polished. Clean filter papers or, preferably, glass plates 7 cm. square were placed on top of the rings.

To confine C. granaria adults to the filter papers and to prevent their climbing up the sides of the rings, the inner surface of the rings was smeared with a very thin film of liquid paraffin. If "Shell Risella" oil was used for this purpose instead of liquid paraffin, many of the Calandra adults were killed. Risella oil appears to be slightly less viscous than liquid paraffin and this difference may account for the toxic action of the lighter oil which is presumably physical rather than physiological and could be due to blockage of the tracheal system.

The use of filter papers is a very convenient method of carrying out biological assays. There is no reason why many other surfaces should not be used provided that they have some sorbtive properties; particularly those more nearly related to the practical aspects of pest control. The author has carried out a few experiments, reported later, using squares of obeche wood 7 cm.

square and 0.5 cm. thick, similar to wood used sometimes in packaging stored food. In using other surfaces, it may be necessary to adjust the composition of the mixed solvent used to dilute the insecticide. In particular, the use of less porous or less sorptive surfaces, such as ground glass, may necessitate reduction in the proportion of Risella oil in the mixture.

After preliminary experiments in which a small number of C. granaria adults were exposed to a wide range of concentrations of pyrethrins to determine the range of concentrations required to give response between 0% and 100%, an assay was carried out in which the insects were exposed on filter paper to a series of concentrations of pyrethrins. The results of this experiment are shown in figure 1 and table 1. The statistical treatment of these results has already been discussed.

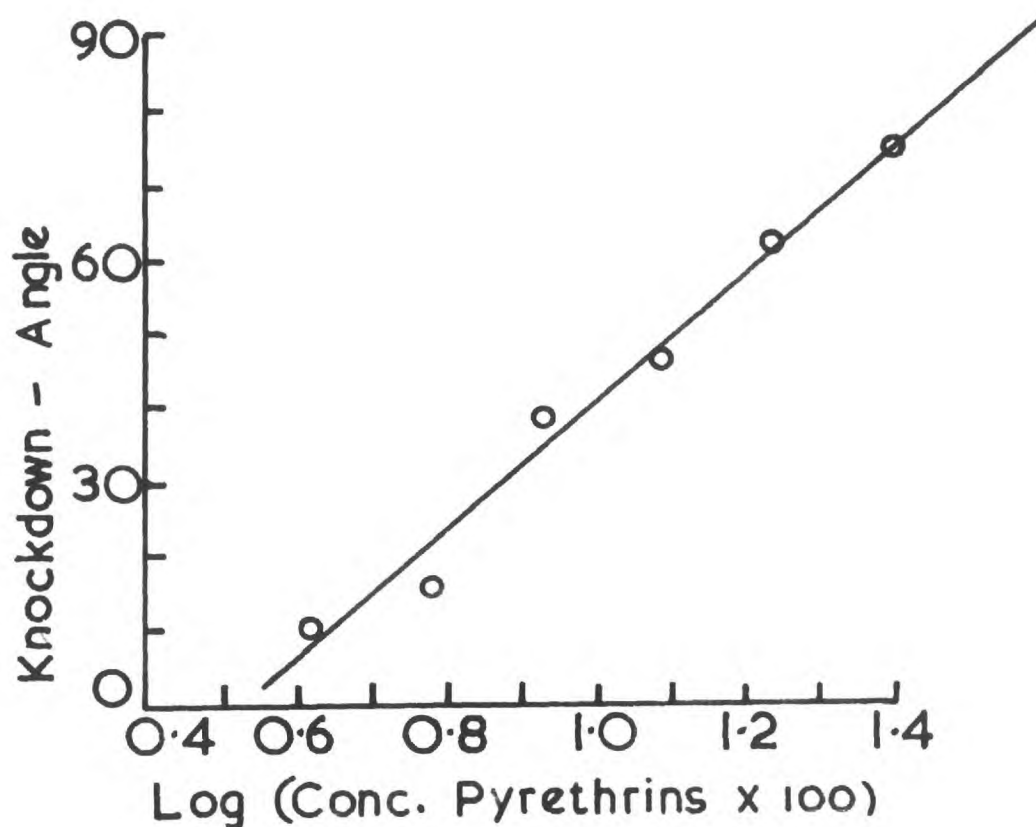
The response of C. granaria adults in these experiments has been estimated as "knockdown". At the end of the exposure period of 24 hours, the beetles were placed in the centre of a 7 cm. filter paper on which was drawn a circle 5.4 cm. diameter (the internal diameter of the brass rings). The paper was illuminated obliquely by a 60 watt electric lamp at a distance of 9 ins., and any insect which failed to crawl away over the pencil line within one minute was counted as knocked down.

In table 2 and graph 2 the result of two further experiments are recorded in which C. granaria adults were exposed to series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide.

From the responses of the insects in these experiments pairs of regression lines have been drawn which clearly show the enhancement of the toxicity of pyrethrins to C. granaria, which is brought about by the addition of the synergist piperonyl butoxide to the solutions. The ~~fraction~~ of synergism at the median response dose has been calculated to be 6.5 in the experiments recorded in table 2.

Graph 1: Exposure of *C. granaria* adults on filter papers impregnated with pyrethrins.
See table 1.

1.



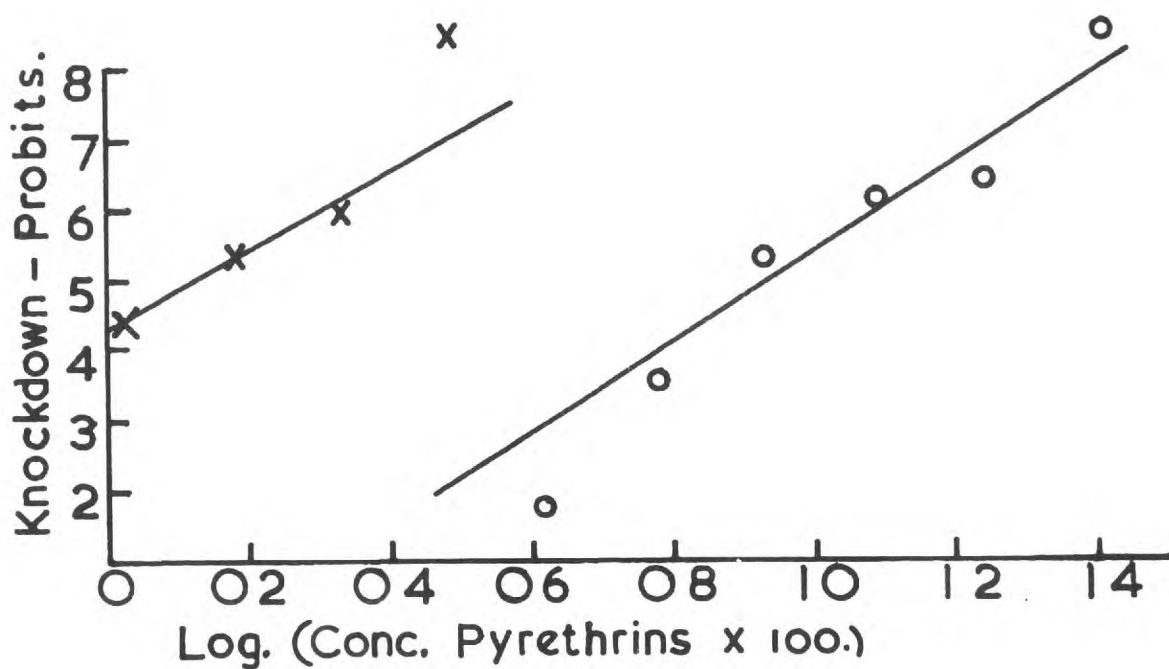
N.B. On all graphs, unless otherwise stated:

O - Treatment with pyrethrins

X - Treatment with pyrethrins plus piperonyl
butoxide.

Graph 2: Exposure of C.granaria adults on filter papers impregnated with pyrethrins, with and without piperonyl butoxide for 24 hours. See table 2.

2.



N.B. On all graphs, unless otherwise stated:

O - Treatment with pyrethrins.

X - Treatment with pyrethrins plus piperonyl
butoxide.

2. Plodia interpunctella.

The fourth instar larvae and newly-emerged adults of Plodia interpunctella (the Indian meal moth) were used in experiments on the biological assay of pyrethrins applied either by contact with a prepared film of the insecticide or by topical application.

Adult moths - Contact with prepared surfaces.

An attempt was made to assay the adult moths using a modification of the technique described above for the assay of Calandra granaria. The surfaces on which the insects were exposed were again 1 inch diameter No. 1 Whatman filter papers, impregnated with pyrethrins by the method already described. The insects were confined on these surfaces by means of the brass rings with the open ends covered with 7 cm. square clear glass plates. Adult moths, not more than four days after emergence were collected from the cultures into 3" x 1" specimen tubes, using an aspirator. When the specimen tubes were gently tapped with the finger the moths fell to the bottom so that the cork could be removed and the moths quickly tipped into the rings and confined there by the small glass plates. About ten insects were placed in each ring but it proved impracticable to have exactly this number on each surface.

The insects were not confined to the poisoned surface only but fluttered about actively within the ring until overcome by the pyrethrins. As they were active it was assumed that, over the period of exposure, the total time each individual insect spent in contact

with the filter paper would be about the same. Because this method of assay may not be reliable, the experiments described here were verified using topical application.

In some of these experiments paralysis was used to determine whether the moths were affected by the insecticide mixtures. After exposure to pyrethrins films for a time, which varied from 10 to 30 minutes, the impregnated filter paper was replaced by a clean one. At a predetermined time after this, the moths in the covered brass rings were placed on the floor of a fly-breeding cage, 18 x 20 inches in area and 18 inches high. The rings and covers were removed from the filter papers, and any moths still on the filter papers after 2 mins. were considered knocked down. To provide some stimulation of the moths the case was placed on a table in the constant temperature room so that the air flow caused by the circulation machinery blew through the muslin walls of the cage and thus across the filter papers. To observe the moths additional lighting was necessary, and the cage was illuminated by a 60 watt bench lamp placed at one side. As this was likely to have some effect on the moths, the same arrangement was always used.

In some other of the experiments the response has been assessed as mortality.

As a test insect P. interpunctella proved to be less satisfactory than Calandra granaria. Results of biological assays in which the moth was used show greater variation and larger control mortalities than the results of experiments using C. granaria.

Figures 3 and 4 (graphs 3 and 4) show the results of two experiments in which adult moths were exposed to series of doses of pyrethrins. Variations in the techniques adopted in the experiments are noted on the tables. Reference to the figures shows that satisfactory regression lines could be obtained using either knockdown or mortality as a response.

In experiments 5, 6, 7 and 8 adult P. interpunctella were exposed on filter papers impregnated with solutions containing series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide in the ratio of 1 part by volume of pyrethrins to 5 parts piperonyl butoxide. From the results of these experiments, pairs of regression lines have been drawn in graphs 5, 6, 7 and 8. These indicate that the enhancement of the toxicity of the pyrethrins towards these moths by piperonyl butoxide is either non-existent or too small to be detected by these experiments.

The fact that adult moths were able to flutter about within the rings during these assays and to settle on surfaces other than the impregnated filter paper rendered the method susceptible to criticism. While it seems likely from observation that the moths were in contact with the filter paper for approximately the same time, an element of uncertainty remained. It was therefore essential to verify the important results obtained by the use of these techniques by employing another method of assay not open to this criticism.

The moths could either be forced to remain on the treated surface or the doses of insecticide could be applied by topical application. The latter method was selected ~~and~~ experiments on the topical application of pyrethrins to moths are described below.

It was found possible to force P. interpunctella adults to stay on the filter papers by confining them in the apparatus designed by Lewis and Hughes (1957) (Figure 2). The dimensions of this apparatus, which was designed for flies, had to be altered to suit the moths. Although this apparatus was found to be suitable for biological assay using P. interpunctella adults, it was not used further.

Adult moths - Topical application.

For obvious reasons a "crawling insect assay" carried out with flying insects as has been described, cannot be considered of any great value unless the results are verified by another technique. Experiments on the topical application of pyrethrins to adult P. interpunctella were therefore carried out. The insecticide was applied using the technique of Kerr and, in later experiments, the micrometer syringe. Kerr's apparatus has been briefly described above and the method of using the micrometer syringe in this work is also described in more detail. When Kerr's apparatus was used 0.05 μ l. of insecticide dissolved in odourless kerosene was applied to the abdomen of the moth. If the micrometer syringe was used 1.0 μ l. of pyrethrins in acetone was applied also to the abdomen.

Newly emerged adult moths were taken from the cultures into

a 2" x 1" or 3" x 1" specimen tube using an aspirator or "pooter". If the insects were used immediately, the aspirator was connected to a cylinder of carbon dioxide which was used to anaesthetise the moths. P. interpunctella adults were sometimes collected prior to dosing and left in the specimen tubes until required, never for more than 45 minutes. In this case the tubes were closed with muslin and held in position by a cork with the centre bored out so that carbon dioxide could be blown into the tube to anaesthetise the insects. The anaesthetised insects could easily be held between forefinger and thumb for dosing and this method was found to be much easier than attempts to hold the moths on the shaped ends of glass tubes using slight suction. After being dosed, the moths were left confined on filter papers by the brass rings and small glass plates at a temperature of 25°C and a relative humidity of 65% $\pm$ 10% until mortality was assessed.

In tables and graphs 9 and 10 the results refer to P. interpunctella adults which have been dosed with series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide using the micro-syringe. Again, when the response of the insects in terms of mortality after the times stated was recorded, it was not possible to demonstrate any enhancement of the toxicity of the pyrethrins in the presence of piperonyl butoxide under the conditions of these experiments.

These experiments did not take into account any differences

in the reactions of male and female moths to pyrethrins. In the experiments recorded in tables and graphs 11, 12 and 13, the mortalities of male and female P. interpunctella after topical application of pyrethrins and in the case of pyrethrins plus piperonyl butoxide have been recorded. Graph 11 and table 12 show a tendency for the males to produce a steeper regression line than the females, but this is not born out by graph 13. At least it can be concluded that there is no great sexual difference in the response of adult P. interpunctella to pyrethrins with or without piperonyl butoxide.

Effect of Pyrethrins on egg laying by P. interpunctella.

One sub-lethal response of P. interpunctella adult females to pyrethrins is a stimulation to egg laying.

In table 14 the number of eggs laid by the female moths used in the experiment recorded in table 13 in the 3 hours after treatment has been recorded. As only three concentrations of insecticide were used in this assay it is not possible to obtain any clear idea of the effect of a range of doses on the number of eggs laid.

In two further experiments P. interpunctella adults were exposed to a series of eight and one of five concentrations of pyrethrins on filter papers. The moths were exposed to the filter papers for 30 minutes and then left on clean filter papers, enclosed by brass rings and glass plates for a further 18 hours. At the end of this time the mortality of the moths was estimated. The number of

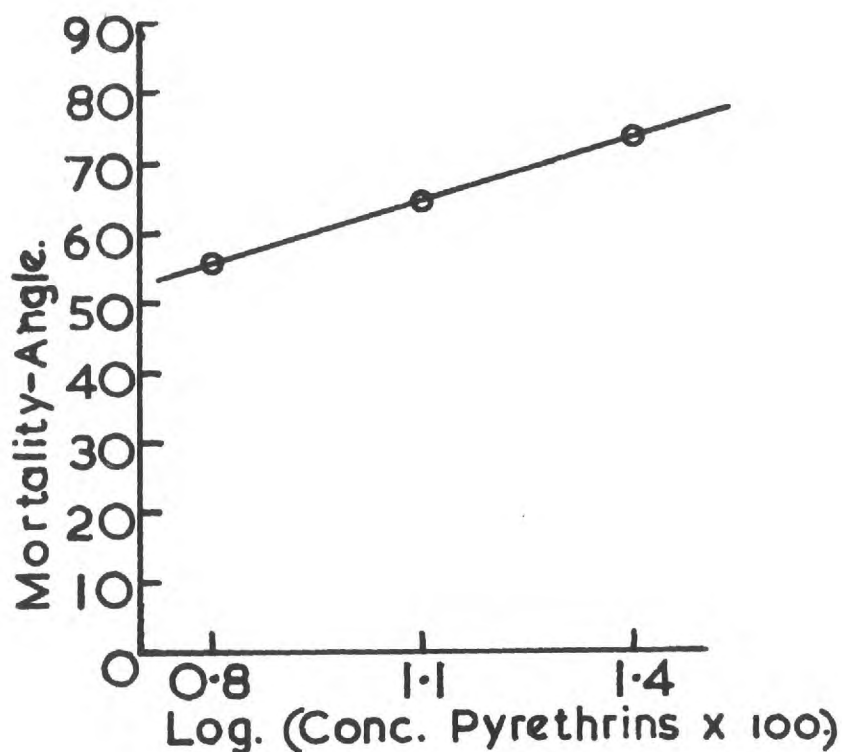
eggs laid during exposure to the pyrethrins films and during the 18 hours on the clean filter papers was counted. Unfortunately no record was taken of the proportions of the sexes, so that these results should be treated with some reserve. However, the proportion should have been nearly constant at all concentrations. The experimental results are fairly clear so that some conclusions may be drawn.

Table 15 and graph 14 show the results of an experiment in which adult moths were exposed to a series of eight concentrations of pyrethrins. The mortality of the moths after 18 hours and the numbers of eggs laid have been recorded. In ^{an} ~~the~~ experiment (table 16 and graph 15) the moths were exposed on papers treated with a series of five concentrations of pyrethrins with and without the addition of piperonyl butoxide; the mortality of the moths after 18 hours, the numbers of eggs laid and the proportion of eggs hatched after a further four days are recorded.

The number of eggs laid is lower in the control, and at the concentrations of pyrethrins giving either complete kill or no kill. It is highest near the median lethal dose. No definite conclusions can be drawn from the figure obtained for viability of eggs in table 16.

Graph 3: Exposure of P. interpunctella adults over filter papers impregnated with pyrethrins. See table 3.

3



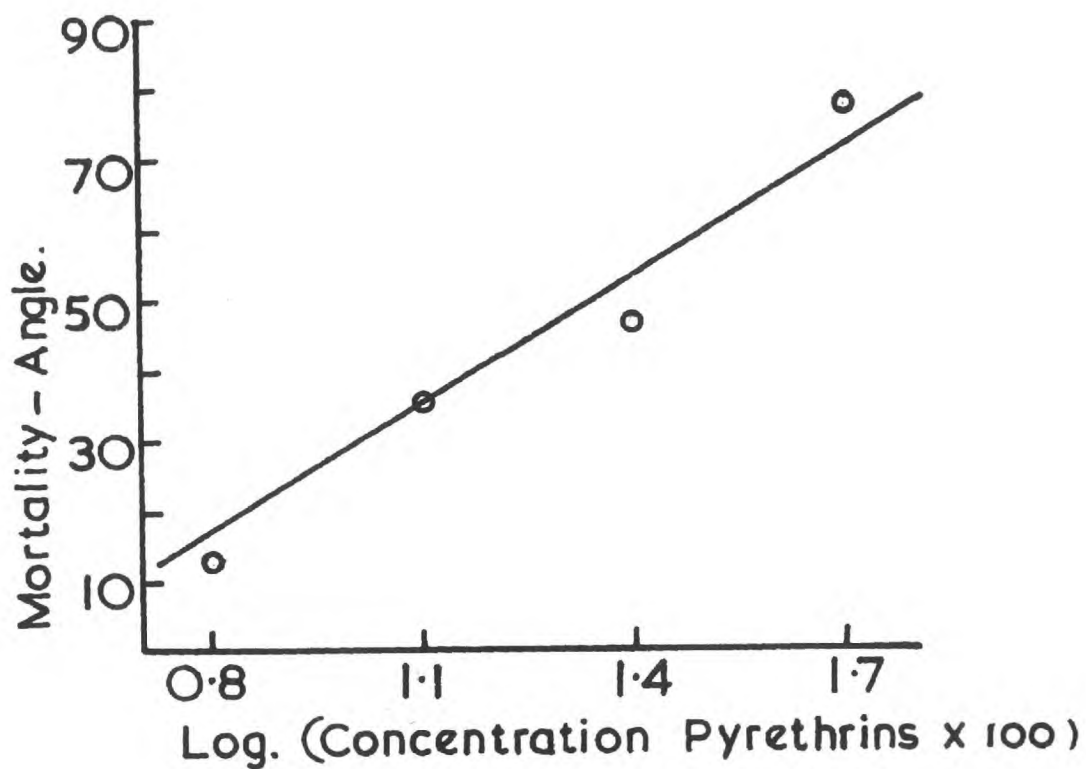
N.B. On all graphs, unless otherwise stated:

O - Treatment with pyrethrins.

X - Treatment with pyrethrins plus piperonyl
butoxide.

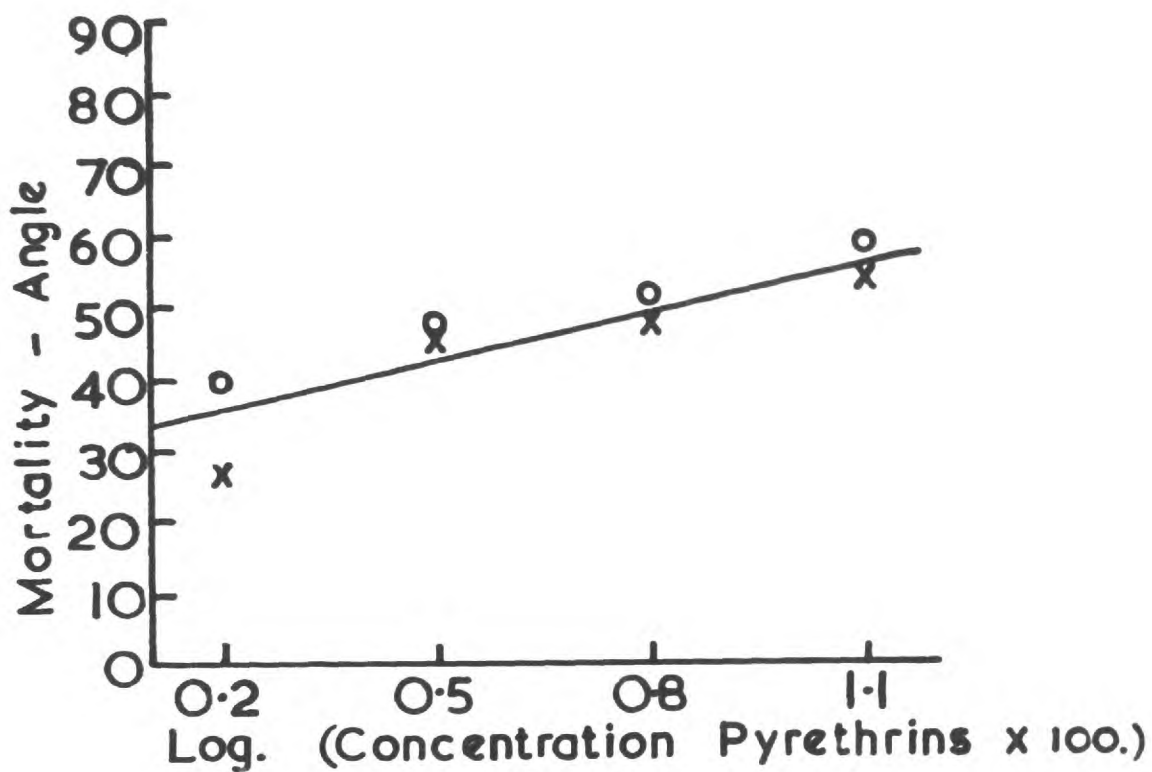
Graph 4: Exposure of P. interpunctella adults over filter papers impregnated with pyrethrins. See table 4.

4.



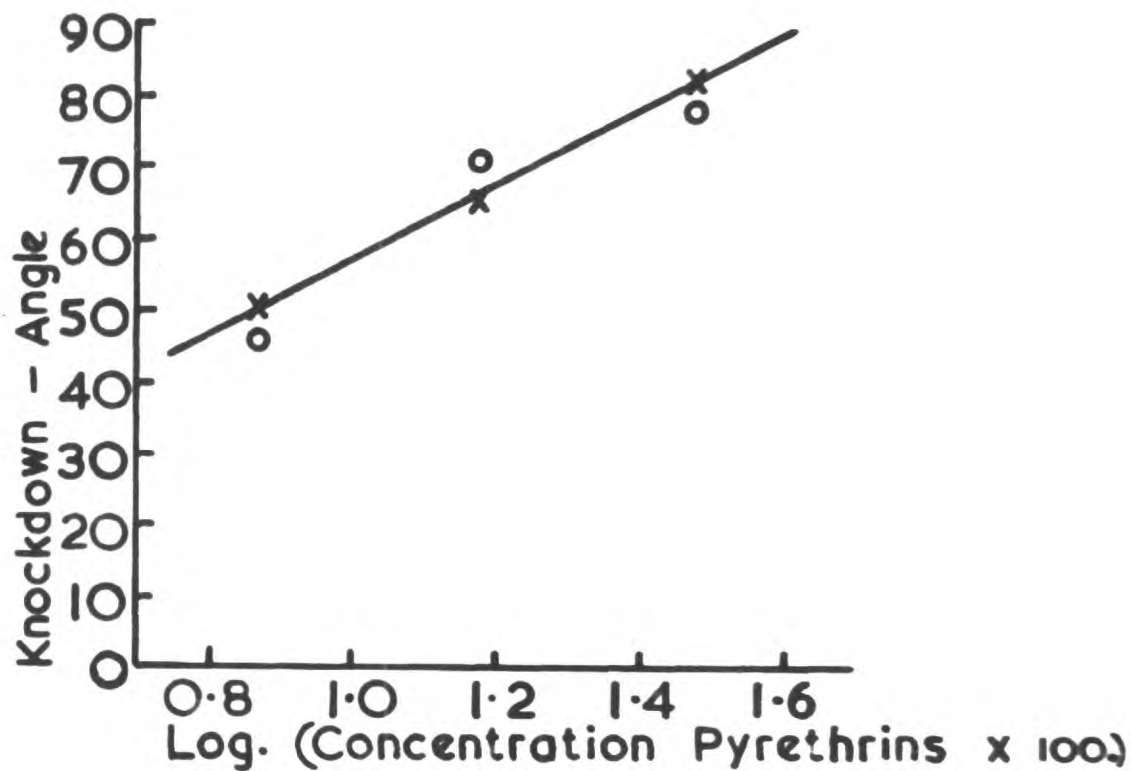
Graph 5: Exposure of *P. interpunctella* adults over filter papers impregnated with pyrethrins, with and without piperonyl butoxide. See table.5.

5.



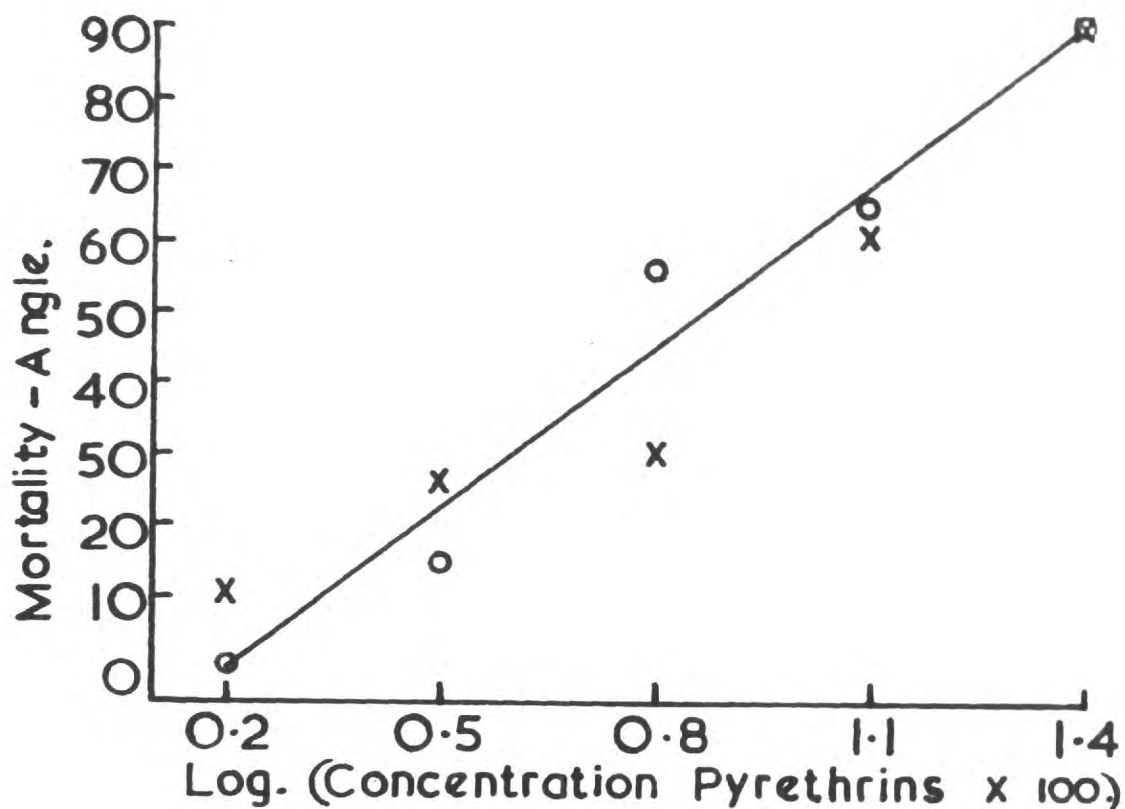
Graph 6: Exposure of P. interpunctella adults over filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 6.

6.



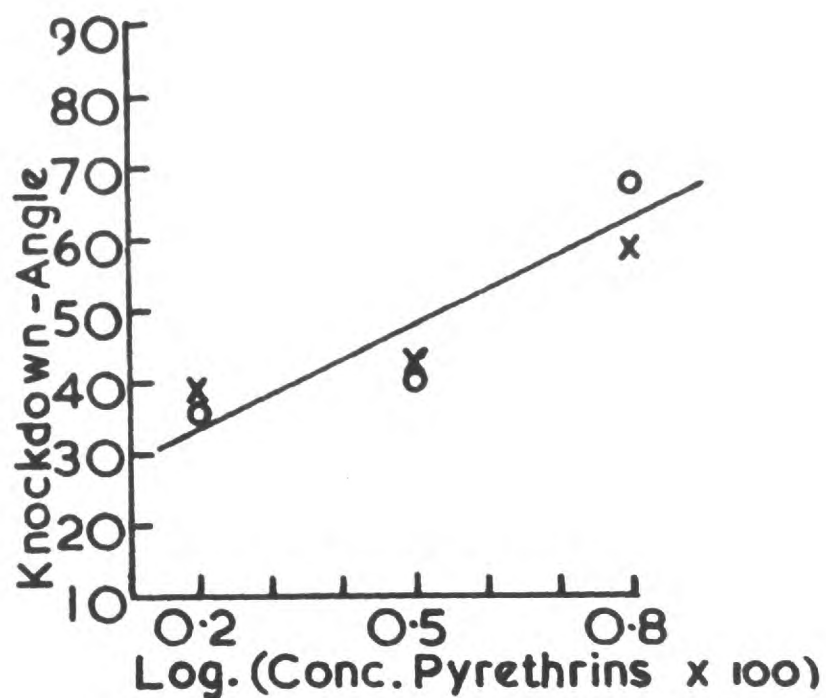
Graph 7: Exposure of P. interpunctella adults over filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 7.

7

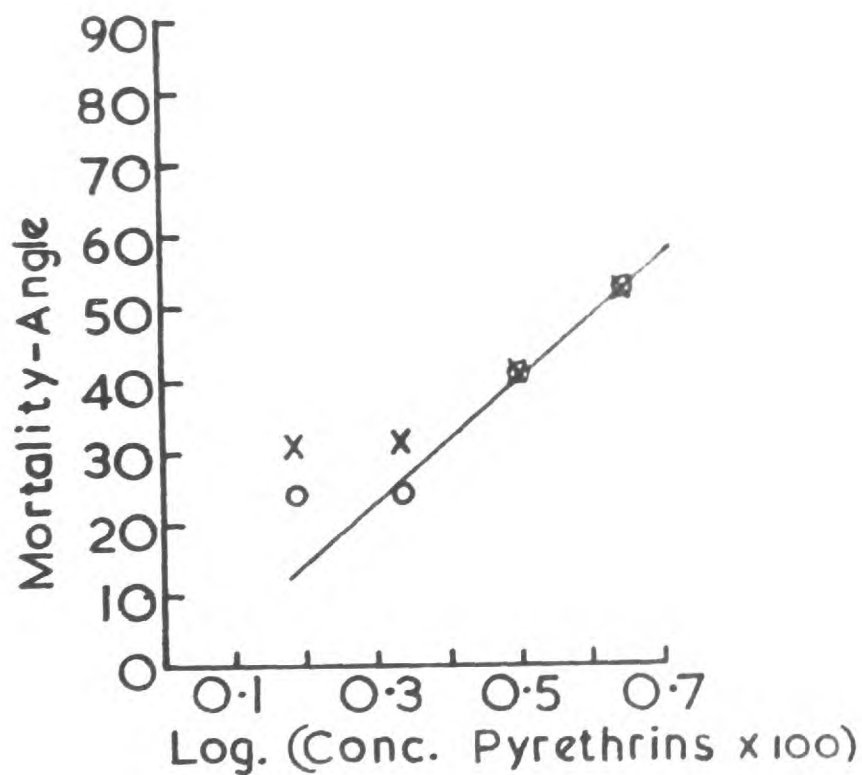


Graph 8: Exposure of P. interpunctella adults over filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 8.

8.

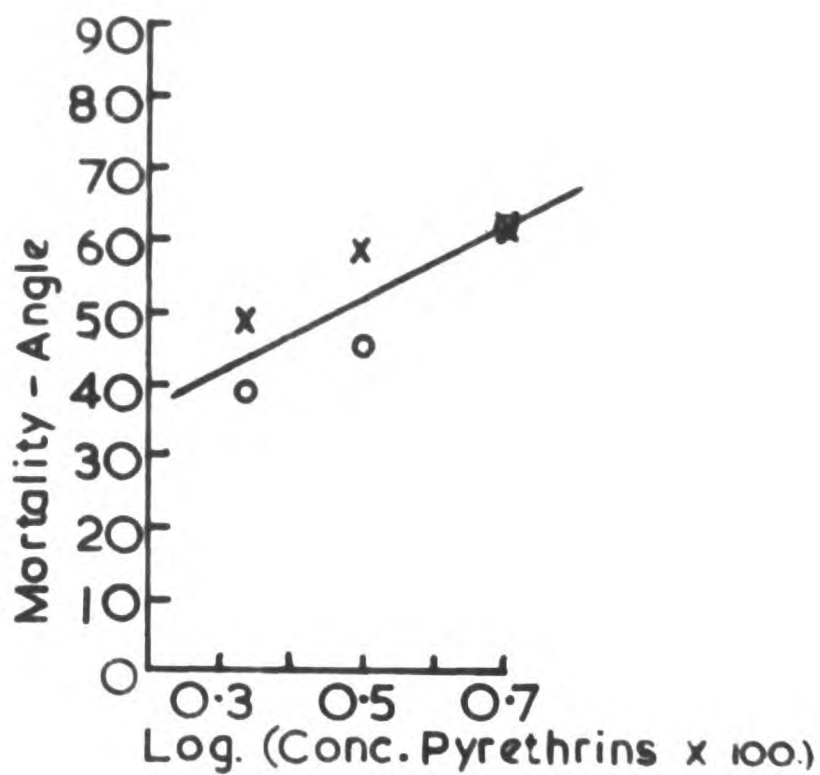


Graph 9: Topical application to P. interpunctella adults of pyrethrins with and without piperonyl butoxide.
See table 9.

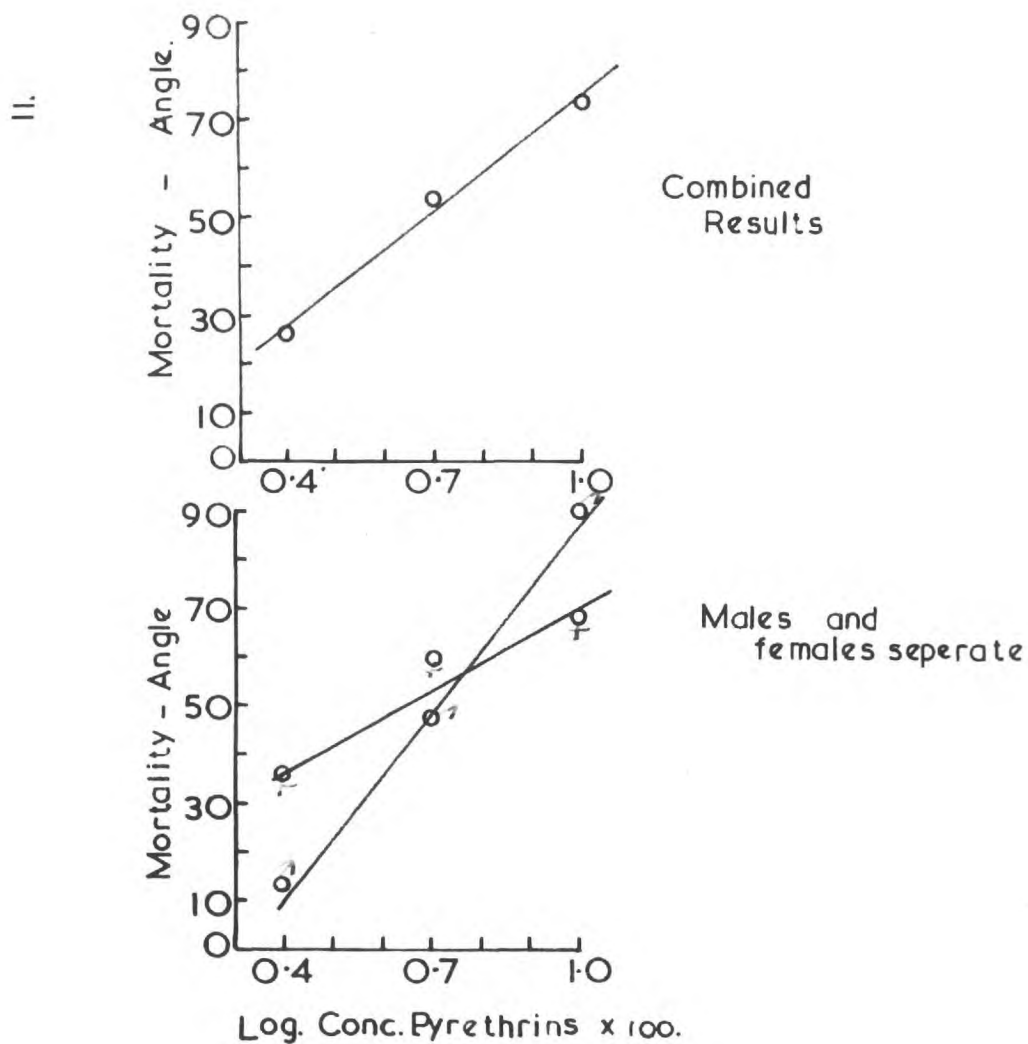


Graph 10: Topical application to P. interpunctella adults of pyrethrins with and without piperonyl butoxide.
See table 10.

10.

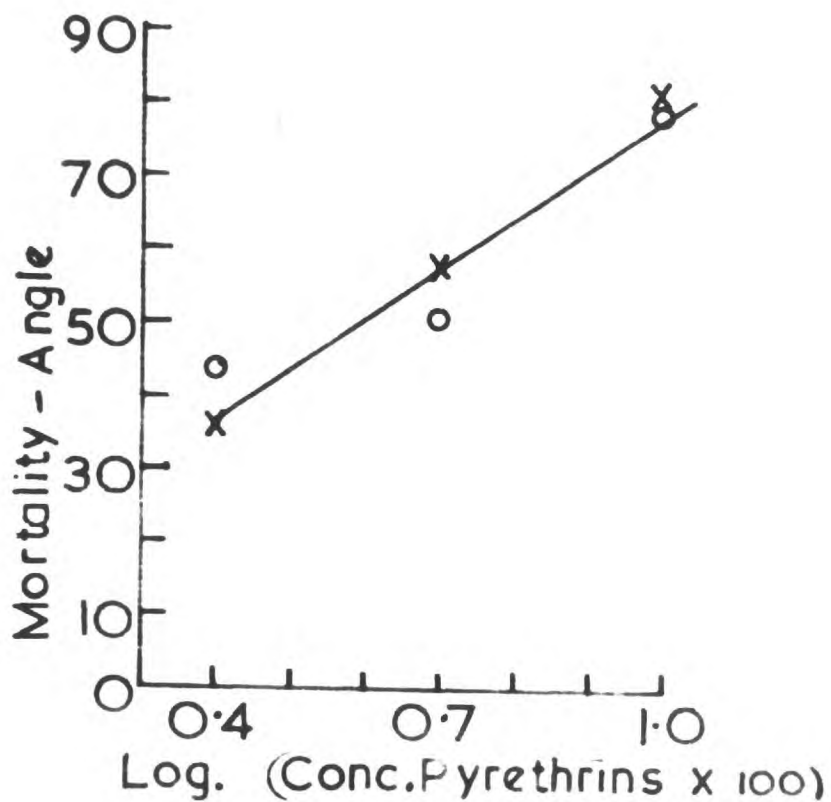


Graph 11: Topical application to P. interpunctella adults of pyrethrins.
See table 11.



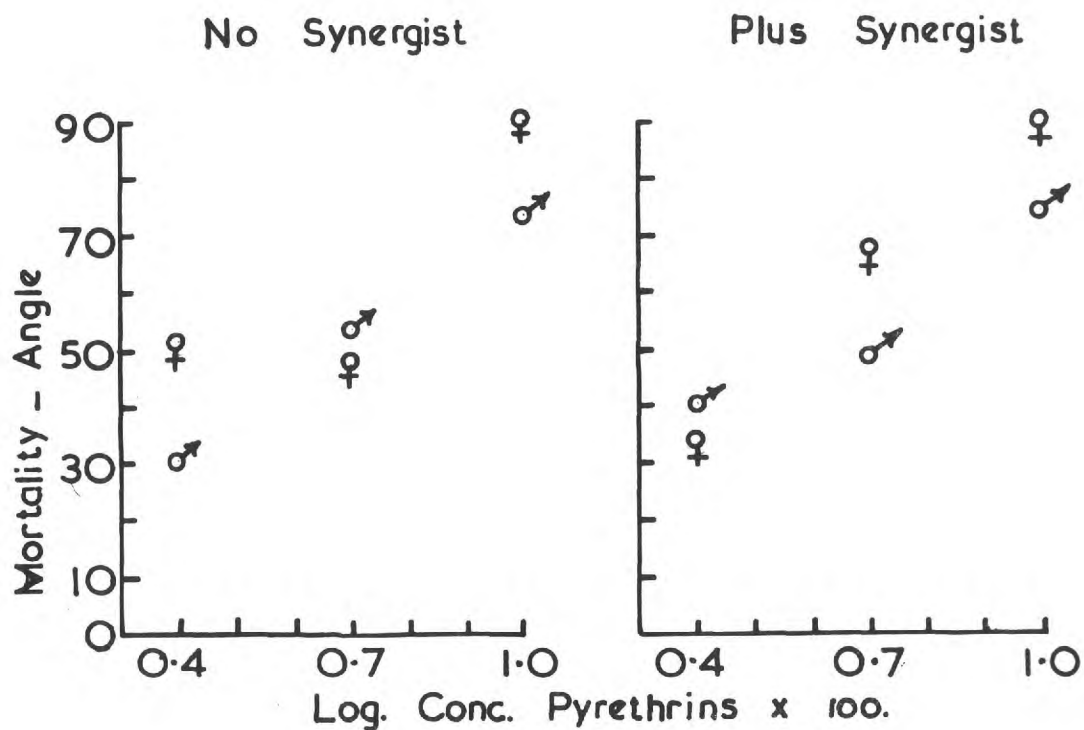
Graph 12: Topical application to P. interpunctella adults of pyrethrins with and without piperonyl butoxide.
See table 13.

12.



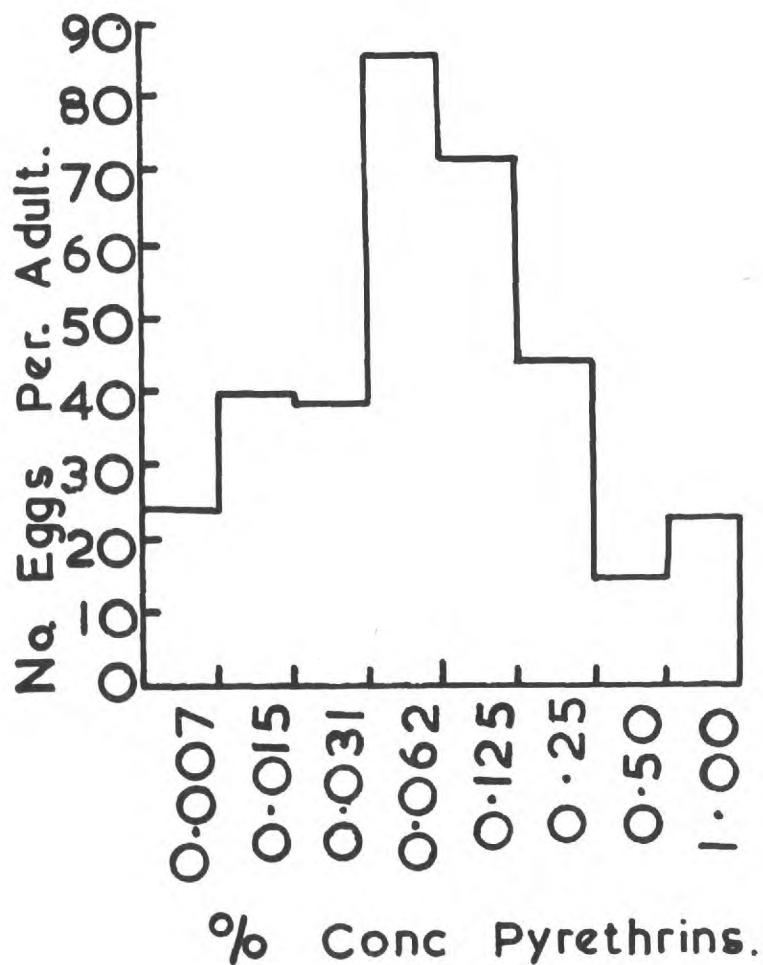
Graph 13: Topical application to P. interpunctella adults of pyrethrins with and without piperonyl butoxide.
See table 13.

13.



Graph 14: Eggs laid by P. interpunctella adults exposed over filter papers impregnated with a series of doses of pyrethrins. See table 15.

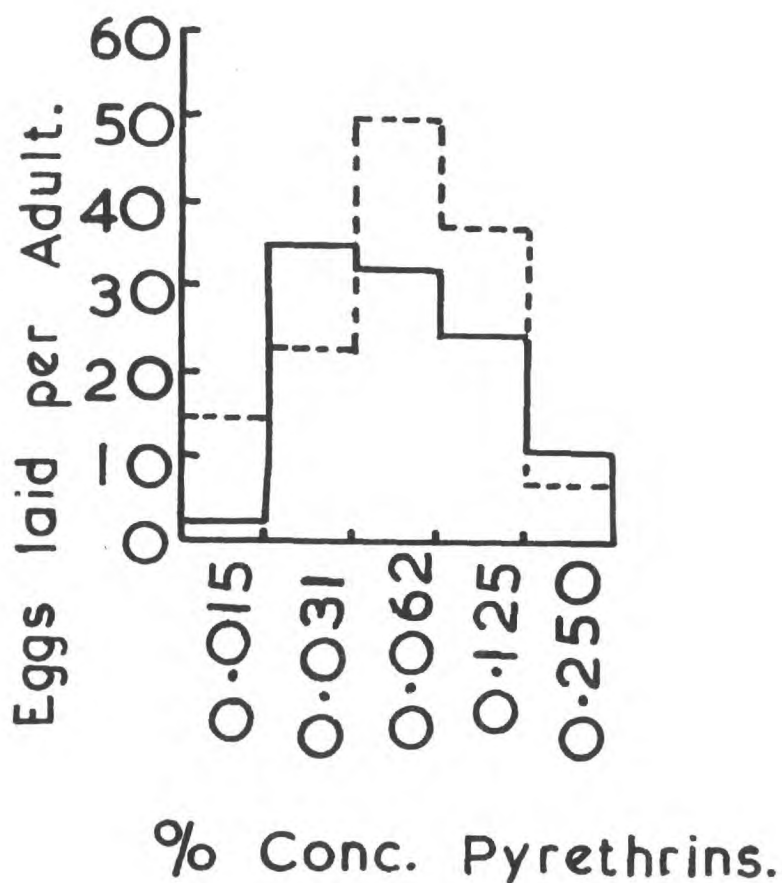
14.



11.9 eggs per adult were laid in the Control.

15.

Graph 15: Eggs laid by P. interpunctella adults exposed over filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 16.



In the control, 2.9 eggs were laid per adult.

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Pyrethrins only

pyrethrins plus piperonylbutoxide.

3. Plodia interpunctella larvae; Contact with prepared surfaces.

The biological assay of P. interpunctella larvae raised some problems not previously encountered. Attempts to assay these insects using the technique adopted with Calandra granaria met with only partial success.

Fourth instar moth larvae were exposed on filter papers impregnated with pyrethrins and enclosed by brass rings and glass plates as previously described. There was a fairly high control mortality and the larvae were able to crawl freely over the inner surfaces of the ring and glass plate as well as the filter paper. Unlike the adult moths, which were also able to settle away from the impregnated filter paper, the larvae were not continually active. After several hours exposure, some of the larvae, which were entering the pre-pupal stage, began to "spin-up", that is to produce a silken cover preparatory to pupation. Therefore there was probably a large variation in the time individual larvae spent actually in contact with the pyrethrins-impregnated surfaces.

Using this method, some results were obtained (table 17) which suggest an absence of synergistic action by piperonyl butoxide. However, analysis of these results showed the distribution of the points on the regression line to be heterogenous and not consistent with their lying about a straight line.

A number of methods of ensuring that P. interpunctella larvae remain on the pyrethrins-treated surfaces during assays have been investigated.

Complete enclosure of larvae in filter paper impregnated with pyrethrins.

Table 18 shows the results of an assay which apparently demonstrated marked synergistic activity by piperonyl butoxide. P. interpunctella larvae were exposed for 30 minutes in corked specimen tubes 2 inches long and 1 inch in diameter, which had been entirely lined with filter paper impregnated with solutions of pyrethrins and of pyrethrins plus piperonyl butoxide. The volume of the solutions pipetted on to the filter papers was calculated to give the same volume per unit area as on the 7 cm. diameter filter papers generally used. The ratio of volume of piperonyl butoxide to pyrethrins was raised to 10 to 1 in the experiment, in an attempt to detect a very low factor of synergism. The response was estimated as failure to have commenced pupation within 5 days after exposure.

Table 18 indicates that the presence of the synergist substantially increased toxicity on this occasion. Because of the short (30 minutes) exposure period, the range of doses of pyrethrins necessary to produce knockdown was high, namely 5.0, 2.5 and 1.25 per cent. As the piperonyl butoxide was added on this occasion at a rate of 10 parts by volume to one part of pyrethrins, the concentrations of the synergist were 50, 25 and 12.5%. At these very high concentrations of synergist, it was difficult to obtain even distribution on the filter papers and the papers remained sticky after the evaporation of the petroleum ether. It seemed likely that the higher knockdown obtained in the presence of the synergist was due on this occasion to a physical effect of the piperonyl butoxide in smothering the larvae

and was not true synergism.

To elucidate this matter, P. interpunctella larvae were exposed on filter papers impregnated with 0.5 ml. of solutions of 50, 25 and 12.5% piperonyl butoxide in light petroleum. The larvae were exposed on the filter papers enclosed by brass rings for 30 minutes and the numbers which pupated within 5 days were again counted. The results (Table 19 and graph 16) indicated that piperonyl butoxide was quite toxic at these concentrations; the effect is probably a physical one.

Because of this toxic action of piperonyl butoxide at high concentrations and because it was not possible to observe the larvae during assay, no further attempts were made to enclose P. interpunctella larvae completely during assay. The assays with P. interpunctella larvae have all been carried out over short exposure periods and hence with high concentrations of pyrethrins and of piperonyl butoxide. It was therefore not possible to use 1:10 mixtures of pyrethrins and piperonyl butoxide in attempts to detect very low synergistic activity.

Use of brass and Fluon rings.

Although Calandra granaria adults can easily crawl up the inside surfaces of the enclosing brass rings during assays, they are prevented from so doing if the metal surface is coated with a very thin film of liquid paraffin. It was found that such a film of liquid paraffin did not deter P. interpunctella larvae at all.

The use of polytetrafluoroethylene known as "Fluon" or P.T.F.E.

as a barrier to insects has been reported. A "Fluon" suspension can be applied like a paint to surfaces and is reported to stop some insects. It is very smooth and slippery and presumably insects are unable to grip the surface. However, when the brass rings used in these experiments were painted with "Fluon" P. interpunctella larvae were still able to crawl up the painted surface. The larvae were also able to escape when enclosed by a short cylinder of "Fluon".

The cylinder of "Fluon" also failed to confine adults of Calandra granaria and Carpophilus dimidiatus.

Liquid barriers.

The use of water or other liquids to serve as moats to isolate the moth larvae on filter papers proved quite unsatisfactory.

The electrified barrier.

Electrified barriers have been used to confine insects during breeding and biological assay. After some experiments the apparatus described below was devised. It was suggested by the method of G.A. Wheatley and S.Z. Moczarski, 1950.

The apparatus is shown in Figure 3 and consists of a platform fitted with terminals on which up to 12 barriers may be accommodated.

Each barrier consists of two brass rings insulated from one another by a thin washer of "Tufnol" and held together with four "Tufnol" screws. The dimensions given in the diagram were satisfactory for confining P. interpunctella larvae and are not critical; they may be varied to suit the particular insect species under test.

The insulated components are connected to a hearing aid battery giving a low current at a potential of 30 volts. Insects attempting to climb up the side of the ring complete the circuit, receive an electric shock and are returned to the treated surface enclosed by the barrier.

In an attempt to reduce the shock received by the insects, a relay switch activated by the alternating mains supply was included in the circuit as an interrupter giving a frequency of 50 pulses per second.

The rings may easily be dismantled for cleaning and, to ensure good electrical contact, the insides of the rings are wiped with alcohol before each assay.

With the barrier, larvae of Plodia interpunctella in the last instar have been used for the biological assay of contact insecticides applied to filter papers. The period of exposure of these insects is generally not longer than 30 minutes. After longer periods, the surface of the ring becomes covered with webbing produced by the larvae, thus insulating them from the current. There is also a tendency for some of the larvae to "spin up" preparatory to pupation (if left on the surface for an extended period) reducing their period of contact with the insecticide. Such difficulties as these may not be encountered when using other insects.

It is possible to treat several different batches of insects simultaneously with a range of concentrations of an insecticide by using a number of the barriers connected in parallel.

The possible detrimental effects of electric shocks on insects has been considered. In experiments with Plodia interpunctella larvae the survival of controls has been satisfactory and similar to the survival of larvae placed in the rings without the current being switched on.

The apparatus could easily be modified to confine insects on surfaces other than filter papers if required. The author has been asked to supply details of the barrier with the thickness of the lower rings greatly reduced, for possible use in the biological assay of ants of the genus Monomorium in Trinidad.

In the experiments shown in graphs 17 and 18 and tables 20 and 21, last instar larvae of P. interpunctella were exposed for 30 minutes in the electrified barrier to filter papers impregnated with a series of four concentrations of pyrethrins. In experiment 20 knockdown after one hour was estimated as the failure of the larvae to crawl off a 7 cm. filter paper when placed in the centre and illuminated from one side by a 60 watt bulb. In experiment 21, mortality after 24 hours has been estimated. In both cases satisfactory regression lines have been obtained from the data.

The use of the electrified barrier enabled P. interpunctella larvae to be kept on the poisoned surfaces during the contact assays described above. The period of exposure was still limited to about 30 minutes so that there was likely to be a considerable variation in the dose of pyrethrins received by individual insects, depending, for example, on the degree of activity of the larvae. Exposure

for longer periods was not practical because some of the larvae might have commenced pupation. In fact these experiments were not considered to be completely satisfactory. The control responses were often very high and the short exposure period necessitated the use of relatively high concentrations of pyrethrins so that a small alteration in the concentrations of insecticides used could have a marked effect on the larvae, giving large proportions of either zero or complete response. Also, because of these high concentrations, any alteration in the level of susceptibility of the insects would have the same effect and regressions would not be obtained.

Although the electrified barrier had been developed for the biological assay of moth larvae, it was used to best advantage for the assay of ants and mustard beetles, as will be explained later.

Moth larvae - Topical application.

Topical application of solutions in odourless kerosene of pyrethrins with and without piperonyl butoxide have been made to P. interpunctella larvae, using the technique of Kerr. As in the experiments described above, the larvae were all in their last instar and had commenced wandering prior to pupation. The dose of insecticide was applied dorsally at about the junction of the thorax and abdomen. The insects were afterwards confined on a clean No. 1 Whatman filter paper by one of the brass rings, with a small clear glass plate as cover. It will be seen that the criterion used for response varied. When knockdown was considered as failure to pupate a loosely folded half circle of 7 cm. diameter Whatman No. 1 filter

paper was put with the larvae to provide sites for pupation.

Graphs 19, 20 and 21 and tables 22, 23 and 24 show the results of experiments in which larvae were treated with a series of concentrations of pyrethrins and with a series of the same concentrations of pyrethrins with added piperonyl butoxide. No synergistic activity of the piperonyl butoxide has been detected.

In table 22 the analysis of the regression using probits and angles has been compared, and it will be seen that the two methods agree well. These results were chosen for the comparison because they show a relatively high control mortality and it was thought that the use of angles might be less reliable under these conditions.

P. interpunctella larvae on wood squares.

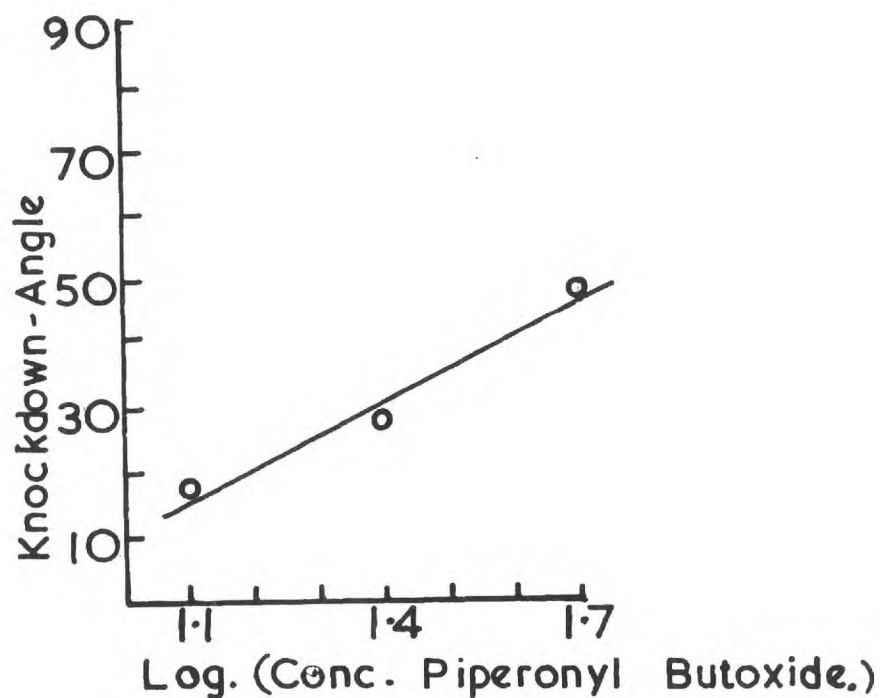
The "crawling insect" assay may be performed on substrates other than filter paper. In particular it is sometimes desirable to obtain results of assays on surfaces similar to those which are sprayed with insecticide in the commercial control of the insect under investigation. As an illustration of this, P. interpunctella larvae were exposed on discs of obeche wood impregnated with pyrethrins. This is a soft wood similar to that often used in packing cases for bulk handling of food.

Pieces of wood were cut 7 cm. square and about 0.4 cm. thick and 0.63 cc. of insecticide, in mixed dilutant, was pipetted on to the wood giving a volume per unit area equivalent to that applied to the 7 cm. diameter filter papers.

The larvae were exposed to these surfaces impregnated with solutions containing series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide. Knockdown (inability to leave a 7 cm. filter paper under oblique illumination in one minute) was determined after 24 hours exposure. The results of this experiment, shown in table 25 and graph 22, indicate again an absence of synergistic activity by piperonyl butoxide against this insect. The electric barrier was not used to prevent the larvae leaving the poisoned surface during assay, but nevertheless a fairly good regression line has been obtained.

Graph 16: Exposure of P. interpunctella larvae to filter papers impregnated with piperonyl butoxide.
See table 19.

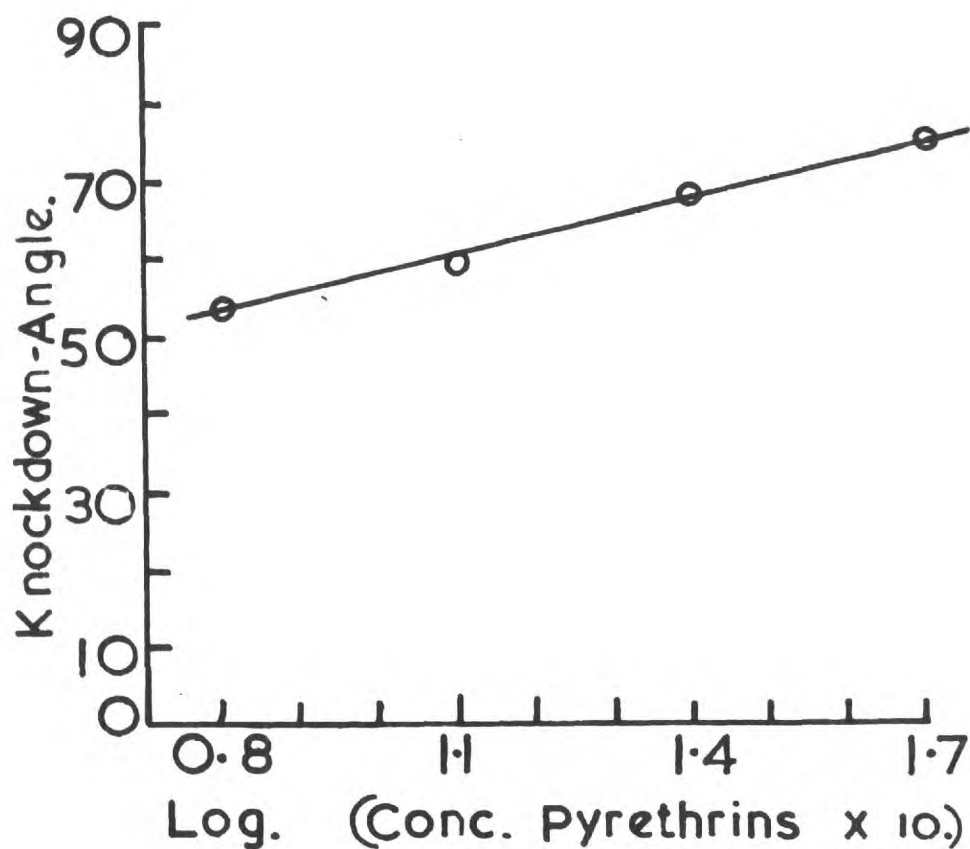
16.



0 Exposed to piperonyl butoxide.

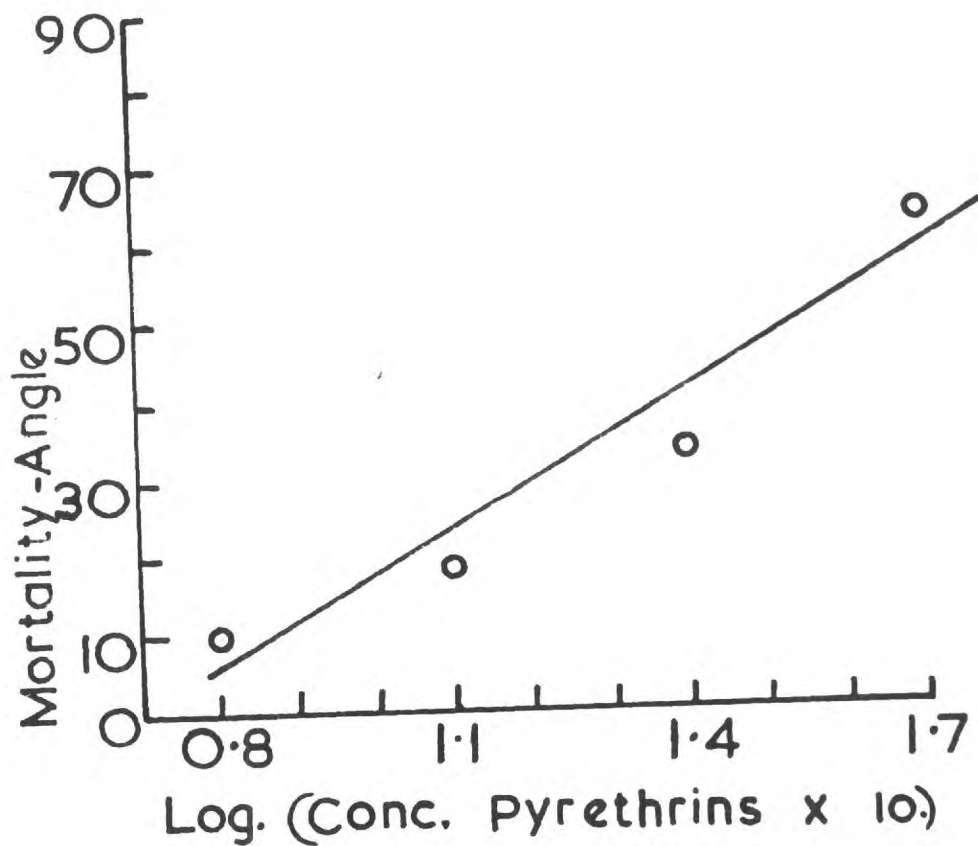
Graph 17: Exposure of P. interpunctella larvae in electric barrier on filter papers impregnated with pyrethrins.
See table 20.

17.



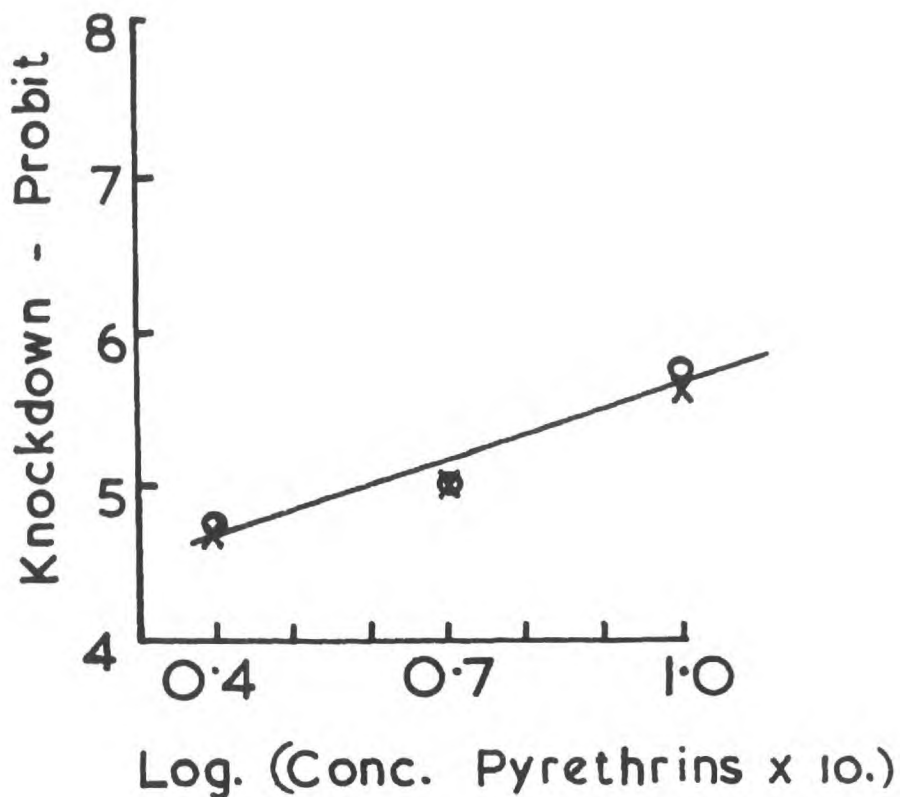
Graph 18: Exposure of P. interpunctella larvae in electric barrier to filter papers impregnated with pyrethrins.
See table 21.

18.



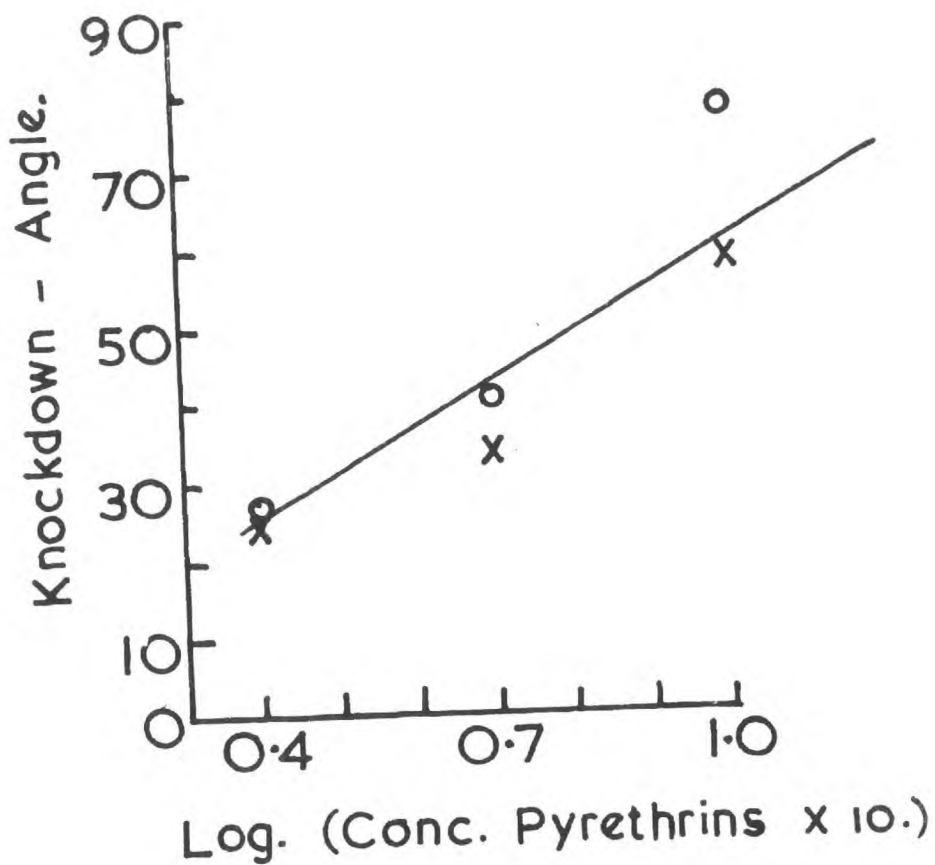
Graph No. 19: Topical application to P. interpunctella larvae of pyrethrins with and without piperonyl butoxide.
See table 22.

19.



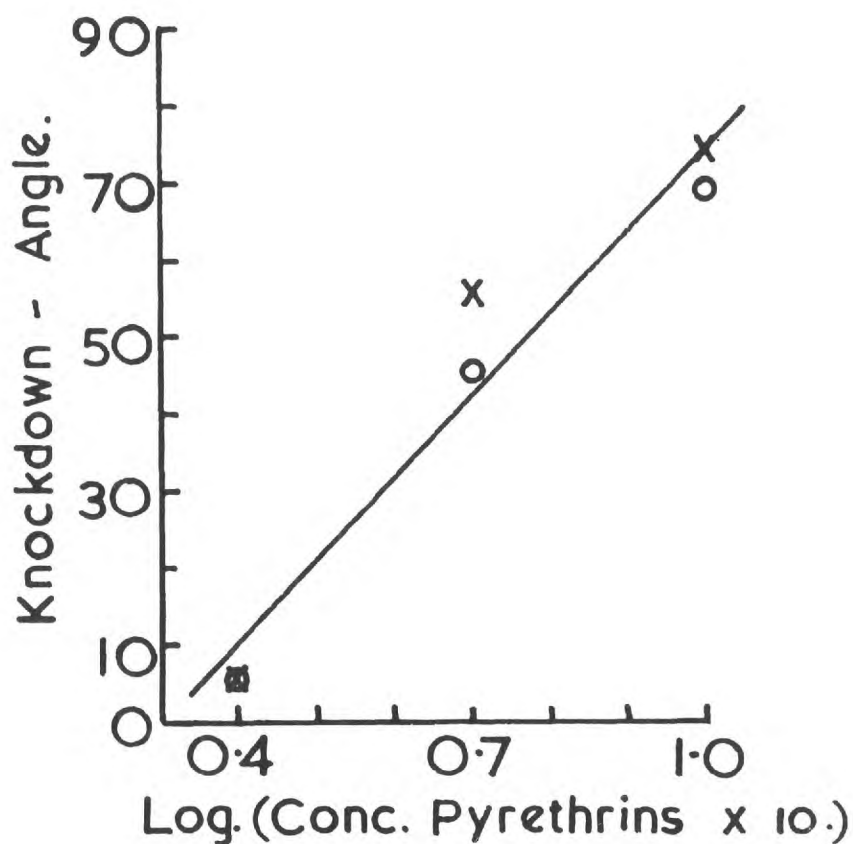
Graph20: Topical application to P. interpunctella larvae of pyrethrins with and without piperonyl butoxide.
See table 23.

20.

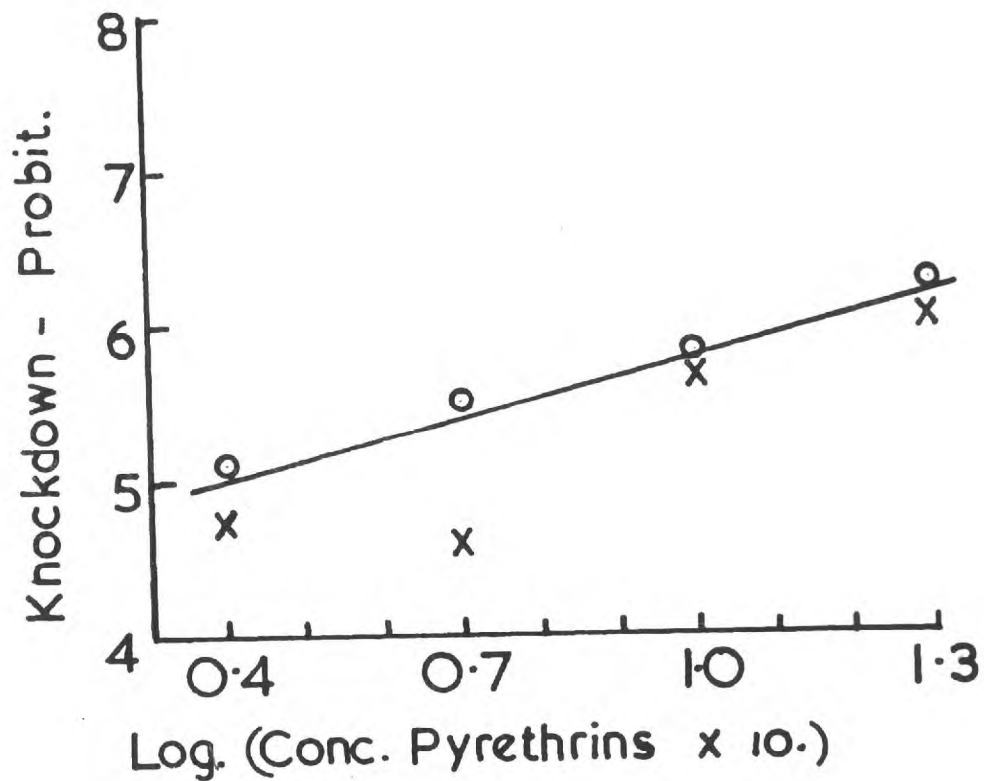


Graph 21: Topical application to P. interpunctella larvae of pyrethrins with and without piperonyl butoxide.
See table 24.

21.



Graph 22: Exposure of P. interpunctella larvae on wooden squares impregnated with pyrethrins with and without piperonyl butoxide. See table 25.



4. Ephestia cautella.

Biological assays with larval and adult Ephestia cautella were carried out using the same techniques as those employed with Plodia interpunctella.

Working under the direction of the author, Miss J. M. Donaldson has carried out biological assays with adult E. cautella. She has examined the effect of irradiation of pyrethrins films on the rate of break-down of the pyrethrins in the presence and absence of piperonyl butoxide. The experiments are described briefly below. Ephestia cautella adults.

Doses of pyrethrins in odourless kerosene have been applied topically to adult E. cautella using the technique of Kerr. The response of the moths used to evaluate these experiments was their mortality after three hours. Table 26 and graph 23 show the results of an experiment in which adult moths were treated with a range of four concentrations of pyrethrins. A satisfactory regression line has been obtained.

Further experiments with Ephestia cautella adults were carried out by Miss J. M. Donaldson under the author's supervision. Solutions of pyrethrins and of pyrethrins plus piperonyl butoxide in odourless kerosene were applied topically to the adult moths at a dosage rate of 0.05 μ l. per moth using Kerr's apparatus. Table 27 and graph 24 show the results of two of these experiments. These results indicate the absence of synergistic activity by the piperonyl

butoxide or, at least, that the synergistic activity is too small to be detected by the experimental method used here.

Miss Donaldson also irradiated pyrethrins and pyrethrins plus piperonyl butoxide with ultra-violet light before using E. cautella adults to assay these solutions. Provided exposure to irradiation was for a sufficient length of time, there was a marked reduction in the toxicity of the pyrethrins solutions to the moths. This reduction in toxicity was also shown when solutions of pyrethrins plus piperonyl butoxide were irradiated. No difference in the amount by which the toxicity of pyrethrins or of pyrethrins plus piperonyl butoxide was reduced could be detected when a regression analysis of the results of these experiments was carried out.

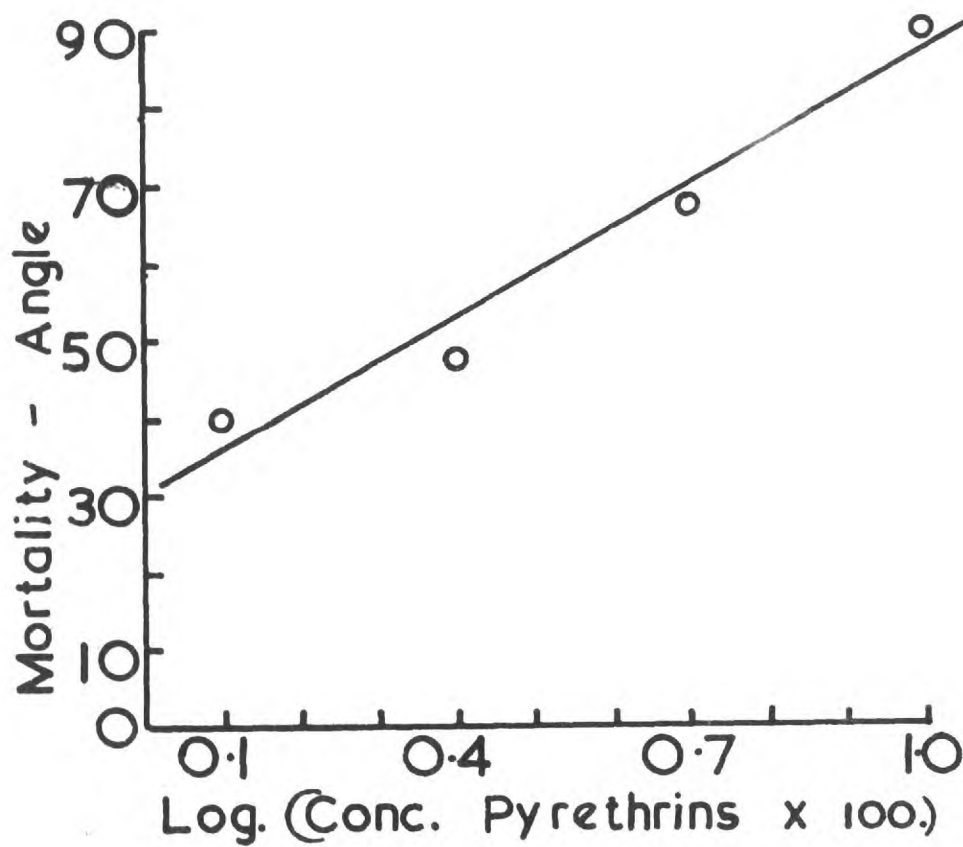
Ephestia cautella larvae.

Crawling insect assays were carried out using E. cautella larvae before the construction of the electrified barrier had been completed. The larvae were exposed on filter papers impregnated with pyrethrins and confined on these by brass rings and small glass plates. They were therefore able to crawl off the poisoned surface and the experiments were subject to the disadvantages mentioned in the description of experiments with P. interpunctella larvae above. Table 28 and graph 25 show the results of an experiment in which E. cautella larvae were left exposed to filter papers impregnated with a range of five concentrations of pyrethrins with and without the addition of piperonyl butoxide for a period

of 24 hours. The knockdown was recorded at the end of exposure. The criterion of knockdown in this experiment was the failure of the larvae to crawl off a clean white 7 cm. filter paper within one minute when placed in the centre and illuminated obliquely. There was no evidence of synergistic action by the piperonyl butoxide.

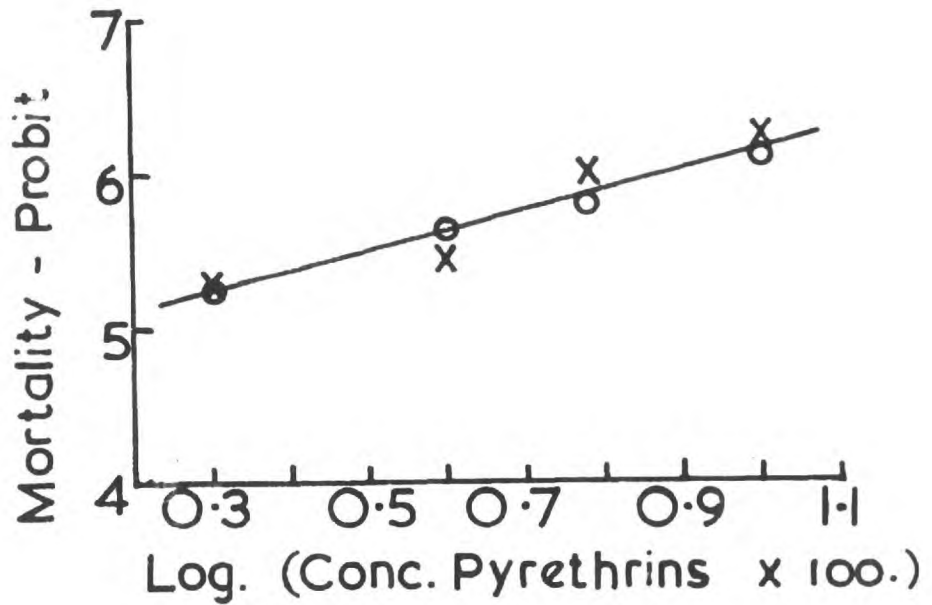
Graph 23: Topical application to E. Cautella adults
of pyrethrins.
See table 26.

23.

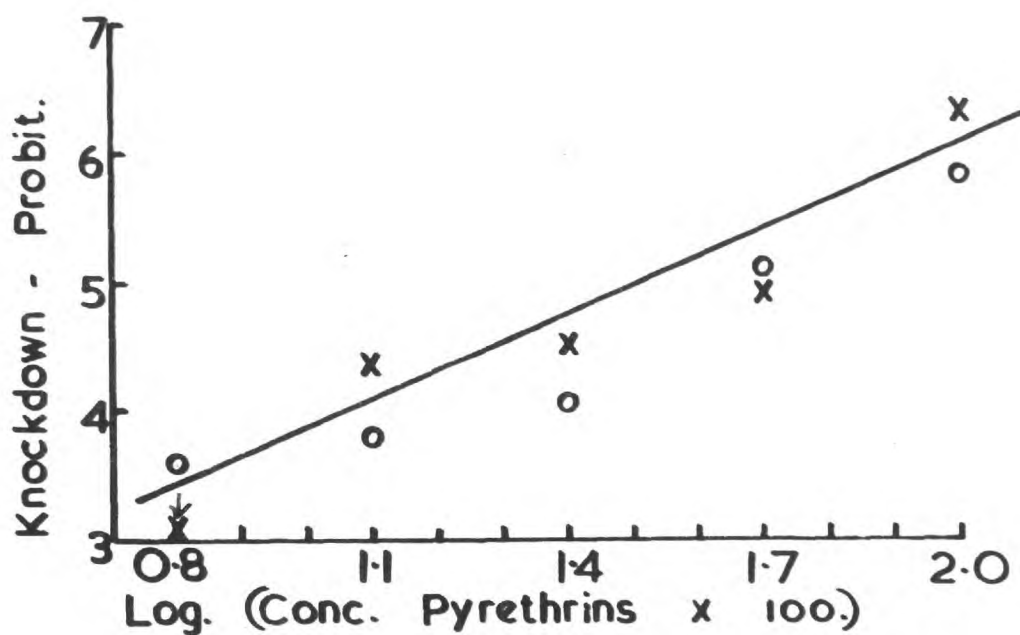


Graph 24: Topical application to E. cautella adults of pyrethrins with and without piperonyl butoxide.
See table 27.

24.



Graph 25: Exposure of E. cautella larvae on filter papers impregnated with pyrethrins with and without piperonyl butoxide.
See table 28.



6. Galleria melonella adults.

Achroia grisella adults.

The wax moths proved more difficult to culture than had been expected. There was never a sufficient number available to undertake all the experiments planned. The results recorded here are therefore incomplete, but do give some idea of the insects' potentialities for biological assay.

Although the intention had been to culture the greater wax moth (Galleria melonella), the original culture was found to be mixed and large numbers of lesser wax moths (Achroia grisella) were present in some of the culture jars, and were developing at the expense of G. melonella. Because of the shortage of material, some experiments were carried out using these lesser wax moth adults.

The use of the crawling insect form of biological assay or any form of contact assay with wax moth adults proved unsuccessful. The experiments mentioned here were all carried out by the topical application methods described above.

The moths are difficult to remove from their cultures and at first escaped very easily. They were collected in lots of two, three or four in 2" x 1" specimen tubes which were then closed with a muslin held in place by a cork with a hole bored in the centre. They were anaesthetised with carbon dioxide blown in through the hole in the cork prior to the application of the insecticide. They required considerably longer exposure in carbon

dioxide before becoming anaesthetised than did Plodia interpunctella adults.

Table 29 and graph 26 show the results of an experiment in which adult Achroia grisella were given topical doses, using the micrometer syringe, of 1.0 μ l. of a series of three concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide dissolved in acetone. It will be seen that one of the three concentrations of pyrethrins alone gave a 100% response. In the absence of a further supply of insects to repeat the experiment, a calculation of the regression has been made using these data and there was found to be no significant enhancement of the toxicity of the pyrethrins when piperonyl butoxide was added to the formulation.

The response used in the biological assay of pyrethrins with adult wax moths was death, which could be easily determined.

Table 30 and graph 27 show the results of a similar experiment in which Galleria melonella adults were given doses of 0.05 μ l. of pyrethrins and of pyrethrins plus piperonyl butoxide in odourless kerosene using Kerr's apparatus. In this case there is again no evidence of synergistic activity by the piperonyl butoxide. Further experiments are necessary to establish this lack of synergism with any certainty.

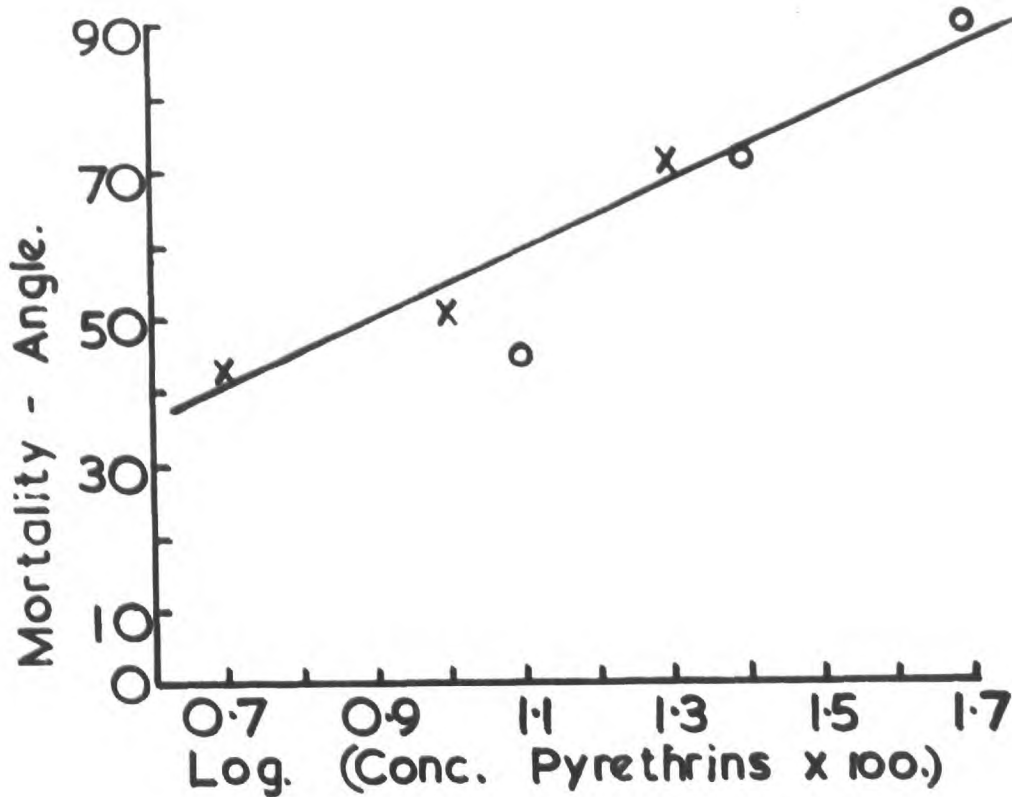
In table 30 the number of eggs laid during the assay by the female moths have been recorded. Pyrethrins stimulated the moths to lay considerable numbers of eggs compared with the control.

To investigate any relationship between concentrations of pyrethrins and numbers of eggs laid it would be necessary to treat the adult moths with a wider range of concentrations of pyrethrins.

The results should be compared with those obtained for the oviposition of P. interpunctella adults stimulated by pyrethrins (Tables 14, 15 and 16).

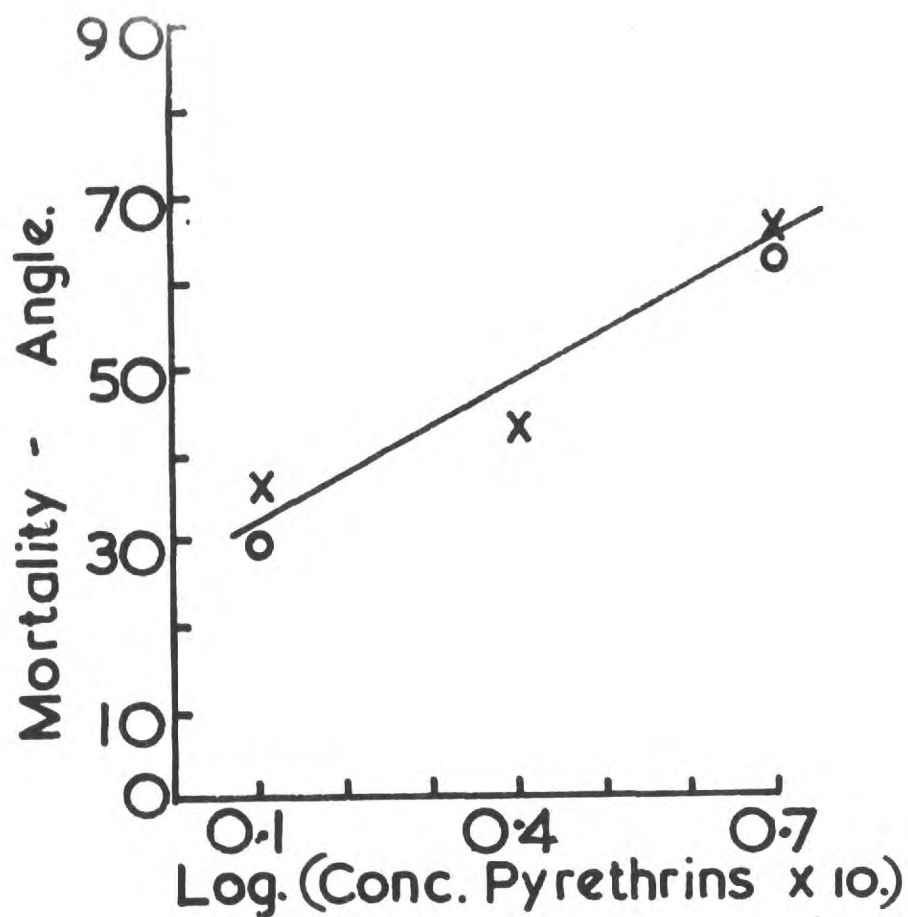
Graph 26: Topical application to A. grisella adults of pyrethrins with and without piperonyl butoxide. See table 29

26.



Graph 27: Topical application to G. melonella adults of pyrethrins with and without piperonyl butoxide.
See table 30.

27.



7. Galleria melonella larvae.

The larvae of the greater wax moth were taken from the cultures for biological assay when they reached the wandering stage in their development prior to pupation.

The "crawling insect" method of biological assay was discarded in favour of topical application of pyrethrins in acetone using the micrometer syringe at a dose rate of $1.0 \mu\text{l}$. solution per insect, placed on the dorsal side of the thorax. It was not necessary to anaesthetise the larvae as they could be easily held between the finger and thumb. After treatment the larvae were left in lots of not more than five on white filter papers enclosed by the brass rings and small glass plates.

Tables 31 and 32 (graphs 28 and 29) show details of experiments in which larvae were treated with doses of a series of three concentrations of pyrethrins and of three concentrations of pyrethrins plus piperonyl butoxide dissolved in acetone. In these experiments the response to the insecticide has been determined as the failure of the larvae to commence pupation within a given time after treatment. The larvae which did not pupate were either dead or dying and there was no evidence that the pyrethrins delayed the onset of pupation.

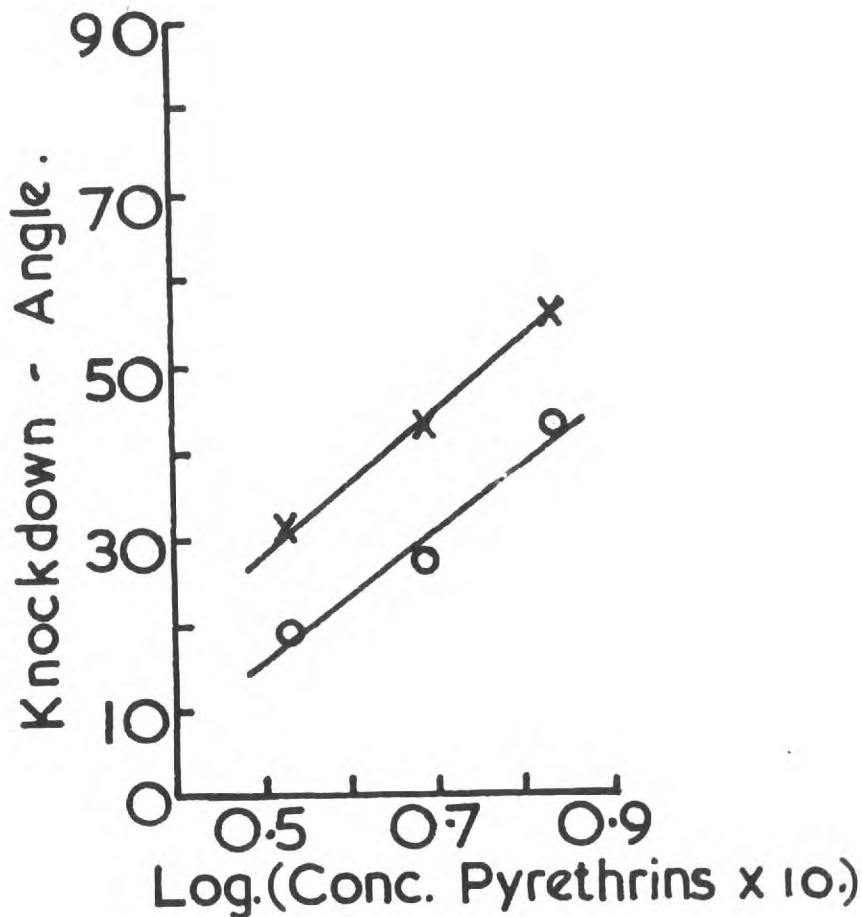
There is evidence of a small degree of synergism from these results and the factor was calculated to be 1.9 in table 32.

After treatment the larvae soiled the filter papers on which they were confined with a liquid excretion. It was obvious from casual observation that this bore some relation to the dosage of pyrethrins given to them. In the experiment recorded in table 32, an attempt has been made to assess the degree of soiling of the filter papers by a system of scoring. There were four filter papers at each concentration and they were classified 1, 2, 3, 4, or 5 according to degree of staining. The results of this assessment together with an explanation of the system by which it was done are given in table 33. A tendency for greater staining and therefore for more excretion at higher dosages is clearly shown. There was no staining in the controls.

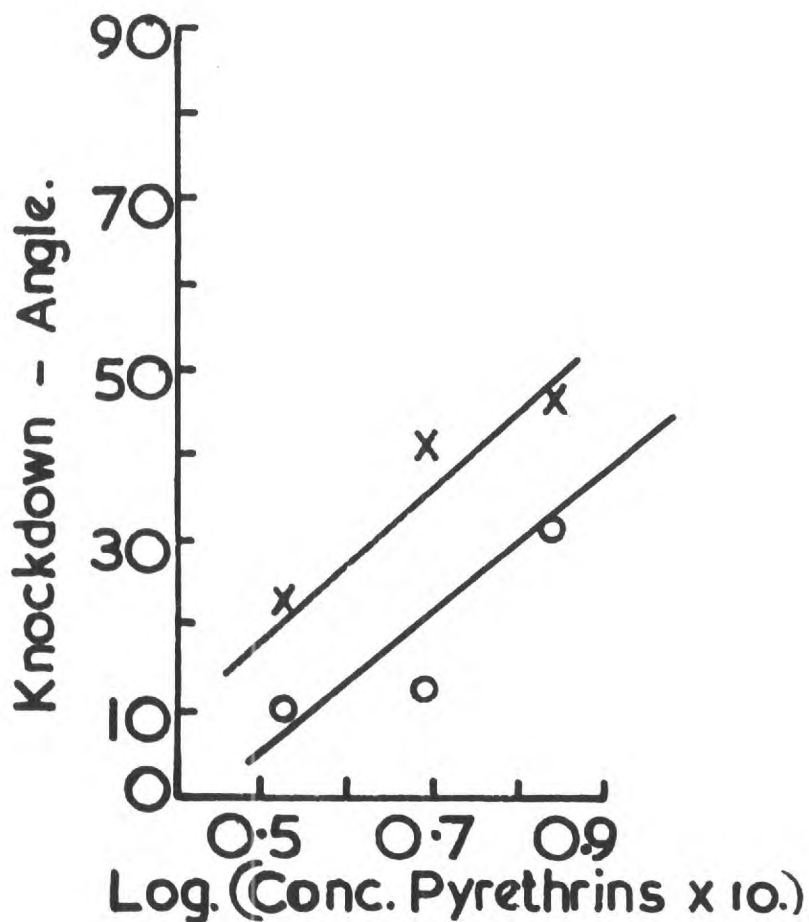
To assess this loss of fluid on a quantitative basis G. melonella larvae were weighed before and after treatment with pyrethrins. In this experiment (table 34) the last instar larvae were taken from the culture prior to their wandering stage and were then treated as in the first experiments described in this section. The results, in terms of pupation failure, are shown in table 34. Also in table 34 the degree of staining of the filter papers (using the same code as in table 33) and the loss in weight are recorded. The increase in degree of staining from larval excretion with increase of concentration of pyrethrins is not as clear cut as in the assessment shown in table 33. The percentage loss of weight of the larvae corresponds roughly with the concentrations of the pyrethrins solutions applied to the insects. When piperonyl butoxide was added to the solutions the correspondence was not so good.

However, the loss of weight in the controls was considerably less than that of insects treated with pyrethrins.

Graph 28: Topical application to G. melonella larvae of pyrethrins with and without piperonyl butoxide.
See table 31.



Graph 29: Topical application to G. melonella larvae of pyrethrins with and without piperonyl butoxide.
See table 32.



8. Pieris brassicae.

Third instar larvae of Pieris brassicae (the Cabbage White Butterfly) were used in a biological assay to compare the toxicity of pyrethrins and of a pyrethrins/piperonyl butoxide mixture towards this insect. Owing to shortage of the insects which, as stated above, were kindly supplied by Dr. B. R. Champ, only a limited number of experiments was possible.

Once again, the crawling insect biological assay technique described for Calandra granaria was used in a slightly modified form, there being no oil film on the inside of the brass rings. The larvae remained fairly active when confined and did not settle on the wall of the ring or on the covering glass plate. During assay the larvae were kept at a temperature of 25°C and at a relative humidity of 65% $\pm$ 10%. The criterion of response to the insecticide used in these experiments was death. The absence of food during the 24 hour exposure period did not appear detrimental judging by the satisfactory low control mortality.

Table 35 (graph 30) shows the results of a biological assay in which P. brassicae larvae were exposed to filter papers impregnated with series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide. It will be seen that the mortality responses in this experiment were erratic, but indicate little or no synergistic action by the piperonyl butoxide. Examination of the filter papers used in this experiment after the exposure period,

showed them to be fairly heavily stained by a green fluid (presumably chlorophyll) excreted by the larvae. It was easy to see from casual observation that there was a gradation in the intensity of the staining, and therefore probably of the quantity of liquid excreted by the larvae, which corresponded roughly with the series of concentrations of pyrethrins.

Table 36 shows the results of an attempt to measure this gradation using the Hilger "Spekker" absorptiometer. Each pair of filter papers representing one of the concentrations of pyrethrins in table 35 was soaked in a known volume of distilled water for 30 minutes. At the end of this time the green coloration had been washed out of the filter paper giving a green solution. In this way 8 solutions were obtained, i.e., from the four concentrations of pyrethrins and from the four concentrations of synergised pyrethrins. The relative intensities of the green colouration in the solutions were measured with the spectrophotometer using a purple filter and the results are shown in table 36.

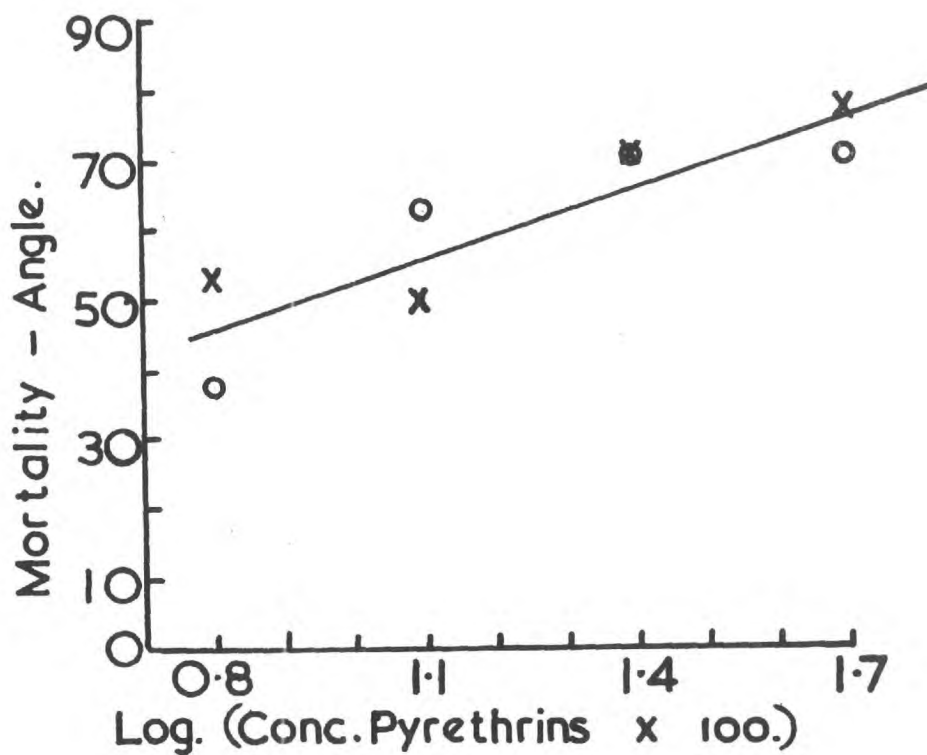
There was very little green staining on the filter papers used in the control and unfortunately the intensity of the colour from these was not measured. In order to get some idea of the relations of these values, it was convenient to give the highest concentration (the solution obtained from the filter paper impregnated with 0.5% pyrethrins plus 2.5% piperonyl butoxide) a value of 1.00, and to adjust the other values accordingly

(table 36). The larvae treated with synergised pyrethrins have excreted rather more than those treated with pyrethrins only.

The two regression lines, based on mortalities, for this experiment (graph 30) are irregular. If they can be interpreted at all, they suggest the absence of any synergistic action by the piperonyl butoxide. Yet the concentrations of the excretions of the affected larvae rise with increasing concentrations of synergists. Also, more has been excreted by the larvae subjected to synergised pyrethrins. This interesting secondary response which can be regarded as a sub-lethal response, is discussed in relation to similar responses by Galleria and Plodia in the in the discussion.

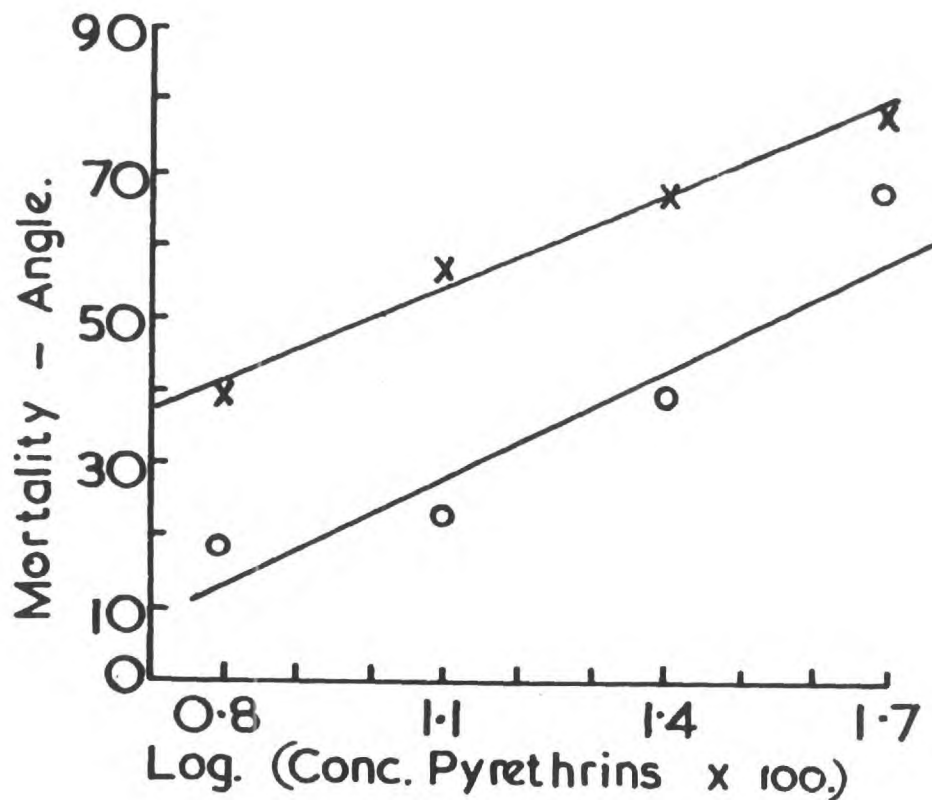
In the experiment shown in table 37 and graph 31, more satisfactory regression lines have been obtained for the toxicities of pyrethrins and of pyrethrins plus piperonyl butoxide towards P. brassicae larvae. The results show a small degree of synergistic activity by the piperonyl butoxide (factor of synergism of 3.5 at the median response dose) contrasting with the results of ^{table 35}~~experiment 8.1.~~

Graph 30: Exposure of P. brassicae larvae on filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 35.



Graph 31: Exposure of P. brassica larvae on filter papers impregnated with pyrethrins with and without piperonyl butoxide.
See table 37.

31.



9. Carpophilus dimidiatus adults.

This beetle is of particular interest as an insect for use in biological assay. It is easy to rear and both the adults and the larvae may be readily handled and used in crawling insect assays.

In culture C. dimidiatus adults emerged within a period of a few days about 6 weeks after the cultures had been set up. They were generally used in experiments when between one and two weeks old. The adult beetles were readily separated from the cultures if the culture, or part of it, was tipped on to the centre of an enamel tray at least 12 ins. x 18 ins. in area. After a few minutes the beetles began to leave the mass of the culture and walk towards the edge of the tray where they were easily collected with an aspirator.

The "crawling insect" technique of biological assay described in the section dealing with Calandra granaria was used in the assay of C. dimidiatus. Adult C. dimidiatus did not climb up the walls of the brass rings as readily as did C. granaria, and a very thin film of paraffin oil was wiped on to the inside surface of the rings to prevent this climbing.

In most of these experiments with both adult and larval C. dimidiatus, a sub-lethal "knockdown" response was used to assess the toxic effect of pyrethrins on the insects, similar to that

described above for C. granaria. In earlier experiments the insects were considered knocked down if, when pushed to the centre of a clean, white, 7 cm. diameter filter paper illuminated from one side by a 60 watt lamp at a distance of 16-20 cms. from the paper, they were unable to crawl off the filter paper in one minute. The insects sometimes tended to cling to the edge of the filter paper and even to walk round the perimeter. In later experiments this tendency was overcome by drawing a pencil circle 5.4 cm. in diameter (the internal diameter of the brass rings) on the filter paper and the insects were then considered knocked down if they were unable to cross this line within one minute. To dispose of the insects after their knockdown had been assessed, the filter paper was placed on the simple piece of apparatus shown in figure 4 so that after leaving the paper the beetles crawled or were pushed in to the 50% alcohol moat around the paper.

Table 38 and graph 32 show the results of an experiment in which adult C. dimidiatus were exposed to filter papers impregnated with pyrethrins at three concentrations. The mortality responses may be represented satisfactorily as a regression line. In this experiment the time of exposure (4 days) was exceptionally long and consequently a lower range of dosages of pyrethrins was used.

Tables 39 and 40 and graphs 33 and 34 show the results of experiments in which C. dimidiatus adults were exposed on filter papers impregnated with series of concentrations of pyrethrins and

of pyrethrins plus piperonyl butoxide, the latter in the ratio of one part of pyrethrins to five parts of piperonyl butoxide by volume. The toxicity of the pyrethrins was enhanced by the piperonyl butoxide and factors of synergism of 5.5 and 10.0 have been calculated at the median response rate in tables 39 and 40 respectively. This large difference in the two values for factors of synergism is surprising. In graph 34 (table 40) the two regression lines are not parallel and there would therefore be large differences in factor of synergism at different response rates. The considerable differences in the slope of the regression line in some of these experiments is difficult to explain.

Since C. dimidiatus proved easy to rear and adults were therefore available in larger numbers than were the other insects used in this work, the opportunity was taken to compare the synergistic activity of other compounds with that of piperonyl butoxide towards this insect. In table 41 and graph 35 the synergistic activity of the compound known as van Dyke 264 (N-Octyl bicycloheptane dicarbonimide) has been examined. The factor of synerism of 1.9 was calculated from the data of this experiment.

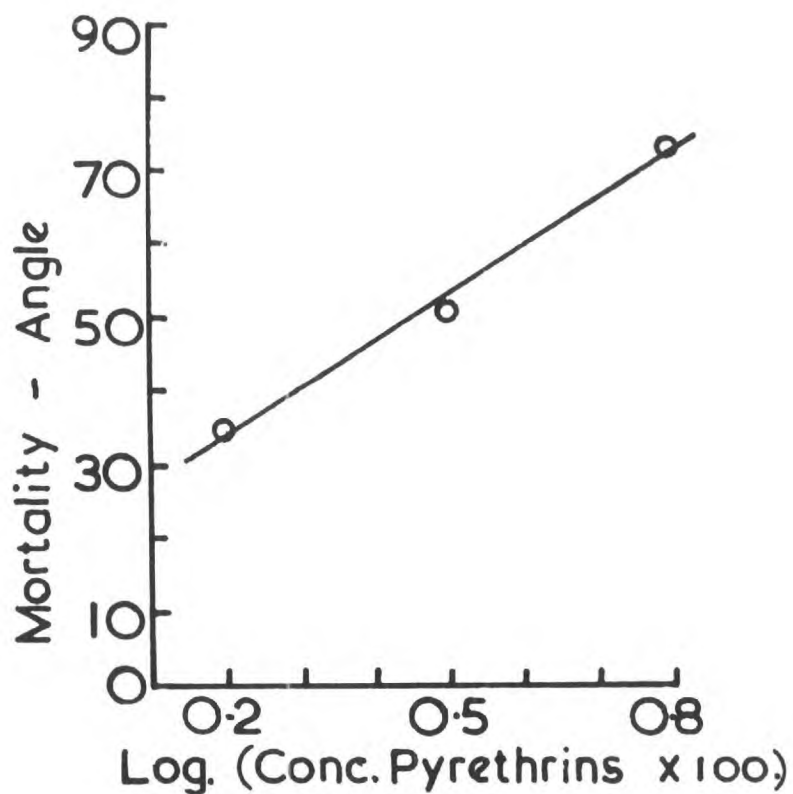
In table 41, the results of two similar experiments are recorded in terms of the mortality response after 24 and 36 hours. The calculations of the regressions have been based on the response after the longer exposure period.

In table 42 and graph 36, the responses obtained in

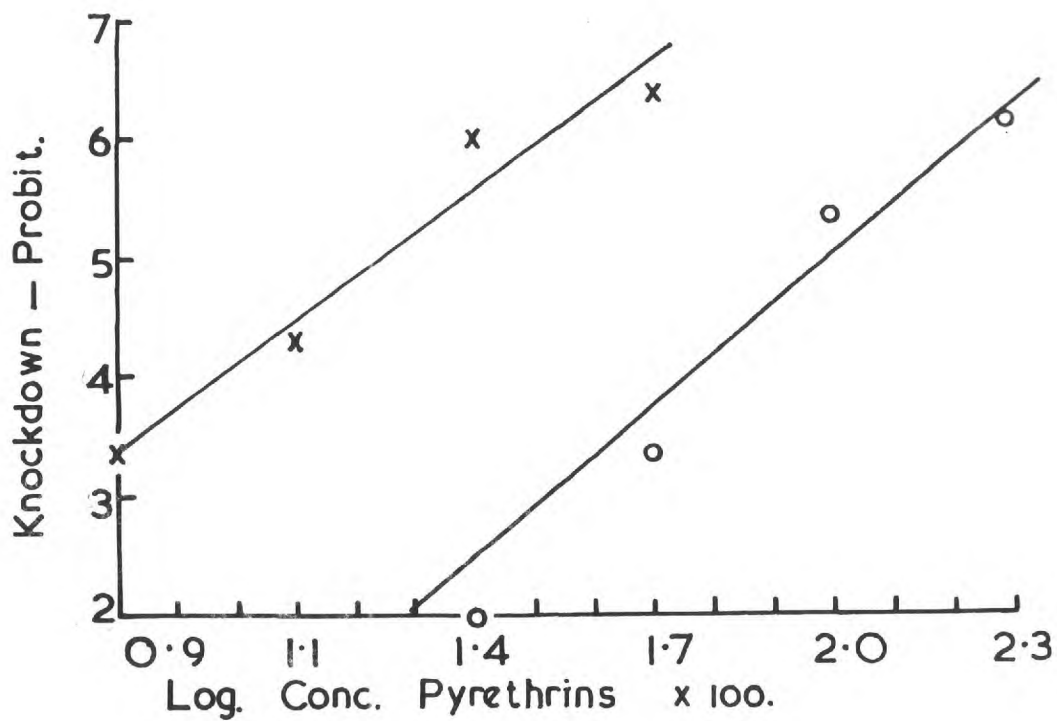
table 41 in the presence of the synergist after 24 and 36 hours exposure have been compared. In the biological assay of fumigants, the dosage is often given as a concentration/time product, that is as the product of the duration of exposure and the concentration of fumigant. Within limits the response to a similar concentration/time product is similar even if the duration and concentration vary. In table 42 concentration/time product values have been obtained by multiplying the duration of exposure to synergised pyrethrins (24 and 36 hours) by the concentrations of pyrethrins. The logarithms of these values were plotted against the angular transform of the response (graph 36) and a straight line has been obtained indicating that, in this experiment, the concentration time relationship held good.

In table 43 a direct comparison has been recorded of the synergistic activity of piperonyl butoxide and van Dyke 264. The results shown in table 43 (graph 37) indicate that a pyrethrins/piperonyl butoxide mixture is about 2.6 times as toxic to adult C. dimidiatus at the median response dose as a comparable pyrethrins/van Dyke 264 mixture.

Graph 32: Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins.
See table 38.

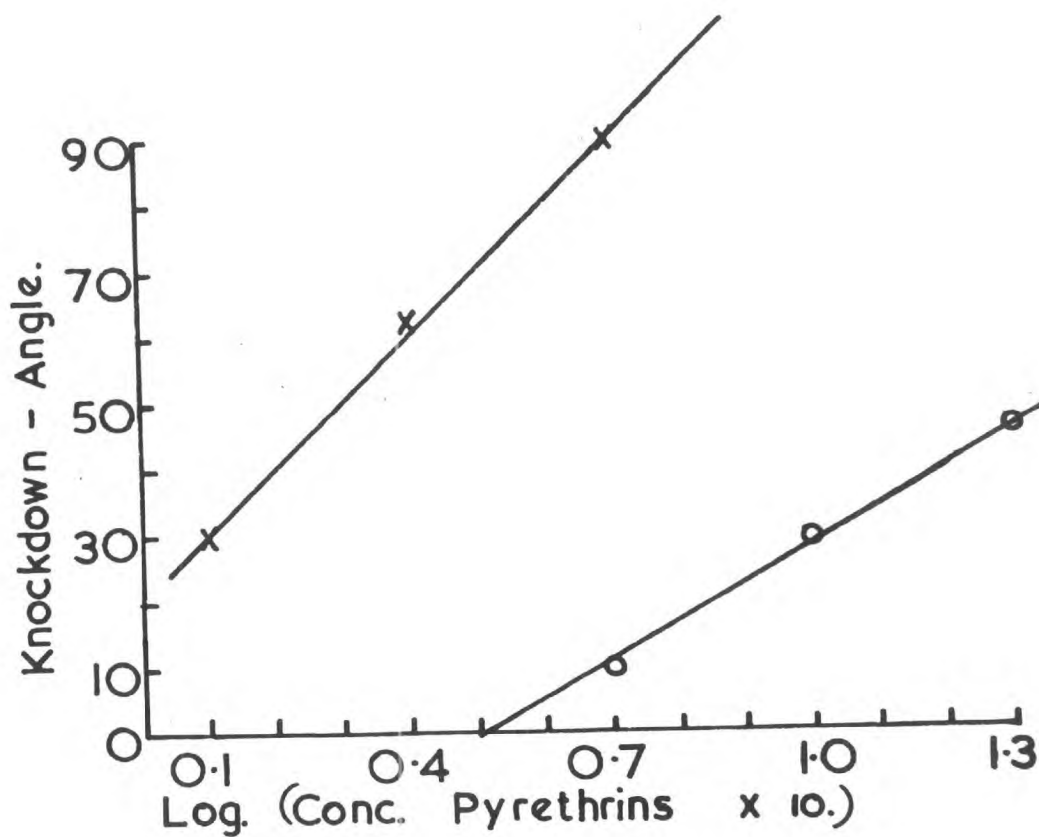


Graph 33: Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 39.



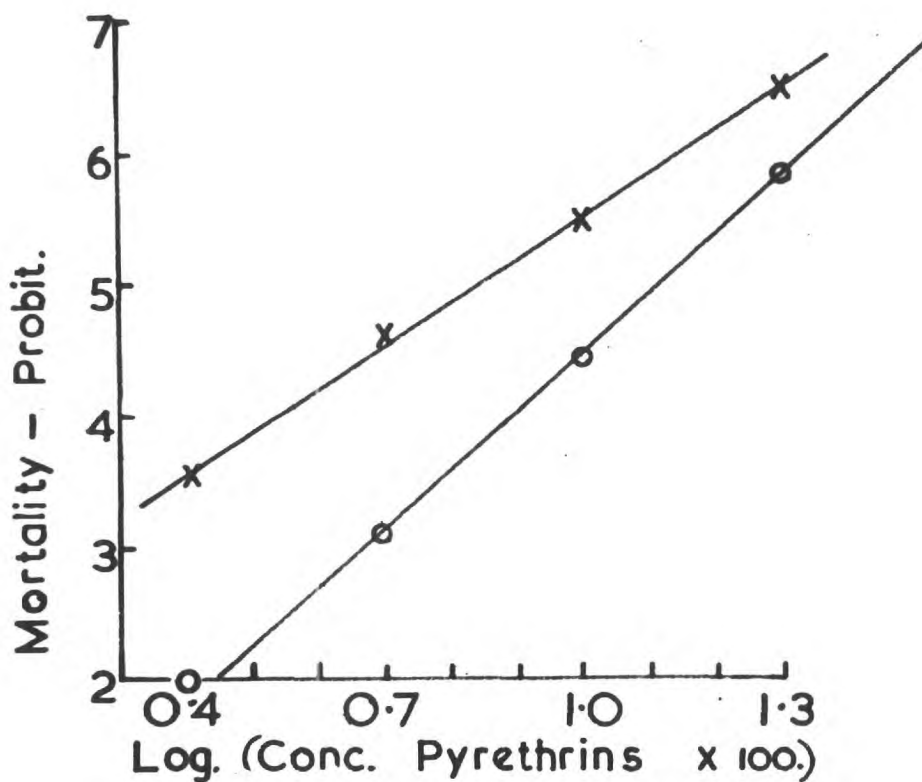
Graph 34; Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins with and without piperonyl butoxide.
See table 40.

34.



Graph 35: Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins with and without "van Dyke 264". See table 41.

35.

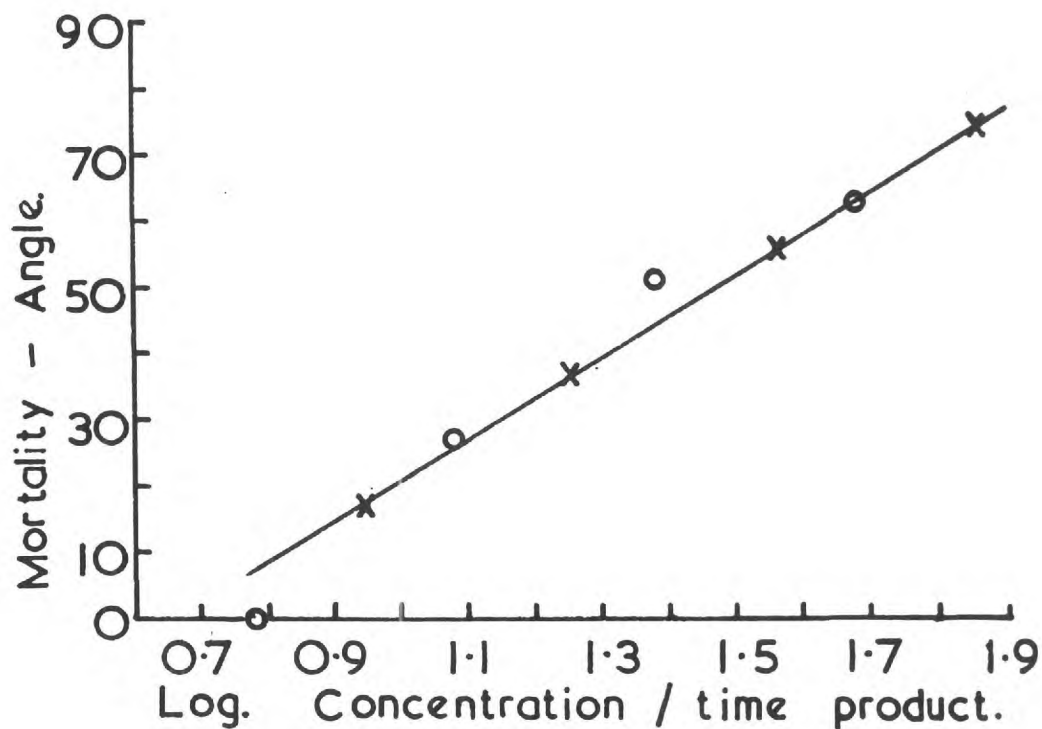


O Pyrethrins only

X Pyrethrins plus "van Dyke 264".

Graph 36: Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins for 24 and 36 hours.
See tables 41 and 42.

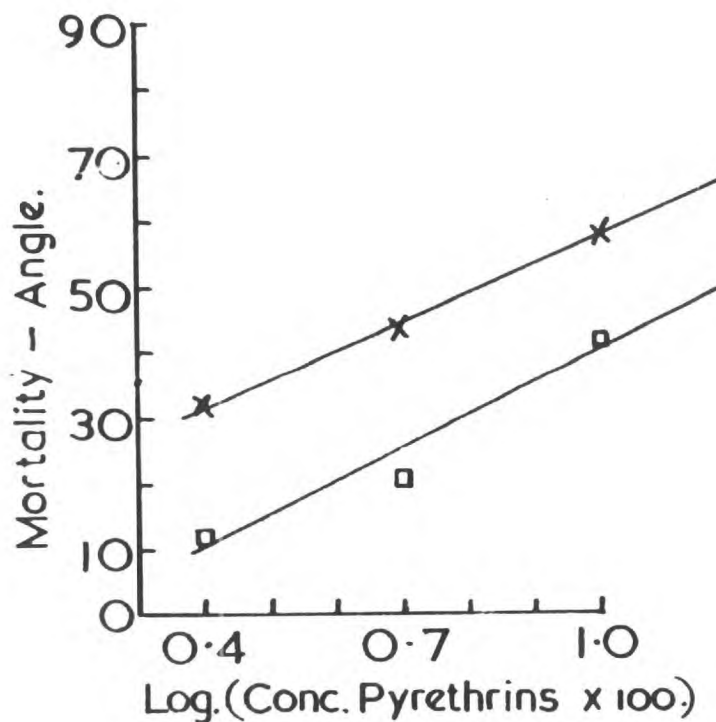
36.



0 - 24 hours exposure
X - 36 hours exposure.

Graph 37: Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins plus piperonyl butoxide and with pyrethrins plus "van Dyke 264".
See table 43.

37.



X Pyrethrins plus piperonyl butoxide.

□ Pyrethrins plus "van Dyke 264".

10. Carpophilus dimidiatus larvae.

Larvae of C. dimidiatus for use in biological assay were taken from the cultures 4-5 weeks after they had been set up. They could be very easily collected using the same method as was employed for collecting adults. The culture was tipped on to the centre of an enamel tray and within a few minutes the larvae began to crawl away across the tray and could be easily collected with an aspirator.

The larvae of this beetle move about freely and are quite unable to climb up most vertical surfaces, including the inside walls of the brass rings which were used to confine them. The method of biological assay was similar to that described above for C. dimidiatus or C. granaria adults, except that it was not necessary to apply a film of oil to the inside surface of the rings.

In all the experiments with C. dimidiatus larvae the toxic action of the pyrethrins has been assessed as knockdown, using the same response as was used with adult C. dimidiatus (see section 9 above), that is, the larvae were placed in the centre of a filter paper illuminated obliquely and those unable to crawl off the filter paper (earlier experiments) or out of a 5.4 in diameter circle drawn on the paper (later experiments) within one minute were counted as knocked down.

Table 44 and graph 38 show the results of two experiments carried out on consecutive days with larvae from the same culture.

In both, the larvae were exposed on filter papers treated with series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide. In the first case the concentrations of synergised pyrethrins used were too high and in the second they were slightly low. This experiment has been included because it demonstrates that larvae taken from the same culture on two succeeding days have about the same susceptibility to pyrethrins. The responses of insects to insecticides alter with age but 24 hours is not a long enough period to alter the susceptibility of C. dimidiatus larvae to pyrethrins. It would therefore be possible to compare results of assays on insects from the same culture made on succeeding days.

In the experiments recorded in table 45 (graph 39) C. dimidiatus larvae were again confined on filter papers treated with doses of pyrethrins and of pyrethrins plus piperonyl butoxide. Factors of synergism at the median response dose have been calculated to be 6.8.

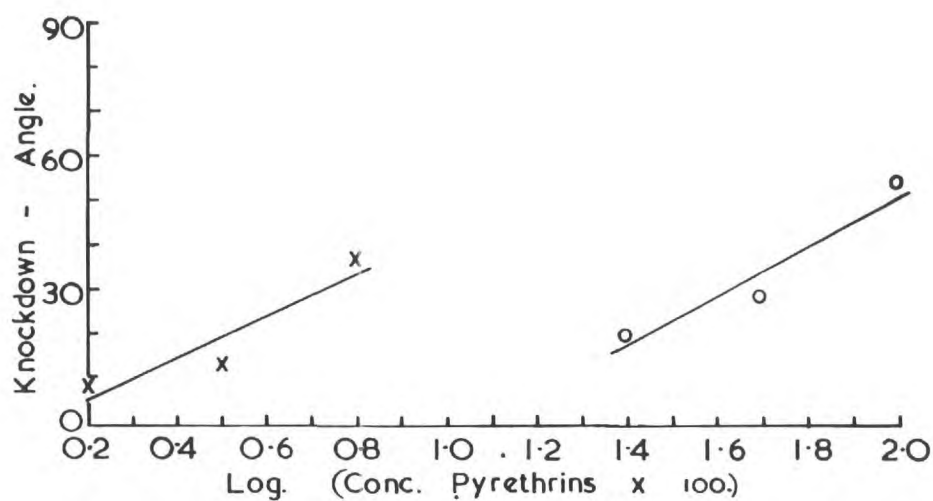
The enhancement of the toxicity of pyrethrins to C. dimidiatus larvae brought about by the addition to the pyrethrins of the compound known as van Dyke 264 has been investigated. In the experiments recorded in tables 46 and 47, C. dimidiatus larvae were confined on filter papers treated with series of doses of pyrethrins and of pyrethrins plus van Dyke 264. The factors of synergism have been calculated to be 7.9 and 2.2 in tables 46 and 47 respectively at the median response doses.

Table 48 has been reported, in spite of the unsatisfactory regression lines, for two reasons. Graph 42 gives some idea of the synergistic action of sesoxane. It also demonstrates an apparent absence of synergistic activity by van Dyke 264 which has been noticed in other experiments not recorded here, and yet contrasts with the results shown in tables 46 and 47.

This large difference in factors of synergism has not been investigated further; it was considered most likely to be due to incomplete solution of the van Dyke 264 in the formulation.

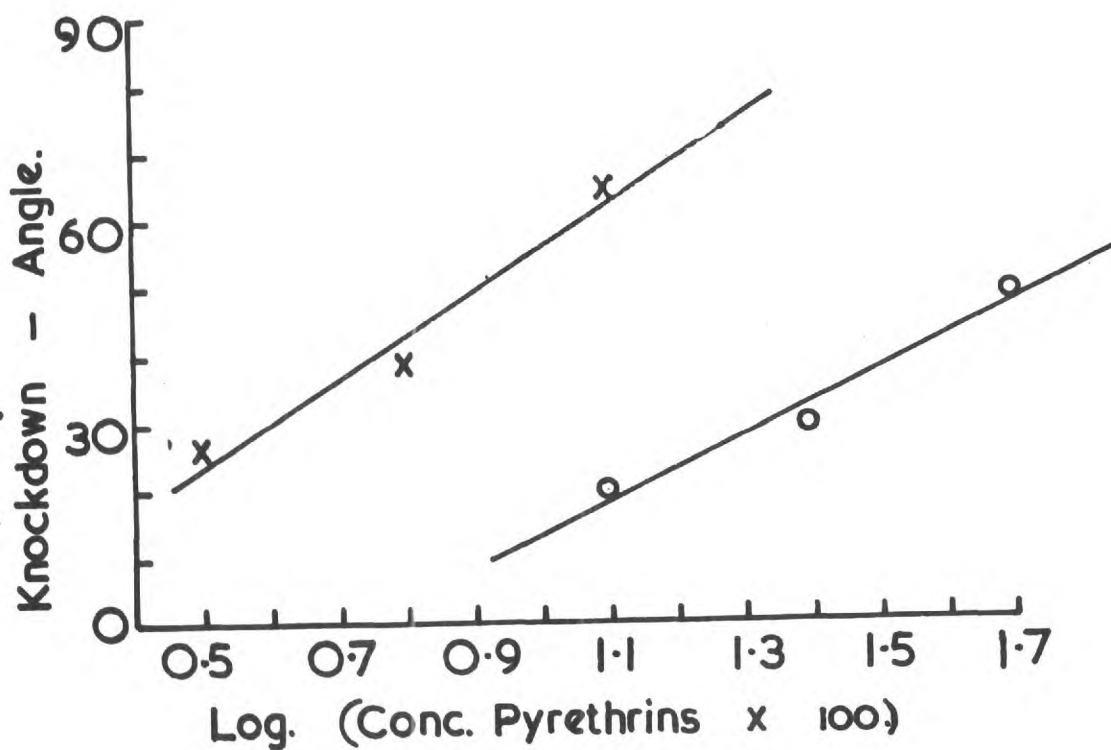
Graph 38: Exposure of C. dimidiatus larvae on filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 44.

38.



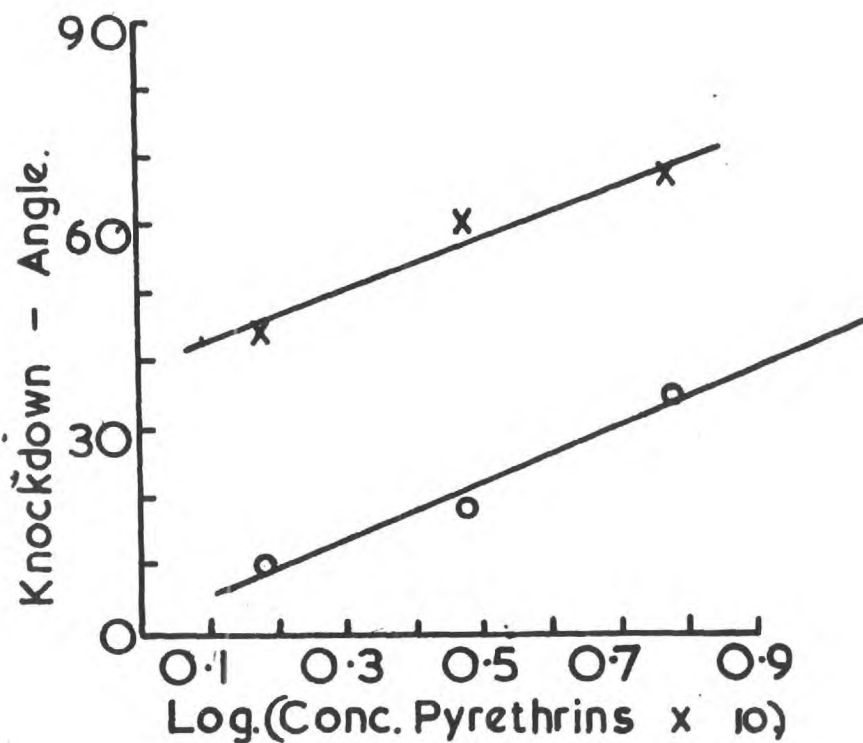
Graph 39: Exposure of C.dimitidatus larvae on filter papers impregnated with pyrethrins with and without piperonyl butoxide
See table 45.

39.



Graph 40: Exposure of C. dimidiatus larvae on filter paper impregnated with pyrethrins with and without "van Dyke 264"
See table 46.

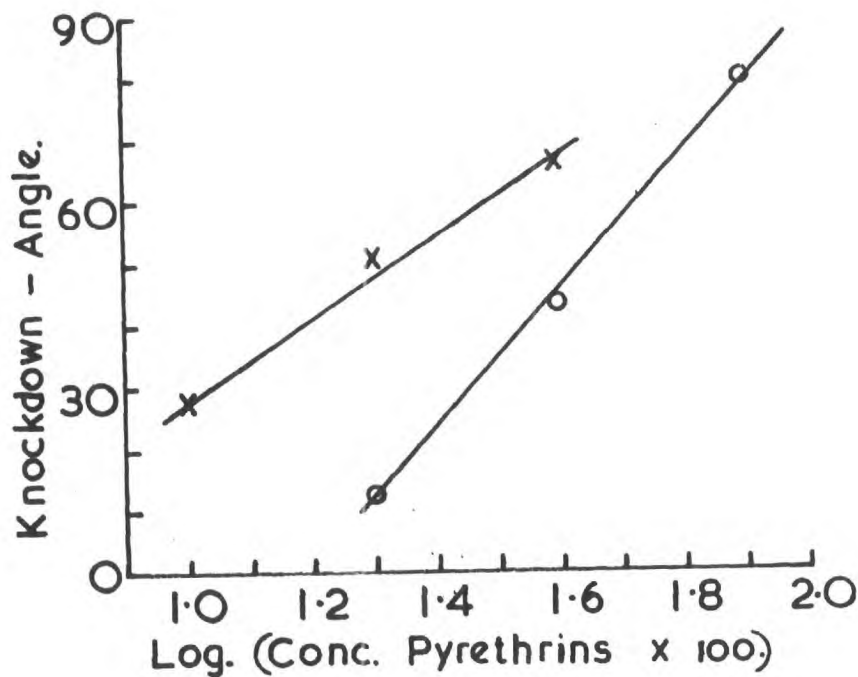
40.



O Pyrethrins only

X Pyrethrins plus "van Dyke 264".

Graph 41: Exposure of C. dimidiatus larvae on filter papers impregnated with pyrethrins with and without "van Dyke 264". See table 47.

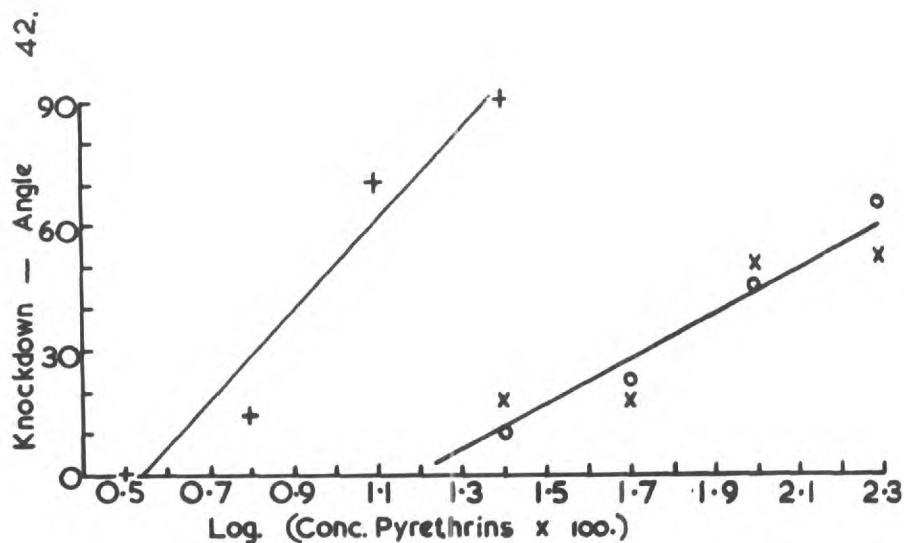


O Pyrethrins only

X Pyrethrins plus "van Dyke 264."

42
Graph 48:

Exposure of C. dimidiatus larvae on filter papers impregnated with pyrethrins, with pyrethrins plus "van Dyke 264", and with pyrethrins plus sesoxane. See table 48.



- O Pyrethrins only
- X Pyrethrins plus "van Dyke 264"
- + Pyrethrins plus sesoxane.

11. Lasius fuliginosus.

As already stated, Lasius fuliginosus was not cultured, but adult workers were collected as required from a colony close to the laboratory.

Worker ants taken from the same nest are likely to be a very homogenous population and it might be expected that this would produce steep regression lines in the results of biological assays. On the other hand, little is known of the age distribution save that some workers live for at least 18 months. A wide range in the age of the test insect may lead to large differences in their susceptibilities in pyrethrins which would tend to reduce the slope of the regression lines. It will be seen from graphs 43-45 that the lines are not in fact particularly steep.

Biological assays with L. fuliginosus workers were carried out using the crawling insect assay method similar to that described above for Calandra granaria. The polished brass rings did not prevent the ants climbing off the filter papers, but they are active insects under these conditions and this shortening of the exposure period was therefore considered to be fairly uniform.

The following points were found to be important in obtaining satisfactory results in assays with these ants, and particularly in reducing the control mortality as much as possible.

1. The use of carbon dioxide to anaesthetise the ants

during collection was impossible because a large proportion (about 40%) did not recover from the anaesthetic.

2. The ants were collected in 2" x 1" specimen tubes using a small camel-hair brush in lots of five and were immediately transferred to the impregnated filter papers. Any delay at this stage increased the control mortality. This may have been due, in part at least, to the absence of filter paper or other absorbant in the tubes to soak up the secretions, presumably formic acid, of the insects which were agitated by being in a confined space. For this reason a clean specimen tube was used for each lot of ants.

3. If too many ants were confined on one filter paper the result of the subsequent fighting and secretion was again a heavy control mortality. It was found best to confine not more than five ants together.

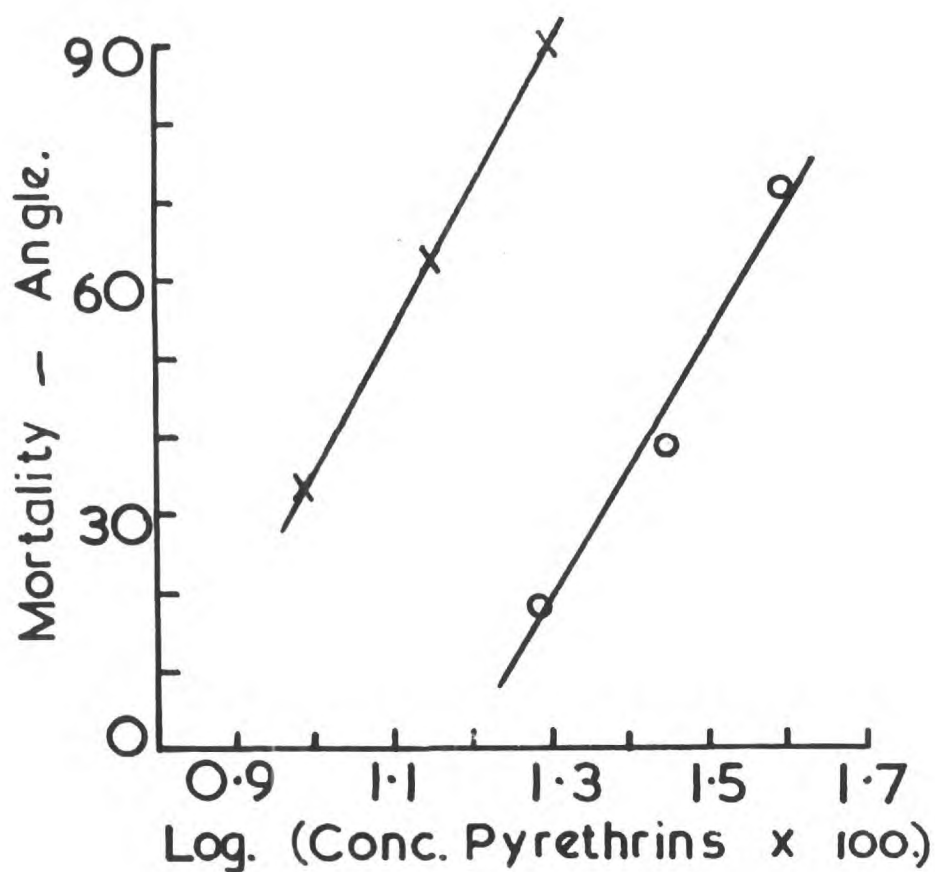
4. High control mortalities were also obtained if the ants were exposed on filter papers for periods of 24 hours. This was overcome by increasing the concentrations of insecticide and reducing the exposure time (table 49) to three hours.

The response was death, which was easily determined. Unaffected ants were found on inspection to be moving actively in the ring, or else they could be very easily stimulated to activity.

The worker ants were exposed on filter papers impregnated with series of doses of pyrethrins and of pyrethrins plus piperonyl butoxide. The results of these experiments in tables 50-52 (graphs 43-45 demonstrate a low level of synergistic activity by the piperonyl butoxide.

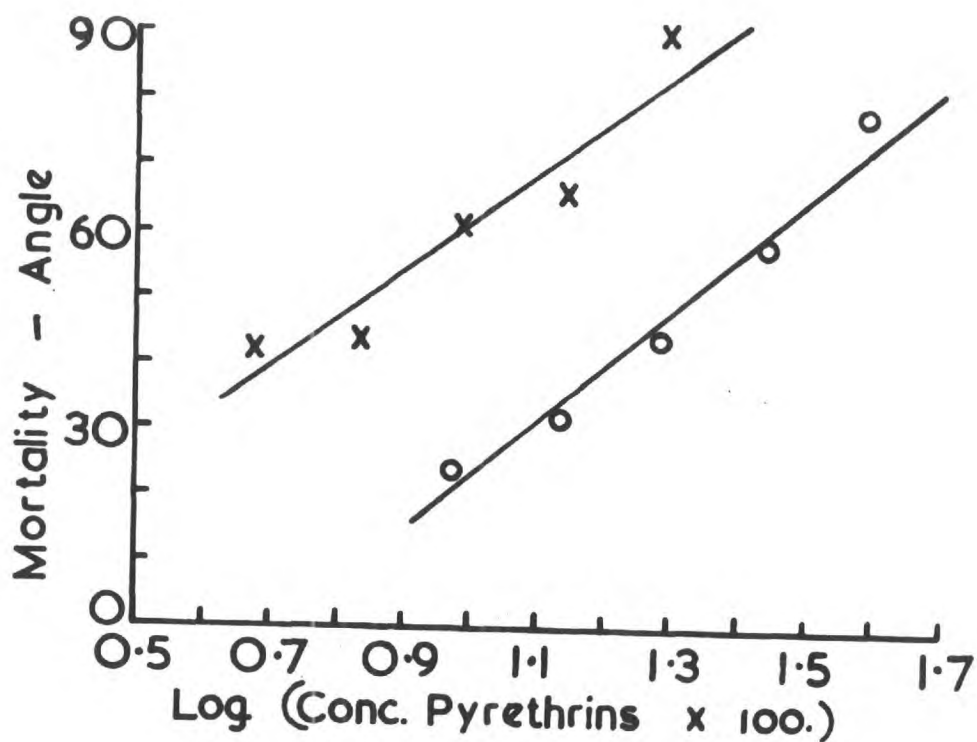
Graph 43: Exposure of L. fuliginosus workers on filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 50.

43.



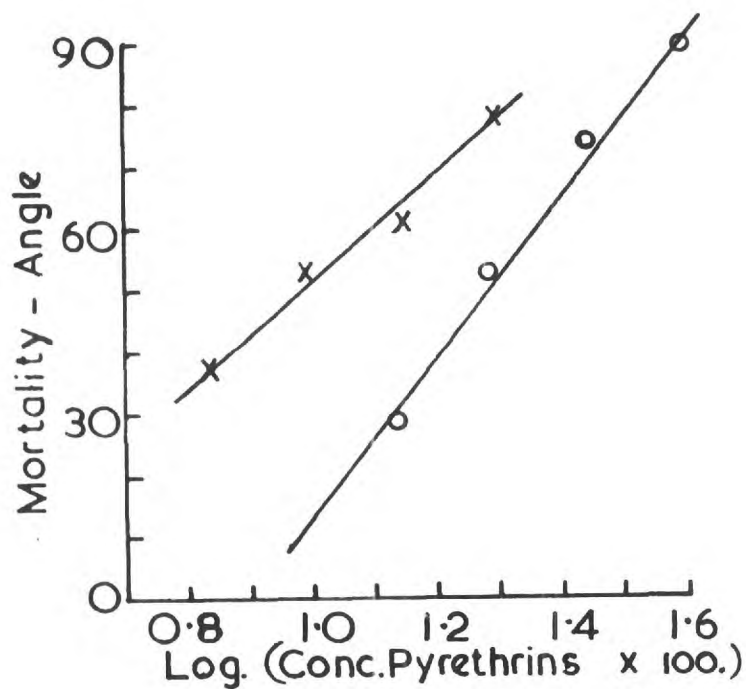
Graph 44: Exposure of *L. fuliginosus* workers to filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 51.

44.



Graph 45: Exposure of *L. fuliginosus* workers on filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 52.

45.



12. Acyrtosiphon pisum.

The results of biological assays using this aphid (cultured by Mr. A. P. Mainwaring), proved to be more satisfactory than had at first seemed possible.

In the final experiments nymphs between 6 and 8 days old were used; they were then in about their 6th instar. Under the conditions of culture they normally required 10 days to become adults.

The response used in all these assays is described as a knockdown. When healthy A. pisum are lightly touched dorsally with a fine camel-hair brush they react by moving sideways slightly and often also continue to walk. It was also often easy to distinguish by their appearance aphids which had responded to pyrethrins. Affected insects would be found with their legs in unusual attitudes. They could often be easily distinguished from aphids dying from other causes because of the asymmetrical disposition of those affected by pyrethrins. While these observations were not used as criteria of response, they were a useful aid in preliminary assessments.

Attempts to carry out crawling insect assays with A. pisum were not successful. Two lots of adults were exposed to filter papers and confined in the electric barrier. One of the filter papers was clean and the other impregnated with the mixed diluent (20% Risella oil, 80% light petroleum), used in previous experiments to leave a deposit of the oil only. The aphids were exposed for

an hour in the laboratory and at the end of this time 2/25 insects on the clean paper failed to respond and 19/19 on the oiled paper failed to respond. The electric barrier was used because the aphids could fairly easily climb up vertical surfaces.

A number of other attempts was made to devise crawling insect assays in which the control response was satisfactory, including confining the aphids on filter papers in petri dishes and some success was obtained as shown in table 53. However, this method of assay was eventually abandoned.

It was concluded that the following conditions were necessary for successful biological assay employing this aphid.

i. The insects should be kept at a constant temperature and humidity for at least 20 minutes before the experiment. They should not be moved from a lower to a higher temperature as the variation in temperature stimulates the aphids to great activity, making them almost impossible to handle.

ii. The survival of A. pisum is influenced by humidity and lowest control responses were obtained at higher humidities. 70% relative humidity was employed in this work.

iii. The aphids should be separated from the host plant for the shortest possible time, and handled as little as possible.

iv. This aphid is extremely sensitive to oil, and eventually none was used in the insecticide solutions.

v. Late instar nymphs were found to be the most convenient stage for biological assay.

After a number of trials taking these considerations into account, the biological assays were carried out by spraying the insects in a spray tower designed by Potter (1952) with pyrethrins dissolved in light petroleum (boiling point 60-80°F).

The aphids were collected from the plants into a large beaker using camel-hair brushes. They were counted into 9 cm. petri dishes having a 9 cm. white filter paper in the bottom. After at least 20 mins. and not more than 30 mins. in the room with the "Potter" tower (temperature 25°C, humidity 65% $\pm$ 10%) they were sprayed using the tower. 2 ml. of solution was used for each spraying. After spraying, the aphids were at once transferred to clean 7 cm. petri dishes containing a 7 cm. filter paper and a freshly picked broad bean leaf. They were left in the same constant temperature room until the response was assessed.

As already stated, insects were considered knocked down if they failed to move when gently touched with a fine camel-hair brush. Most of the surviving insects of an assay (about 70-90%) would be found on the bean leaf.

When two lots of A. pisum nymphs were sprayed, one with 2 cc. of mixed diluent and the other with 2 cc. of light petroleum, 4 out of 8 of the former were knocked down, while all (10) of the latter were unaffected 18 hours after exposure.

Table 53 shows the results of an experiment in which four lots of A. pisum nymphs were sprayed with a series of concentrations

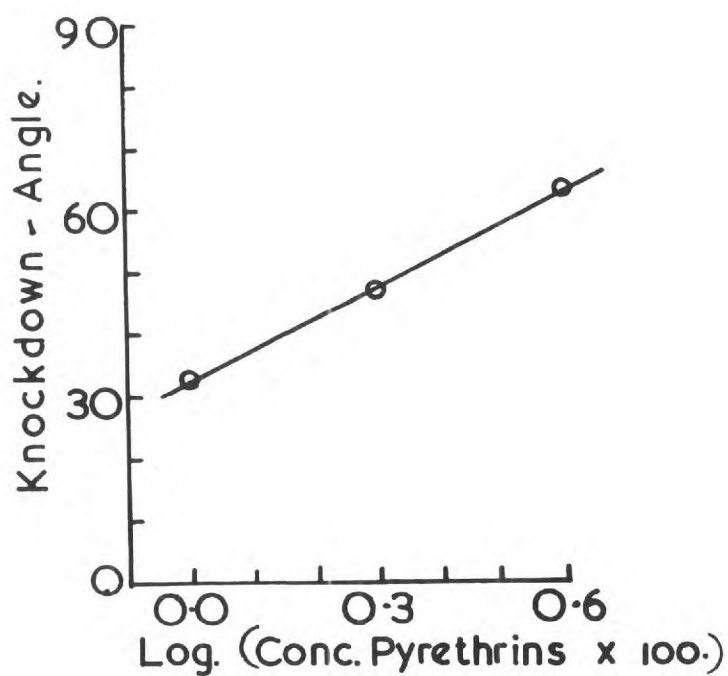
of pyrethrins and two lots were exposed to concentrations of pyrethrins on filter papers, prepared as previously described for Calandra granaria, in petri dishes for 45 minutes. The knockdown at 3, 17 and 40 hours after the end of treatment has been recorded. There has been a marked recovery from the initial knockdown effect of the pyrethrins, as is commonly found with this insecticide. However, on this occasion there was a remarkable (9/10) control response for the sprayed insects after 3 hours; this observation could not be repeated.

Table 54 and graph 46 show the results of an experiment similar to that described above in which late instar nymphs of A. pisum were sprayed with three concentrations of pyrethrins. The response was assessed 21 hours after treatment. Four lots of small 1st-3rd instar nymphs were sprayed at the same time with the same solutions. After 21 hours the responses of these smaller nymphs were 21/21, 3/3 and 5/5, corresponding to concentrations of pyrethrins in the sprays of 0.04, 0.02 and 0.01% respectively. The control knockdown was 9/11 showing that this form of assay is unsuitable for younger A. pisum nymphs.

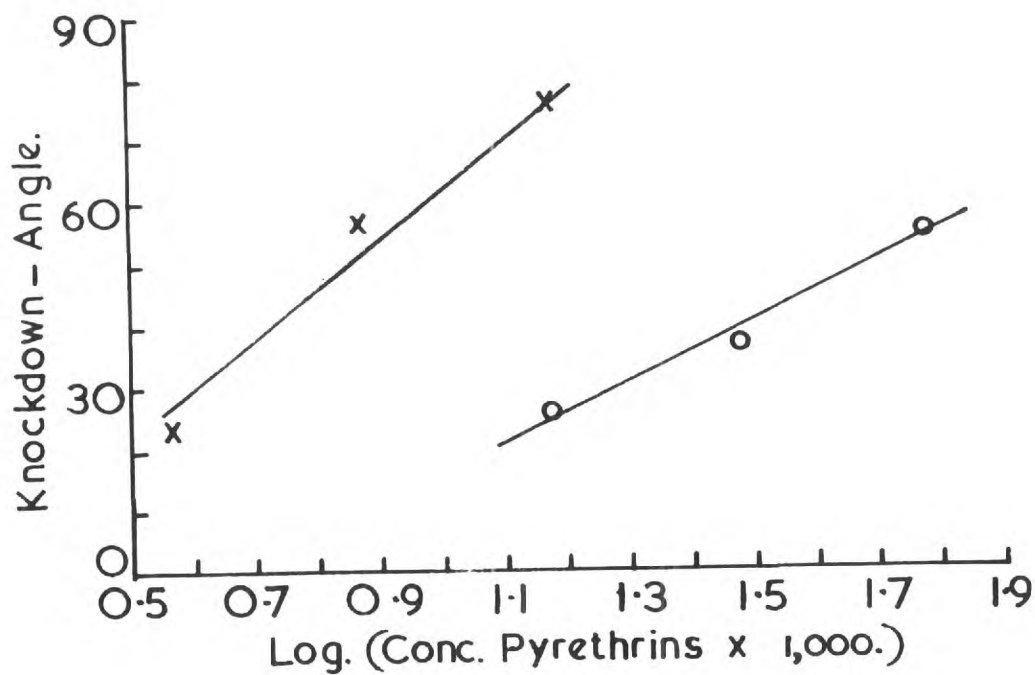
In the experiments recorded in tables 55 and 56 (graphs 47 and 48), A. pisum nymphs between 6 and 8 days old have been sprayed with solutions of pyrethrins and of pyrethrins plus piperonyl butoxide in light petroleum. Factors of synergism have been calculated to be 7.2 and 6.4 at the 50% response level.

Graph 46: A. pisum sprayed with pyrethrins.
See table 54.

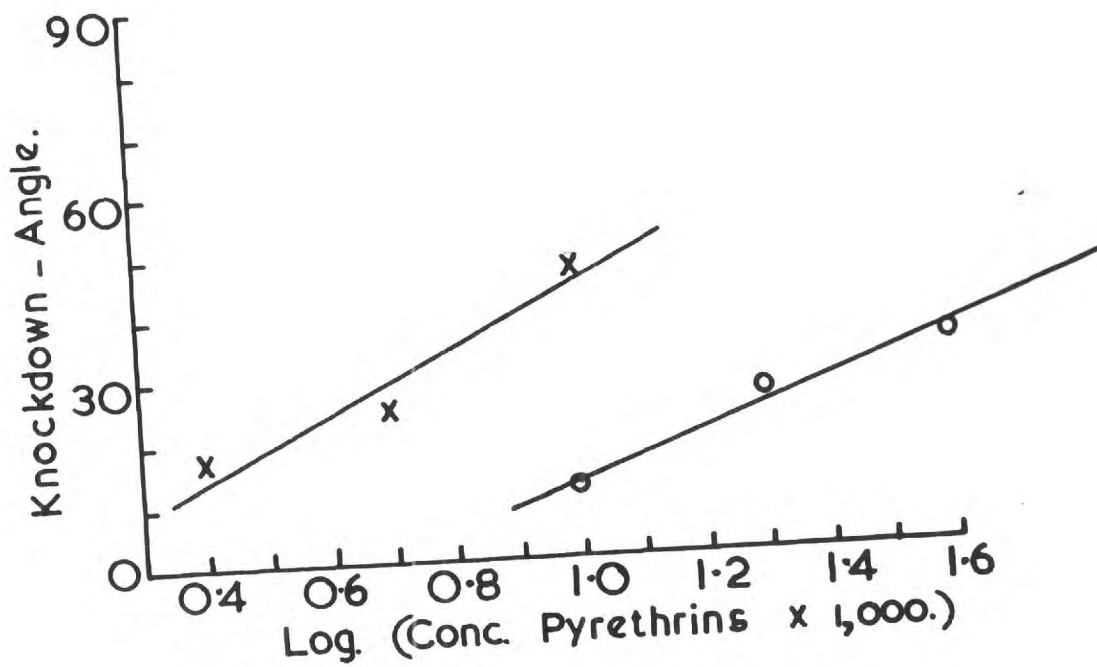
46.



Graph 47: A. pisum sprayed with pyrethrins with and without piperonyl butoxide.
See table 55.



Graph 48: A. pisum sprayed with pyrethrins with and without piperonyl butoxide.
See table 56.



13. Phaedon cochleareae.

When the original egg laying period in cultures of Phaedon cochleareae (the mustard beetle) had been restricted to 48 hours, the adults all emerged within a few days of one another. The adult beetles lived in culture for several weeks and were used in these experiments 7-10 days after emergence.

Adult P. cochleareae were used to assay series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide by confining them on filter papers impregnated with the insecticide solutions by the method already described.

The adult beetles can climb up vertical surfaces fairly easily but were confined to the filter papers by electrified barriers.

In the last experiment (table 60) of this series, the response to the insecticide recorded was mortality. This is an easily determined response; the insects' legs fold against the abdomen and thorax and the insects generally roll over onto their backs.

A knockdown response has also been used - the criterion being the inability of the adults to leave a 5.4 diameter pencil circle on a clean filter paper within one minute when placed on the centre of the paper and illuminated obliquely with a 60 watt bulb.

During the period between the end of exposure to the insecticide and before assessment of response, the beetles were confined over clean filter paper by brass rings, not by the electrified barrier and were given a small piece of cabbage leaf.

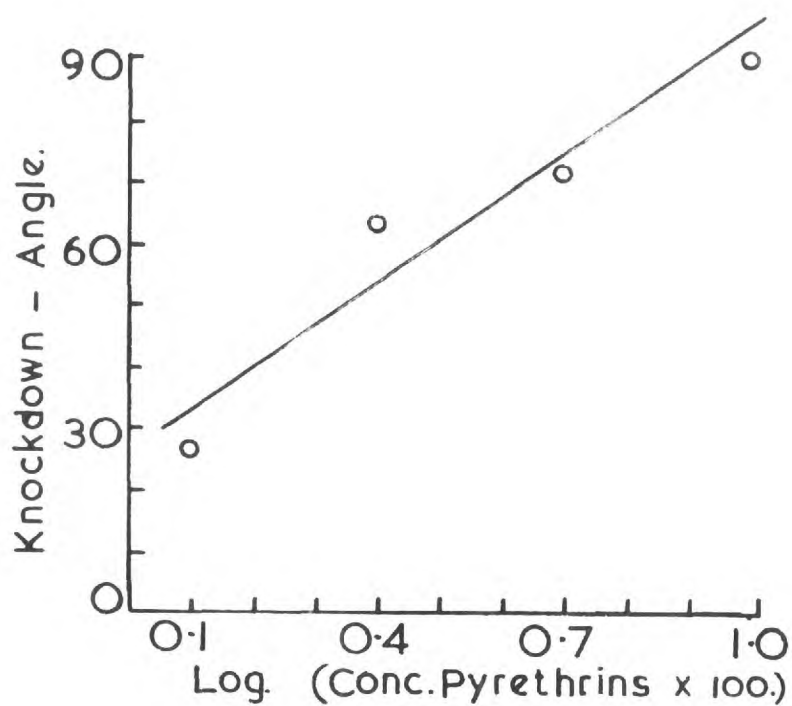
In the experiment recorded in graph 49 and table 57, the P. cochleareae were exposed on filter papers impregnated with a series of four concentrations of pyrethrins. The response is knockdown.

In experiments recorded in tables 58-60, the adult beetles have been used in the assay of series of doses of pyrethrins and of pyrethrins plus piperonyl butoxide. In table 58, the wrong range of concentrations of pyrethrins plus piperonyl butoxide was chosen and a regression line was not obtained. In table 59 shortage of insects reduced the number of individuals at each point to 10, which cannot be considered sufficient; in spite of this a remarkably good pair of regression lines was obtained (graph 50) which compare well with those shown in table 60 based on 20 insects per point. The results in tables 58 and 59 are based on knockdown, and those of 60 on mortality. Factors of synergism were calculated as 2.3 in table 60 and estimated as 2.9 in table 59.

The larvae of the mustard beetle crawl freely over their host plant during feeding and would probably be satisfactory tests insects for biological assay. The author has not carried out any experiments using these larvae.

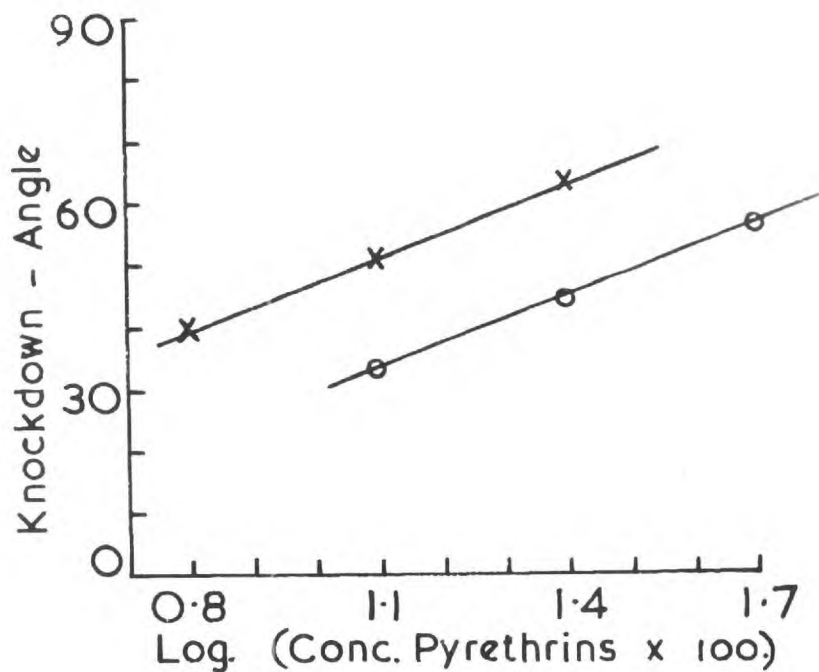
Graph 49: Exposure of P. cochleariae adults on filter papers impregnated with pyrethrins.
See table 57.

49.



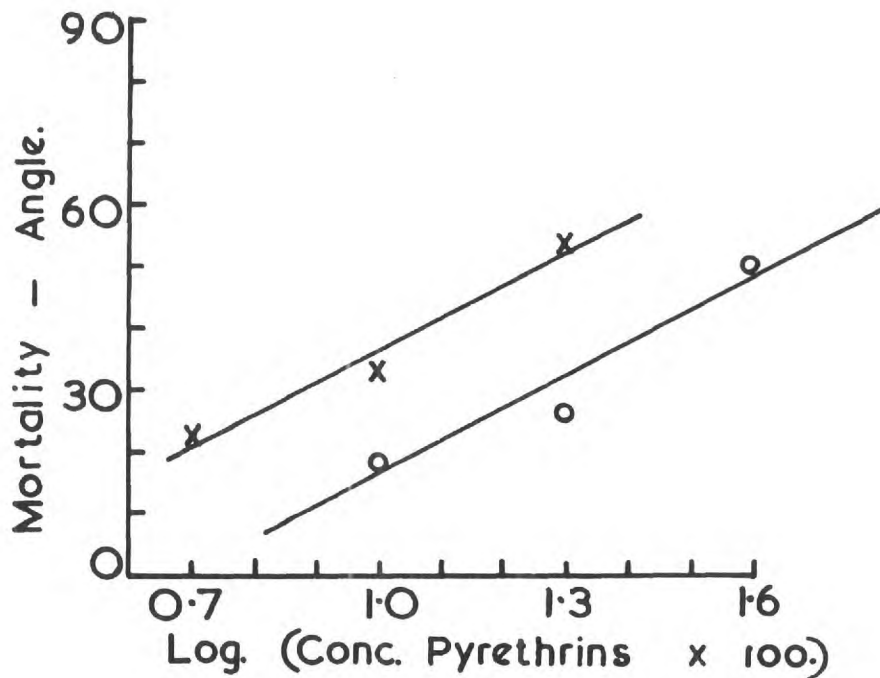
Graph 50: Exposure of P. cochleariae adults on filter papers impregnated with pyrethrins with and without piperonyl butoxide.
See table 59.

50.



Graph 51: Exposure of P. cochleariae adults on filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 60.

51.



14. Dysdercus fasciatus.

Although Dysdercus fasciatus (the cotton stainer) is considered easy to rear in culture, the author had some difficulty in rearing insects for these experiments and there was seldom a sufficient number available for biological assay. Consequently only a limited number of experiments could be carried out.

The fourth nymphal instar was chosen for assay. In the absence of any other considerations this was the most convenient size for experiment. The nymphs were confined over filter papers with the brass rings and small glass plates during exposure. They could easily climb over the surfaces of the ring and glass plate; but as they were active and the exposure period was relatively long, it was assumed that the overall time spent in contact with the insecticide-impregnated filter paper would be approximately the same for each individual insect. Because of the insect's size and long legs the electrified barrier in the dimensions described above was useless in preventing the nymphs from leaving the filter papers.

The response used in these experiments is termed mortality. This was fairly easy to determine, the insects were found to be either able to walk, even if only slowly, or else unable to walk and nearly always completely motionless. There was thus a surprisingly clear distinction between affected and unaffected insects.

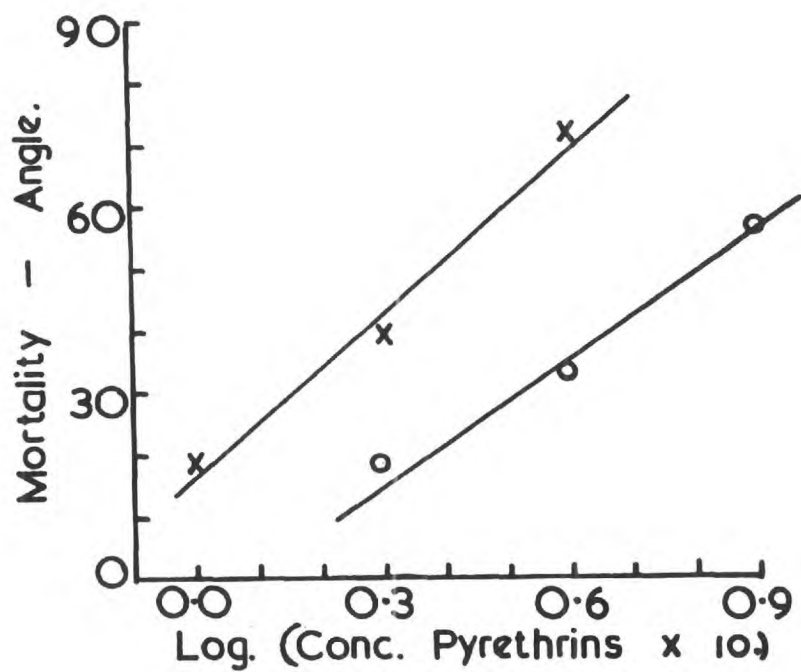
Tables 61 and 62 show the results of biological assays

in which fourth instar nymphs of D. fasciatus were exposed to filter papers impregnated with series of concentrations of pyrethrins and of pyrethrins plus piperonyl butoxide for 22 hours. In experiment 62, the factor of synergism has been calculated to be 2.6.

A preliminary experiment in which 0.05 μ l. of pyrethrins in odourless kerosene was topically applied at two concentrations (and control) to lots of 6 5th instar nymphs of D. fasciatus using Kerr's method, suggested that this form of assay could easily be carried out using the cotton stainer as test insect.

Graph 52: Exposure of D. fasciatus nymphs on filter papers impregnated with pyrethrins with and without piperonyl butoxide. See table 62.

52.



SECTION II.

THE BIOLOGICAL ASSAY OF PYRETHRINS RESIDUES USING THE BRINE SHRIMP ARTEMIA SALINA.

The use of biological assay methods to determine residues of pesticides and other poisons in plant and animal tissues has been considerably developed in recent years, and it has been found possible to obtain quantitative estimates of concentrations of such residues by reference to standards containing known concentrations of the compound being assayed. Dewey (1958) summarised these methods.

Michael, Thompson and Abramovitz (1956) described the use of the brine shrimp, Artemia salina in the detection of insecticide residues. An attempt has been made in the present work to demonstrate the potentialities of the brine shrimp as a test organism for the detection of pyrethrum residues in insects, in the belief that experiments of this nature might provide some information about the mode of action of pyrethrins synergists. Two important considerations commend the brine shrimp's use in biological assay; first the eggs like those of Aedes aegypti may be stored for months until required and secondly, if the first instar nauplii are used for biological assay, the culturing technique is very simple.

Because of the complex chemistry of the pyrethrins it seemed particularly desirable to demonstrate a sensitive method of biological assay of these principles. The unstable nature

of pyrethrins necessitated a number of special precautions during those assays for which residual doses of the insecticide needed to be determined after extraction from insect tissue. A simpler procedure may be satisfactory in assaying other more stable insecticides.

Biological assay using *Aedes aegypti* larvae.

The most thoroughly investigated of the methods of biological assay of pesticide residues is that of the Yellow Fever mosquito, *Aedes aegypti*, which has been described by Burchfield et al. (1952, 1955). A few experiments were carried out using the mosquito larvae method of assay to provide a comparison with the brine shrimp technique.

Eggs of *A. aegypti* laid on filter paper can be kept in the dry state for long periods and still retain their viability. When larvae are required, the eggs are placed in water when they hatch in a few days. The developing larvae are fed on an infusion of dry biscuit and "Bemax" which is added to the water as required. In this experiment a suspension in water of the substance to be assayed was obtained and the mosquito larvae were released into this suspension. After a predetermined period of exposure in the solution, the mortality or paralysis of the larvae was estimated, generally as the proportion failing to show the normal negative phototropism when illuminated obliquely.

One Carpophilus dimidiatus adult was killed by contact with a filter paper impregnated with pyrethrins. It was then washed in acetone, and thoroughly macerated in about 10 cc. of acetone. The resulting suspension was added to 50 cc. distilled water containing fourth instar A. aegypti larvae. After one minute the knockdown (failure to respond to light) was 19/35 and after ten minutes it was 35/35. A control treatment at the same time gave knockdown values of 2/30 after five minutes and 19/30 after 45 minutes exposure of the larvae.

Table 63 shows the knockdown responses obtained when 0.5 cc. of an acetone solution containing 0.00025% pyrethrins was added to 50 cc. distilled water containing fourth instar A. aegypti larvae. The table also shows results obtained when a solution of pyrethrins plus piperonyl butoxide was added and demonstrates the sensitivity of A. aegypti larvae to the pyrethrins.

Culture of Artemia salina.

Preliminary experiments were carried out with adult A. salina, later the first instar nauplii were used exclusively.

A. Culture of adult brine shrimps.

The eggs of A. salina are imported from the United States and are commonly used as food for tropical fish. Eggs may be obtained from suppliers of biological materials and from many pet stores. Usually 60-70% of the eggs hatched but a number of non-viable batches were purchased.

The brine shrimps were bred in an artificial saline solution having the following formula:

Sodium chloride	60 grams.
Calcium sulphate	3.4 "
Magnesium sulphate	6.4 "
Potassium chloride	1.6 "
Sodium bromide	0.2 "
Distilled water	2000 ml.

This is the formula of Michael et al. except that they also include 17 g. of magnesium chloride and adjusted the pH to 9.5 with sodium hydroxide. This adjustment could not be made because at pH 9.5 the magnesium was precipitated and the instruction is presumably a misprint. If the pH of the above solution was adjusted to 8 with sodium hydroxide it was found to be quite satisfactory for rearing A. salina.

The shrimps were reared in glass bowls 16 cms. in diameter filled to a depth of 4 cms. The eggs were left floating on the surface of the saline, confined by a hollow, floating plastic ring, for 48 hours to allow the nauplii to hatch and were then removed. The cultures were kept covered by a glass plate at 27°C. and the level of liquid was maintained by topping up with distilled water when necessary. The food provided was bakers' yeast which was added to the cultures very sparingly. Any excess of food quickly caused the putrefaction and death of the culture. Under these conditions adult shrimps were obtained in about three weeks. The

adults to be used in biological assay were transferred to clean saline 24 hours before the experiment.

No attempt has been made to maintain self-perpetuating cultures.

B. Culture of first and second instar nauplii of the brine shrimp.

After preliminary experiments the use of adult A. salina in biological assay was abandoned in favour of the use of 24-48 hour nauplii. These nauplii were obtained by floating the eggs in a covered beaker of saline kept at 25°C for 24-48 hours to allow the nauplii to hatch. No food was required and the technique was therefore very simple and convenient.

Biological assay using adult Artemia salina.

The method suggested by Michael et al. was used for biological assays with adult A. salina. About 15 minutes before the experiment was started the adult shrimps were transferred to double strength saline solution; this was possible because of the wide range of salt concentrations which this animal will tolerate. Batches of five adult shrimps were placed in 2 ml. of double strength saline solution in a series of test tubes 7 mm. in diameter and 75 mm. in length. To each of these tubes was added 1 ml. of pyrethrins extract also in double strength saline solution. At intervals the shrimps were examined and the response was estimated as the number of shrimps which had ceased active swimming movements. Because of the high salt concentration of the solution, any inactive shrimps float to the surface where Michael et al. claim they may be fairly easily counted. In practice this was found to be quite difficult as healthy shrimps also cease active swimming from time to time and float to the surface. To distinguish between "resting" and knocked down adult A. salina, the tube had to be quite violently shaken and the end point was not clear.

Tables 64 and 65 (graphs 53 and 54) show the results of experiments in which A. salina were exposed to pyrethrins under these conditions for series of times, and a regression of knockdown with time of exposure has been obtained.

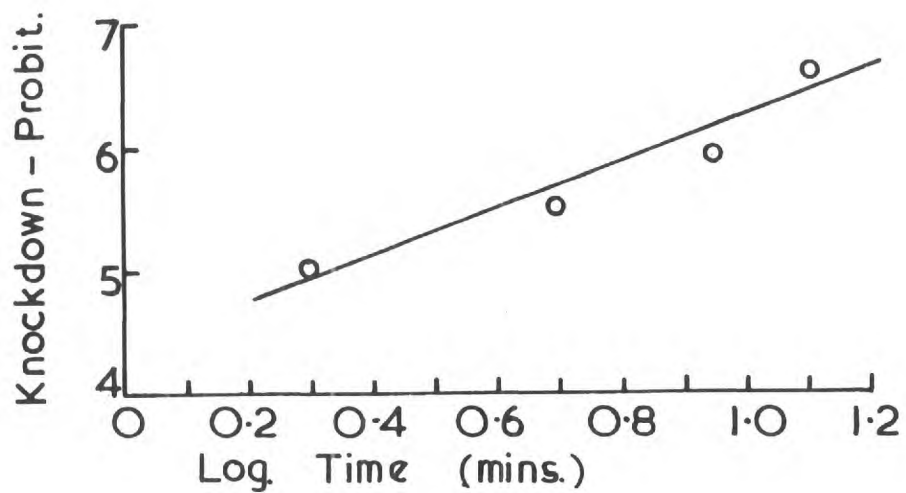
An experiment was carried out in which the toxicities of pyrethrins and of piperonyl butoxide to adult A. salina were compared. Three lots of 30 adult shrimps in double strength saline were exposed to 0.2 parts per million pyrethrins, 1.0 ppm. piperonyl butoxide and 0.5 ppm piperonyl butoxide respectively; there was also a control. Each batch of 30 adults was divided into six replicates of five. The numbers of paralysed shrimps floating on the surface of the saline were recorded at intervals and are shown in table 66 (graph 55). It will be seen that piperonyl butoxide alone has some toxic effect on adult A. salina.

In all experiments with A. salina adults and nauplii, except the one last quoted, estimates of response after different periods of exposure to toxicants were not carried out on the same lot of shrimps. The response obtained after a given time was therefore not influenced by previous counts and did not prejudice results obtained after longer periods of exposure.

No further experimental work was undertaken with A. salina adults.

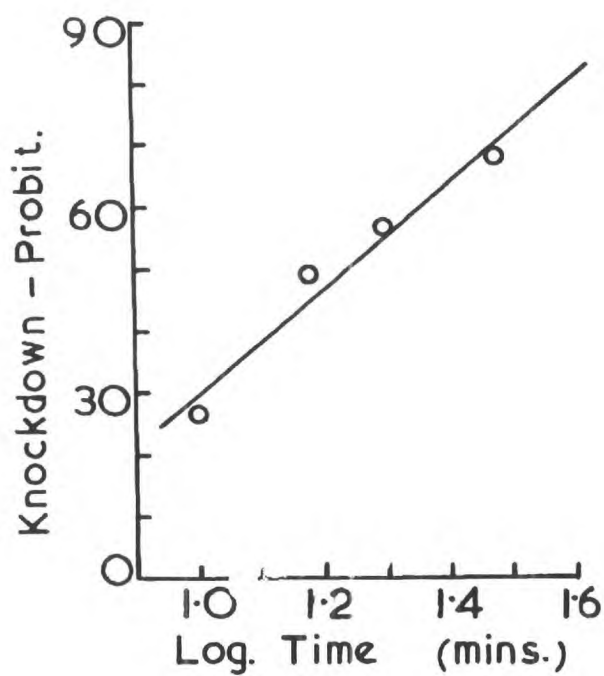
Graph 53: Exposure of A. salina adults in saline containing 1 p.p.m pyrethrins.
See table 64.

53.



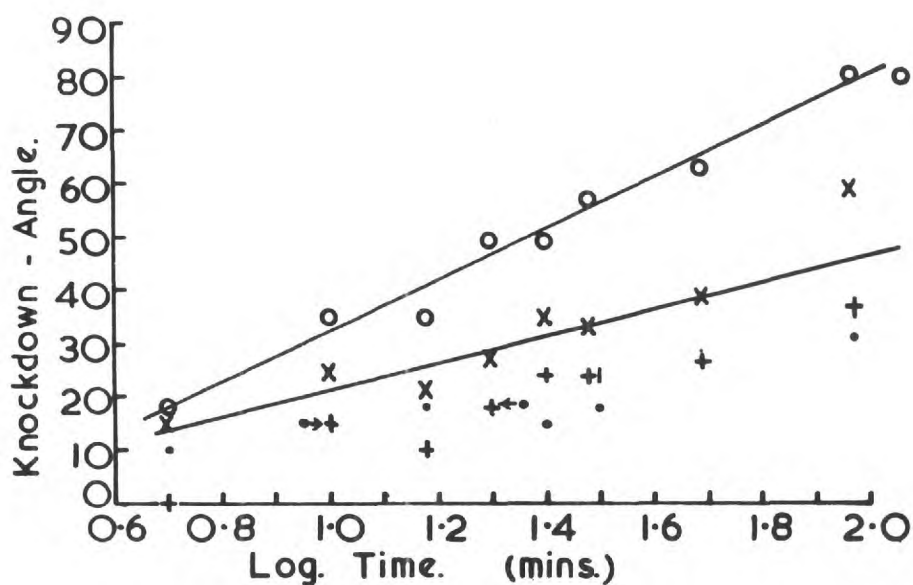
Graph 54: Exposure of A. salina adults in saline containing 0.01 p.p.m. pyrethrins. See table 65.

54.



Graph 55: Exposure of A. salina adults in saline containing pyrethrins or piperonyl butoxide.
See table 66.

55.



O pyrethrins.

X 1.0 p.p.m. piperonyl butoxide.

+ 0.5 p.p.m. piperonyl butoxide.

. Control.

Biological assay using Artemia salina nauplii.

Michael et al. mention in passing that besides the adults, the newly-hatched nauplii of A. salina may be useful in biological assay. The author decided to investigate the possible use of the nauplii for the biological assay of pyrethrins residues in the hope of throwing some light on the differential action of synergists already noted.

The investigation to be described shows that, for the assay of pyrethrins residues, the use of the shrimp nauplii has a number of advantages over the use of the adults. As already mentioned, very little culturing of the nauplii is needed and there is no need to feed them at all. Nauplii are easily available in much larger numbers than adults and also occupy much less space, one small beaker containing hundreds of nauplii whereas several bowls would be needed to accomodate a similar number of adults. It is easier to define a knockdown response of pyrethrins of the nauplii than of the adults, and also easier to determine this response quickly.

The method of estimating knockdown or mortality of A. salina nauplii was the same throughout these experiments.

During exposure to pyrethrins and other poisons the nauplii were kept in small test tubes about 7 mm in diameter and 75 mm in length having a capacity of slightly more than 3 ml. These tubes were marked at the 2 ml. level. Two methods of adding the saline solution containing the insecticide or insecticide residue were used.

In earlier experiments the nauplii and saline were placed in the tubes and the volume adjusted to 2 ml., 1 ml. of the saline containing insecticide was then added from a pipette, the tube was immediately shaken gently to mix the saline and insecticide thoroughly and was put in a constant temperature cabinet at 25°C until the response was estimated. In later experiments the nauplii were put in the tube in about 0.5 ml. of saline. 1 ml. of saline containing the insecticide or residue extract was then added from a pipette. The volume was immediately made up to 2 ml. with pure saline, the tube was shaken and put into a constant temperature cabinet at 25°C. until the response was estimated.

It was convenient to place the tubes in holes drilled in a wooden board during the assays.

In many of these experiments there is considerable variation in the number of A. salina nauplii per tube. To reduce this variation as far as possible, without making the time required to count out the nauplii unduly long, the apparatus shown in diagram form in figure 5 was devised. The micrometer head from an "Agla" micrometer syringe was used in conjunction with an ordinary 5 ml. syringe to push the nauplii in saline slowly through a glass tube drawn out as a capillary. If the capillary was illuminated by direct light against a black background the nauplii could be fairly easily counted and it was possible to place

approximately the required number of nauplii in the tubes without too much waste of time. The apparatus was recharged by turning the capillary tube downwards into the culture of nauplii and drawing the nauplii up with the syringe.

At the end of exposure the mortality or knockdown of the nauplii was estimated by pouring the contents of the tube into a counting tray and observing the nauplii with the low power of a binocular dissection microscope. The counting tray was similar in design to trays used at Imperial College Field Station to count nematode cysts. It consisted of a block of perspex 17 cm x 7 cm. x 0.7 cm. in which a continuous groove 6 mm. wide and about 2-3 mm deep in the centre was cut running back and forth across the block, giving a total length of about 140 cms.

The response was sometimes estimated as mortality but more generally as knockdown. Observation showed that healthy A.salina nauplii swim continuously with a regular beat of the limbs and that when affected by pyrethrins this regular rhythm was broken and the motion became disjointed with frequent pauses and changes of frequency. This irregularity of beat was easily observed with a binocular microscope and nauplii showing it were considered knocked down.

A.salina nauplii in a beaker of saline were seen to be negatively phototropic, and attempts were made to use this response in biological assay as is done with Aedes aegypti larvae mentioned above. The reaction of the shrimp nauplii is not as clear cut as

that of A. aegypti larvae; and after trying a number of different sets of conditions including wide variation in the intensity of the light, the use of this form of response to biological assay was abandoned.

Attempts were also made to measure the response of A. salina larvae to pyrethrins as failure to react to a gradient of temperature. These experiments were also unsuccessful.

Before A. salina nauplii could be used in biological assay of pyrethrins, it was necessary to determine their tolerance of organic solvents in which extracts of pyrethrins could be made. A solvent was required which was miscible with water and which would readily dissolve pyrethrins. Acetone was found to be too toxic to the shrimps but they were able to tolerate up to 1% alcohol. The nauplii also seemed able to tolerate up to 10% propylene glycol; however, this solvent was not used. In these experiments the solvent used was ethyl alcohol, most of ten at a concentration of 0.66% or 1% in saline.

Biological assay of pyrethrins.

To determine the sensitivity of A. salina nauplii to pyrethrins, a number of biological assays were carried out in which pyrethrins in alcohol was added to the saline containing the nauplii.

Tables 67 and 68 (graphs 56 and 57) show the results of experiments in which 36 hour nauplii were exposed to concentrations

of 0.67, 0.33 and 0.17 parts per million of pyrethrins in 0.67% alcohol in saline for different periods of time. The results may be expressed as regression lines and show that increasing the time of exposure caused an increase in the response rate which is more marked in graph 57 than in graph 56.

In the experiment shown in table 69 and graph 58, A. salina nauplii were exposed to 0.67 and 0.17 parts per million pyrethrins for different times and the results are similar to those shown in the previous experiment. In graph 58 the results are shown as a regression line using the logarithm of the concentration time product (CTP) - that is the product of the exposure time and the concentration of pyrethrins - as the x axis. It is known that a dose of a fumigant to insects may be expressed as a concentration time product and that within fairly broad limits the response of the insects will be similar if the duration of exposure and concentration of fumigant are varied while keeping the concentration time product constant. It seemed likely that this rule would apply also to nauplii in a solution of pyrethrins and the regression line shown in graph 58 illustrates this relationship.

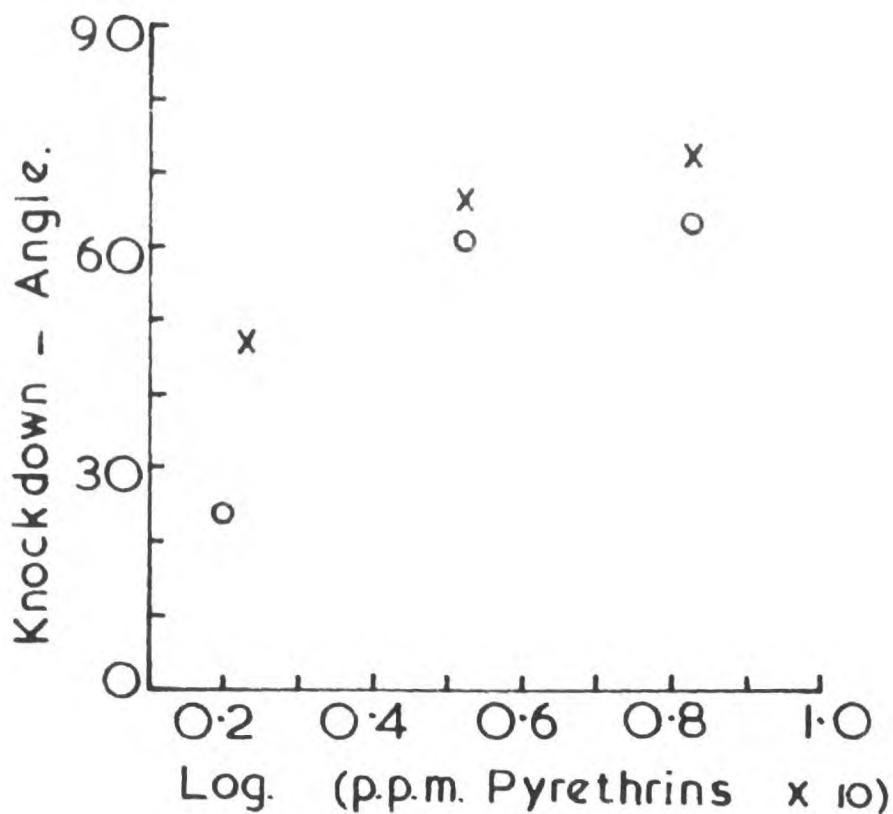
Table 70 shows the result of biological assays in which the shrimp nauplii were exposed in solutions containing pyrethrins and pyrethrins plus piperonyl butoxide. The presence of piperonyl butoxide considerably increased the response of the nauplii whether pyrethrins were present or not. This toxicity of piperonyl butoxide to A. salina has already been demonstrated to affect

adult shrimps in graph 55.

It has been assumed in these assays that the sorbtion of pyrethrins by the nauplii was negligible with respect to the large volume of solution present. The alkalinity of the saline (about pH 8) is less than that recommended by Michael et al. (pH 9) and it was thought that this pH did not adversely affect the pyrethrins. This assumption appears to be justified by the results obtained, but in any further work this form of assay of pyrethrins it would be well to check this.

Graph 56: Exposure of A. salina nauplii in
saline containing pyrethrins
See table 67.

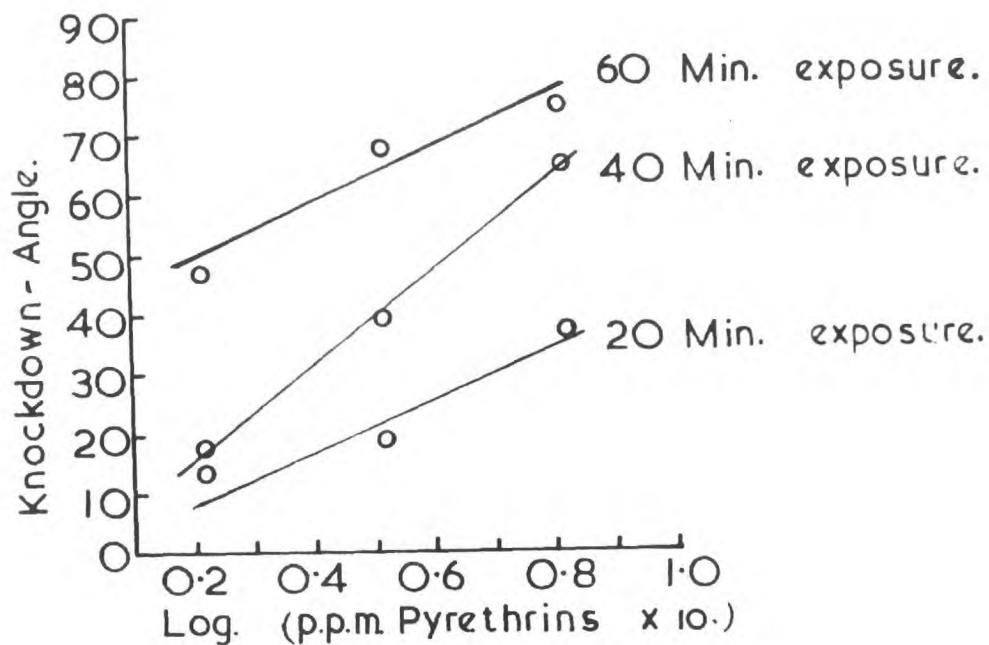
56.



o 20 minutes exposure
x 30 minutes exposure.

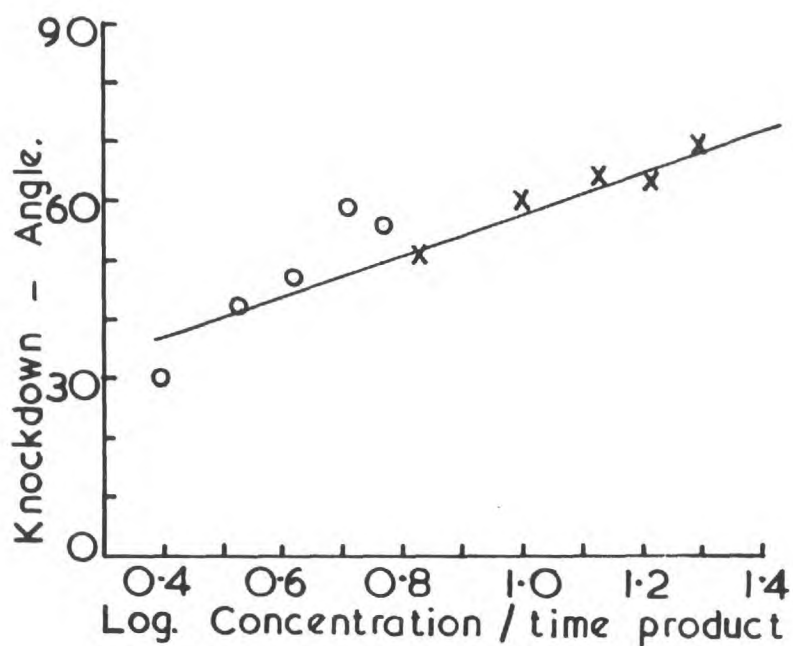
Graph 57: Exposure of A. salina nauplii to pyrethrins.
See table 68.

57.



Graph 58: Exposure of A. salina nauplii to saline containing pyrethrins.
See table 69.

58.



O exposed in 0.17 p.p.m. pyrethrins.

X exposed in 0.67 p.p.m. pyrethrins.

Biological assay of pyrethrins residues in insects, using *Artemia salina* nauplii.

The system of biological assay with *Artemia salina* nauplii described above seemed well suited for the estimation of toxic residues in insects treated with insecticides. In particular, this form of assay seemed likely to provide a means of investigating the detoxification of pyrethrins in insects treated with that insecticide. It has been suggested that synergists function by preventing detoxification of the pyrethrins or by reducing the rate of detoxification. The *A. salina* assay could also be used to investigate this in the light of results reported in the first part of this work.

To determine some idea of the most satisfactory insect for use in this sort of experiment, extracts of adult and larval *Carpophilus dimidiatus* and of larval *Plodia interpunctella* and *Galleria melonella*, poisoned with pyrethrins, have been made and assayed with *A. salina* nauplii. Blood taken from fifth instar *Schistocerca gregaria* poisoned with pyrethrins has also been used.

In early experiments the method of extraction of the pyrethrins residues varied but the following method was used in all the later work.

A known number of insects was treated with pyrethrins as specified in each experiment. At a predetermined time after treatment the insects in 1 ml. of absolute ethyl alcohol, were homogenised in a Potter-Elvehjem homogeniser. After homogenisation

the contents of the homogeniser were washed with more alcohol into a coarse sintered glass filter funnel lined with No. 1 Whatman filter paper. Using reduced pressure, the alcohol was drawn through the filter into a specimen tube and the macerated insect material was washed by the addition of more alcohol. The filter paper was included because it greatly simplified subsequent cleaning of the sintered glass filter funnel. If the filter paper was used in conjunction with a Buchner funnel, the paper became perforated.

The alcohol containing the pyrethrins residues was transferred from the specimen tube to a distillation flask and the specimen tube was washed out with a little more alcohol. The total volume of alcohol used was about 20 ml. and this was reduced by distillation under reduced pressure to 0.5 ml. The use of reduced pressure for this distillation was necessary because of the chemical instability of the pyrethrins molecules, the heating which would be required in the absence of pressure reduction being likely to cause some chemical breakdown.

The remaining 0.5 ml. of alcohol extract was transferred to a 25 ml. volumetric flask where the volume of solution was made up to 25 ml. with saline. This extract was assayed by adding 1 ml. of the extract to 1 ml. of saline containing nauplii in the small test tubes already mentioned. Any increase in the concentration of alcohol led to high control responses due to the toxic effect of the alcohol.

Biological assay of extracts of *Carpophilus dimidiatus* adults.

Tables 71 and 72 and graphs 59 and 60 show the results of assays, using *A. salina* nauplii, of extracts of *C. dimidiatus* adults poisoned with pyrethrins.

C. dimidiatus adults were treated with pyrethrins by exposure on impregnated filter papers in the manner already described. Topical application, whilst being a possible procedure, was inconvenient because of the small size of the insects. Spraying in the Potter tower was found convenient with other insects used in these assays, but the adult beetles recovered quickly from the anaesthetic effects of carbon dioxide and, being very active, escaped from the spray platform.

The extractions were made by the method already described. Using the results shown in tables 71 and 72, it has been possible to draw regression lines, shown in graphs 59 and 60, of time of exposure against percentage response of the nauplii. The increase of response with time in the controls is presumably due to the presence of 1% alcohol in the saline.

The extract of adult beetles treated with only piperonyl butoxide (table 72) is no more toxic to *A. salina* nauplii than control extract. This result, which has also been obtained with extracts of other insects, provides an interesting contrast with the results shown in table 70 where piperonyl butoxide solutions were shown to be toxic to *A. salina* nauplii and will be dealt with later in the discussion.

The results of assay of extracts from insects treated with pyrethrins plus piperonyl butoxide (table 72) are erratic. They suggest that this extract has a greater toxicity to shrimp nauplii than does the extract of beetles treated with pyrethrins only. This result is at variance with the findings of other experiments in which the toxicity of extracts of insects treated with synergised pyrethrins was no greater than the toxicity of extracts of insects treated with pyrethrins alone.

Biological assay of extracts of *Carpophilus dimidiatus* larvae.

To assay extracts of *C. dimidiatus* larvae with *A. salina* nauplii, the larvae were dosed with pyrethrins by spraying them in the Potter tower. Before being sprayed the insects were anaesthetised with carbon dioxide which immobilised them during the period of spraying. The pyrethrins were diluted with the mixed diluent (80% light petroleum and 20% Risella oil) used for dilution in previous experiments. Each batch of larvae was sprayed with 2 ml. of solution.

In table 73 (graph 61) are given the results of an experiment in which extracts of *C. dimidiatus* larvae after spraying with pyrethrins were assayed using *A. salina* nauplii. The results have been recorded as the responses of batches of nauplii after different times and a regression line (graph 61) has been obtained.

It is more usual in biological assay to present the results as a regression of response on concentration. This procedure has been followed in the experiment recorded in table 74. *C. dimidiatus* larvae were sprayed with three concentrations of pyrethrins. Extracts of the larvae were assayed with *A. salina* nauplii and the responses counted at two exposure times which were approximately the same for extracts from insects treated at each of the three concentrations. From these results two regression lines (graph 62) have been drawn representing the regression of response of the nauplii against the original

concentrations of the pyrethrins spray after the two periods of exposure of the nauplii. As would be expected, the regression line obtained after the longer period of exposure is the higher of the two.

The effect of introducing piperonyl butoxide into the spray on the results of subsequent assays of extracts of the larvae with A. salina nauplii has been examined. The result of two experiments are given in tables 75 and 76. The results from table 75 have been plotted in graph 63. When C. dimidiatus larvae were sprayed in the Potter tower with 2 ml. of 2% pyrethrins, the knockdown response one hour after spraying was about 60%. With the addition of the synergist to the spray, the response was of course greater. However, the responses of nauplii to extracts of larvae were similar whether the larvae had been treated with pyrethrins only or with pyrethrins plus piperonyl butoxide. If any difference can be detected from these results, there is a slight suggestion that the extract of larvae treated with pyrethrins only is the more toxic of the two.

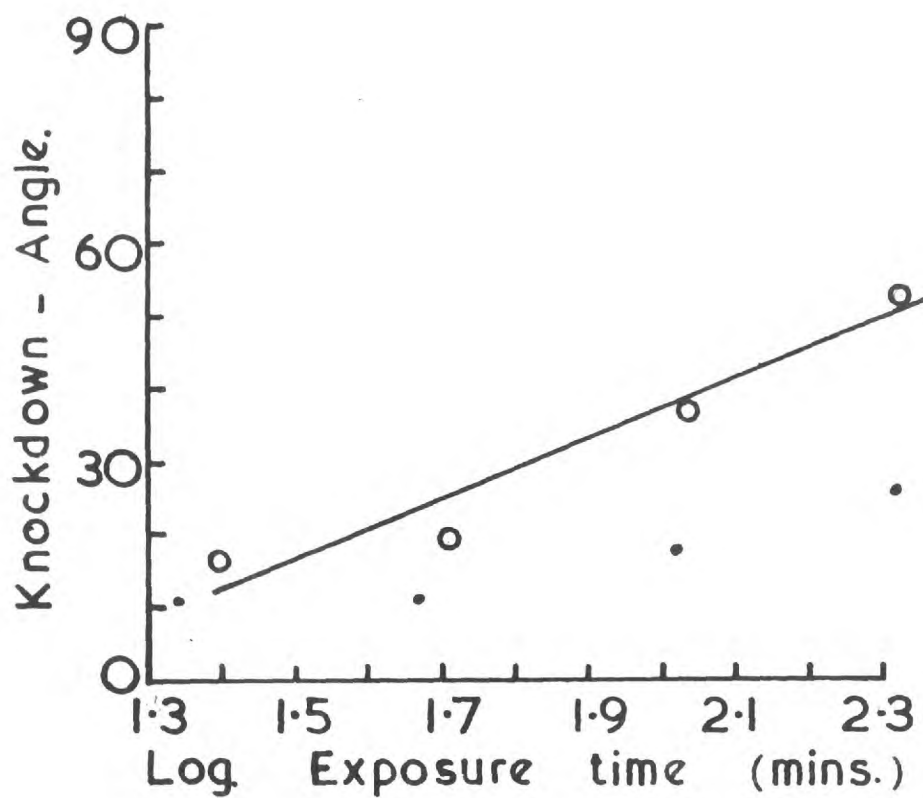
As already stated, the micro-biological assay technique may provide a method of estimating the rate of detoxification of pyrethrins in treated insects. Tables 77 and 78 show the results of experiments in which extracts of C. dimidiatus larvae treated with pyrethrins were made at intervals after spraying so as to allow time for any detoxification process to function. In each experiment the toxicity of the extracts to the nauplii has been

compared with the toxicity of extracts made immediately after spraying. Reference to tables 77 and 78 and to graphs 64, 65a and 65b shows that no appreciable detoxification had taken place if the larvae were left up to 130 minutes after treatment with pyrethrins.

A similar result was obtained when the larvae had been sprayed with pyrethrins and piperonyl butoxide (graph 65b).

Graph 59: Artemia nauplii assay of C. dimidiatus
adults treated with pyrethrins.
See table 71.

59.

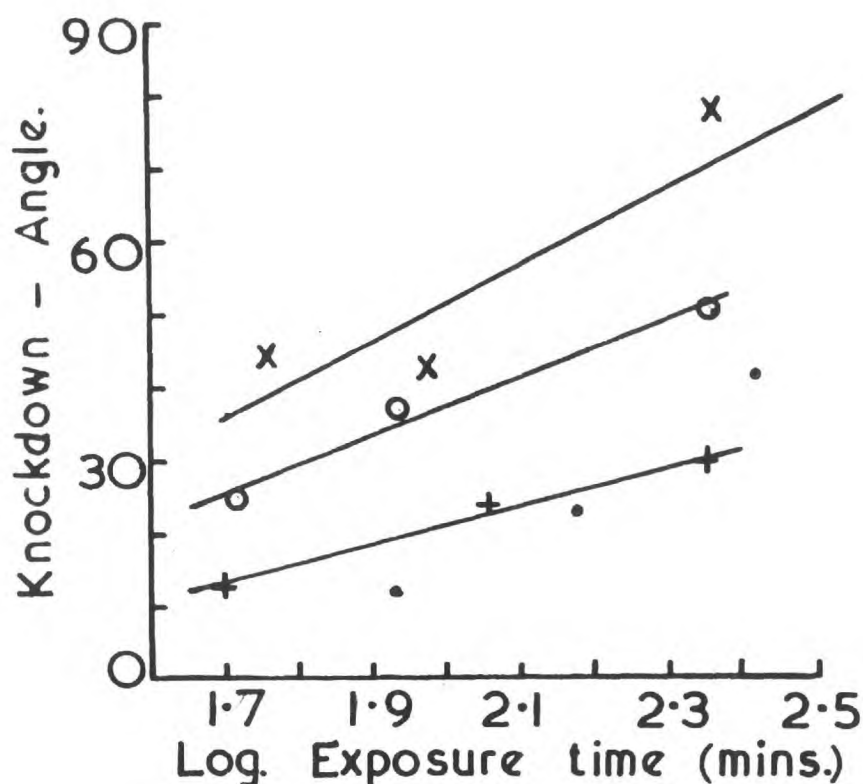


○ Beetles treated with pyrethrins.

• Control.

Graph 60: Artemia assay of extracts of C. dimidiatus adults treated with pyrethrins, piperonyl butoxide, and pyrethrins plus piperonyl butoxide.
See table 72.

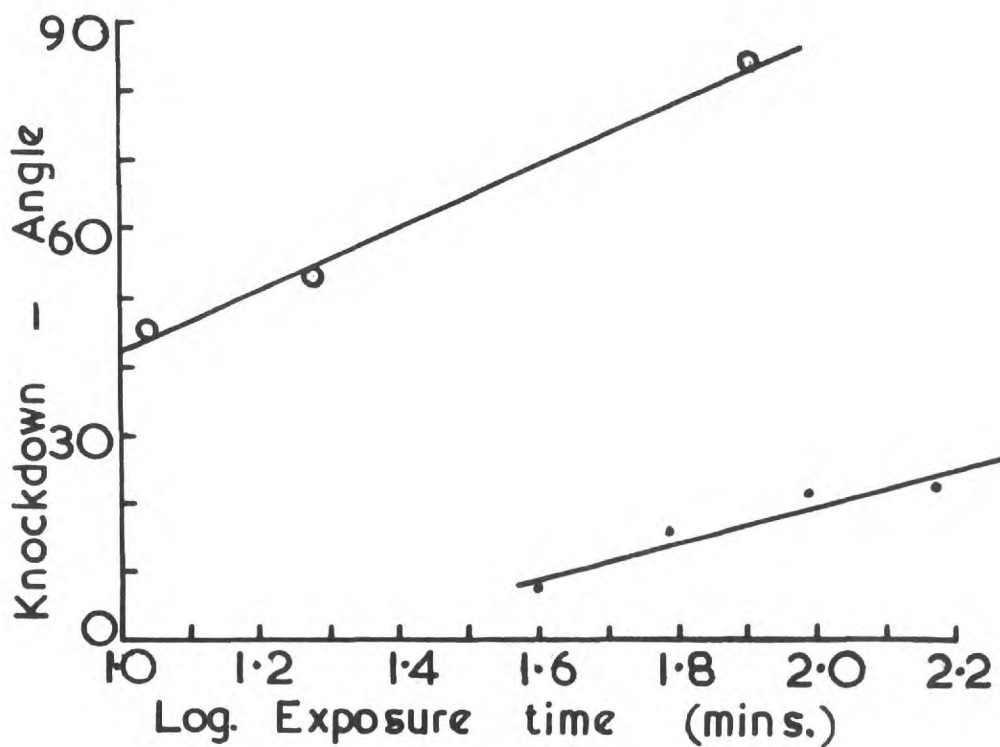
60.



- O Beetles treated with pyrethrins
- X Beetles treated with pyrethrins, plus piperonyl butoxide.
- + Beetles treated with piperonyl butoxide.
- Control.

Graph 61: Artemia assay of extracts of C. dimidiatus
larvae treated with pyrethrins
See table 73

61.

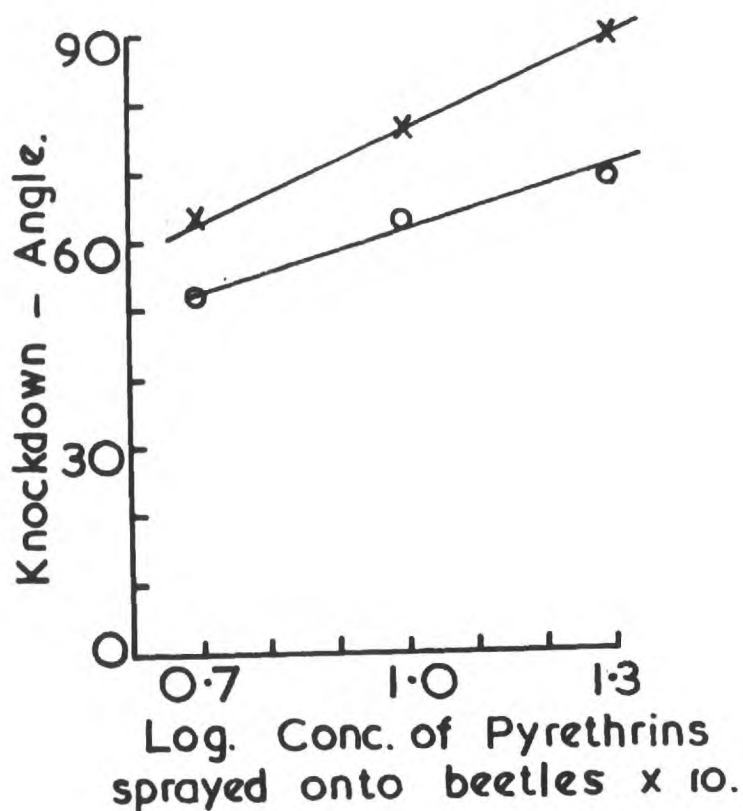


○ Beetles treated with pyrethrins.

• Control.

Graph 62: Artemia assay of extracts of C. dimidiatus larvae treated with pyrethrins.
See table 78.74.

62.

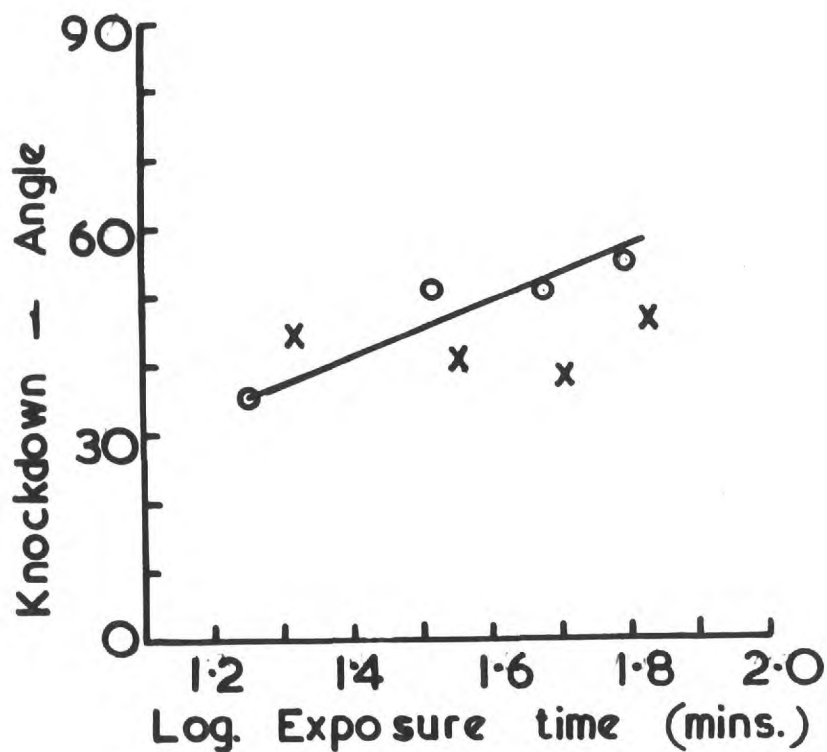


O Exposures of Artemia for 189, 190, 198 mins.

X Exposures of Artemia for 244, 241, 250 mins.

Graph 63: Artemia assay of extracts from C. dimidiatus larvae treated with pyrethrins, and with pyrethrins plus piperonyl butoxide. See table 75.

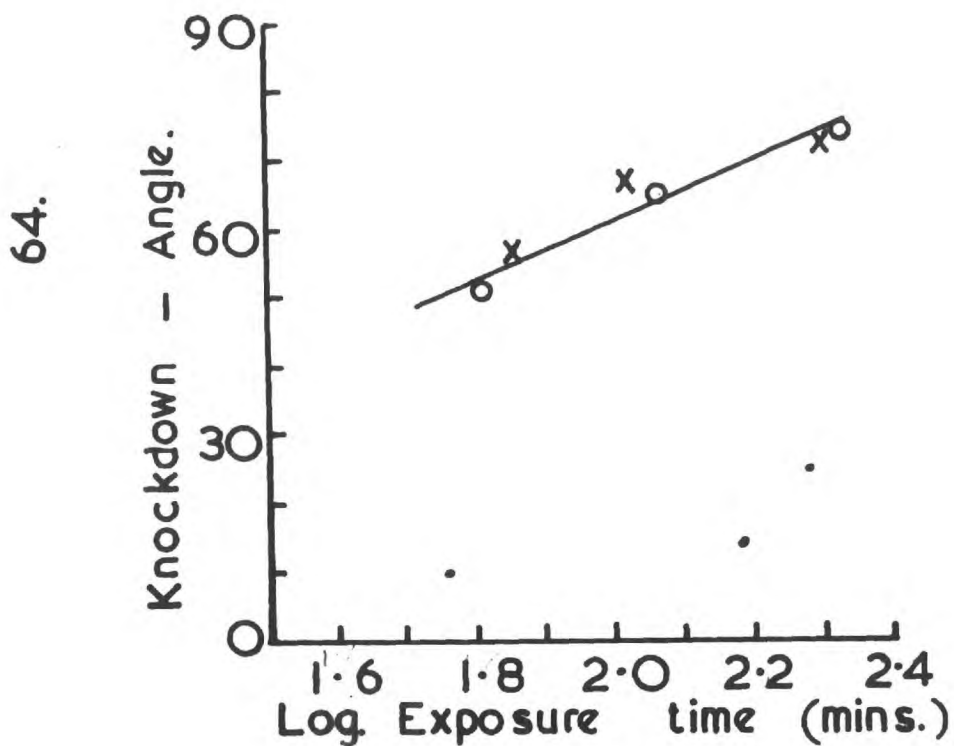
63.



O Larvae treated with pyrethrins.

X Larvae treated with pyrethrins and synergist.

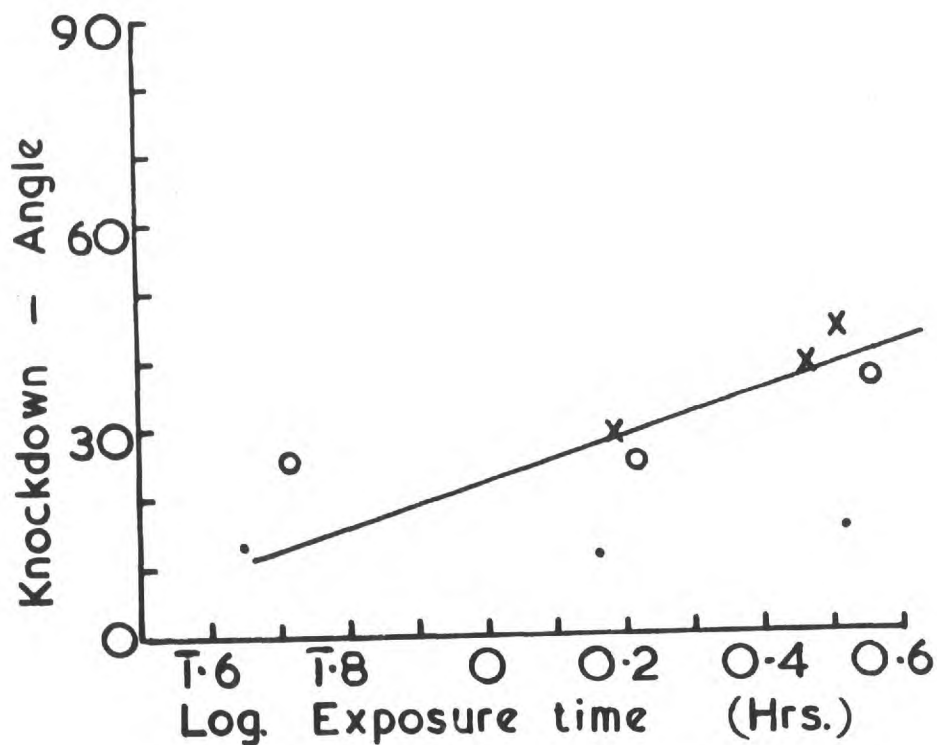
Graph 64: Artemia assay of extracts from C. dimidiatus larvae treated with pyrethrins.
See table 77.



- Extracted immediately after spraying
- × Extracted 20 minutes after spraying
- . Control.

Graph 65a: Artemia assay of extracts of C. dimidiatus larvae treated with pyrethrins.
See table 78.

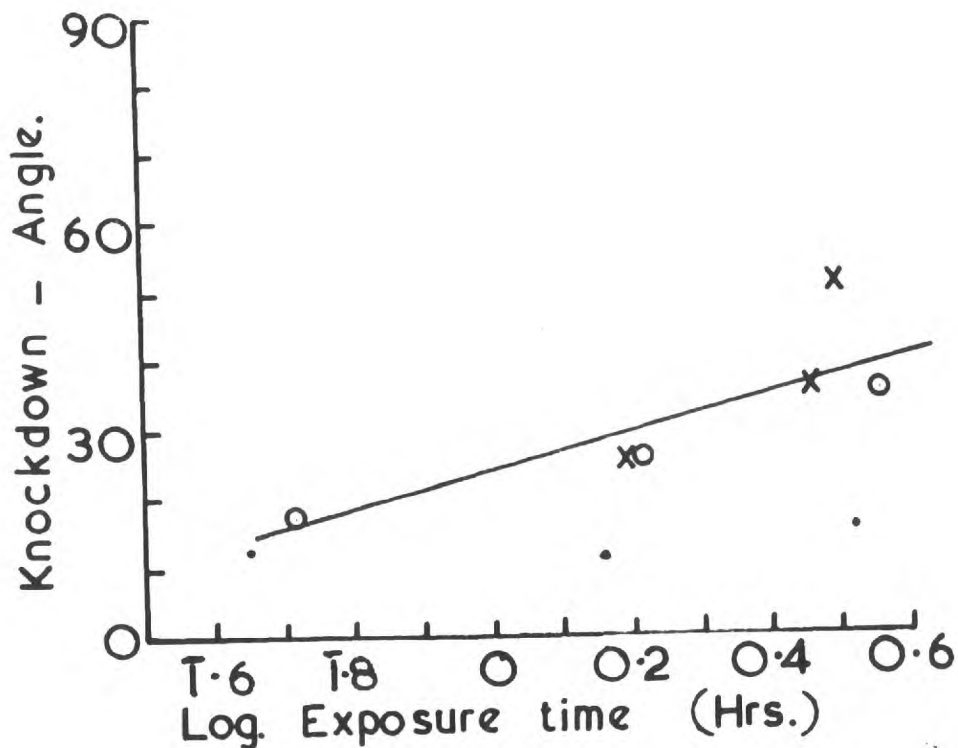
65a.



- O Extracted immediately after being sprayed.
- X Extracted 130 minutes after being sprayed.
- Control

Graph 65b: Artemia assay of extracts of C. dimidiatus larvae treated with pyrethrins plus piperonyl butoxide.
See table 78.

65b.



- O Extracted immediately after being sprayed.
- X. Extracted 130 minutes after being sprayed.
- Control.

Biological assay of extracts of Plodia interpunctella larvae.

It was thought that the different reactions of various species of insect to treatment with synergised pyrethrins might be reflected in their ability to detoxify pyrethrins. Experiments with Carpophilus dimidiatus, an insect showing a fairly high factor of synergism, had failed to demonstrate detoxification of pyrethrins in up to 130 minutes after treatment. As Plodia interpunctella had been shown to be unaffected by synergists, this insect was chosen for further experiments.

In the experiment recorded in table 79 and graph 66, an attempt was made to relate the toxicity to A. salina of an extract of P. interpunctella sprayed with pyrethrins to the toxicities of solutions of known concentrations of pyrethrins. Because there were two sets of controls in this experiment, separate corrections were made for control responses before plotting graph 66. The apparent absence of the difference in toxicity of solutions of 0.33 ppm. and 0.17 ppm. pyrethrins is noteworthy. In a subsequent experiment (table 68) differences in toxicities of these concentrations were found to be more clearly marked. Data taken from table 68 has been plotted in graph 67.

Extracts were also made of P. interpunctella larvae which had been treated with pyrethrins and with pyrethrins plus piperonyl butoxide. The toxicities of these two extracts to A. salina nauplii were compared (table 80) and it will be seen that the

extract of larvae sprayed with synergised pyrethrins was slightly more toxic to the nauplii than that of larvae sprayed with pyrethrins only.

The results of experiments in which the toxicities of extracts of P. interpunctella larvae made immediately after treatment with pyrethrins were compared with the toxicities of extracts made some time after treatment are given in tables 81, 82 and 83 and from these results graphs 69, 70 and 71 have been drawn. In these graphs there is no definite indication of detoxification of the pyrethrins. The experiment recorded in table 83 (graph 71) has been included in spite of the erratic results because it suggests confirmation of the lack of detoxification. Taking these three experiments together, it may be concluded that extracts made some time after treatment with pyrethrins have about the same toxicity to A. salina nauplii as extracts made immediately after treatment.

In the experiment recorded in table 81, the treatment of the P. interpunctella larvae with pyrethrins was carried out using topical application. This method of dosage has the very great advantage that the exact dose of insecticide received by each insect is known. From the information in table 81 it is possible to gain some idea of the efficiency of the extraction procedure.

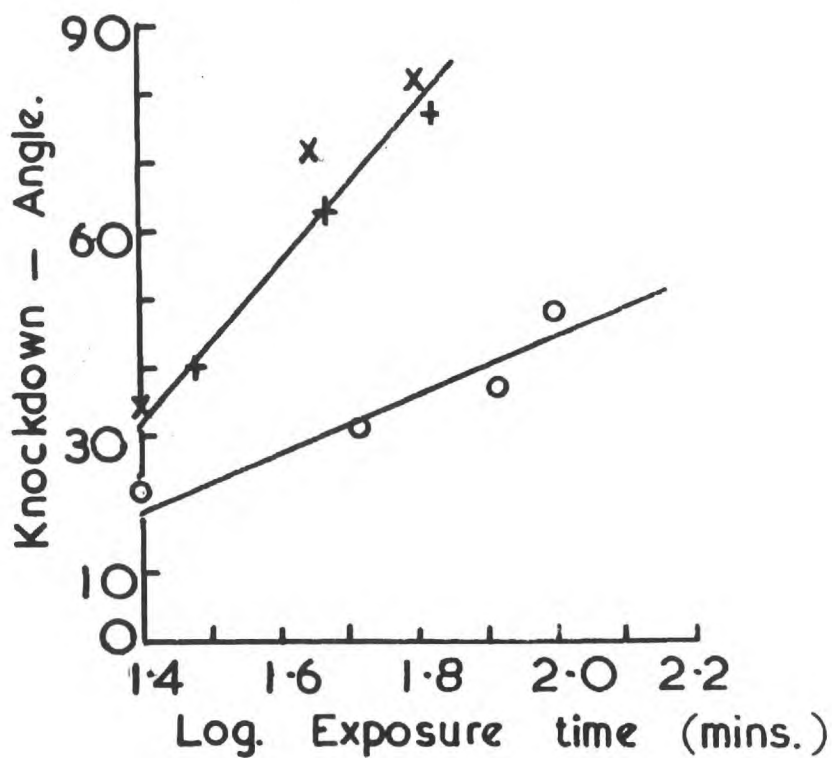
Nauplii, of the same batch as those used in the assay shown in table 81, were exposed to 0.33 and 0.67 parts per million pyrethrins. The results of this experiment (table 84) were

compared with those in table 81 (graphs 69 and 72).

Reference to graph 69 shows that after 100 minutes exposure (2.0 on the $\times$ axis), the response of the nauplii to the extract of P. interpunctella larvae was equivalent to an angular transform value of 37. Graph 72 shows that the response after 100 minutes of nauplii exposed to 0.13 ppm. pyrethrins was equivalent to an angle of 40, and to 0.67 ppm. pyrethrins was equivalent to an angle of 79. There was, therefore, the equivalent of about 0.13 ppm. pyrethrins present in the extract. If there was no loss of pyrethrins during extraction, a dose of 1 ~~ml~~ of 0.3% pyrethrins applied to 20 larvae would give a concentration of 1.2 ppm. in the saline containing the nauplii. Thus the extract is about one tenth as toxic to the nauplii as the quantity of pyrethrins applied to the larvae. This loss of toxicity may be due to the inefficiency of the extraction process or to break down of the pyrethrins and is discussed further below.

Graph 66: Artemia assay of solutions of pyrethrins,
and of extracts of P. interpunctella larvae
treated with pyrethrins.
See table 79.

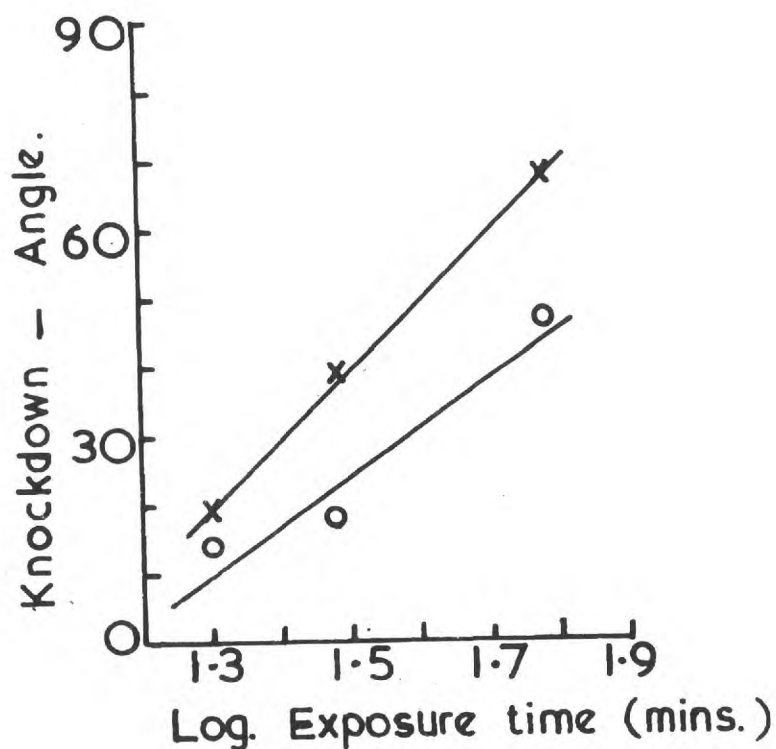
66.



- o Extract of treated P. interpunctella
 x 0.33 p.p.m. pyrethrins
 + 0.17 p.p.m. pyrethrins.

Graph 67: Artemia assay of solutions of pyrethrins
in saline.
Data from table 68. Compare table 79, graph 66.

67.

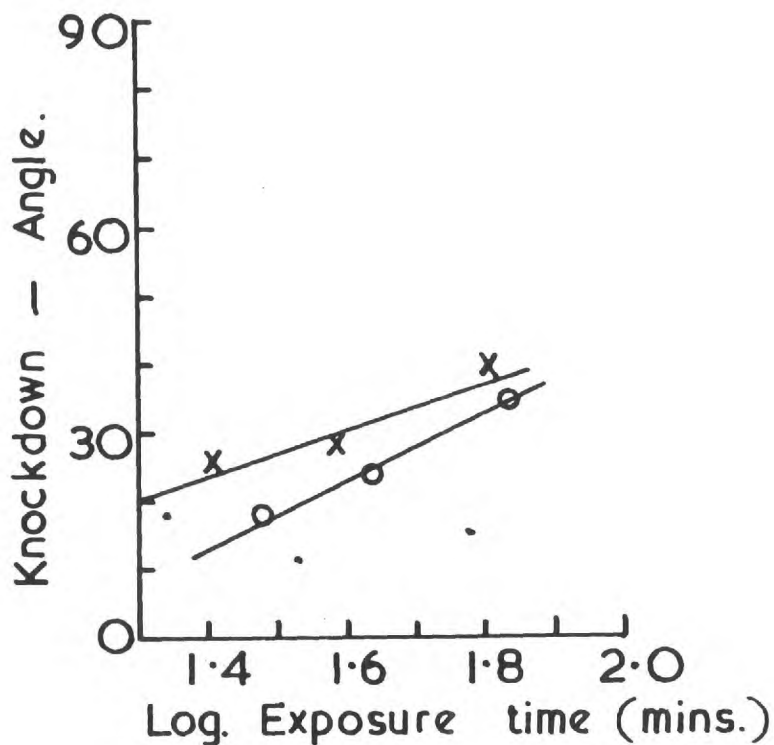


O - 0.17 p.p.m. pyrethrins.

X - 0.33 p.p.m. pyrethrins.

Graph 68: Artemia assay of extracts from P. interpunctella larvae treated with pyrethrins and with pyrethrins plus piperonyl butoxide. See table 80.

68.

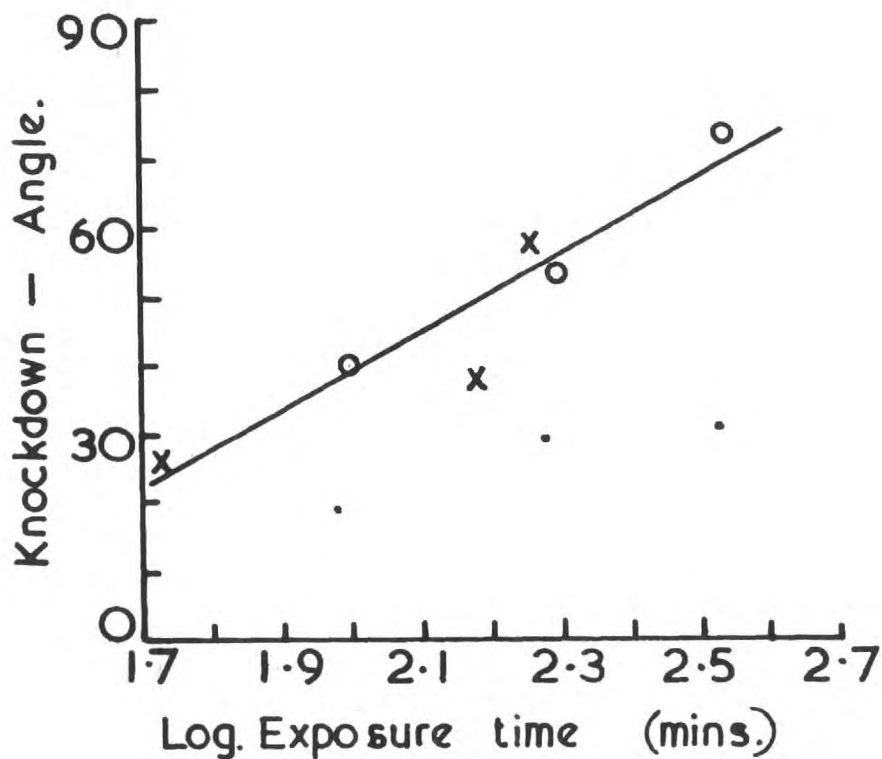


O Larvae treated with pyrethrins.

X Larvae treated with pyrethrins plus synergists.

Graph 69: Artemia assay of extracts of P. interpunctella larvae treated with pyrethrins.
See table 81.

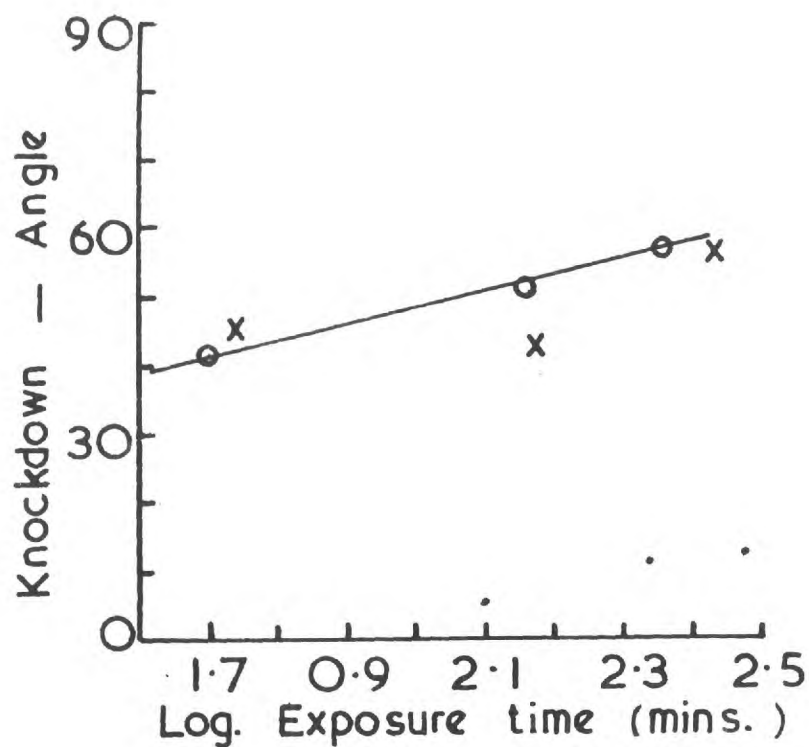
69.



- O Extract made immediately after treatment
 X Extract made 30 minutes after treatment.
 • Control.

Graph 70: Artemia assay of extracts of P. interpunctella larvae treated with pyrethrins.
See table 82.

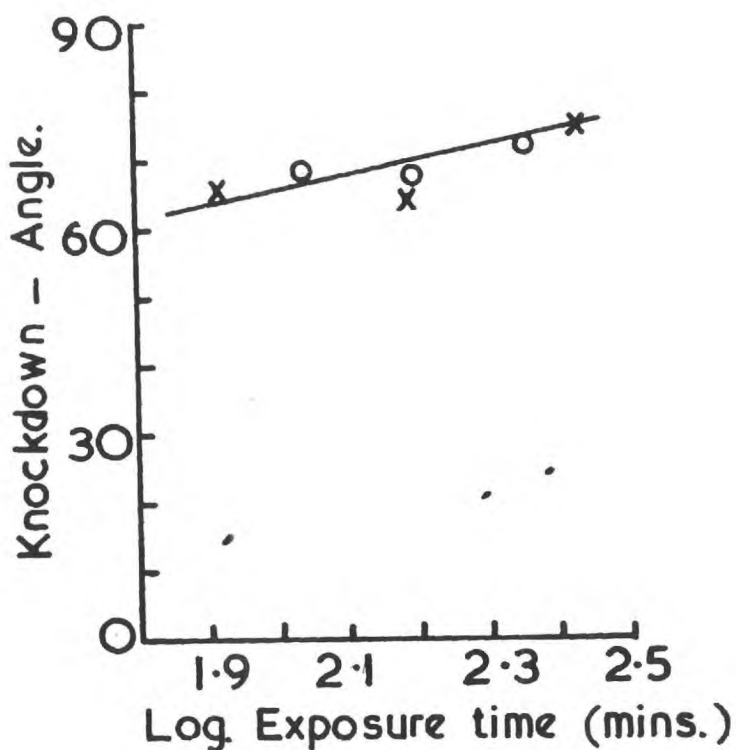
70.



- O Extraction made immediately after treatment.
 X Extraction made 2 3/4 hours after treatment.
 • Control.

Graph 71: Artemia assay of extracts from P. interpunctella larvae treated with pyrethrins.
See table 83.

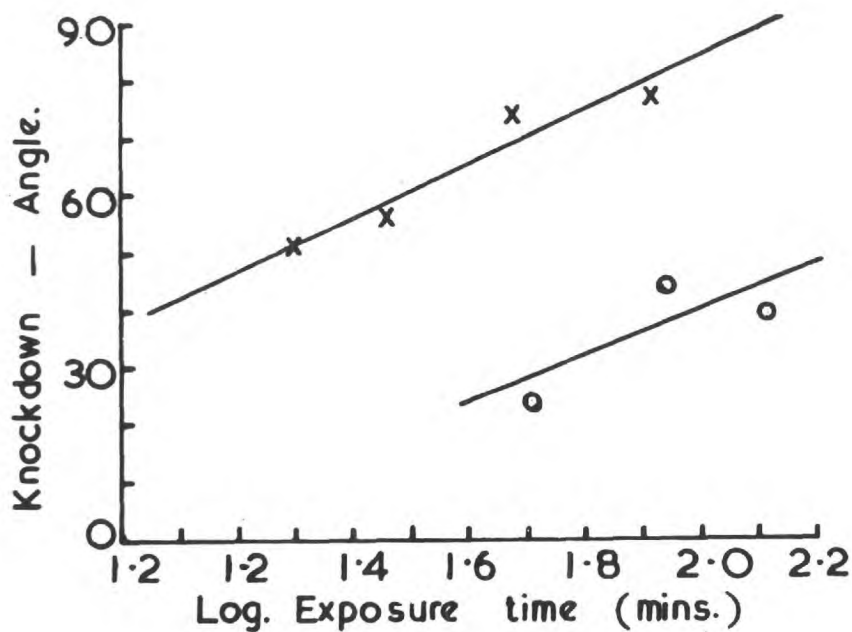
71.



- o Extraction made immediately after treatment.
- x Extracted $2\frac{1}{2}$ hours after spraying.
- . Control.

Graph 72: Artemia assay of solutions of pyrethrins.
See tables 84 and 81: also graph 69.

72.



O 0.13 p.p.m. pyrethrins.

X 0.67 p.p.m. pyrethrins.

Assay of extracts of *Galleria melonella* treated with pyrethrins.

A small number of experiments were carried out in which extracts of last instar larvae of *Galleria melonella* treated with pyrethrins were assayed with *Artemia salina* nauplii. Due to the large size of the wax moth larvae, only small numbers could be used in each extraction.

In the experiment recorded in table 85 five *G. melonella* larvae were dosed by topical application each with 1.0 μ l. of 0.7% pyrethrins in acetone. An alcohol extraction of the larval tissues was then made using the method already described. As a control 5 larvae were treated with doses of 1.0 μ l. of acetone and an extraction made. Table 89 shows that the extract from the larvae treated with pyrethrins is considerably more toxic than the control extract.

Table 86 and graph 73 show the results of an assay carried out with shrimp nauplii on an extract of 10 *G. melonella* larvae which had been injected with 1.0 μ l. of 0.1% pyrethrins in 1.0% alcohol in Ringer's solution. The pyrethrins in alcohol in Ringer's solution formed an unstable emulsion but was used immediately after preparation and before the emulsion broke. In plotting the regression line obtained from this experiment, corrections were made for responses in the controls.

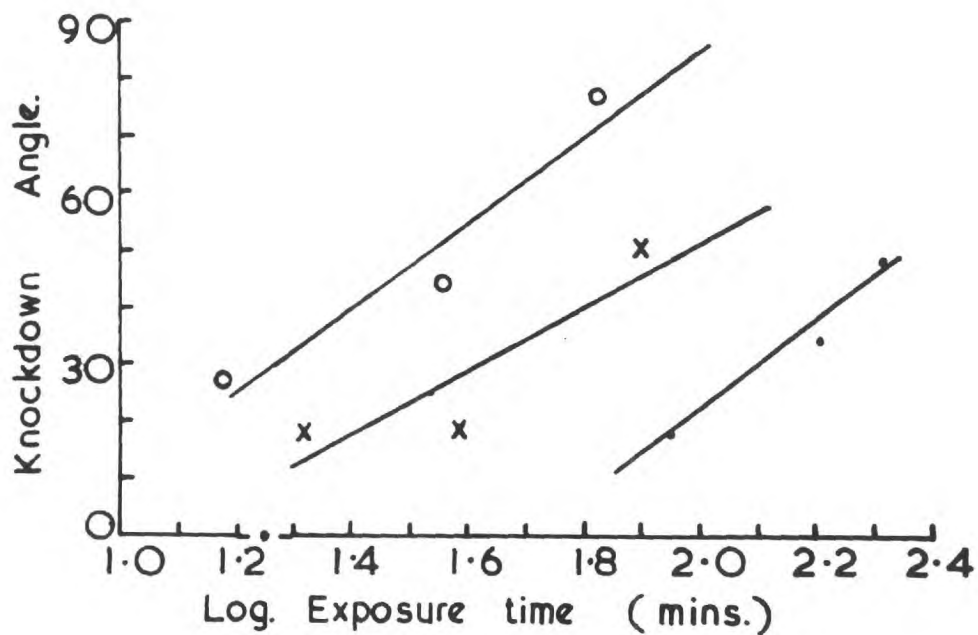
To investigate the efficiency of extraction from *G. melonella*, *A. salina* nauplii from the same culture as that used in the

experiment recorded in table 24, were exposed to solutions containing 0.13 and 0.33 ppm. pyrethrins (table 87). The results of this experiment were rather erratic, and they have been plotted on graph 73 together with the results from table 86.

It has been shown in previous experiments that if the time of exposure of the nauplii is constant, a straight regression response against concentration of pyrethrins is obtained after angular transformation. Using the lines drawn on graph 73, the knockdown after 200 minutes, when the nauplii were exposed to 0.13 and 0.33 ppm. pyrethrins, have been determined. From this determination the toxicity of the extract has been estimated to be equivalent to roughly 0.05 ppm. pyrethrins. If there had been no loss of pyrethrins during extraction, a dose of 1 μ l. of 0.1% pyrethrins applied to 10 larvae would give a concentration of 0.2 ppm. in tubes containing the nauplii. The extraction process is thus slightly more efficient in this case than with P. interpunctella larvae. This matter is further discussed below.

Graph 73: Artemia assay of extract of G. melonella larvae injected with pyrethrins, also assay of solutions of pyrethrins. See tables 86 and 87.

73.



- Extract of G. melonella
- O 0.33 p.p.m. pyrethrins.
- X 0.13 p.p.m. pyrethrins.

Assay of the blood of *Schistocerca gregaria* after treatment with pyrethrins.

To demonstrate possible further applications of *Artemia salina* nauplii in biological assay, the blood of the fifth instar of the locust *Schistocerca gregaria* (Forsk.) has been assayed after injection of the locusts with pyrethrins.

This work has been carried out in conjunction with Dr. G. F. Howden of Imperial College Field Station, who kindly injected the locusts. When the locust hoppers were injected with 5 μ l. of 0.2% pyrethrins in acetone, they were immediately paralysed completely, but after about $\frac{3}{4}$ hour they began to show signs of recovery. This recovery from the knockdown caused by pyrethrins is well known and suggests that some form of detoxification mechanism may exist in the insect. In the experiment described here the toxicity to *A. salina* nauplii of locust blood taken immediately after injection of the nymphs with pyrethrins has been compared with the toxicity of the blood taken one hour after injection.

Table 88 and graph 74 show the results of an experiment in which 5 μ l. of 0.2% pyrethrins in acetone was injected into each of eight *S. gregaria* fifth instar hoppers.

Immediately after injection about 0.15 ml. of blood was taken from each of two locusts and added to saline to give a total volume of 5 ml. As a replicate blood was taken from another

set of two treated locusts in the same way. 1 ml. of the solution of blood in saline was added to 1 ml. of saline containing the A. salina nauplii so that there were two sets of four tubes of nauplii treated with the solution of blood (A and B in table 88 and graph 74). 75 minutes after injection blood was taken from the four remaining hoppers in the same way to give two further sets of four tubes of A. salina nauplii in solutions of blood in saline (c and D). A control (E) was also prepared using two locusts injected with 5.0 μ l. of acetone. Blood was withdrawn from the locusts using a Pasteur pipette, marked so that a constant volume could be used; this volume was not known accurately but was about 0.15 ml. per insect.

It will be seen from table 88 and graph 74 that blood taken from the locusts immediately after injection appears to be more toxic to the shrimps than blood taken 75 minutes after injection. The responses of the nauplii have been plotted using the angular transformation in graph 74 and although scattered show some evidence of regression on time.

Table 89 and graphs 75 and 76 show the results of a further experiment on the toxicity of blood from locusts injected with pyrethrins to A. salina nauplii. Batches of five fifth instar female S. gregaria were injected with 5.0 μ l of solutions in acetone of;

- A. 1.0% piperonyl butoxide
- B. Control
- C. 0.2% pyrethrins
- D. 0.2% pyrethrins
- E. 0.2% pyrethrins plus 1.0% piperonyl butoxide.
- F. 0.2% pyrethrins plus 1.0% piperonyl butoxide.

Hoppers which would moult to become adult females were used because they have a larger volume of blood available than have hoppers which would moult to male adults.

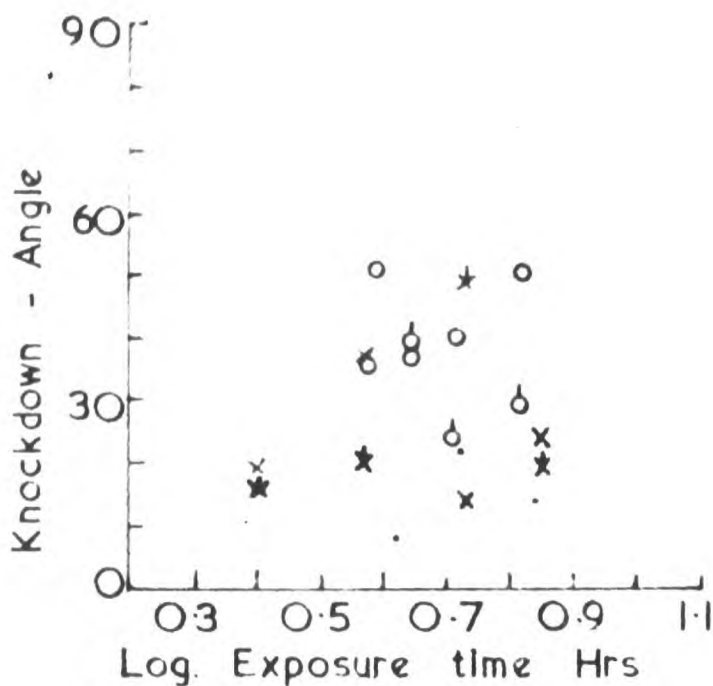
Blood was taken from batches A, B, C and E immediately after injection (about 0.15 ml. blood per insect) and this was added to saline to give total volume of 10 ml. Blood was taken from batches D and F 80 minutes after injection when the locusts injected with pyrethrins only (D) were showing signs of recovery but those injected with synergised pyrethrins (F) were not.

Blood taken immediately from hoppers injected with pyrethrins or with pyrethrins plus piperonyl butoxide immediately after injection was considerably more toxic to A. salina nauplii than was blood taken from the controls. Blood taken after 80 minutes did not appear to differ in toxicity to the shrimps from blood taken from the controls. The presence of piperonyl butoxide alone in locust blood did not alter its toxicity to the nauplii.

These results show that 80 minutes after injection, the quantity of toxic material in the blood of the locusts has been considerably reduced. This may be due to bio-chemical detoxification or to the adsorption of the pyrethrins from the blood on the tissues.

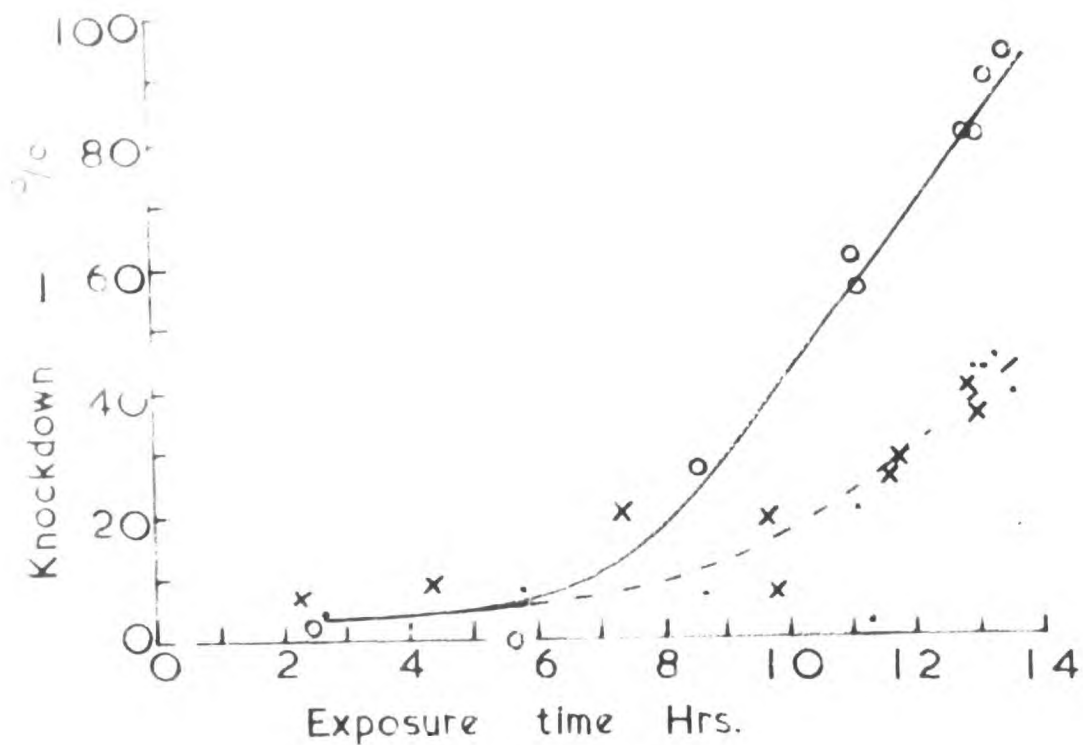
The pyrethrins/piperonyl butoxide mixture appeared to be more toxic to the locusts than pyrethrins alone, but the toxicity of the blood from these two batches to A. salina nauplii was not sufficiently different to be detected by these experiments.

Graph 74: Artemia assay of blood from S. gregaria
 injected with pyrethrins.
 See table 88.



- Blood withdrawn immediately after injection
Sample A
- Blood withdrawn immediately after injection
Sample B.
- X Blood withdrawn 75 minutes after injection
Sample C.
- * Blood withdrawn 75 minutes after injection
Sample D.
- Control.

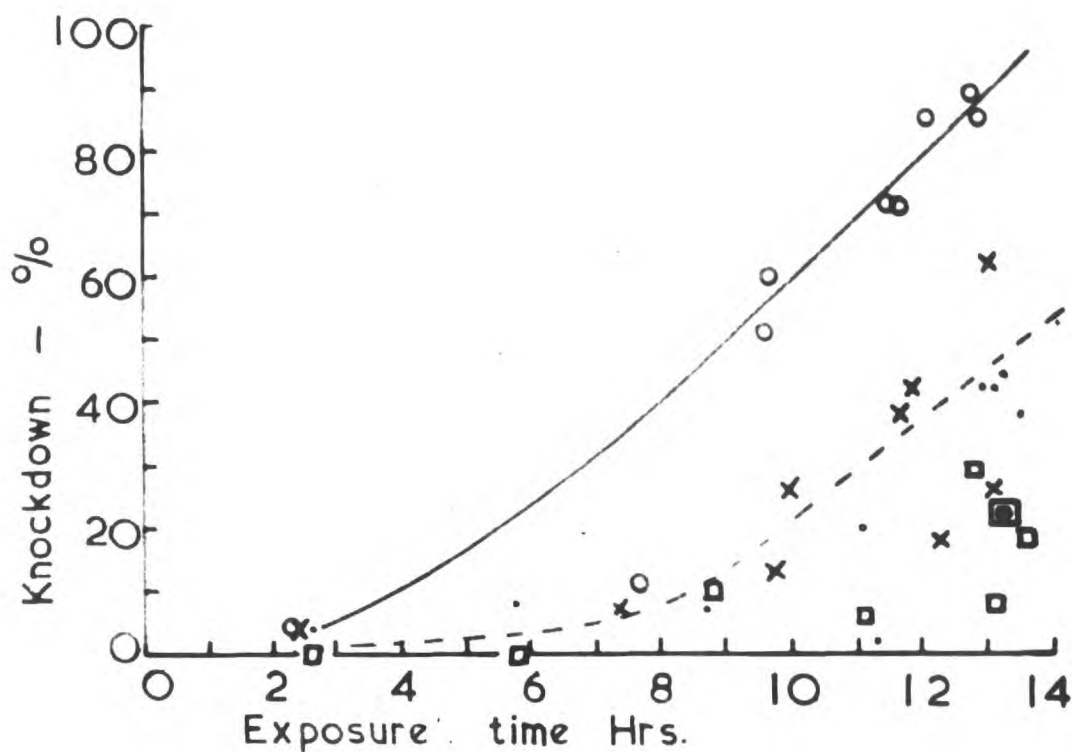
Graph 75: Artemia assay of blood from S. gregaria
nymphs injected with pyrethrins.
See table 89.



- O Blood withdrawn immediately after injection
- X Blood withdrawn 80 minutes after injection
- Control.

Graph 76: Artemia assay of blood from S. gregaria nymphs injected with pyrethrins plus piperonyl butoxide and with piperonyl butoxide. See table 89.

76.



- O Blood withdrawn immediately after injection
- X Blood withdrawn 80 minutes after injection
- Control.
- Blood from locusts injected with piperonyl butoxide.

DISCUSSION.

Synergism.

The rapid action of pyrethrins in bringing about paralysis or knockdown of treated insects is well known, and is generally thought to be due to the insecticides' immediate action on the nervous system where impulse transmission may be blocked. If the insect receives a sufficiently large dose of pyrethrins, paralysis is followed by death. However, sub-lethal doses of pyrethrins may also cause paralysis followed by the recovery of the insect within a few hours.

In insects poisoned with pyrethrins symptoms of paralysis which last for more than a few hours are generally followed eventually by death. In selecting a response for use in the biological assays these knockdown effects have been frequently chosen. It is often difficult to determine whether an insect treated with pyrethrins is dead or not. A definite, if arbitrary, definition of knockdown may be made (e. g. ability to leave a filter paper within a given time) which can easily be determined and leaves no doubt in the mind of the experimenter.

Metcalf (1955) has summarised theories proposed to account for the action of pyrethrin synergists, and useful summaries are given by Negherbon (1959):

A. Synergists may increase the proportion of the applied dose of poison which is received by the insect:

1. By narrowing the range of spray droplet size
2. By reducing rate of knockdown
3. By stimulating flight activity.
4. By preventing deterioration of the toxicant.

B. They may increase the effectiveness of the dose of poison received.

The action of synergists in increasing the dose received by the insect is physical rather than physiological. A1 and A3 apply only to atomised sprays while A2 may apply also to surface assays. Reduction in the rate of knockdown would enable insects to remain flying in a pyrethrins spray for a longer period and to pick up a lethal dose rather than a smaller dose causing only temporary paralysis.

Whether or not synergists prevent deterioration of deposits is the subject of conflicting evidence. It is more convenient to discuss this topic later in connection with Miss Donaldson's experiments on Ephesia cautella adults.

When all the factors mentioned in section A above have been eliminated, synergistic action of pyrethrins formulations may still be demonstrated as increased effectiveness of the applied dosage of poison. It has been suggested that synergists act by retarding a pyrethrins detoxification process in the insect.

Chamberlain (1950), Blum and Kearns (1956) and Wilson (1949) have shown that when pyrethrins and various synergists were applied

to insects at different locations, or at different times up to eight hours apart, marked synergistic effects were observed.

Chamberlain showed that insects exposed briefly on panels sprayed with piperonyl butoxide and then on panels sprayed with pyrethrins showed synergistic effects but there was very little effect if contact with pyrethrins came first. He also showed that the hydrolysis of pyrethrins by lipase from Periplaneta americana, Musca domestica and the rat was partially inhibited in the presence of piperonyl butoxide.

Lindquist et al. (1947) also demonstrated that if houseflies were sprayed first with a synergist and then with pyrethrins after intervals of 30 seconds, 1, 2 and 4 hours the synergists still enhanced the toxicity of the pyrethrins considerably. If the pyrethrins spray was applied first this enhancement was only apparent if the interval between application of pyrethrins and synergist was 30 seconds. No effect was observed if the interval was one hour or more.

Blum and Kearns (1956) claimed to show that if houseflies were treated first with pyrethrins by topical application and then with piperonyl butoxide either by topical application or by injection after intervals of one to five hours, there was considerable synergistic action. This is the reverse of the findings quoted above. However, Blum and Kearns quote Lindquist et al. as having shown that "application of the synergist after the pyrethrum produced a response equal to application of the materials

simultaneously; the reverse of the order of application produced no mortality when the interval exceeded 30 seconds". In view of this misquotation, it is probably unwise to take these contradictory observations seriously.

As a result of experiments using radiotracer techniques, Winteringham (1952) concluded that Musca domestica possessed a mechanism for the detoxification of pyrethrins which was inhibited by piperonyl cyclonene.

It therefore seems likely that, at least in the insects examined by these workers, synergists act by inhibiting the detoxification of pyrethrins. In the absence of a synergist the pyrethrins are quickly detoxified by the insect, and subsequent treatment of a synergist is too late to prevent this detoxification. However, the synergist remains in the insect for some time after application and is thus available to prevent or reduce detoxification of pyrethrins applied subsequently. The dose of pyrethrins applied in these experiments was sub-lethal, in the absence of a synergist. With higher doses of pyrethrins the insect is killed before detoxification becomes effective. When an insect is given a sub-lethal dose of pyrethrins, sufficient to cause temporary paralysis but not death, the time the insect remains paralysed may reflect, to some extent, the time required for detoxification of the pyrethrins. If this were so, treatment with a synergist while the insect was still paralysed might prevent subsequent recovery. It would be interesting to examine this possibility.

The suggestion of Page and Blackith (1949) and Blackith (1953) that molecules of pyrethrins and synergist form a loose molecular complex at a surface which is more toxic than pyrethrins alone does not seem likely in view of the above observations.

However, synergists may act they do not appear to alter the mode of action of pyrethrins but only to enhance it. The reactions of insects to synergised pyrethrins are similar to their reaction to pyrethrins alone. This further supports the theory that synergists function by inhibiting detoxification rather than by forming a complex with pyrethrins which may well act in a slightly different way. Regression lines representing the responses of insects to synergised pyrethrins are generally parallel, indicating a similar reaction of the insect population but at a different level.

It is difficult, therefore, to explain the results of some experiments, for example with Carpophilus dimidiatus and Acyrtosiphon pisum in which the regression lines were not parallel, particularly as the differences of the slopes of the lines do not always correspond in different experiments with the same species.

Wilson (1949) dosed Musca domestica and Calliphora vomitoria with pyrethrins in deodorised kerosene by topical application. He also applied piperonyl cyclonene and piperonyl butoxide to the flies both with the pyrethrins and separately to different parts of the insects. When either of these compounds was applied to a part different from that on which pyrethrins was applied, the effect was nearly as great as that produced by the same compound applied together

with the pyrethrins.

It might be concluded from this evidence that the pyrethrins and synergist penetrate the cuticle at the sites of application and meet inside the insect body where the synergist prevents detoxification of the pyrethrins. However, Lewis (private communication) has investigated the fate of small quantities of saturated oil applied to the cuticle of houseflies by incorporating a small quantity of unsaturated oil combined with radio-iodine 131 with the saturated oil. Autoradiographs of the treated flies showed that the oil had spread over most of the cuticle surface very quickly.

It is possible, therefore, that in Wilson's experiments the pyrethrins and synergist mixed with one another while still on the cuticle.

Other workers have pointed out that many pyrethrins synergists, including piperonyl butoxide, incorporate the methylene dioxyphenyl group in their molecule. Beroza (1956) examined 66 compounds containing this group and concluded that almost all those which were ethers, acetals or sulphonates showed some synergistic activity and that some would repay further investigation. The exact significance of this grouping is not apparent.

In this work no attempt has been made to separate the pyrethrins complex into its constituents or to examine these constituents individually.

Variation of factors of synergism.

The experiments described in the first section of this work show the variation between species in the enhancement of the toxicity of pyrethrins due to the presence of the synergist piperonyl butoxide. This enhancement of the toxicity of pyrethrins has been calculated as the factor of synergism - a term defined in the introduction to this work. In table 90 the results of these experiments have been summarised and the factors of synergism obtained for the insects examined are recorded. In the case of Ephestia cautella and Plodia interpunctella the difference in toxicity between synergised and unsynergised pyrethrins was too small to be detected by the experimental method employed.

Brooke (1958) reported very low factors of synergism when Ephestia elutella larvae and adults were treated with pyrethrins and piperonyl butoxide. His results are shown in table 91. The failure to detect synergistic action in Ephestia cautella and Plodia interpunctella, both closely related to E. elutella systematically, is slightly surprising. However, there is no reason at the present state of knowledge why the two species of Ephestia should behave exactly alike. The author (1958), commenting on Brooke's paper, quoted the results with P. interpunctella and E. cautella and pointed out that the standard error of the median response dose in the author's experiments was such that only those preparations of which one was twice as toxic as the other could be readily distinguished. For this reason it

is better to state that the factors of synergism for P. interpunctella and E. cautella are too small to be detected by the experimental methods employed rather than to state that they have a value of 1.0.

The variation in the values obtained for factors of synergism for the insects examined here may be explained in terms of the detoxification theory. There seem to be two possibilities:

1. The efficiency of the detoxification process may vary between species.
2. Piperonyl butoxide may vary in its ability to detoxify pyrethrins in different insect species.

Suppose the pyrethrins-detoxification process was more efficient in species A than in species B. If piperonyl butoxide was equally effective in both species, partial inhibition of the detoxification process in species A would leave the insect able to detoxify more pyrethrins than species B after a similar partial inhibition of detoxification. Therefore, the synergist would be expected to have a smaller effect on species A, giving a smaller factor of synergism. In this case insects showing small or negligible synergism, such as P. interpunctella, would be expected to have a particularly effective detoxification mechanism which is unaffected by the presence of piperonyl butoxide. This theory implies that when synergistic action takes place, the insect is able to detoxify the pyrethrins almost instantaneously, otherwise the inhibitory effect of the synergist would have some effect. Except when a very large dose of pyrethrins is administered,

the death of the insect is preceded by a period of paralysis.

If the dose is small enough the insect recovers from paralysis and possibly detoxification takes place during paralysis. This reaction is common to all insects including P. interpunctella and E. cautella and would be unlikely in insects if the detoxification process were instantaneous. It seems improbable, therefore, that variation in efficiency of detoxification mechanisms between species accounts for variation in factor of synergism.

Variation of the ability of piperonyl butoxide to detoxify pyrethrins seems a more likely explanation. Whatever the site of detoxification, the work of Chamberlain and Lindquist shows that the synergist and insecticide must both be present at this site if detoxification is to be prevented. In this connection, the rate at which a synergist penetrates into the insect is likely to be important.

Any information regarding the rate at which pyrethrins loses its toxicity when inside the insect is of importance in investigating the action of pyrethrins synergists. A biological assay for the determination of pyrethrins residues in insects is preferable in some ways to a chemical determination. The pyrethrins molecules may be slightly changed in the insect without becoming detoxified not only in the course of a detoxification process. These changes may or may not be detected by chemical assay, whereas biological determination makes the distinction between toxic and non-toxic residues which is required in investigations of this sort.

For this reason biological assays of residues of pyrethrins in poisoned insects were undertaken using the brine shrimp as a test organism. It is convenient to defer discussion of these experiments, and to mention first some further points arising from the investigations into the variation of the factor of synergism between species.

The differences in factors of synergism noted here must reflect differences in the physiological make-up of the insects. A comparison of these differences between groups of insects would be of great interest. To be of any value, such a comparison would have to be based on the examination of a very much larger selection of species than has been made here or in any other set of investigations. No great differences in synergistic activity have been reported between the species of Diptera or of Coleoptera which have been extensively used in investigations on pyrethrins synergism.

In the present work a comparison is provided between the synergistic activity of one species of butterfly and three species of moth. (Table 90).

Biological assay.

The first part of this work involved the modification and development of techniques of biological assay to suit the insects to be examined and to provide the information required.

Of the three basic types of assay adopted (topical application, crawling insect assays and spraying), topical application is probably the most satisfactory provided the insects are large enough to be easily handled. The micro-syringe delivery drops of $1.0\ \mu\text{l.}$ of insecticide in acetone was easier and quicker to use than Kerr's apparatus which delivered drops of $0.05\ \mu\text{l.}$ of insecticide in odourless kerosene and had to be used in a darkened room. Unless very small insects are used, when the difficulty of holding them renders topical application difficult anyway, the volume of $1.0\ \mu\text{l.}$ of solvent used with the micro-syringe did not affect the test insects adversely. In future work it would be better to substitute a less volatile solvent such as octane for acetone. If the micro-syringe were left for any time between applications, evaporation of the acetone took place. The use of the apparatus already described (fig. 1) to measure the dose delivered by the micro-syringe semi-automatically considerably improved the accuracy and speed of the operation. This apparatus was obviously not as quick to operate as more complicated applicators such as that described by Hewlett (1954). It was, however, suitable for the type of experiment described here.

Provided the test insects are fairly active under experimental conditions, but do not leave the poisoned surface, the crawling insect type of assay is also very satisfactory. Carpophilus dimidiatus is ideally suited to this type of assay. The use of the electrified barrier enabled extensions of the crawling insect assay to other, less suitable species to be made. Before the electric barrier is used very extensively its effects on the insects ought to be investigated more fully than has been done here. The author was satisfied that control responses as determined in the experiments were not affected. However, there may be considerable influence on the behaviour of the insects.

The use of a "crawling insect" assay with flying moths gave surprisingly good results, probably because these insects were fairly active during exposure.

A spray technique was only used for the assay of Aphids when other methods proved less suitable. The actual dose received from a spray by an insect in flight must be very variable. However, the aphids were sprayed while on a surface and in an apparatus designed to give a particularly even spray deposit.

Hurst (1943) and many others have drawn attention to the enormous effect of carriers, solvents and conditions of application on the toxicity of insecticides. Bearing this in mind, the important results obtained with Plodia interpunctella as the test insect using crawling insect assays were verified using topical application techniques.

In assessing results of work of this sort, the variation in susceptibility of insects to insecticides must be remembered. Susceptibilities vary not only as between species but also as between strains of the same species or even cultures of the same strain. These differences are demonstrated in the results shown here. They may be minimised if care is taken to ensure uniformity of cultural methods. In particular, overcrowding is to be avoided. The relative susceptibilities of insect species to synergised and to unsynergised pyrethrins do seem to be more constant.

It is important to verify the assumption that the synergist is non-toxic. In the experiments reported in tables 18 and 19, P. interpunctella larvae were exposed on filter papers to high concentrations of piperonyl butoxide. Under these conditions the piperonyl butoxide was toxic to the larvae and an apparent case of synergistic activity was demonstrated. The action of the piperonyl butoxide was probably mechanical, covering the insects and blocking their trachea.

Biological assays with Carpophilus dimidiatus of pyrethrins synergised with N-octyl bicycloheptane dicarbonimide (which has been referred to by its old trade name of "van Dyke 264" for convenience) apparently showed this synergist to vary greatly in its effectiveness. These results may probably be explained as being due to incomplete solution of the synergist in the mixed diluent during preparation of the test solutions. This result serves as a reminder of the care which must be taken to ensure proper solution and even

dispersal of insecticides for biological assay.

The simple proportionality of toxic effect and the product of insecticidal concentration, and duration of exposure has been shown to hold good with respect to Carpophilus dimidiatus exposed to films of pyrethrins (table 42), as well as for Artemia salina nauplii immersed in pyrethrins in saline. When exposing insects to fumigants the dosage is expressed as the product of the concentration of fumigant and the time of exposure. Within limits the concentration and time may be altered so as not to alter the value of the product of the two and the response of the insects remains unchanged. The graphs and tables quoted suggest that this also applies to C. dimidiatus and A. salina under the conditions mentioned and presumably has wider applications. Such a result suggests that the insects continue to absorb insecticide from the deposit, so that the amount acquired is a constant for a given concentration/time product.

Sub-lethal responses.

Sub-lethal responses of insects to insecticides, that is responses other than death, may or may not be followed by the death of the treated insect. For example, the paralysis which is one of the first responses of insects to pyrethrins is followed by the death of the insect if a sufficiently large dose of the insecticide has been received, otherwise the treated insect may recover.

Sub-lethal responses can affect the commercial value of insecticides in controlling pests. For instance, a proportion of pyrethrins is often added to fly sprays to produce a quick knockdown or paralysis after spraying which lasts long enough to keep the insect inactive until the slower acting insecticide becomes effective.

A study of an insect's reactions to sub-lethal dosage may provide valuable evidence regarding the mode of action of an insecticide. The possibility is discussed below that the period of paralysis following a sub-lethal dose of pyrethrins may represent the time required by the insect to detoxify the pyrethrins it has received.

Great use has been made in this work, as well as by other authors, of sub-lethal effects in determining the responses of insects in biological assay. It has been stated already that the use of death as a response to assays can be very unsatisfactory,

due to the difficulty of deciding whether or not an insect is actually dead. Sub-lethal responses may be accurately, even if arbitrarily, defined for particular bio-assay systems and hence remove any subjectivity from the determination of responses.

As a result of poisoning with pyrethrins, Calandra granaria, Carpophilus dimidiatus, Plodia interpunctella, Ephestia cautella and Aedes aegypti showed disruption of their normal phototropic reactions. These reactions were used to estimate response in biological assay. C. dimidiatus larvae and adults, and P. interpunctella and E. cautella larvae were unable to crawl off a circle of filter paper away from a light if affected by pyrethrins. If the size of the circle on the filter paper, the time allowed for the insects to crawl off, and the intensity and distance of the light were specified, it was possible to make estimates of response to the insecticide which were quite objective and to use these estimates in the assays. The loss of ability of A. aegypti to swim away from a light source over a fixed distance in a fixed time was also used as a means of estimating response to biological assay.

Other sub-lethal effects used in this work to estimate responses were the loss of the reaction of Acyrtosyphon pisum to move sideways on mechanical stimulation and the inhibition by pyrethrins poisoning of the regular swimming rhythm of Artemia salina.

Wallis and De Bishop (1957) reports what they describe as "death stresses" in Aedes canadensis adults. When subjected to

tobacco smoke, the adult mosquitoes were stimulated to lay eggs before dying. It is possible to make a distinction between these death stresses and sub-lethal responses which are followed by the recovery of the insect. Stimulation of P. interpunctella adults by treatment with pyrethrins promoted egg laying. If the dose of pyrethrins was small the moth may have been temporarily paralysed but recovered subsequently. If the dose was larger, the moths died during or after oviposition which in this case may be considered as a death stress.

Experiments described here showed that adults of Plodia interpunctella and Galleria melonella were stimulated to lay eggs on being poisoned with moderate doses of pyrethrins. This reaction may have an important practical application in the case of P. interpunctella. If a pyrethrins film in a warehouse is not maintained by regular spraying the effective concentration of pyrethrins falls owing to breakdown of the insecticide, as well as accumulation of dust, etc. Adult moths landing on such a film may take longer to die than on a properly maintained surface and be stimulated to oviposit first or they may only be stimulated to oviposit. The moths' activity may increase due to the pyrethrins and a large proportion of the eggs laid may be viable and laid on surfaces which are fairly free of insecticide. However, pyrethrins probably stimulates the rate of oviposition rather than increases the total number of eggs laid. The survival of the larvae hatching from the eggs depends largely on whether the eggs were deposited near a food supply. Mature larvae are less sensitive to pyrethrins

than adults but this may not apply to newly hatched larvae, in which case the film of pyrethrins which stimulates the adult to oviposit may be sufficiently powerful to kill the young larvae.

Thus a sub-lethal dose of pyrethrins may conceivably increase a population of insects. It would be interesting to investigate the relative importance of the factors mentioned above and so elucidate this question.

Results (tables 15 and 16) of experiments in which P. interpunctella adults were exposed to a series of concentrations of pyrethrins showed that the number of eggs laid reached a peak at about the median response dose. There appear to be two factors operating here. On exposures to concentrations of pyrethrins up to a certain level, the number of eggs laid rises with increase in concentration of pyrethrins due, presumably, to increased stimulation of the moths by the insecticide. Beyond this critical level of concentration, increase in dose of pyrethrins causes quicker paralysis and death to the moths so that they are unable to lay eggs. The number of eggs laid therefore falls with increasing concentration of pyrethrins.

The stimulation of excretion demonstrated with Galleria melonella and Pieris brassicae larvae went up with increasing concentrations of pyrethrins within the limited range of doses used. Unlike the oviposition response, a peak was not reached at about the median response rate. The loss of weight of G. melonella larvae (table 74) associated with stimulation to excretion is, of course,

to be expected.

If larvae are subjected to massive overdoses of pyrethrins, death may not be associated with this excretion and loss of weight. On the other hand the convulsions caused by the pyrethrins may lead to sudden excretion and loss of weight also.

Both oviposition and weight loss (which must be principally water loss) may be considered as arising from stimulation and derangement of the nervous system due to pyrethrins poisoning. The loss of water due to pyrethrins poisoning may be a very important subsidiary factor in the mode of action of pyrethrins. By exposure of insects to pyrethrins, in high humidity or under other low water-loss conditions, it may be possible to reduce the rate at which the insects die. If such experiments gave negative results it should not be concluded that water loss has no effect on the death rate because, as has already been said, the water loss is brought about by stimulation of excretion. Ingram (1955) showed that flies and cockroaches treated with pyrethrins lost water but did not consider this to be a direct cause of death. He suggested that water loss was due to interference of a mechanism controlling epidermal secretion.

Stabilisation of pyrethrins films by piperonyl butoxide.

It has been suggested that one of the ways in which pyrethrins synergists act is to prevent the deterioration of the toxicant, especially deterioration caused by light. With the biological assay techniques used in some of the author's experiments, Miss J. M. Donaldson has investigated this problem. Her work, using Ephestia cautella adults (tables 26 and 27), indicated that the presence of piperonyl butoxide does not protect pyrethrins films from breakdown on irradiation with ultra-violet light. Ephestia cautella (or Plodia interpunctella) were considered to be particularly suitable for the investigation of this problem. As the moth is unaffected by the presence of piperonyl butoxide in a pyrethrins formulation, the results of assays are intended to demonstrate loss of toxicity of pyrethrins due to irradiation in the presence or absence of piperonyl butoxide will not be confounded by the synergistic action of the piperonyl butoxide.

The previous evidence on this subject is confusing. Phipers and Wood (1957) using chemical methods of assay claimed that stabilisation of pyrethrins by piperonyl butoxide did occur. Blackith (1953) concluded that there was no stabilisation. If stabilisation occurs at all it is probably only to a very limited extent. Blackith pointed out that in a film containing both pyrethrins and synergist, the breakdown of the pyrethrins is not accompanied by breakdown of synergist. As the pyrethrins is removed the ratio of synergist to pyrethrins increases giving greater synergistic action,

so that the film may retain its toxicity to insects susceptible to pyrethrins synergists.

More detailed investigation of this point would require the refined use of methods of biological and chemical assay in conjunction.

The use of Artemia salina in biological assay.

Dewey (1958) reviewed the use of biological assay in the determination of insecticide residues and mentioned a number of species suitable for these assays. The most thoroughly investigated of these species is the yellow fever mosquito - Aedes aegypti.

Burchfield and Hartzell (1955) described a biological assay method using 4th instar Aedes aegypti larvae as the test organism and making use of their negative phototropic reaction to evaluate the response. To compare Burchfield's method with assays using A. salina described here, an assay with mosquito larvae was carried out (table 63).

Michael et al (1956) described the use of Artemia salina adults in biological assay and mentioned in passing the possibility of using the first stage nauplii. The use of the nauplii for the biological assay of pyrethrins residues has been investigated. The relative merits of the adult shrimp and of the nauplii for this work have already been discussed.

Some assaying of insecticide residues may well be done using micro-chemical methods. The essential point about these experiments was to distinguish between toxic and non-toxic residues. Slight alterations in the chemical structure of an insecticide may render it non-toxic to the insect but may not be easily distinguished by chemical techniques. A biological assay such as that described here might be more sensitive or more convenient than a micro-chemical

assay and might more easily yield information on the availability of pyrethrins in insect tissue. Chemical assay is outside the scope of this work, but any further extension of it would be greatly facilitated if done in conjunction with chemical assay.

The sensitivity of *A. salina* nauplii to pyrethrins and other insecticides.

The sensitivity of *A. salina* nauplii to pyrethrins compares favourably with that of *A. aegypti*. If a particularly sensitive method of assay such as this is available, it may be used with advantage even though it may not be very simple or accurate. It is often sufficient, at this stage of knowledge, to be able to demonstrate the presence or absence of insecticidal activity.

A comparison of the different methods of biological assay used in this work show the following concentrations to be easily detected:

	<u>Parts per million pyrethrins.</u>
<u>Calandra granaria</u> (contact)	5,000
<u>Plodia interpunctella</u> (contact)	500
<u>Plodia interpunctella</u> (topical)	20
<u>Aedes aegypti</u> larvae	2
<u>Artemia salina</u> nauplii	0.01

These figures show approximately the levels of concentration of pyrethrins which were easily detected in experiments described here. A very cursory examination of the use of *A. aegypti* was made and further investigation would doubtless reveal a greater sensitivity as described by Burchfield.

In using Artemia salina which is a Crustacean for the biological assay of insecticides it must be remembered that the physiological structure and functioning of the Crustacea and Insecta are not necessarily the same. There are wide differences among the insects in their susceptibilities to the different insecticides and it would not be at all surprising to find that compounds toxic to some Crustacea are not toxic, or are much less toxic to insects and vice versa. Piperonyl butoxide alone is not toxic to insects at the concentrations normally used in insecticide formulations but it has been shown to be poisonous to A. salina. In undertaking any work on the biological assay of insecticides with A. salina these considerations should not be forgotten.

In this connection the toxicities of some other insecticides to A. salina nauplii have been briefly investigated. The nauplii were found to be sensitive to 0.5 parts per million of dieldrin and to 0.5 parts per million of benzene hexachloride. The solutions in saline for these experiments were made up by diluting solutions of dieldrin and benzene hexachloride in acetone and alcohol. However, under certain conditions the nauplii appeared to be completely insensitive to dieldrin. If nauplii in saline were put in a glass dish containing crystals of dieldrin they were unaffected. This was presumably due to the insolubility of dieldrin in water. Addition of up to 2% alcohol did not apparently bring sufficient dieldrin into solution.

The efficiency of the extraction method.

The choice of a solvent with which to make extractions of the macerated insect tissues is important. The solvent must be non-toxic to A. salina at the concentrations present after saline has been added to the extract. It must be a good solvent for the insecticide residue and it must be miscible with saline. The choice is therefore limited.

When acetone was used it was found to be too toxic to the nauplii and alcohol was therefore chosen but was not generally used at a concentration exceeding 0.67% in saline. This placed a limit on the amount by which the saline could be diluted with alcoholic extract. A distillation under reduced pressure was used to reduce the volume of alcohol, but had to be carried out with care because of the unstable nature of pyrethrins. Again work in conjunction with a chemist may result in improvements in the technique and efficiency of the extraction process. Propylene glycol was also examined as a possible solvent but alcohol was thought to be more satisfactory.

Further work may show that more efficient extraction results when the extract is made using a solvent (e.g. light petroleum) in which pyrethrins are very soluble but which is not miscible with water. The dissolved residue could then be transferred to another solvent, more suitable for mixture with saline.

The toxicity of the extracts from Plodia interpunctella and Galleria melonella has been shown to be about one tenth of the

toxicity of the pyrethrins applied to the insects. This loss of toxic material may be due largely to the inefficiency of the extraction procedure. It may also be due to chemical changes of the pyrethrins in the insects. In table 74 it is shown that when C. dimidiatus larvae were sprayed with three different concentrations of pyrethrins, the toxicity of extracts of these beetles varied in proportion to the original dose of pyrethrins. Although a large part of the pyrethrins was not recovered in the extract, the toxic material recovered was proportional to the dose of pyrethrins.

Concentration time product.

The simple proportionality of the toxic effect of pyrethrins to A. salina and the product of duration of exposure and insecticidal concentration has already been pointed out. This proportionality is a well known feature of the fumigation of insects and in this respect the exposure of nauplii in a solution containing pyrethrins is analogous to fumigation. The system corresponds to the very simple case of fumigation of insects at a constant concentration at any one exposure since the removal of poison by the nauplii is, presumably, too small to reduce the concentration in the aqueous alcoholic solution materially.

Detoxification of pyrethrins.

The results of the experiments with Artemia are not entirely compatible with the detoxification of any large proportion of pyrethrins in the tissues of insects. Although only a small proportion of the original dose of pyrethrins was shown to be present in extracts of treated insects, this toxic material did not decrease markedly if the insects were left for up to two hours before extraction as would be expected if a massive detoxification were operating. In this respect no difference was detected between extracts of P. interpunctella (not affected by synergists) and C. dimidiatus (having a fairly large factor of synergism).

The loss of toxic materials from locust blood 80 minutes after treatment does not necessarily mean that detoxification has taken place. It may be that the pyrethrins are sorbed onto other body tissues during this time.

Although piperonyl butoxide in solution in saline was shown to be toxic to Artemia salina, extracts of P. interpunctella and C. dimidiatus treated with piperonyl butoxide were not toxic to the nauplii, and extracts of insects treated with pyrethrins and piperonyl butoxide were generally not more toxic than were extracts of insects treated with pyrethrins only. At first this result was assumed to be due to the inefficiency of the extraction process in failing to remove the synergist from the insect tissue. However, in the experiments with locust blood, an extraction process was not employed and again the presence of piperonyl butoxide was not detected by

the Artemia nauplii assay. It therefore seems possible that the synergist is very strongly held by the insect tissue in such a way that it cannot be released into solution or suspension in saline.

The work of Chamberlain and others already mentioned demonstrated that if piperonyl butoxide was applied to insects some time before the application of pyrethrins, a synergistic response was obtained, whereas if the pyrethrins were applied first little or no synergism was observed. This suggests that the detoxification of pyrethrins in the insect is rapid.

It may be that the small amount of toxic material detected by the Artemia assay in extracts made of insects immediately after treatment with pyrethrins is partly due to a considerable amount of detoxification already having taken place. The fact that similar results were obtained in the presence of piperonyl butoxide is difficult to explain. The inhibitory effect of the piperonyl butoxide may be removed during the maceration of the insect tissues. On the other hand, synergistic action may include other processes besides the prevention of detoxification of pyrethrins molecules.

Final Considerations.

There is considerable scope for further work as a result of the experiments described above, in particular the experiments in which Artemia assays were used to follow the disappearance of insecticide from treated individuals.

The investigation into the action of piperonyl butoxide as a pyrethrins synergist on a selection of insects has shown the variation in the effectiveness of the synergist between species. The physiological basis of these variations is not well understood though they may reflect the partial inhibition by the synergist of different rates of pyrethrins detoxification obtaining in different species. A detailed comparative study in species known to vary in their response to synergised pyrethrins is clearly required. As an additional point of interest, the species employed in such a comparative investigation might be chosen to disclose possible phylogenetic correlations with the physiological factors involved in ability to respond to synergised pyrethrins.

It would also be of interest to repeat these experiments using other synergists.

The work described here in which a method of microbiological assay using Artemia salina has been developed must be considered as largely introductory. Further information on the efficacy and accuracy of the method would be of great value. The assay of separate tissues of insects poisoned with pyrethrins would yield information about the fate of the insecticide after entry into

the insect. It has been shown that the toxicity of the blood of poisoned locusts fell when the locusts were left for a time after treatment before withdrawing the blood. This may be due to the transfer of the toxic residue to other tissues or possible to loss of toxicity of the residue.

A further investigation into the apparent disappearance of piperonyl butoxide in extracts and into the possibility of other synergists behaving similarly, may provide valuable information about the function of synergists.

The importance to commercial users of insecticides of establishing the toxicity of a particular formulation when applied in a particular manner to a particular species which has to be controlled is obvious. It is surprising, therefore, that the enhancement of toxicity to species of beetles produced by including piperonyl butoxide in formulations of pyrethrum was for several years assumed to obtain also against species of moths, without any evidence being produced to support this assumption. An immediate outcome of this investigation has been the decision to continue to use pyrethrum formulations without the addition of piperonyl butoxide for the control of P. interpunctella and E. cautella by residual film in warehouses containing dried fruit imported from Australia. For this purpose piperonyl butoxide acts merely as an expensive diluent.

SUMMARY.

1. Methods of biological assay have been developed to investigate the susceptibility of eleven species of insect to pyrethrins and to pyrethrins to which the synergist piperonyl butoxide has been added. The increase in the toxicity of the pyrethrins brought about by the addition of the synergist varies between species and has been expressed as the factor of synergism. The results of these experiments are summarised in table 90.
2. An electrified barrier to confine insects during biological assay and a semi-automatic method of delivering known volumes from a micrometer syringe have been described.
3. The responses of male and female Plodia interpunctella to pyrethrins have been compared.
4. The stimulation of Plodia interpunctella adults to lay eggs when treated with pyrethrins has been briefly examined.
5. The stimulation of larvae of Galleria melonella and Pieris brassicae to exude fluids when treated with pyrethrins is reported.
6. The enhancement of the toxicity of pyrethrins to Carpophilus dimidiatus caused by the addition of piperonyl butoxide to the insecticide formulation is compared with the enhancement caused by addition of the synergist known as van Dyke 264.
7. The exposure of Plodia interpunctella larvae on wooden

squares treated with pyrethrins is compared with exposure on filter papers.

8. Experiments which demonstrate that piperonyl butoxide does not stabilise pyrethrins are reported. These experiments were carried out by Miss J. M. Donaldson using Ephesia cautella adults.

9. The development of a method of biological assay of insecticide residues using nauplii of the brine shrimp Artemia salina is described. Extracts of Carpophilus dimidiatus, Plodia interpunctella and Galleria melonella which had been treated with pyrethrins and with synergised pyrethrins have been assayed using the nauplii. If insects treated with pyrethrins were left for a time before extraction the toxicity of the extract to A. salina was not conspicuously less than the toxicity of extracts of insects made immediately after treatment of the insects. An experiment in which blood from locusts (Schistocerca gregaria) poisoned with synergised pyrethrins was assayed with A. salina is reported.

10. The significance of these results is discussed.

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Figure 1a: Apparatus for topical application of measured doses of insecticides - isometric view.

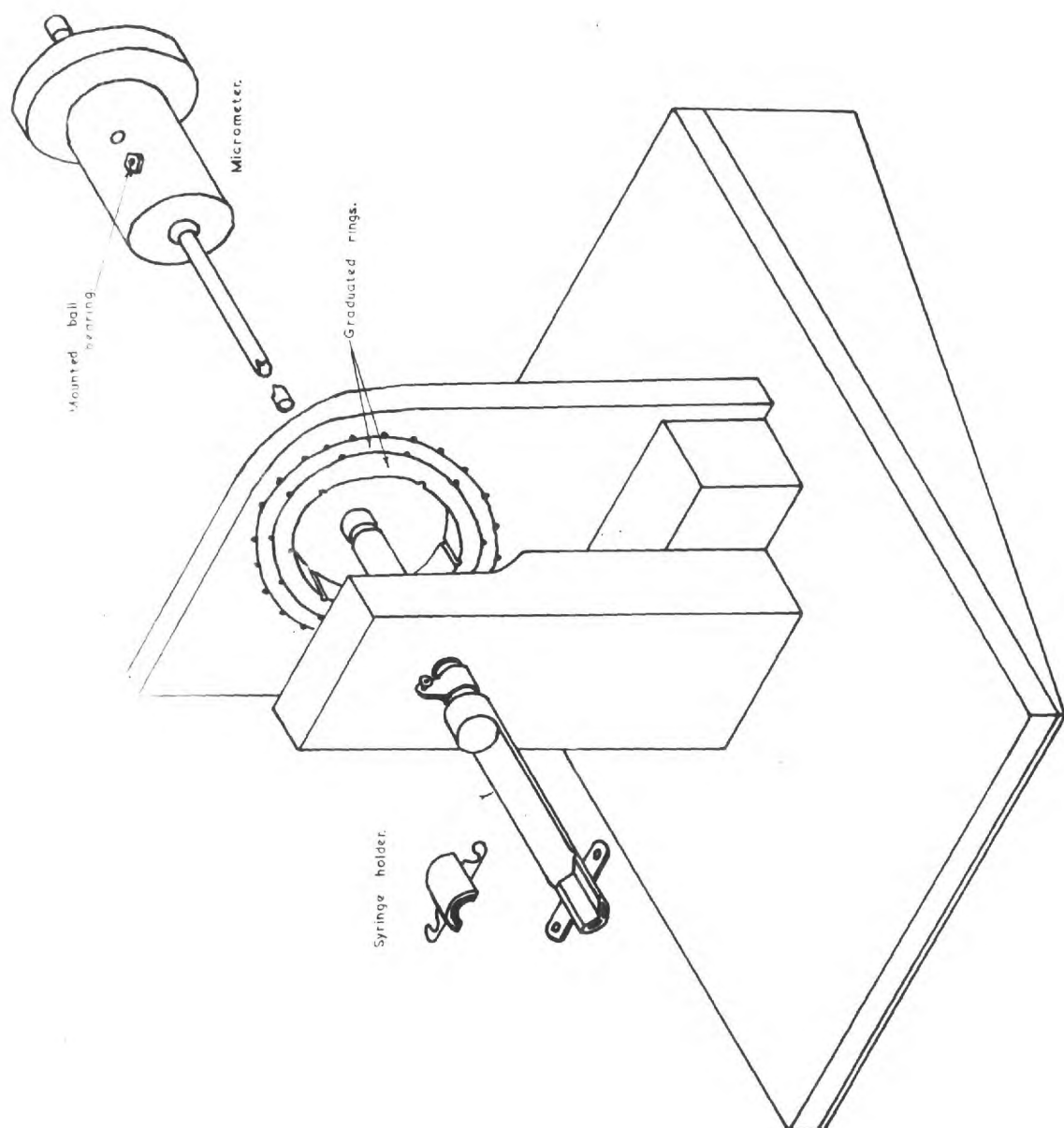


Figure 1b:

Apparatus for topical application of measured doses of insecticides - Exploded diagram and plan to show arrangement of rings.

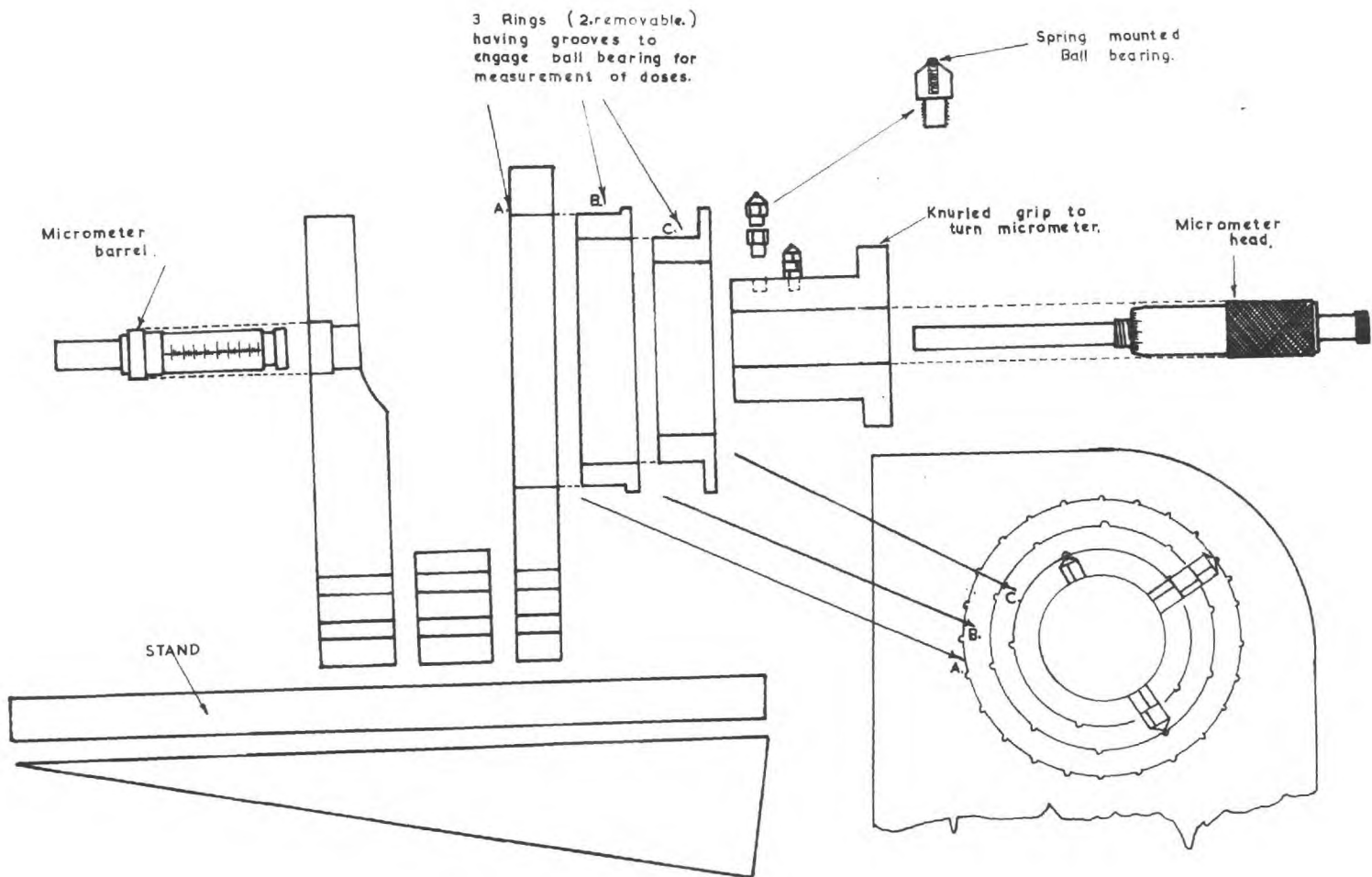
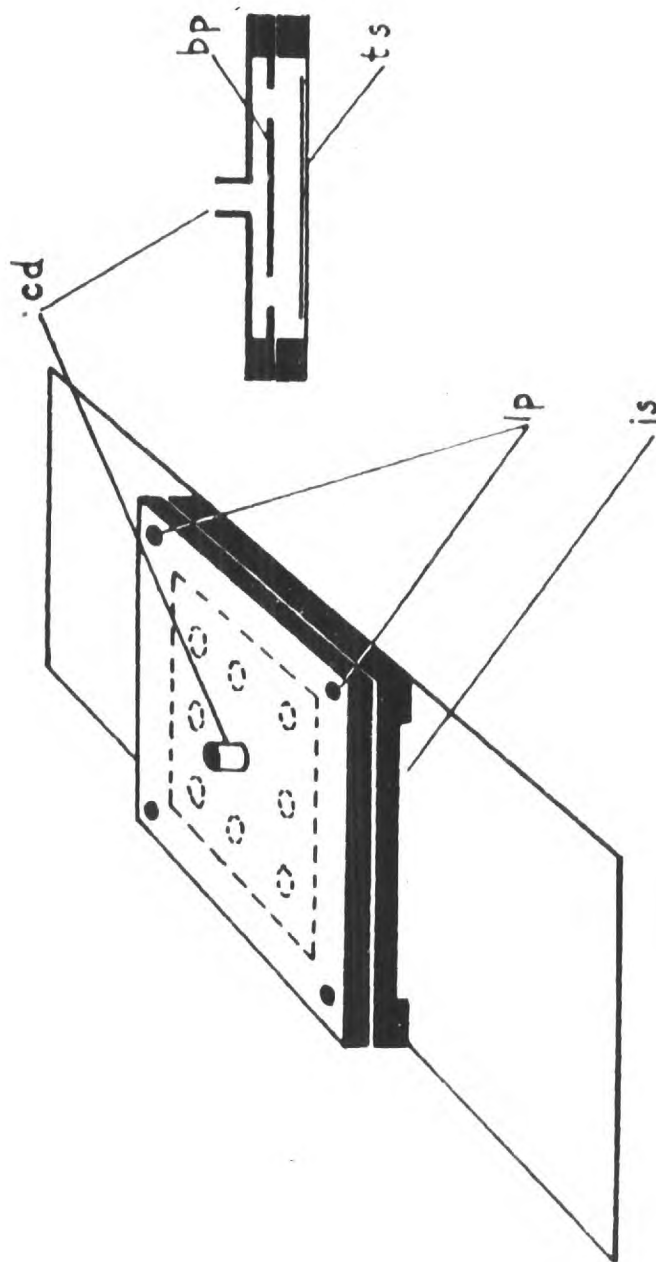


Figure 2: Apparatus to confine flying insects on deposits (After Lewis & Hughes, 1957)



—The apparatus for exposing flies to deposits: bp, baffle plate;
cd, carbon-dioxide duct; is, slot for insertion of treated surfaces;
ip, locating pins for lid of chamber; ts, treated surface.

Figure 3: Electrified barrier to confine insects during "crawling insect" biological assays.

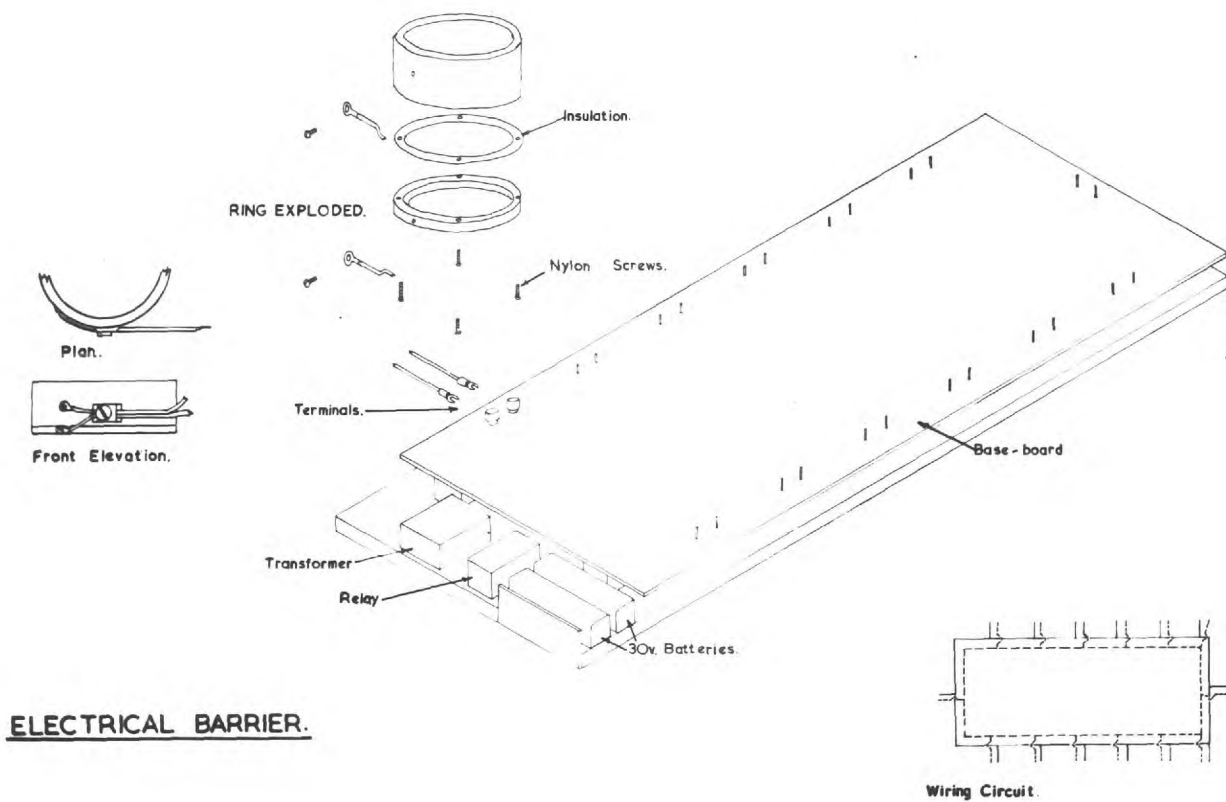
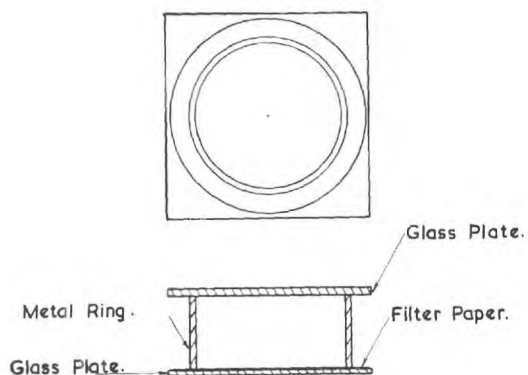


Figure 4: A: Arrangement of metal ring and glass plates used to confine insects during assay.



B. Liquid barrier to trap insects after estimation of knockdown.

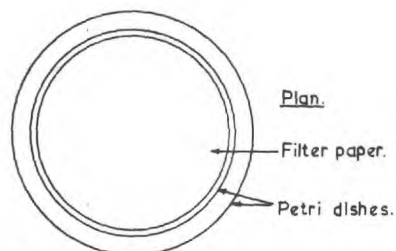
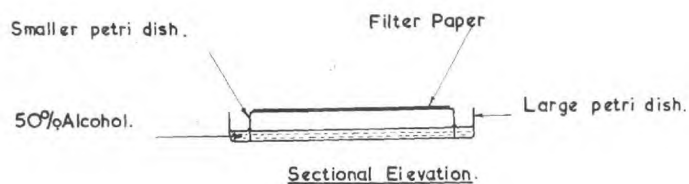


Figure 5: Apparatus to count Artemia salina nauplii.

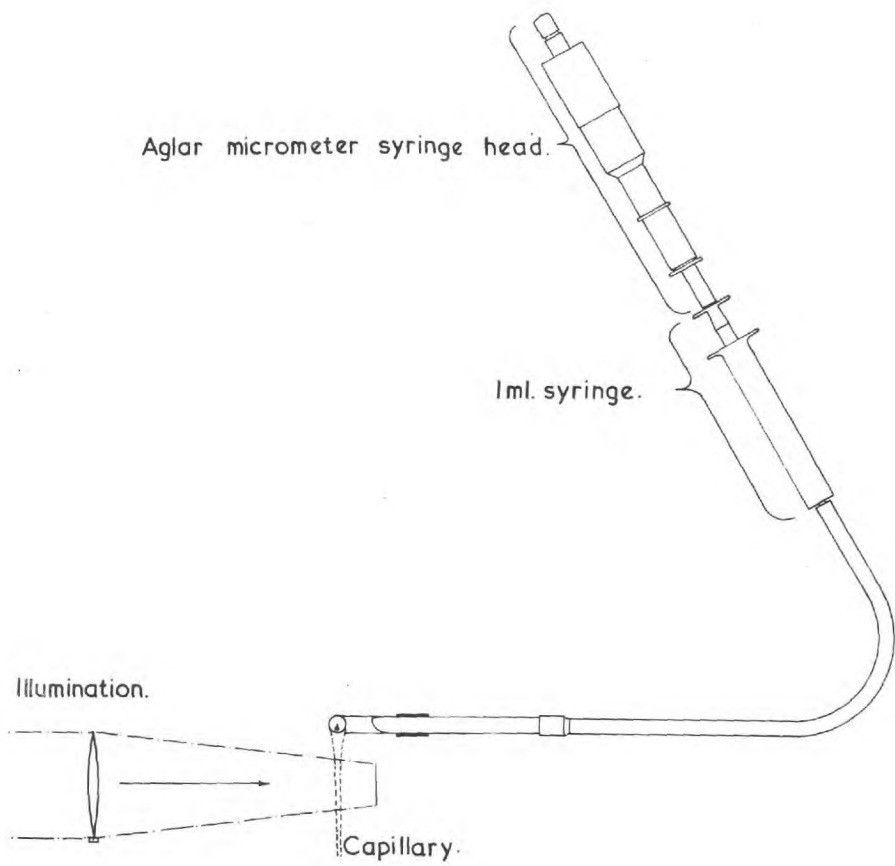


TABLE 1. Calandra granaria adults exposed on filter papers impregnated with pyrethrins for 24 hours. Insects confined by oiled brass rings.
 Response: Failure to leave 5.4 cm. diameter pencil circle in 1 minute.
 See graph 1.

Concentration %	Pyrethrins (Log.(Conc. x100)	Total	Knockdown % ^x	Angle
0.25	1.40	28/30	93	74.7
0.175	1.24	24/30	78	62.0
0.1225	1.09	17/30	53	46.7
0.0857	0.93	13/30	39	38.6
0.0600	0.78	4/30	7	15.3
0.0420	0.62	3/30	3	10.0
0	-	2/30	-	-

x Correction made for control mortality.

$$\chi^2_{\text{for regression}} = 2.21; \quad = 0.7 > P > 0.5.$$

RD 50 (median response dose) = 0.127% pyrethrins.

TABLE 2. *C. granaria* adults on filter papers impregnated with pyrethrins (+ piperonyl butoxide) for 24 hours. Insects confined by oiled brass rings.
Response: Failure to leave 5.4 cm. circle in 1 minute.
See graph 2.

Conc. %	pyrethrins Log. (Concl. x 100)	% Conc. Pip. butoxide	Total	Knockdown %	Probit
0.250	1.40	0	30/30	100	∞
0.175	1.24	0	28/30	93	6.47
0.122	1.09	0	26/30	87	6.13
0.086	0.93	0	19/30	63	5.33
0.060	0.78	0	2/28	7	3.52
0.042	0.62	0	0/30	0	
0.062	0.80	0.312	30/30	100	∞
0.044	0.64	0.218	30/30	100	∞
0.031	0.48	0.153	30/30	100	∞
0.021	0.33	0.107	25/30	83	5.95
0.015	0.18	0.075	19/30	63	5.33
0.010	0.02	0.052	8/30	27	4.39

χ^2_4 for regression (pyrethrins only) = 5.88; $0.3 > P > 0.2$

χ^2_2 for regression (+ synergist) = 1.74; $0.5 > P > 0.3$

Factor of synergism = 6.5 at RD 50.

TABLE 3. P. interpunctella adults exposed for 10 minutes on filter papers impregnated with pyrethrins.

Response assessed as mortality 2 hours after end of exposure.

See graph 3.

Conc. pyrethrins % Log. (Conc. x 100)		Total	Mortality %	Angle
0.25	1.4	34/37	92	73.6
0.125	1.1	30/37	81	64.2
0.062	0.8	21/31	68	55.6
0	-	0/34	-	-

TABLE 4. P. interpunctella adults exposed on filter papers impregnated with pyrethrins for 30 minutes.

Response: Mortality 17 hours after end of exposure.

See graph 4.

Conc. pyrethrins % Log. (Conc. x 100)		Total	Mortality % ^x	Angle
0.5	1.7	21/22	95	77.1
0.25	1.4	11/20	53	46.7
0.125	1.1	8/22	33	35.1
0.062	0.8	2/21	5	12.9
0	-	1/25	-	-

x Correction made for control response.

χ^2 for regression - 0.94; 0.7/p > 0.5

TABLE 5. *P. interpunctella* adults exposed on filter papers for 10 minutes impregnated with pyrethrins - piperonyl butoxide (1:5).

Response: Mortality 24 hours after end of exposure.

See graph 5.

Conc. %	Pyrethrins Log.(Conc. x 100)	% Conc. Pip.butoxide	Total	Mortality % ^x	Angle
0.125	1.1	0.625	20/30	64	53.1
0.062	0.8	0.312	15/26	54	47.3
0.031	0.5	0.156	16/29	51	45.6
0.015	0.2	0.078	9/34	20	26.6
0.125	1.1	0	20/27	72	58.1
0.062	0.8	0	16/25	61	51.4
0.031	0.5	0	17/29	55	47.9
0.015	0.2	0	13/29	40	39.2
0	-	-	3/36	-	-

^x Correction made for control response.

χ^2 for regression, pyrethrins only - 0.15; 0.95 > p > 0.90

χ^2 for regression, pyrethrins plus synergist - 2.03; 0.5 > p > 0.3

χ^2 for regression, combined results - 5.44; 0.5 > p > 0.3

TABLE 6. *P. interpunctella* adults exposed on filter papers treated with pyrethrins + piperonyl butoxide (1:5) for 10 minutes.

Response: Failure to leave filter papers on removal of rings within 2 minutes, 1 hr. 10 mins. after end of exposure.

See graph 6.

Conc. pyrethrins		%	Pip. butoxide	Total	Knockdown	
%	Log.(Conc.x 100)				%	Angle
0.300	1.48		1.50	26/27	98	81.9
0.150	1.18		0.75	21/25	82	64.9
0.075	0.87		0.37	18/31	59	50.2
0.30	1.48		0	29/31	95	77.1
0.150	1.18		0	33/37	89	70.6
0.075	0.87		0	17/33	51	45.6
0	-		-	0/30	-	-

χ^2 for regression, pyrethrins only. - 2.65 0.2)P>0.1
 χ^2 for regression, pyrethrins, plus synergist - 0.047 0.9)P>0.8
 χ^2 for regression, combined results - 2.87 0.7)P>0.5

TABLE 7. Exposure of *Plodia interpunctella* adults to pyrethrins and piperonyl butoxide films on filter paper for 30 minutes.

See graph 7.

Concentration of pyrethrins % Log(Conc. x 100)		Concentration of piperonyl butoxide %	Total	Mortality %	Angle
0.250	1.4	-	20/20	100	90.0
0.125	1.1	-	16/19	82	64.9
0.062	0.8	-	16/22	68	55.6
0.031	0.5	-	3/18	6	14.2
0.015	0.2	-	2/18	0	0
0.250	1.4	1.250	29/29	100	90.0
0.125	1.1	0.625	11/14	76	60.7
0.062	0.8	0.310	8/24	25	30.0
0.031	0.5	0.155	5/18	19	25.8
0.015	0.2	0.075	3/21	3	10.0
Control		-	2/18	-	-

$$\begin{aligned} X^2_3 \text{ for regression (pyrethrins only)} &= 4.52; 0.3 > P > 0.2 \\ X^2_3 \text{ for regression (+ synergist)} &= 6.30; 0.10 > P > 0.05 \\ X^2_8 \text{ for regression (combined results)} &= 10.92; 0.3 > P > 0.2 \end{aligned}$$

TABLE 8. Exposure of P. interpunctella adults on filter papers impregnated with pyrethrins + piperonyl butoxide (1:5) for 30 minutes.

Response: Failure to fly off papers in 2 minutes after removal of rings and plates 24 hours after end of exposure (see text).

See graph 8.

Pyrethrins		Pip.butoxide	Knockdown		
% Conc.	Log.(Conc. x 100)	% Conc.	Total	%	Angle
0.062	0.8	0	22/26	85	67.2
0.031	0.5	0	9/22	41	39.8
0.015	0.2	0	8/24	33	35.1
0.062	0.8	0.312	22/30	73	58.7
0.031	0.5	0.156	10/22	45	42.1
0.015	0.2	0.078	11/29	38	38.1
0	-	-	0/22	-	-

$$\chi^2_{\text{for regression (pyrethrins only)}} = 2.35; 0.2 \rangle P \rangle 0.1$$

$$\chi^2_{\text{for regression (plus synergist)}} = 0.704; 0.5 \rangle P \rangle 0.3$$

$$\chi^2_{\text{for regression (combined results)}} = 3.83; 0.5 \rangle P \rangle 0.3$$

TABLE 9. *P. interpunctella* adults dosed with 1.0 μ l pyrethrins + piperonyl butoxide in acetone solution.

Response: Mortality after 5 hours.

See graph 9.

Pyrethrins		Pip. butoxide	Mortality		
% Conc.	Log.(Conc.x 100)	% Conc.	Total	%	Angle
0.0045	0.65	0	13/20	63	52.5
0.0031	0.50	0	9/20	42	40.4
0.0022	0.34	0	4/20	16	23.6
0.0015	0.19	0	4/20	16	23.6
0.0045	0.65	0.0225	13/20	63	52.5
0.0031	0.50	0.0155	9/20	42	40.4
0.0022	0.34	0.0110	6/20	26	30.7
0.0015	0.19	0.0075	6/20	26	30.7
0	-	-	1/20	-	-

* Correction made for control response.

$\chi^2_{\text{for regression (pyrethrins only)}} = 1.51; 0.5 > p > 0.3$

$\chi^2_{\text{for regression (plus synergist)}} = 1.72; 0.5 > p > 0.3$

$\chi^2_{\text{for regression (combined results)}} = 4.10; 0.7 > p > 0.5$

TABLE 10. Topical application to P. interpunctella adults of 1.0 μ l pyrethrins - piperonyl butoxide in acetone.

Response: Mortality after 6 hours.

See graph 10.

Pyrethrins		Pip. butoxide % Conc.	Mortality		
% Conc.	Log.(Conc. x 100)		Total	%	Angle
0.0045	0.65	0	16/20	78	62.0
0.0031	0.50	0	11/20	50	45.0
0.0022	0.34	0	7/20	39	38.6
0.0045	0.65	0.0225	16/20	78	62.0
0.0031	0.50	0.0155	15/20	72	58.1
0.0022	0.34	0.0120	12/20	55	47.9
0	-	-	2/20	-	-

* Correction made for control response.

χ^2 for regression (pyrethrins only) = 0.32; $0.7 > P > 0.5$

χ^2 for regression (plus synergist) = 1.82; $0.2 > P > 0.1$

χ^2 for regression (combined results) = 3.15; $0.7 > P > 0.5$

TABLE 11. Topical application to *P. interpunctella* adults of 0.05 μ l. pyrethrins in odourless kerosene.

Response: Mortality after 6 hours.

See graph 11.

Pyrethrins		Mortality								
% Conc. Log. (Conc. x 100)		Males			Females			Combined		
		Total	%	* Angle	Total	%	Angle	Total	%	* Angle
0.100	1.0	12/12	100	90.0	12/14	86	68.0	24/26	92	73.6
0.050	0.7	9/16	54	47.3	17/23	74	59.3	26/39	66	54.3
0.025	0.4	2/19	5	12.9	7/21	33	35.1	9/40	20	26.6
0	-	1/18	-	-	0/22	-	-	1/40	-	-

* Correction made for control response.

χ^2 for regression of males and females combined = 0.54; $0.5 > P > 0.3$

TABLE 12. Topical application to P. interpunctella adults of 0.05 μ l. pyrethrins in odourless kerosene (using Kerr apparatus).

Response: Failure to leave filter papers on removal of rings within 2 minutes
5 hours after end of exposure (see text).

Pyrethrins		Pip. butoxide % Conc.	Males			Knockdown Females			Combined		
% Conc.	Log. (Conc. x 100)		Total	%	*Angle	Total	%	*Angle	Total	%	*Angle
0.050	0.7	0	18/23	73	58.7	13/21	60	50.8	31/44	66	54.3
0.025	0.4	0	10/25	26	30.7	6/17	32	34.4	16/42	30	33.2
0.050	0.7	0.250	19/22	83	65.6	16/22	71	57.4	35/44	77	61.3
0.025	0.4	0.125	6/20	14	22.0	7/19	34	35.7	13/39	24	29.3
0	-	-	4/21	-	-	1/21	-	-	5/42	-	-

* Correction made for responses in controls.

TABLE 13. Topical application to P. interpunctella adults of 0.05 μ l. pyrethrins + piperonyl butoxide in odourless kerosene (Kerr's method).

Response: Mortality after 3 hours.

See graphs 12 and 13.

Pyrethrins % Conc. Log.(Conc. x 100)		Pip. butoxide % Conc.				Mortality			Combined		
			Total	<u>Males</u>		Total	<u>Females</u>		Total	<u>%*</u>	
				%*	Angle		%*	Angle		%*	Angle
0.100	1.0	0.	20/21	92	73.6	23/23	100	90.0	43/44	96	78.5
0.050	0.7	0	16/20	65	53.7	14/20	54	47.3	30/40	59	50.2
0.025	0.4	0	8/14	26	30.7	15/20	61	51.4	23/34	47	43.3
0.100	1.0	0.500	25/26	93	74.7	31/31	100	90.0	56/57	97	80.0
0.050	0.7	0.250	15/20	57	49.0	18/20	85	67.2	33/40	71	57.4
0.025	0.4	0.125	10/15	42	40.4	10/18	31	33.8	20/33	34	35.7
0	-	0	8/19	-	-	7/20	-	-	15/39	-	-

* Corrections made for responses in controls.

TABLE 14. Eggs laid by Plodia interpunctella treated with pyrethrins and pyrethrins plus piperonyl butoxide using Kerr's apparatus. Mortality of adults is recorded in table 13.

% Concentration of pyrethrins.	No. ♀♀	Eggs laid	Eggs laid /female
0.1	23	401	17.4
0.05	20	1087	54.3
0.025	20	1029	51.4
0.1 *	31	401	12.9
0.05 *	20	544	27.2
0.025 *	18	959	53.3
0	20	899	44.9

* Treated with pyrethrins plus piperonyl butoxide.

TABLE 15. Exposure of P. interpunctella adults on filter papers impregnated with pyrethrins for 30 mins.

Response: Mortality 18 hours after end of exposure.

See graph 14.

Pyrethrins		Total	Mortality		Total	Eggs laid per insect
% Conc.	Log. (Conc. x 1000)		% *	Angle		
1.000	3.0	29/29	100	90.0	653	22.5
0.500	2.7	21/22	94	75.8	317	14.4
0.250	2.4	25/25	100	90.0	1101	44.0
0.125	2.1	7/11 ⁺	59	50.2	791	71.9 ϕ
0.062	1.8	3/15	11	19.4	514	85.6 ϕ
0.031	1.5	3/21	4	11.5	813	38.7
0.015	1.2	1/23	0	0	916	39.8
0.007	0.9	1/24	0	0	574	23.9
0	-	2/20	-		239	11.9

* Correction made for response in controls

ϕ Eggs were counted from only 1 of 2 replicates

+ One of two replicates lost.

TABLE 16. Exposure of P. interpunctella adults on filter papers impregnated with pyrethrins + piperonyl butoxide for 30 mins.

Response; Mortality 18 hours after end of exposure.

See graph 15.

Pyrethrins		Pip.butoxide	Total	Mortality		Eggs laid		Eggs hatched after 4 days	
% Conc.	Log.(Conc.x 100)	% Conc.		% *	Angle	Total	per adult	Total	%
0.250	1.4	0	20/20	100	90.0	402	10.1	30/80	37
0.125	1.1	0	16/19	82	64.9	456	24.0	31/80	39
0.062	0.8	0	16/22	68	55.6	700	31.8	28/40	70
0.031	0.5	0	3/18	6	14.2	625	34.7	36/80	45
0.015	0.2	0	2/18	0	0	38	2.1	20/29	69
0.250	1.4	1.25	29/29	100	90.0	192	6.6	31/80	39
0.125	1.1	0.62	11/14	76	60.7	516	36.9	22/80	27
0.062	0.8	0.31	8/24	25	30.0	1183	49.2	32/80	40
0.031	0.5	0.15	5/18	19	25.8	409	22.7	10/40	25
0.015	0.2	0.07	3/21	3	10.0	304	14.5	40/69	58
0	-	0	2/18	-	-	53	2.9	27/39	69

* Correction made for control response.

TABLE 17. Exposure of *P. interpunctella* larvae on filter papers impregnated with pyrethrins + piperonyl butoxide for 24 hours.

Response: Failure to crawl off illuminated filter paper within one minute.

Pyrethrins % Conc. Log. (Conc. x 100)		Pip. butoxide % Conc.	Total	Knockdown %*	Probit
1.00	2.0	0	19/20	94	
0.50	1.7	0	16/20	75	
0.25	1.4	0	4/20	0	
0.12	1.1	0	9/20	31	
0.06	0.8	0	7/20	19	
1.00	2.0	5.00	11/20	44	
0.50	1.7	2.50	16/20	75	
0.25	1.4	1.25	12/20	50	
0.12	1.1	0.62	6/20	12	
0.06	0.8	0.31	5/20	6	
0	-	0	4/20	-	-

* Correction made for response in control.

χ^2_3 for regression (Pyrethrins only) = 21.19; not significant.

TABLE 18. Exposure of P. interpunctella larvae in 2" x 1" specimen tube completely lined with filter paper impregnated with pyrethrins - piperonyl butoxide for 30 minutes.

Response: Failure of larvae to have commenced pupation 5 days after exposure.

Pyrethrins		Pip. butoxide		Total	Knockdown	
% Conc.	Log. (Conc.%)	%	Conc.		% *	Angle
5.00	0.7	25.0		30/30	100	90.0
2.50	0.4	12.5		30/30	100	90.0
1.25	0.1	6.2		20/30	57	49.0
5.00	0.7	0.		26/30	85	67.2
2.50	0.4	0		15/30	35	36.3
1.25	0.1	0		8/30	5	12.9
0	-	0		7/30	-	-

* Correction made for response in control.

TABLE 19. Exposure of P. interpunctella larvae to filter papers impregnated with piperonyl butoxide for 30 minutes.
 Response: Failure to have pupated 5 days after exposure.
 See graph 16.

Piperonyl butoxide.		Knockdown.		
% Conc.	Log. Conc.	Total	%*	Angle
50.0	1.7	20/30	56	48.4
25.0	1.4	12/30	22	28.0
12.5	1.1	9/30	9	17.5
0	-	7/30	-	-

* Correction made for response in control.

TABLE 20. Exposure of P. interpunctella larvae within electrified barrier to filter papers impregnated with pyrethrins for 30 mins.
 Response: Failure to leave illuminated filter paper within one minute, 1 hr. after end of exposure.
 See graph 17.

Pyrethrins		Total	Knockdown	
% Conc.	Log.(Conc. x 10)		%*	Angle
5.00	1.7	28/30	93	74.7
2.50	1.4	26/30	86	68.0
1.25	1.1	22/29	74	59.3
0.62	0.8	20/30	64	53.1
0	-	2/30	-	-

* Correction made for response in control.

TABLE 21. Exposure of P. interpunctella larvae within electric barrier to filter papers impregnated with pyrethrins for 30 minutes.

Response: Mortality after 24 hours.

See graph 18.

Pyrethrins		Total	Mortality	
% Conc.	Log. (Conc. x 10)		%	Angle
5.00	1.7	24/30	80	63.4
2.50	1.4	9/30	30	33.2
1.25	1.1	3/30	10	18.4
0.62	0.8	1/30	3	10.0
0	-	0/30	-	-

TABLE 22. Topical application to P. interpunctella larvae of 0.05 μ l. pyrethrins + piperonyl butoxide in odourless kerosene.

Response: Inability to crawl off illuminated filter paper in one minute, 2 hours after treatment.

See graph 19.

Pyrethrins		Pip.butoxide		Knockdown		Probit
% Conc.	Log(Conc. x 10)	% Conc.	Total %	%	Angle *	
1.00	1.0	5.00	24/31	73	58.7	5.61
0.50	0.7	2.50	16/28	49	44.4	4.97
0.25	0.4	1.25	15/31	38	38.1	4.70
1.00	1.0	0	25/31	77	61.3	5.73
0.50	0.7	0	18/31	50	45.0	5.00
0.25	0.4	0	15/30	40	39.2	4.75
0	-	0	5/32	-	-	-

* Correction made for response in control.

Using angles:

X_r for regression (pyrethrins only) = 0.49; 0.5)P)0.3

X_r for regression (with synergist) = 0.34; 0.7)P)0.5

X_r for regression (combined results) = 0.89; 0.95)P)0.90

Using Probits.

X_r for regression (pyrethrins only) = 0.53; 0.5)P)0.3

X_r for regression (with synergist) = 0.30; 0.7)P)0.5

X_r for regression (combined results) = 0.84; 0.95)P)0.90.

TABLE 23. Topical application to P. interpunctella larvae of 0.05 μ l. pyrethrins + piperonyl butoxide in odourless kerosene.

Response: Failure to have pupated within 6 days.

See graph 20.

Pyrethrins		Pip. butoxide	Total	Knockdown	
% Conc.	Log. (Conc. x 10)	% Conc.		%*	Angle
1.00	1.0	0	22/23	95	77.1
0.50	0.7	0	12/25	42	40.4
0.25	0.4	0	7/25	20	26.6
1.00	1.0	5.00	18/24	72	58.1
0.50	0.7	2.50	9/24	30	33.2
0.25	0.4	1.25	6/24	17	24.4
0	-	0	2/20	-	-

* Correction made for responses in controls.

χ^2 for regression (pyrethrins only) = 2.69; 0.2 ∇ P > 0.1

χ^2 for regression (with synergist) = 1.19; 0.3 ∇ P > 0.2

χ^2 for regression (combined results) = 6.80; 0.2 ∇ P > 0.1

TABLE 24. Topical application to P. interpunctella larvae of 0.05 μ l. of pyrethrins in odourless kerosene.

Response: Failure to have commenced pupation four days after treatment.

See graph 21.

Pyrethrins		Pip. butoxide		Total	Knockdown	
% Conc.	Log. (Conc. x 10)	% Conc.			%*	Angle
1.00	1.0	0		18/20	87	68.9
0.50	0.7	0		12/19	51	45.6
0.25	0.4	0		5/20	1	5.7
1.00	1.0	5.00		19/20	93	74.7
0.50	0.7	2.50		15/20	68	55.6
0.25	0.4	1.25		5/20	1	5.7
0	-	0		4/17	-	-

* Correction made for response in control.

χ^2 for regression (pyrethrins only) = 1.64; $P = 0.2$
 χ^2 for regression (with synergist) = 3.52; $0.10 > P > 0.05$.
 χ^2 for regression (combined results) = 6.42 $0.20 > P > 0.10$

TABLE 25. Exposure of P. interpunctella larvae on wooden squares impregnated with pyrethrins + piperonyl butoxide for 24 hours.

Response: Failure to crawl off illuminated filter paper in one minute at end of exposure period.

See graph 22.

Pyrethrins % Conc. Log. (Conc. x 10)		Pip. butoxide % Conc.	Knockdown		Probit
			Total	%	
2.00	1.3	0	18/20	90	6.28
1.00	1.0	0	16/20	80	5.84
0.50	0.7	0	14/20	70	5.52
0.25	0.4	0	11/20	55	5.12
2.00	1.3	10.0	17/20	85	6.03
1.00	1.0	5.00	15/20	75	5.67
0.50	0.7	2.50	7/20	35	4.61
0.25	0.4	1.25	8/20	40	4.74
0	-		0/20	-	-

χ^2_1 for regression (pyrethrins only) = 0.02; P = 0.98

χ^2_1 for regression (with synergist) = 2.91 0.3/P>0.2

χ^2_2 for regression (combined results) = 7.68; 0.3/P>0.2

TABLE 26. Topical application to E. cautella of 0.05 μ l. pyrethrins in odourless kerosene.

Response: Mortality after 3 hours.

See graph 23.

Pyrethrins		Mortality		
% Conc.	Log. (Conc. x 100)	Total	% *	Angle
0.1	1.0	19/19	100	90.0
0.05	0.7	18/21	85	67.2
0.025	0.4	10/18	54	47.3
0.0125	0.1	10/23	41	39.8
0	-	1/24	-	-

* Correction made for response in control

$$\chi^2_{\text{for regression}} = 1.44; 0.5 > P > 0.3$$

TABLE 27. Topical application to E. cautella adults of 0.05 μ l. pyrethrins + piperonyl butoxide in odourless kerosene.

Response: Mortality after 5 hours.

See graph 24.

N.B. 30 insects were dosed at each point.

Pyrethrins		Pip. butoxide	Mortality	
% Conc.	Log.(Conc. x 100)	% Conc.	% *	Probit
0.09	0.95	0	86	6.08
0.06	0.78	0	78	5.77
0.04	0.60	0	73	5.61
0.02	0.30	0	59	5.23
0.09	0.95	0.45	89	6.23
0.06	0.78	0.30	84	5.99
0.04	0.60	0.20	67	5.44
0.02	0.30	0.10	61	5.28

* Correction made for 9.7% response in control.

χ^2 for regression (pyrethrins only) = 1.08; 0.7)P>0.5

χ^2 for regression (with synergist) = 1.10; 0.7)P>0.5

χ^2 for regression (combined results) = 1.10; 0.99)P>0.98

TABLE 28. Exposure of *E. cautella* larvae on filter papers impregnated with pyrethrins + piperonyl butoxide for 24 hours.

Response: Failure to crawl off illuminated filter paper within one minute at end of exposure.

See graph 25.

Pyrethrins % Conc. Log.(Conc. x 100)		Pip. butoxide % Conc.	Total	Knockdown %*	Probit
1.000	2.0	5.00	26/28	91	6.34
0.500	1.7	2.50	18/31	47	4.92
0.250	1.4	1.25	13/29	31	4.50
0.125	1.1	0.62	12/30	25	4.32
0.062	0.8	0.31	5/32	0	- 0
1.000	2.0	0	25/30	79	5.81
0.500	1.7	0	19/30	54	5.10
0.250	1.4	0	10/30	17	4.05
0.125	1.1	0	9/31	11	3.77
0.062	0.8	0	8/30	8	3.59
0	-	0	5/25	-	-

* Correction made for response in control.

χ^2 for regression (pyrethrins only) = 1.19; 0.8)P)0.7

χ^2 for regression (with synergist) = 4.33; 0.3)P)0.2

χ^2 for regression (combined results) = 6.14; 0.7)P)0.5

TABLE 29. Topical application to *A. grisella* adults of 1.0 μ l. pyrethrins + piperonyl butoxide in acetone.

Response: Mortality after 18 hours.

See graph 26.

Pyrethrins		Pip. butoxide	Total	Mortality	
% Conc.	Log. (Conc. x 1000)	% Conc.		%	Angle
0.0050	1.7	0	20/20	100	90.0
0.0025	1.4	0	18/20	90	71.6
0.00125	1.1	0	10/20	50	45.0
0.002	1.3	0.010	18/20	90	71.6
0.001	1.0	0.005	12/20	60	50.8
0.0005	0.7	0.0025	9/20	45	42.1
0	-	0	0/20	-	-

$\chi^2_{\text{for regression (pyrethrins only)}} = 0.34; 0.7 \rangle P) 0.5$

$\chi^2_{\text{for regression (with synergist)}} = 0.60; 0.5 \rangle P) 0.3$

$\chi^2_{\text{for regression (combined results)}} = 5.39; 0.3 \rangle P) 0.2$

TABLE 30. Topical application to G. melonella adults of 0.05 μ l. pyrethrins + piperonyl butoxide in odourless kerosene.

Response: Mortality after 4 hours.

See graph 27.

Pyrethrins		Pip.butoxide	Mortality				Eggs laid	
% Conc.	Log.(Conc.x 10)	% Conc.	Total	♀♀	%*	Angle	Total	per female
0.500	0.7	0	16/20	12/15	79	62.7	516	31.9
0.125	0.1	0	5/18	5/17	24	29.3	2332	183.2
0.500	0.7	2.50	17/20	12/13	84	66.4	1404	108.0
0.250	0.4	1.25	10/20	9/16	47	43.3	547	34.2
0.125	0.1	0.62	8/21	5/17	35	36.3	1159	68.2
0	-	0	1/20	0/14	-	-	17	1.2

* Correction made for response in control.

TABLE 31. Topical application to G. melonella larvae of 1.0 μ l. pyrethrins + piperonyl butoxide in acetone.

Response: Failure to have commenced pupation 24 hours after treatment.

See graph 28.

Pyrethrins		Pip. butoxide	Knockdown		
% Conc.	Log.(Conc.x 10)	% Conc.	Total	%*	Angle
0.70	0.84	0	10/20	47	43
0.49	0.69	0	5/20	21	27
0.34	0.53	0	3/20	11	19
0.70	0.84	3.50	14/20	68	56
0.49	0.69	2.45	10/20	47	43
0.34	0.53	1.71	6/20	26	31
0	-	0	1/20	-	-

* Correction made for response in control.

TABLE 32. Topical application to G. melonella larvae of 1.0 μ l. pyrethrins + piperonyl butoxide in acetone.

Response: Failure to have commenced pupation 5 days after treatment.

See table 33 and graph 29.

Pyrethrins		Pip. butoxide	Total	Knockdown	
% Conc.	Log. (Conc. x 10)	% Conc.		%*	Angle
0.70	0.84	0	14/40	28	31.9
0.49	0.69	0	6/40	5	12.9
0.34	0.53	0	5/40	3	10.0
0.70	0.84	3.50	23/40	53	46.7
0.49	0.69	2.45	19/40	42	40.4
0.34	0.53	1.71	7/30	15	22.8
0	-	0	4/40	-	-

* Correction made for response in control.

χ^2 for regression (pyrethrins only) = 2.05; $0.2 > P > 0.1$

χ^2 for regression (with synergist) = 0.86; $0.5 > P > 0.3$

χ^2 for regression (combined results) = 22.39; Not significant.

Factor of synergism at RD 50 = 1.9

TABLE 33. Soiling of filter papers by excretion of G. melonella larvae after topical application of pyrethrins + piperonyl butoxide recorded in table 32.

Score rating system for degree of soiling of filter papers:

1. Clean, i.e. no excretion.
2. Less than 10 spots.
3. Mostly clean, 70% surface clear.
4. Half-covered.
5. Heavily stained.

Pyrethrins % Conc.	Pip. Butoxide % Conc.	Soiling Score rating of papers.	Mean rating
0.70	0	4, 5, 5, 4, 3, 4, 3, 2	3.7
0.49	0	3, 2, 3, 3, 3, 4, 3, 4	3.1
0.34	0	1, 1, 2, 3, 2, 1, 2, 3	1.9
0.70	3.50	3, 4, 3, 4, 2, 3, 4, 3	3.2
0.49	2.45	4, 2, 2, 3, 3, 3, 3, 3,	2.9
0.34	1.71	2, 2, 1, 2, 3, 3	2.2
0	0	1, 1, 1, 1, 1, 1, 1, 1	1.0

N. B. Five larvae were confined on each filter paper.

TABLE 34. Topical application to G. melonella larvae of 1.0 μ l. of pyrethrins plus piperonyl butoxide in acetone.

Response: Failure to have commenced pupation 3 days after treatment.

Also: i. Soiling of filter papers by larvae - see table 33 for scoring system.

ii. Average loss of weight of larvae: Average weight 3 days after treatment expressed as percentage of weight immediately before treatment.

Pyrethrins % Conc.	Pip.butoxide % Conc.	Knockdown Total	Soiling Score rating of papers	Mean rating	Av.wt.loss %
0.70	0	20/20	4, 3, 3, 2	3.0	55
0.49	0	14/20	3, 5, 4, 5	4.25	47
0.34	0	9/20	3, 4, 4, 3	3.5	36
0.70	3.50	20/20	4, 3, 4, 2	3.25	53
0.49	2.45	19/20	2, 3, 3, 2	2.5	47
0.34	1.71	18/20	3, 4, 3, 3	3.25	48
0	0	1/20	1, 1, 1, 1	1.0	19

N. B. There were 5 larvae on each filter paper.

TABLE 35. Exposure of P. brassicae larvae on filter papers impregnated with pyrethrins + piperonyl butoxide for 24 hours.

Response: Mortality at end of exposure.

See table 36 and graph 30.

Pyrethrins		Pip.butoxide	Total	Mortality %*	Angle
% Conc.	Log.(Conc.x 100)	% Conc.			
0.500	1.7	0	18/20	89	70.6
0.250	1.4	0	18/20	89	70.6
0.125	1.1	0	16/20	79	62.7
0.062	0.8	0	8/20	37	37.5
0.500	1.7	2.50	19/20	95	77.1
0.250	1.4	1.25	18/20	89	70.6
0.125	1.1	0.62	12/20	58	49.6
0.062	0.8	0.31	13/20	63	52.5
0	-	0	1/20	-	-

* Correction made for response in control.

TABLE 36. Relative concentrations of green coloration from filter papers used in Experiment 35 determined on Spectrophotometer, using Purple filter.

See table 35.

Concentration of pyrethrins on papers.	Concentration of synergist on papers.	Reading of Spectrophotometer	Arbitrary scale of concentrations
0.5%	-	0.239	0.60
0.25	-	0.178	0.44
0.125	-	0.114	0.28
0.062	-	0.058	0.14
0.5	2.5	0.406	1.00
0.25	1.25	0.244	0.60
0.125	0.625	0.098	0.24
0.062	0.312	0.105	0.26

TABLE 37. Exposure of *P. brassicae* larvae on filter papers impregnated with pyrethrins $\pm$ piperonyl butoxide for 24 hours.

Response: Mortality at end of exposure.

See graph 31.

Pyrethrins		Pip butoxide	Total	Mortality	
% Conc.	Log. (Conc. x 100)	% Conc.			Angle
0.500	1.7	0	17/20	85	67.2
0.250	1.4	0	8/20	40	39.2
0.125	1.1	0	3/20	15	22.8
0.062	0.8	0	2/20	10	18.4
0.500	1.7	2.50	19/20	95	77.1
0.250	1.4	1.25	17/20	85	67.2
0.125	1.1	0.62	14/20	70	56.8
0.062	0.8	0.31	8/20	40	39.2

χ^2 for regression (pyrethrins only) = 4.19; $0.2 > P > 0.1$

χ^2 for regression (with synergist) = 3.43; $0.2 > P > 0.1$

Factor of synergism (R.D.50) = 3.5

Mortality in controls - 0/20.

TABLE 38. Exposure of *C. dimidiatus* adults on filter papers impregnated with pyrethrins for 4 days.

Response: Mortality at end of exposure.

See graph 32.

Pyrethrins		Total	Mortality	
% Conc.	Log. (Conc. x 10)		%	Angle
0.62	0.8	23/25	92	73.6
0.31	0.5	15/25	60	50.8
0.15	0.2	8/25	32	34.4
0	-	6/25	-	-

TABLE 39. Exposure of *C. dimidiatus* adults on filter papers impregnated with pyrethrins + piperonyl butoxide for 24 hours.

Response: Knockdown (see text), failure to leave filter paper.

See graph 33.

Pyrethrins		Pip. butoxide	Total	Knockdown	
% Conc.	Log.(Conc.x 100)	% Conc.		%*	Probit
2.0	2.3	0	36/41	87	6.13
1.0	2.0	0	26/40	63	5.33
0.5	1.7	0	4/40	5	3.35
0.25	1.4	0	0/40	0	$-\infty$
0.5	1.7	2.5	36/39	91	6.34
0.25	1.4	1.25	32/38	83	5.95
0.125	1.1	0.62	12/42	24	4.29
0.062	0.8	0.31	4/40	5	3.35
0	-	0	2/40	-	-

* Correction made for response in control.

χ^2 for regression (pyrethrins only) = 3.63; $0.2 > P > 0.1$

χ^2 for regression (with synergist) = 3.66; $0.2 > P > 0.1$

Factor of synergism (R.D.50) = 5.5

TABLE 40. Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins + piperonyl butoxide for 24 hours.

Response: Knockdown (see text), failure to leave filter paper.

See graph 34.

Pyrethrins		Pip. butoxide		Knockdown	
% Conc.	Log. (Conc. x 10)	% Conc.	Total	%*	Angle
2.0	1.3	0	22/41	51	45.6
1.0	1.0	0	11/39	24	29.3
0.5	0.7	0	3/36	3	10.0
0.50	0.7	2.50	38/38	100	90.0
0.25	0.4	1.25	35/44	78	62.0
0.125	0.1	0.62	12/42	25	30.0
0	-	0	2/40	-	-

* Correction made for response in control.

χ^2 for regression (pyrethrins only) = 0.08; $0.8 > P > 0.7$

χ^2 for regression (with synergist) = 0.14; $0.8 > P > 0.7$

Factor of synergism (R.D.50) = 10.0

TABLE 41. Exposure of *C. dimidiatus* adults to filter papers impregnated with pyrethrins + van Dyke 264 for 36 hours.

Response: Mortality after 24 and 36 hours.

See graph 35.

Pyrethrins % Conc. Log.(Conc.x 10)		Van Dyke 264 % Conc.	Mortality after 36 hours.		
			Total	% *	Probit
2.0	1.3	0	48/60	80	5.84
1.0	1.0	0	18/60	29	4.45
0.5	0.7	0	3/60	3	3.12
0.25	0.4	0	0/60	0	- ∞
2.0	1.3	10.0	56/60	93	6.47
1.0	1.0	5.0	41/60	69	5.49
0.5	0.7	2.5	22/60	36	4.64
0.25	0.4	1.25	6/60	8	3.59
0	-	0	1/60	-	
Mortality after 24 hours					
2.0	1.3	0	4/24	11	
1.0	1.0	0	6/30	14	
0.5	0.7	0	0/28	0	
0.25	0.4	0	0/30	0	
2.0	1.3	10.0	23/28	81	
1.0	1.0	5.0	21/33	61	
0.5	0.7	2.5	8/30	21	
0.25	0.4	1.25	1/29	0	
0	-	0	2/30	-	

* Correction made for response in control.

For 36 hour exposure

χ^2 for regression (pyrethrins only) = 0.01 ; $P > 0.99$

χ^2 for regression (with synergist) = 0.20 ; $P > 0.99$

Factor of synergism (R.D.50) = 1.95.

TABLE 42. Exposure of C. dimidiatus adults on filter papers impregnated with pyrethrins plus van Dyke 264 for 24 and 36 hours.

Response: Mortality at end of exposure.

N.B. Data from table 41.

See graph 36.

Pyrethrins plus van Dyke 264				Mortality	
% Conc.	Exposure	C.T.P. ^o	Log. C.T.P.	% *	Angle
Pyrethrins	hrs.				
0.25	24	6	0.78	0	0
0.50	24	12	1.08	21	27.3
1.0	24	24	1.38	61	51.4
2.0	24	48	1.68	81	64.2
0.25	36	9	0.95	8	16.4
0.50	36	18	1.25	36	36.9
1.0	36	36	1.56	69	56.2
2.0	36	72	1.86	93	74.7

* Correction made for responses in controls.

^o C.T.P = Concentration time product.

TABLE 43. Exposure of *C. dimidiatus* adults on filter papers impregnated with pyrethrins plus piperonyl butoxide and with pyrethrins plus van Dyke 264 for 24 hours.

Response: Mortality at end of exposure.

See graph 37.

Pyrethrins		Pip.butoxide	van Dyke 264	Total	Mortality	
% Conc.	Log.(Conc.x 10)	% Conc.	% Conc.		%	Angle
1.0	1.0	10.0	0	18/25	72	58.1
0.5	0.7	5.0	0	12/25	48	43.9
0.25	0.4	2.5	0	7/25	28	31.9
1.0	1.0	0	10.0	11/25	44	41.6
0.5	0.7	0	5.0	3/25	12	20.3
0.25	0.4	0	2.5	1/25	4	11.5
0	-	0	0	0/25	-	-

TABLE 44. Exposure of *C. dimidiatus* larvae on filter papers impregnated with pyrethrins + piperonyl butoxide for 18 hours.

Knockdown: Failure to leave 5.4 cm. diameter circle on filter paper within one minute.

See graph 38.

Pyrethrins % Conc. Log. (Conc. x 100)		Pip. butoxide % Conc.	Total	Knockdown % *	Angle
<u>1st Assay</u>					
1.0	2.0	0	33/40	81	64
0.5	1.7	0	15/43	31	34
0.25	1.4	0	4/43	4	11
0.5	1.7	5.0	38/38	100	90
0.25	1.4	2.5	39/39	100	90
0.125	1.1	1.25	41/41	100	90
0	-	0	2/41	-	-
<u>2nd Assay</u>					
1.0	2.0	0	27/39	67	55
0.5	1.7	0	12/42	24	29
0.25	1.4	0	7/41	13	20
0.062	0.8	0.62	8/20	37	37
0.031	0.5	0.31	4/39	5	13
0.015	0.2	0.15	3/40	2	8
0	-	0	2/39	-	-

For second assay:

χ^2 for regression (pyrethrins only) = 2.70; $P = 0.1$.

χ^2 for regression (with synergist) = 2.92; $0.1 > P > 0.05$

Factor of synergism (R.D.50) = 5.6

* Correction made for responses in controls

TABLE 45. Exposure of *C. dimidiatus* larvae on filter papers impregnated with pyrethrins + piperonyl butoxide for 24 hours.

Knockdown: Failure to leave 5.4 cm. diameter circle on filter paper within one minute.

See graph 39.

Pyrethrins % Conc. Log. (Conc. x 100)		Pip. butoxide % Conc.	Total	Knockdown %* Angle	
0.5	1.7	0	24/40	57	49.0
0.25	1.4	0	12/40	24	29.3
0.125	1.1	0	7/40	11	19.4
0.125	1.1	0.62	33/40	81	64.2
0.062	0.8	0.31	17/39	39	38.6
0.031	0.5	0.15	10/40	19	25.8
0	-	0	3/40	-	-

* Correction made for response in control.

χ^2 for regression (pyrethrins only) = 0.75, 0.5 > P > 0.3

χ^2 for regression (with synergist) = 1.27, 0.3 > P > 0.2

Factor of synergism (a.D.50) = 6.8

TABLE 46. Exposure of *C. dimidiatus* larvae on filter papers impregnated with pyrethrins + van Dyke 264 for 24 hours.

Response: Knockdown (see text), failure to leave pencil circle.

See graph 40.

Pyrethrins		Van Dyke 264	Knockdown		
% Conc.	Log.(Conc. x 10)	% Conc.	Total	%*	Angle
0.60	0.78	0	10/29	32	34.4
0.30	0.48	0	4/31	10	18.4
0.15	0.18	0	2/30	3	10.0
0.60	0.78	3.0	26/30	85	67.2
0.30	0.48	1.5	23/30	75	60.0
0.15	0.18	0.7	15/29	49	44.4
0	-	0	1/30	-	-

* Correction made for response in controls.

χ^2 for regression (pyrethrins only) = 0.34; $0.7 > P > 0.5$

χ^2 for regression (with synergist) = 0.41; $0.7 > P > 0.5$

Factor of synergism (R.D.50) = 7.9

TABLE 47. Exposure of C. dimidiatus larvae on filter papers impregnated with pyrethrins + van Dyke 264 for 24 hours.

Response: Knockdown (see text), failure to leave pencil circle.

See graph 41.

Pyrethrins		van Dyke 264	Knockdown		
% Conc.	Log.(Conc.x 100)	% Conc.	Total	%*	Angle
0.8	1.9	0	38/39	97	80.0
0.4	1.6	0	19/38	47	43.3
0.2	1.3	0	4/40	5	12.9
0.4	1.6	2.0	35/40	84	66.4
0.2	1.3	1.0	25/40	60	50.8
0.1	1.0	0.5	10/40	21	27.3
0	-	0	2/40	-	-

* Correction made for response in control.

I_1^2 for regression (pyrethrins only) = 1.30; 0.3) P) 0.2

I_1^2 for regression (with synergist) = 0.34; 0.7) P) 0.5

Factor of synergism (R.D.50) = 2.24

TABLE 48. Exposure of *C. dimidiatus* larvae on filter papers impregnated with pyrethrins, pyrethrins plus van Dyke 264 and pyrethrins plus sesoxane.

Response: Knockdown (see text), failure to leave pencil circle.

See graph 42.

Pyrethrins		van Dyke 264 Sesoxane		Knockdown		Angle
% Conc.	Log.(Conc.x 100)	% Conc.	% Conc.	Total	% *	
2.0	2.3	0	0	34/40	82	64.9
1.0	2.0	0	0	23/40	50	45.0
0.5	1.7	0	0	11/40	15	22.8
0.25	1.4	0	0	7/40	3	10.0
0.250	1.4	0	1.25	40/40	100	90.0
0.125	1.1	0	0.62	33/40	79	70.6
0.062	0.8	0	0.31	8/40	6	14.2
0.031	0.5	0	0.15	1/40	0	0
2.0	2.3	10.0	0	27/40	62	51.9
1.0	2.0	5.0	0	26/40	59	50.2
0.5	1.7	2.5	0	9/39	9	17.5
0.25	1.4	1.25	0	9/40	9	17.5
0	-	0	0	6/40	-	-

* Correction made for response in control.

TABLE 49. Survival of worker ants (Lasius fuliginosus) confined by the brass rings on clean filter papers.

Time of exposure (hours)	No. of ants per ring.	Total Mortality
3	1	0/5
3	2	0/10
3	3	0/15
3	4	0/20
3	5	0/25
24	1	3/5
24	2	6/10
24	3	8/15
24	4	10/20
24	5	7/25

TABLE 50. Exposure of L. fuliginosus workers over filter papers impregnated with pyrethrins + piperonyl butoxide for three hours.

Response: Mortality at end of exposure.

See graph 43.

Pyrethrins		Pip. butoxide	Mortality		
% Conc.	Log.(Conc. x 100)	% Conc.	Total	%	Angle
0.400	1.60	0	18/19	90	72
0.280	1.45	0	8/20	40	39
0.196	1.29	0	2/20	10	18
0.200	1.30	2.00	20/20	100	90
0.140	1.15	1.40	16/20	80	63
0.098	0.99	0.98	6/20	30	33
0	-	0	0/20	-	-

TABLE 51. Exposure of L. fuliginosus workers to filter papers impregnated with pyrethrins + piperonyl butoxide for 3 hours.

Response: Mortality at end of exposure.

See graph 44.

Pyrethrins		Pip. butoxide	Mortality		
% Conc.	Log. (Conc. x 100)	% Conc.	Total	%	Angle
0.400	1.60	0	24/25	96	78
0.280	1.45	0	18/25	72	58
0.196	1.29	0	12/25	48	44
0.137	1.14	0	7/25	28	32
0.096	0.98	0	4/24	17	24
0.200	1.30	2.00	25/25	100	90
0.140	1.15	1.40	21/25	84	66
0.098	0.99	0.98	19/25	76	61
0.069	0.84	0.69	12/25	48	44
0.048	0.68	0.48	11/25	44	42
0	-	0	0/25	-	-

X^1_3 for regression (Pyrethrins only) = 2.93; 0.5) P) 0.3

X^2_3 for regression (with synergist) = 2.68; 0.5) P) 0.3

Factor of synergism (R.D.50) = 3.02

TABLE 52. Exposure of L. fuliginosus workers to filter papers impregnated with pyrethrins + piperonyl butoxide for 3 hours.

Response: Mortality at end of exposure.

See graph 45.

Pyrethrins		Pip. butoxide		Mortality		
% Conc.	Log.(Conc.x 100)	% Conc.	Total	%	Angle	
0.400	1.60	0	25/25	100	90	
0.280	1.45	0	23/25	92	74	
0.196	1.29	0	16/25	64	53	
0.137	1.14	0	6/25	24	29	
0.200	1.30	2.00	24/25	96	78	
0.140	1.15	1.40	19/25	76	61	
0.098	0.99	0.98	16/25	64	53	
0.069	0.84	0.69	9/25	36	37	
0	-	0	0/25	-	-	

χ^2 for regression (pyrethrins only) = 3.99; $0.2 > P > 0.1$

χ^2 for regression (with synergist) = 0.63; $0.8 > P > 0.7$

Factor of synergism (R.D.50) = 2.4

TABLE 53. A. pisum sprayed with pyrethrins in petroleum ether and exposed on filter papers impregnated with pyrethrins for 45 minutes.

Response: Knockdown (see text), at different times after end of treatment.

	Dose % Pyrethrins	3	Knockdown 17	40 hours
<u>Sprayed</u>	-	9/10	1/10	0/11
	0.1	10/10	10/10	7/9
	0.05	10/10	4/10	4/10
	0.025	8/10	0/10	2/10
	0.0125	4/10	0/10	0/10
<u>Surface</u>	-	0/10	0/9	1/9
	1.0	9/10	5/10	6/10
	0.5	0/10	4/10	1/9

TABLE 54. A. pisum sprayed with pyrethrins in light petroleum.

Response: Knockdown (see text) after 21 hours.

See graph 46.

Pyrethrins		Knockdown		
% Conc.	Log. (Conc. x 100)	Total	%*	Angle
0.04	0.6	19/23	80	63.4
0.02	0.3	12/20	54	47.3
0.01	0.0	8/21	29	32.6
0	-	4/31	-	-

* Correction made for response in control.

TABLE 55. A. pisum sprayed with pyrethrins $\pm$ piperonyl butoxide in light petroleum.

Response: Knockdown (see text) after 20 hours.

See graph 47.

Pyrethrins		Pip. butoxide	Knockdown		
% Conc.	Log. (Conc. x 1000)	% Conc.	Total	%	Angle
0.060	1.78	0	8/12	67	54.9
0.030	1.48	0	5/14	36	36.9
0.015	1.18	0	3/17	18	25.1
0.0150	1.18	0.0750	15/16	94	75.8
0.0075	0.87	0.0375	11/16	69	56.2
0.0037	0.57	0.0185	2/13	15	22.6
0	-	0	0/17	-	-

$X^1_{\text{for regression (pyrethrins only)}} = 0.10; 0.8 \text{ P} > 0.7$

$X^2_{\text{for regression (with synergist)}} = 0.65; 0.5 \text{ P} > 0.3$

Factor of synergism (R.D.50) = 6.4

TABLE 56. A. pisum sprayed with pyrethrins $\pm$ piperonyl butoxide in light petroleum.

Response: Knockdown (see text) after 20 hours.

See graph 48.

Pyrethrins		Pip. butoxide		Knockdown	
% Conc.	Log.(Conc. x 1000)	% Conc.	Total	%*	Angle
0.04	1.6	0	7/19	31	33.8
0.02	1.3	0	8/31	19	25.8
0.01	1.0	0	4/34	4	11.5
0.0100	1.0	0.0500	24/42	53	46.7
0.0050	0.7	0.0250	8/34	17	24.4
0.0025	0.4	0.0125	4/26	8	16.4
0	-	0	2/25	-	-

* Correction for response in control.

I^2 for regression (pyrethrins only) = 0.22; $0.7 > P > 0.5$

I^2 for regression (with synergist) = 0.23; $0.7 > P > 0.5$

Factor of synergism (R.D.50) = 7.2

TABLE 57. Exposure of P. cochleareae adults on filter papers impregnated with pyrethrins for 3 hours.

Response: Failure to crawl off 5.4 cm. diameter circle on illuminated filter paper in one minute 17 hours after end of exposure.

See graph 49.

Pyrethrins		Total	Knockdown	
% Conc.	Log. (Conc. x 100)		%	Angle
0.100	1.0	10/10	100	90
0.050	0.7	9/10	90	71.6
0.025	0.4	8/10	80	63.4
0.012	0.1	2/10	20	26.6
0	-	0/10	-	-

TABLE 58. Exposure of P. cochleareae adults on filter papers impregnated with pyrethrins + piperonyl butoxide for 3 hours.

Response: Failure to crawl out of 5.5 cm. diameter pencil circle on illuminated filter paper in one minute 18 hours after end of exposure.

Pyrethrins		Pip.butoxide % Conc.	Total	Knockdown	
% Conc.	Log. (Conc. x 100)			%*	Angle
0.500	1.7	0	20/21	95	77.1
0.250	1.4	0	20/24	82	64.9
0.125	1.1	0	12/25	45	42.1
0.250	1.4	2.5	26/26	100	90.0
0.125	1.1	1.25	28/28	100	90.0
0.062	0.8	0.62	24/25	96	78.5
0	-	0	1/20	-	-

* Correction made for response in control.

TABLE 59. Exposure of P. cochleareae adults on filter papers impregnated with pyrethrins + piperonyl butoxide for 3 hours.

Response: Failure to crawl out of 5.4 cm. diameter pencil circle on illuminated filter paper in one minute 18 hours after end of exposure.

See graph 50.

Pyrethrins		Pip. butoxide	Total	Knockdown	
% Conc.	Log. (Conc. x 100)	% Conc.		%	Angle
0.500	1.7	0	7/10	70	56.8
0.250	1.4	0	5/10	50	45.0
0.125	1.1	0	3/10	30	33.2
0.250	1.4	2.50	8/10	80	63.4
0.125	1.1	1.25	6/10	60	50.8
0.062	0.8	0.62	4/10	40	39.2
0	-	0	0/10	-	-

Estimated factor of synergism (n.D.50) = 2.9.

TABLE 60. Exposure of P. cochleareae adults on filter papers impregnated with pyrethrins + piperonyl butoxide for 30 minutes.

Response: Mortality 17 hours after end of exposure.

See graph 51.

Pyrethrins % Conc. Log.(Conc. x 100)		Pip. butoxide % Conc.	Total	Mortality % Angle	
0.40	1.6	0	12/20	60	50.8
0.20	1.3	0	4/20	20	26.6
0.10	1.0	0	2/20	10	18.4
0.20	1.3	1.00	13/20	65	53.7
0.10	1.0	0.50	6/20	30	33.2
0.05	0.7	0.25	3/20	15	22.8
0	-	0	0/15	-	-

I^2 for regression (Pyrethrins only) = 0.80; $0.5 > P > 0.3$

I^2 for regression (with synergist) = 0.39; $0.7 > P > 0.5$

Factor of synergism (R.D.50) = 2.34

TABLE 61. Exposure of D. fasciatus nymphs on filter papers impregnated with pyrethrins + piperonyl butoxide for 22 hours.

Response: Mortality at end of exposure period.

Pyrethrins % Conc. Log.(Conc.x 10)		Pip. butoxide % Conc.	Total	Mortality %* Angle	
0.8	0.9	0	11/12	90	84.3
0.4	0.6	0	6/12	40	46.4
0.2	0.3	0	2/12	0	0
0.4	0.6	2.0	8/12	60	56.2
0.2	0.3	1.0	4/12	20	32.6
0.1	0.0	0.5	2/12	0	0
0	-	0	2/12	-	-

* Correction made for response in control.

TABLE 62. Exposure of D. fasciatus nymphs on filter papers impregnated with pyrethrins + piperonyl butoxide for 22 hours.

Response: Mortality at end of exposure period.

See graph 52.

Pyrethrins % Conc. Log. (Conc. x 10)		Pip. butoxide % Conc.	Mortality		
			Total	%*	Angle
0.8	0.9	0	17/23	70	56.8
0.4	0.6	0	9/23	30	33.2
0.2	0.3	0	5/23	10	18.4
0.4	0.6	2.0	21/23	90	71.6
0.2	0.3	1.0	11/23	40	39.2
0.1	0.0	0.5	5/23	10	18.4
0	-	0	3/23	-	-

* Correction made for response in controls.

I^2 for regression (pyrethrins only) = 0.34; $0.7 > P > 0.5$

I^2 for regression (with synergist) = 0.62; $0.5 > P > 0.3$

Factor of synergism (R.D.50) = 2.6

TABLE 63. Exposure of A. aegypti larvae in 50 ml. distilled water containing 0.5 ml. of 2.5 parts per million pyrethrins and 2.5 parts per million pyrethrins plus 12.5 parts per million piperonyl butoxide.

	Exposure time. Mins.	Knockdown Total
Pyrethrins only	1	8/28
	5	13/28
	10	12/28
	20	24/28
Pyrethrins plus piperonyl butoxide	1	11/45
	5	22/45
	10	32/45
	20	45/45

TABLE 64. Exposure of A. salina adults in saline containing 1 part per million pyrethrins.

See graph 53.

Exposure time		Control response	Response to 1 ppm. pyrethrins		
Mins.	Log.(mins.)		Total	%*	Probit
0	-	0/12	0/24	0	
2	0.30	0/12	12/24	50	5.0
5	0.70	1/12	17/24	68	5.5
9	0.95	3/12	21/24	83	5.9
13	1.11	3/12	23/24	95	6.6

* Correction made for response in controls.

TABLE 65. Exposure of A. salina adults in saline containing 0.01 ppm. pyrethrins.

See graph 54.

Exposure time		Control response	Response to 0.01 ppm. pyrethrins		
Mins.	Log.(mins.)		Total	%*	Probit
10	1.00	0/30	6/30	20	26.6
15	1.18	0/30	17/30	57	49.0
20	1.30	3/30	22/30	70	56.8
30	1.48	3/30	26/30	86	68.0

* Correction made for response in controls.

TABLE 66. Exposure of A. salina adults in saline containing pyrethrins or piperonyl butoxide.

See graph 55.

	Exposure time		Total	Response	Angle
	Mins.	Log. mins.		%	
0.2 ppm. pyrethrins	5	0.70	3/30	10	18
	10	1.00	10/30	33	35
	15	1.18	10/30	33	35
	20	1.30	17/30	57	49
	25	1.40	17/30	57	49
	30	1.48	21/30	70	57
	49	1.69	24/30	80	63
	94	1.97	29/30	97	80
1.0 ppm. piperonyl butoxide	5	0.70	2/30	7	15
	10	1.00	5/30	17	24
	15	1.18	4/30	13	21
	20	1.30	6/30	20	27
	25	1.40	10/30	33	35
	30	1.48	9/30	30	33
	49	1.69	12/30	40	39
	94	1.97	22/30	73	59
0.5 ppm. piperonyl butoxide	5	0.70	0/30	0	0
	10	1.00	2/30	7	15
	15	1.18	1/30	3	10
	20	1.30	3/30	10	18
	25	1.40	5/30	17	24
	30	1.48	5/30	17	24
	49	1.69	6/30	20	27
	94	1.97	11/30	37	37
Control	5	0.70	1/30	3	10
	10	1.00	2/30	7	15
	15	1.18	3/30	10	18
	20	1.30	3/30	10	18
	25	1.40	2/30	7	15
	30	1.48	3/30	10	18
	49	1.69	7/30	23	29
	94	1.97	8/30	27	31

TABLE 67. Exposure of *A. salina* in saline to 0.67% alcohol containing pyrethrins.

See graph 56.

Exposure time (mins)	Conc. pyrethrins.		Knockdown		
	ppm.	Log.(ppm.x 10)	Total	%	Angle
20	0.67	0.83	107/135	79	63
20	0.33	0.52	133/173	77	61
20	0.17	0.23	34/207	16	24
30	0.67	0.83	250/279	90	72
30	0.33	0.52	91/110	83	66
30	0.17	0.23	48/90	53	47
40	0	-	12/279	4	11

TABLE 68. Exposure of A. salina nauplii to pyrethrins.

See graphs 57 and 67.

Exposure time. mins.	Conc. pyrethrins.		A	B	C	Knockdown		%	Angle
	ppm.	Log.ppm x 10.				Total			
20	0.67	0.82	40/90	39/85	40/99	119/274	43.4	37	
20	0.33	0.52	39/155	16/78	31/168	86/401	21.4	19	
20	0.17	0.22	5/82	53/310	9/35	67/427	15.7	14	
40	0.67	0.82	23/26	83/97	59/74	165/197	83.7	65	
40	0.33	0.52	73/182	45/78	74/163	192/423	45.4	39	
40	0.17	0.22	42/182	35/240	35/164	112/586	19.1	18	
60	0.67	0.82	54/57	106/113	204/217	364/387	94.0	75	
60	0.33	0.52	86/105	151/179	446/495	683/779	87.7	68	
60	0.17	0.22	26/103	190/358	273/387	489/848	57.7	47	
20	0	-	16/51	4/39	-	10/90	11.1	-	
40	0	-	4/37	7/86	3/43	14/166	8.4	-	
60	0	-	6/58	5/38	5/93	16/189	8.5	-	

TABLE 69. Exposure of A. salina nauplii in 0.67% alcohol in saline containing pyrethrins.

See graph 58.

Exposure time. mins.	Conc. pyrethrins ppm.	C.T.P.	Log. C.T.P.	Knockdown		
				Total	% *	Angle
10	0.67	6.7	0.83	106/165	60	51
15	0.67	10.0	1.00	121/158	74	60
20	0.67	13.4	1.13	213/257	81	64
25	0.67	16.7	1.22	74/90	80	63
30	0.67	20.1	1.30	53/60	87	69
15	0.17	2.5	0.40	31/94	25	30
20	0.17	3.4	0.53	32/63	45	42
25	0.17	4.2	0.62	44/76	53	47
30	0.17	5.1	0.71	151/199	73	59
35	0.17	5.9	0.77	110/154	68	56
15	0	-		6/50	-	-
20	0	-		8/90	-	-
30	0	-		5/66	-	-

* Correction made for response in controls assuming an overall average of 10%.

TABLE 70. Exposure of *A. salina* nauplii to pyrethrins + piperonyl butoxide in 0.67% alcohol in saline, together and separately.

Exposure time. mins.	Pyrethrins ppm.	Pip. butoxide ppm.	Knockdown	
			Total	%
15	0.067	0	4/26	15
48	0.067	0	4/31	13
98	0.067	0	4/30	13
25	0.033	0	1/22	4
51	0.033	0	0/5	0
15	0.067	0.67	9/23	39
27	0.067	0.67	26/49	53
79	0.067	0.67	37/47	79
16	0.033	0.33	18/47	38
29	0.033	0.33	139/149	93
80	0.033	0.33	53/77	69
17	0	0.67	32/101	32
30	0	0.67	37/45	82
83	0	0.67	72/87	83
25	0	0	0/37	0
91	0	0	1/37	3

TABLE 71. Artemia nauplii assay of extract from 100 C. dimidiatus adults which had been exposed on filter papers impregnated with 4% pyrethrins for 30 minutes by which time they were dead.

See graph 59.

	Exposure time of <u>A. salina</u>		Knockdown of <u>Artemia</u>		
	Mins.	Log. mins.	Total	%	Angle
<u>Carpophilus</u>					
Exposed to pyrethrins	25	1.40	11/142	8	16
	51	1.71	18/170	11	19
	111	2.04	55/147	37	37
	215	2.33	122/192	63	53
Control	22	1.34	7/189	4	11
	47	1.67	6/156	4	11
	105	2.02	17/164	10	18
	211	2.32	29/144	20	26

TABLE 72. Artemia nauplii assay of extract from batches of 100 C. dimidiatus adults which had been exposed to filter papers impregnated with 4% pyrethrins and/or 20% piperonyl butoxide for 30 minutes (sufficient time to kill beetles on pyrethrins $\pm$ piperonyl butoxide).

See graph 60.

<u>C. dimidiatus</u>	Exposure time mins.	<u>Artemia salina</u>		Knockdown	
		Log. mins.	Total	%	Angle
Exposed on pyrethrins	52	1.72	85/465	18	25
	88	1.94	112/309	36	37
	229	2.36	139/228	61	51
Exposed on pyrethrins + piperonyl butoxide	58	1.76	156/316	49	44
	95	1.98	145/318	46	43
	233	2.37	321/335	96	78
Exposed on piperonyl butoxide	50	1.70	14/264	5	13
	116	2.06	26/167	16	24
	224	2.35	42/166	25	30
Control	86	1.93	3/67	4	12
	153	2.18	19/121	16	23
	262	2.42	70/154	45	42

TABLE 73. Artemia nauplii assay of extract from 100 C. dimidiatus larvae which had been sprayed with 2 ml. 2% pyrethrins in the Potter Tower.

See graph 61.

	Exposure time of nauplii		Knockdown of nauplii		
	Mins.	Log. mins.	Total	%	Angle
<u>Carpophilus</u>	11	1.04	47/94	50	45
Treated with pyrethrins	19	1.28	94/146	64	53
	81	1.91	84/85	99	84
Control	40	1.60	4/169	2	8
	60	1.79	4/49	8	16
	98	1.99	7/50	14	22
	149	2.17	11/75	15	23

TABLE 74. Artemia, nauplii assay of extract from lots of 50 C. dimidiatus larvae which had been sprayed with 2 ml. of solutions of pyrethrins.

See graph 62.

Conc. Pyrethrins sprayed on larvae		<u>Artemia</u> Exposure time (mins)	Knockdown of nauplii		
%	Log.(Conc.x 10)		Total	%	Angle
0.5	0.7	189	234/380	62	52
1.0	1.0	190	114/145	79	63
2.0	1.3	198	64/88	87	69
0.5	0.7	244	115/144	80	63
1.0	1.0	241	239/255	94	76
2.0	1.3	250	108/108	100	90
0	-	127	4/60	7	
0	-	194	5/110	4	
0	-	247	14/140	10	

TABLE 75. *Artemia* nauplii assay of extracts from lots of 50
C. dimidiatus larvae sprayed with 2 ml. pyrethrins and/or
 piperonyl butoxide solutions.

See graph 63.

Conc. solution sprayed on larvae	<i>Artemia</i> Exposure time		Knockdown of nauplii		
	Mins.	Log.mins.	Total	%	Angle
2% Pyrethrins	18	1.25	45/137	33	35
	33	1.52	53/87	61	51
	48	1.68	41/68	60	51
	63	1.80	69/103	67	55
2% Pyrethrins + 10% Pip.butoxide	21	1.32	43/89	48	44
	36	1.56	49/120	41	40
	51	1.71	31/81	38	38
	67	1.83	43/82	52	46
10% Pip.butoxide	21		3/196	1	
	37		1/125	1	
	52		12/312	4	
	67		7/284	2	
Mixed diluent	24		7/205	3	
	39		5/233	2	
	55		5/181	3	
	71		6/313	2	

TABLE 76. *Artemia*, nauplii assay of extracts from lots of 50 *C. dimidiatus* larvae sprayed with 2 ml. 1% pyrethrins and 1% pyrethrins plus 10% piperonyl butoxide.

Conc. solution sprayed on larvae	<i>Artemia</i> . Exposure time, mins.	Knockdown of nauplii	
		Total	%
1% Pyrethrins	29	3/59	5
	46	13/95	14
	87	29/56	52
	173	33/43	77
1% Pyrethrins + 10% Pip. butoxide	26	2/80	2
	50	6/55	11
	82	22/57	39
	169	41/72	57
Mixed diluent	22	5/126	4
	53	0/81	0
	78	4/67	6
	165	8/41	19

TABLE 77. Artemia, nauplii assay of extracts from batches of 50 C. dimidiatus larvae which had been sprayed with 2 ml. 2% pyrethrins and

A. extracted at once

B. extracted 20 minutes after spraying.

See graph 64.

	Exposure time of nauplii		Knockdown of nauplii		
	Mins.	Log. mins.	Total	%	Angle
A	65	1.81	62/103	60	51
	118	2.07	98/120	82	65
	213	2.33	73/79	92	74
B	73	1.86	50/72	69	56
	104	2.02	204/246	83	66
	200	2.30	124/137	90	72
Control	58	1.76	1/33	3	10
	153	2.18	2/33	6	14
	238	2.38	7/38	18	25

TABLE 78. *Artemia*, nauplii assay of extracts from batches of 40 *C. dimidiatus* larvae which had been sprayed with 2 ml. pyrethrins and/or piperonyl butoxide solutions.

- A. extracted at once.
- B. extracted 130 mins. after spraying.
- C. sprayed with 0.7% pyrethrins
- D. sprayed with 0.7% pyrethrins + 3.5% piperonyl butoxide.

See graphs 65a, 65b.

	Exposure time of nauplii		Knockdown of nauplii		
	Hrs.	Mins. Log. Hrs.	Total	%	Angle
A, C.	0.31	T.72	14/79	18	25.1
	1.39	0.22	17/97	18	25.1
	3.36	0.56	34/93	36	36.9
B, C.	1.33	0.19	28/122	23	28.7
	2.58	0.47	52/134	39	38.6
	3.17	0.51	49/101	48	43.9
A, D.	0.32	T.72	8/86	9	17.5
	1.40	0.22	18/94	19	25.8
	3.36	0.56	38/116	33	35.1
B, D.	1.33	0.19	20/110	18	25.1
	2.53	0.46	45/131	34	35.7
	3.11	0.50	102/166	61	51.4
Control	0.27	T.65	7/147	5	12.9
	1.27	0.16	5/111	4	11.5
	3.20	0.52	7/105	7	15.3

TABLE 79. Artemia, nauplii assay of extract from 20 P. interpunctella larvae sprayed with 2 ml. 2% pyrethrins in Potter Tower and also Artemia assay of pyrethrins in 0.67% alcohol in saline.

See graphs 66, 67.

	Exposure time of nauplii		Knockdown of nauplii		
	Mins.	Log mins.	Total	% *	Angle
A. Extract of sprayed larvae	25	1.40	21/127	14	22
	53	1.72	129/457	26	31
	83	1.92	162/406	37	37
	101	2.00	58/99	56	48
B. Control extract	26	1.41	1/56	-	
	53	1.72	6/123	-	
	80	1.90	0/56	-	
	102	2.01	14/218	-	
C. 0.33 ppm. pyrethrins	25	1.40	141/455	31	34
	45	1.65	135/150	89	71
	63	1.80	132/135	98	82
D. 0.17 ppm. pyrethrins	30	1.48	207/475	42	40
	47	1.67	140/173	80	63
	66	1.82	207/218	95	77
E. Control	30	1.48	1/85	-	
	37	1.57	2/67	-	
	79	1.90	114/397	-	
	91	1.96	10/100	-	

* Correction made for responses in controls.

TABLE 80. Artemia assay of extracts from batches of 30 P. interpunctella larvae sprayed with pyrethrins + piperonyl butoxide.

See graph 68.

	Exposure time of nauplii		Knockdown of nauplii		
	Mins.	Log. mins.	Total	%	Angle
A. Larvae sprayed	30	1.48	21/243	9	17.5
with 2%	44	1.64	30/186	16	23.6
pyrethrins	69	1.84	36/111	32	34.4
B. Larvae sprayed	26	1.41	19/102	19	25.8
with 2% pyrethrins	39	1.59	68/311	22	28.0
+ 10% Pip.	64	1.81	41/99	41	39.8
butoxide					
C. Control.	22	1.34	14/133	10	18.4
	34	1.53	8/206	4	11.5
	61	1.78	15/204	7	15.3

TABLE 81. Artemia assay of extracts from batches of 20
P. interpunctella larvae;

A. Extract made immediately after treatment.

B. Extract made 30 minutes after treatment.

See graph 69.

	Exposure time of nauplii		Knockdown of nauplii		
	Mins.	Log. mins.	Total	%	Angle
A. 1.0 μ l of 0.3% pyrethrins per larva.	101	2.00	54/129	42	40
	198	2.30	100/155	64	53
	344	2.54	171/185	92	74
B. 1.0 μ l of 0.3% pyrethrins per larva.	54	1.73	30/157	19	26
	150	2.18	35/90	39	38
	183	2.26	80/110	73	58
Control.	94	1.98	8/72	11	19
	191	2.28	22/96	23	29
	337	2.53	25/92	27	31

TABLE 82. Artemia assay of extracts from batches of 10
P. interpunctella larvae which had been sprayed with
 2 ml. of 2% pyrethrins and

A. extracted at once.

B. extracted after 2 $\frac{1}{2}$ hours.

See graph 70.

	Exposure time		Knockdown		
	Mins.	Log.mins.	Total	%	Angle
A.	50	1.70	63/142	44	41.6
	144	2.16	107/173	62	51.9
	230	2.36	79/111	71	57.4
B.	55	1.74	74/149	50	45.0
	153	2.18	102/223	46	42.7
	273	2.44	192/280	69	56.2
Control.	125	2.10	1/163	1	3.7
	217	2.34	7/175	4	11.5
	306	2.48	12/254	5	12.9

Using variance ratio test, no significant difference was detected in the positions of the two regression lines.

TABLE 83. Artemia assay of batches of 5 P. interpunctella larvae which had been sprayed with 2 ml. of 2% pyrethrins and

A. extracted at once.

B. extracted after $2\frac{1}{2}$ hours.

See graph 71.

	Exposure time		Knockdown		Angle
	Mins.	Log. mins.	Total	%	
A.	111	2.04	83/96	86	68.0
	160	2.20	92/108	85	67.2
	232	2.36	95/94	90	71.6
B.	83	1.92	82/100	82	64.9
	157	2.19	82/102	80	63.4
	272	2.43	73/78	93	74.7
Control.	81	1.91	5/88	6	14.2
	187	2.27	17/108	16	23.6
	235	2.37	18/95	19	25.8

TABLE 84. Artemia assay of solutions containing 0.13 and 0.67 ppm. pyrethrins.

See graph 72 also compare table 81 and graph 69.

	Exposure Time		Knockdown		Angle
	Mins.	Log. mins.	Total	%	
0.13 ppm. pyrethrins	51	1.71	12/72	17	24
	90	1.95	52/107	49	44
	132	2.12	29/73	40	39
0.67 ppm. pyrethrins	20	1.30	27/45	60	51
	29	1.46	44/64	69	56
	48	1.68	73/79	92	74
	83	1.92	41/43	95	77
Control	19	1.28	4/124	3	10
	47	1.67	5/38	6	14
	83	1.92	4/154	3	10

TABLE 85. *Artemia* assay of extract from 5 *G. melonella* larvae topically dosed with 1.0 μ l. of 0.7% pyrethrins in acetone.

	Exposure time	Knockdown	
	mins.	Total	%
Larvae treated with pyrethrins	120	4/59	7
	219	9/57	16
	291	18/70	26
Control	117	4/61	6
	216	7/58	12
	283	8/70	11

TABLE 86. *Artemia* assay of extracts from 10 *G. melonella* larvae injected with 1.0 μ l of 0.1% pyrethrins.

See graph 73 and compare table 87.

	Exposure time		Knockdown		Angle
	Mins.	Log. mins.	Total	%*	
Larvae injected with pyrethrins	18	1.25	5/139	0	0
	89	1.95	27/211	10	18
	164	2.21	76/213	31	34
	210	2.32	143/235	55	48
Control	14	1.15	7/153	-	
	65	1.93	6/181	-	
	159	2.20	13/180		
	215	2.33	27/207		

* Correction made for control responses.

TABLE 87. Artemia assay of solutions containing 0.33 and 0.13 ppm. pyrethrins.

See graph 73 and compare table 86.

	Exposure time		Knockdown		Angle
	Mins.	Log. mins.	Total	%	
0.33 ppm. pyrethrins	15	1.18	54/255	21	27
	36	1.56	152/314	48	44
	68	1.83	296/313	95	77
0.13 ppm. pyrethrins	21	1.32	27/278	10	18
	39	1.59	24/232	10	18
	79	1.90	59/100	59	50
Control.	25	1.40	3/333	1	6
	46	1.66	4/465	1	6
	85	1.93	5/152	3	10

TABLE 88. *Artemia* assay of blood from V instar *S. gregaria* after injection with 5.0 μ l. of 0.2% pyrethrins.

A & B. Blood taken immediately after injection.

C & D. Blood taken 75 minutes after injection.

E. Control.

See graph 74.

	Exposure time of nauplii		Knockdown of nauplii		
	Hrs.	Log. Hrs.	Total	%	Angle
A.	3.88	0.59	30/50	60	51
	4.50	0.65	19/51	37	37
	5.20	0.72	20/48	42	40
	6.68	0.82	29/48	60	51
B.	3.83	0.58	19/54	35	36
	4.45	0.65	23/59	39	39
	5.15	0.71	10/57	17	24
	6.65	0.82	13/54	24	29
C.	2.53	0.40	7/63	11	19
	3.70	0.57	15/41	37	37
	5.35	0.73	3/49	6	14
	7.12	0.85	3/19	16	24
D.	2.53	0.40	5/59	8	16
	3.70	0.57	5/42	12	20
	5.35	0.73	17/30	57	49
	7.12	0.85	5/45	11	19
E.	4.08	0.61	1/55	2	8
	5.25	0.72	6/44	14	22
	6.95	0.84	3/51	6	14

TABLE 89. Artemia assay of blood from V instar S. gregaria injected with 5.0 μ l. of pyrethrins and/or piperonyl butoxide in acetone.

- A. Blood taken from 5 locusts immediately after injection of 1.0% piperonyl butoxide.
 B. Blood taken after injection of acetone.
 C. Blood taken immediately after injection of 0.2% pyrethrins.
 D. Blood taken 80 minutes after injection of 0.2% pyrethrins.
 E. Blood taken immediately after injection of 0.2% pyrethrins plus 1.0% piperonyl butoxide.
 F. Blood taken 80 minutes after injection with 0.2% pyrethrins plus 1.0% piperonyl butoxide.

See graphs 75 and 76.

	Exposure time of nauplii	Total	Knockdown.
	Hrs.		%
A.	2.58	0/48	0
	5.83	0/91	0
	8.78	7/70	10
	11.07	3/53	6
	12.95	16/58	28
	13.13	6/74	8
	13.28	13/60	22
	13.32	12/54	22
	13.61	11/62	18
B.	2.57	2/55	4
	5.80	5/60	8
	8.75	4/56	7
	11.08	10/50	20
	11.35	2/84	2
	12.97	22/52	42
	13.12	20/47	42
	13.32	21/48	44
	13.62	21/55	38
C.	2.52	1/61	2
	5.73	0/64	0
	8.63	13/48	27
	11.07	33/57	60
	11.23	40/72	55
	12.93	45/57	79
	13.08	54/68	79
	13.30	53/60	88
	13.60	56/61	92

TABLE 89. (Continued)

	Exposure time of nauplii	Total	Knockdown
	Hrs.		%
D.	2.30	6/89	7
	4.42	5/53	9
	7.43	12/59	20
	9.73	11/58	19
	9.83	6/84	7
	11.58	15/59	25
	11.77	19/67	28
	12.92	24/61	39
	12.97	25/71	35
E.	2.30	2/56	4
	7.27	6/53	11
	9.70	44/86	51
	9.75	48/80	60
	11.57	44/61	72
	11.75	40/56	71
	12.22	51/60	85
	12.93	58/65	89
	12.98	72/85	85
F.	2.42	2/59	3
	7.38	4/54	7
	9.82	11/84	13
	9.97	18/68	26
	11.67	22/58	38
	11.87	27/64	42
	12.33	14/77	18
	13.10	34/55	62
	13.12	18/69	26

TABLE 91. Effect of pyrethrins and piperonyl butoxide against Ephestia elutella.
(Taken from Brooke 1958).

Test Method	Ratio of pyrethrins to piperonyl butoxide	Factor of synergism		No. of replicate test	No. of insects per test.
		L.D.50	L.D.80		
Larva Topical application	1 : 10	1.1	1	5	20
Adult Space spray	1 : 10	1.3	1.3	4	50
Adult Space spray	1 : 1	1.5	1.3	4	50
Adult Deposit	1 : 10	1.6	1.9	3	25
Adult Topical application	1 : 10	1.2	1.3	6	25

AUTUMNAL POPULATIONS OF WASPS NESTS

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INTRODUCTION

This note draws attention to the wide range of composition of autumnal populations of wasps nests of the ground-building species of the genus *Vespula*.

Apart from the data of WEYRAUCH (1935) the published records are scanty, both in respect of the numbers of the different forms and stages of wasps and of their parasites. Moreover, as RICHARDS and RICHARDS (1951) indicate, WEYRAUCH's records for *V. vulgaris* L. are the least satisfactory in that the nests were taken too early in the season for the sexual forms to have matured.

We have recently examined two nests of *V. vulgaris* in the grounds of the Imperial College Field Station. Both nests were in stacks of sods cut and stored under large trees, and separated by some 100 yards. They were taken within a short period of one another (18th. September and 3rd. October, 1957) and were as likely to be comparable as naturally occurring nests ever are, yet the proportions of the inhabitants were quite different. Both nests were taken by pouring some 100 ml. of liquid hydrogen cyanide into the entrance hole, and blocking this hole with a cotton wool plug loosely tamped so as to allow sufficient gas to diffuse through the plug to kill returning foragers flying into the space between the plug and the external entrance. In this way, up to 100 workers have been taken at each nest, having spent the night outside the nest, when, as in this instance, the entrance is blocked after dark the previous evening. Towards the end of the season these straying workers will return in diminishing numbers from first light until midday (BLACKITH, 1957).

Should the passage through the earth be more than about a foot, most of the wasps and their parasites will recover from the cyanide on prolonged airing when the nest is dug out, though this recovery may take up to 24 hr... Doubtless the strong sorption of hydrogen cyanide on damp soil is responsible for the low concentrations reaching the nest; with a short passage it is difficult to judge the dosage so as to avoid killing the nest contents.

POPULATIONS OF WASPS

Table I shows the number of wasps of different castes and stages found in each nest. The adult counts are probably accurate to a few individuals, those of the larvae and pupae are rather more difficult to classify and may be biassed. In any event there is a considerable overlap between the maturation of the last virgin queens and males to be produced and the nest-leaving of those which mature earliest, so that nothing but a census of wasps leaving the nest throughout the daylight hours for some weeks would ensure accuracy in this respect. Our taking of the nests was timed

by a third nest kept in a beehive fitted with an observation entry tube in which the various groups of wasp could be distinguished as they left the nest. We are glad to acknowledge the collaboration of Mr. N. G. M. Hague in this part of the experiment.

Nest A is noteworthy for the excessive number of males, and the entire absence of those virgin queens which are conveniently called gynes (BRIAN, 1957). An obvious explanation for the excess of males would be the death or disfunction of the queen. We were able to recover her from the nest and she laid an egg whilst recovering from the cyanide anaesthesia, and then took some sugared water. An ample supply of ovarian eggs was found on dissection, but unfortunately the queen died during the night before dissection, so that our inability to find sperm in the spermatheca could have been due to the death of the sperm (RICHARDS and RICHARDS, 1951). On the other hand, sperm may have been absent either because the original fertilisation was inadequate, as RUTTNER (1956) found to occur in a queen honey-bee if the sperm is not pressed home by the muscles surrounding the oviduct, or the wasp queen may have exhausted her supply.

The large number of immature workers found in nest B is the chief abnormality in this autumn population, since the workers cease to be produced, or so it is generally supposed, when the sexual forms are laid down (See, for example, RICHARDS, 1953). The physiological age of all but a few of these workers was younger than that of the immature sexual forms, most of which had passed into the advanced stages subsequent to the sealing of the cell. The possibility exists that the worker larvae were neglected for those of the sexual brood, but had yet been laid before the sexual cells were constructed. However this may be, the presence of large numbers of worker larvae is difficult to reconcile either with the view that the change to sexual brood is associated with a particular worker-larvae proportion, or, alternatively, with the idea that transition to the production of sexual brood is irreversible. In either event, some of the later sexual brood must have matured whilst an overwhelming proportion of the worker larvae needed attention from the adult foragers and nurses.

THE DISTRIBUTION OF WASPS IN THE NEST

One feature of the records which we have not seen recorded in the literature is the substantial degree of segregation of the adult forms within the nest. In nest B, whose population was very rapidly killed by the cyanide, almost all the workers were found in or on the envelope. Of the 526 males found in the nest, only 47 were in the top 7 combs, the remainder lying in the bottom of the envelope or in the lowest three combs. The adult queens were found, on the contrary, to be mainly confined to the top combs, especially numbers 3-7 inclusive. This segregation bore no apparent relationship to the cells from which the different forms had

emerged. We have in fact observed on an earlier occasion, with adult virgin queens of *V. germanica* F. that striking grouping of these adults takes place if the illumination is reduced and the temperature is dropped. Rosettes of queens form with their heads facing the centre of the rosette, sometimes as many as four ranks forming up in this way. The centre of the rosette seems to be fortuitously selected, since, if the orientation be dispersed by raising the temperature, and the cooling repeated, the rosettes form at quite different parts of the cage in which the wasps are confined. No workers or males were seen in these groups.

One other form of non-randomness among the queens remains to be noted. TAKAMATSU (1949) found that the queens of *V. lewisii* (Cameron) leave the nest in the autumn in two very marked periods some 10 days apart separated by a lull in activity, neither the peaks nor the lulls being related to the prevailing weather. It is possible, therefore, that the development of the queens of *Vespula* is not a steady process, but may occur in waves, either because the eggs are laid irregularly or because the immature stages are not uniformly tended. Such irregular emergence, from whatever cause, has a bearing on the problem of differential queen infestation by parasites, as noted below.

Lastly, in this section, we have noted that the proportion of foraging workers in nest A is 968 out of a total of 2,160. We were able to capture these foragers separately because the nest entrance was so long (about 3 metres) that the workers leaving the nest had an opportunity to reach the plug before being anaesthetised.

THE PARASITE POPULATION

Wasps' nests in this locality are usually replete with parasites at the end of the season. The external parasites include woodlice, millepedes, numerous larvae of *Volucella* spp. and muscid larvae. Of the internal parasites the beetle *Metoecus paradoxus* (L.) occurs sporadically; we have not found it since 1949. Furthermore, a large parasitic nematode was found to occur irregularly and to be of importance in the vicinity. In a personal communication, Dr. H. E. Welch informed us that these worms were larval mermithids. He has provisionally identified the worm as *Mermis pachysoma* Linstow, 1905, and deals with the taxonomic status of this species in a paper immediately following this one (P 353 to 355).

The importance of this nematode lies in the fact that it is found in the majority of cases in the virgin queens, and only exceptionally in the males and workers. As far as the evidence goes, it is not species specific, being found, as Table 2 shows, in three of the four species of *Vespula* that nest in the ground in Southern England. No parasitised individuals of *V. rufa* L. have been recorded, but the absence of the parasite in this species may be fortuitous.

The differential infestation of queens and workers in the two nests for

which proper counts are available suggests that the treatment given to the maturing castes is different in some respect. Several parasitised queens of *V. sylvestris* (Scop.) were found in a nest originally founded in a beehive. This nest had been entirely out of contact with the ground during its development (Table 2) and was reared on the staging of a greenhouse. This observation, in collaboration with Mr. N. G. M. Hague, establishes that the worms must be taken into the nest by the foragers, either adventitiously or as part of the load of food, and do not invade the nest from the soil. The problem is then to decide the origin of infestation. The occasional finding of worms in the workers or males implies that the worms are introduced to the larvae of all groups but may fail to develop except in the queens.

In order to discover whether the worms are to be found in the larval or pupal queens, a sample of 135 of these forms from nest B was examined, but no parasites were found. If this sample were representative of the whole queen population of the nest, the absence of worms would exclude the possibility that they develop within the larvae; on the other hand, any sample of the immature stages is necessarily different from those queens which have already emerged, since we have already discussed the waves of emergence of the queens. Indeed, it was evident, from the fact that queens from some parts of the nest were more often infected than were other queens, that the infestation is non-random even within this caste, although it was not practicable to relate the source of infested wasps to any particular comb as the queens were removed from the nest and laid out for examination.

An exact test of the significance of the differential infestation of males and workers in the nest of *V. germanica* shows that the hypothesis of equal infestation rates for the two groups is effectively disproved ($P = 0.014$). Thus different rates of infestation exist for queens, males and workers in this nest. Evidently, the process of infestation is related to some feature of the development of the wasps, but one needs to remember that a sudden, temporary, influx of parasites into a nest where each group is developing at its appropriate rate can easily give rise to such apparently different rates of parasitism. Nevertheless, the fact that the infestation pattern in the nests of *V. germanica* and *V. vulgaris* is essentially the same tends to support the suggestion that the nematodes are introduced with the larval diet.

Summary.

Although only preliminary results are given, they are sufficient to draw attention to the diversity of the autumnal populations of wasps' nests, whether in respect of the proportions of the larvae and adults, or of the castes and sexes. This diversity is demonstrated by observations made on similar nests, and is also shown by the parasite population.

TABLE I. — CONTENTS OF NESTS OF *Vespula vulgaris* DEVELOPED UNDER COMPARABLE CONDITIONS.

	NEST A.	NEST B.
Item of census.	—	—
Date taken.....	18.ix.'57	3.x.'57
No. of combs	6	11
<i>Adults.</i>		
Functional queen	1	1
Virgin queens (gynes)	0	710
Males	1,197	526
Workers	2,160	1,582
Total adult population	3,358	2,819
<i>Immature forms.</i>		
Males and queens	1,979	987
Workers	0	2,471
Total of immature forms	1,979	3,458
Total of all forms.....	5,337	6,277
Actual worker/larva ratio, counting pupae as larvae.....	1.09 : 1	0.46 : 1
Males emerged/queens emerged..	—	0.74 : 1

TABLE II. — DIFFERENTIAL PARASITISM OF WASPS BY *Mermis pachysoma*.

SPECIES.	GROUP.	SEASON.	PARASITISED.	NOT PARASITISED.	SAMPLE TOTAL.
—	—	—	—	—	—
<i>V. germanica</i>	{ Queens	1955	65	123	188
	{ Males		1	392	393
	{ Workers		3	64	67
<i>V. vulgaris</i> (Nest B of Table I)	{ Queens	1957	65	645	710
	{ Males		0	226	226
	{ Workers		0	218	218
<i>V. sylvestris</i>	{ Queens	1955	5	25 (1)	30
	{ Males		not sampled		
	{ Workers		not sampled		
<i>V. vulgaris</i> (2)	Queens ?	1932	8	23	31

(1) Died from other causes.

(2) Data from Beck (1937).

Notwithstanding some taxonomic difficulties, a brief account of the distribution of a larval mermithid, parasitic in several species of social wasp, is presented. These worms occur but rarely in the males and workers, although sporadically they may attain considerable rates of infestation in the virgin queens.

Sommaire.

Bien que des résultats de caractère préliminaire seulement soient donnés, ceux-ci suffisent à faire ressortir la diversité de la population automnale des guêpiers. Cette diversité se manifeste soit par la proportion des castes et des sexes observée à l'état de larve ou d'adulte dans des guêpiers semblables, soit par la population des parasites.

On a noté dans plusieurs espèces de guêpes sociales la présence, à l'état larvaire, d'un helminthe parasite, bien que sa taxonomie soit difficile. Ce ver ne se trouve que rarement dans les mâles et les ouvrières; il atteint parfois un pourcentage considérable de parasitisme dans les reines vierges.

Riassunto.

Benché solo pochi risultati di carattere preliminare siano presentati, essi sono sufficienti per far risaltare la diversità della popolazione autunnale dei vespai.

Questa diversità è mostrata sia dalla proporzione delle caste e dei sessi osservata nelle larve e negli adulti in vespai simili, sia dalla popolazione dei parassiti.

Si è constatata, in parecchie specie di vespe sociali, la presenza allo stato larvale di un nematode parassitico, nonostante certe difficoltà tassonomiche. Questo verme, di rado presente nei maschi e nelle operaie, raggiunge talvolta una percentuale considerabile di parassitismo nelle regine vergini.

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**THE EFFECT OF PYRETHRINS AND PIPERONYL
BUTOXIDE AGAINST THE MOTHS
Plodia interpunctella AND *Ephestia cautella***

SIR,—Brooke¹ has described the low level of synergistic activity shown by piperonyl butoxide/pyrethrins mixtures towards the Cacao moth, *Ephestia elutella* Hb., while Lloyd and Hewlett² note that a film of 1.3% pyrethrins was conspicuously more toxic to *Ephestia cautella* larvae than a film containing 0.3% pyrethrins and 3.0% piperonyl butoxide.

The results of work carried out at this Field Station using the moths *Plodia interpunctella* Hb. and *Ephestia cautella* Walk. indicate that this lack of synergistic action extends to other species. Adult and larval moths have been exposed on filter paper to deposits of pyrethrins, and of pyrethrins plus 5 times the volume of piperonyl butoxide, and the larvae have been exposed to deposits on wood. The larvae of *Plodia* have also been treated by topical application using the micro-burette method of Kerr.³

In none of the experiments noted above have we succeeded in demonstrating a significant enhancement of the toxicity of pyrethrum films by the addition of piperonyl butoxide.

In our experiments, the standard error of a median response dose was such that only those preparations of which one was at least twice as toxic as another, could be reliably distinguished. Biological assay with moths has proved to be consistently less precise than with beetles. Since we doubt whether the accuracy of these assays, which was not greatly improved by increasing them to 10(5 + 5) points, can be readily increased, our results imply that factors of synergism of less than two should seldom be treated as significant.

I am, Sir, etc.,

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THE DISTRIBUTION OF METHYL BROMIDE IN BARGES CONTAINING DRIED FRUIT

By J. H. STEVENSON



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THE DISTRIBUTION OF METHYL BROMIDE IN BARGES CONTAINING DRIED FRUIT

By J. H. STEVENSON

The distribution of methyl bromide in barges during the fumigation of dried fruit has been examined. By altering the stowage of the cases of fruit, a more even distribution of the fumigant has been obtained, while the rate of loss of methyl bromide from the barges has been substantially reduced by sealing off hatch covers and other openings in the deck.

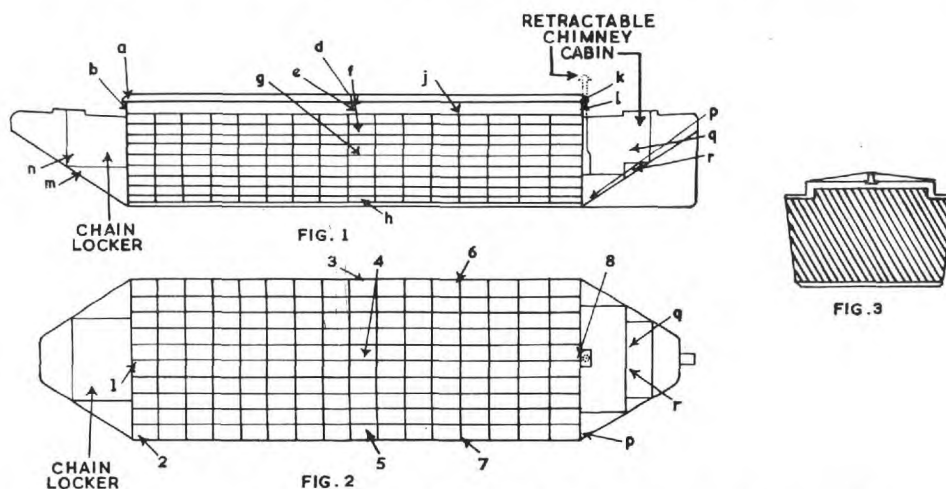
Introduction

During 1957 some 34,694 tons of currants, sultanas and raisins were imported into the United Kingdom from Australia by the Australian Dried Fruits Board. Of this total about half was brought into the Port of London. On arrival, Australian dried fruit is fumigated with methyl bromide to remove any insect infestation. To prevent subsequent reinfestation, a film of pyrethrins in oil is maintained on the stacked cases of fruit during storage.

In London the fumigation is carried out in the barges used to transport the fruit from ship to wharf. Burns Brown¹ has described the fumigation of fruit in barges using ethylene oxide. This paper describes an investigation into the loss and distribution of methyl bromide during fumigation and improvements in technique which have now been made.

Description of barges

The barges used in the Port of London for the transport of dried fruit have a carrying capacity between 5000 and 10,000 cu. ft. They are divided into three compartments (Figs. 1-3). At the forward end there is a chain locker entered through a hatch in the deck and usually having, also in the deck, at least one hole through which an anchor chain may be passed. At the after



FIGS. 1-3.—Diagrams showing stowage of cases in the barges and positions of sampling points
Fig. 1. Side elevation Fig. 2. Plan Fig. 3. End elevation

end of the barge is the cabin with a similar hatch. In some barges there are vents from the deck into the fore and aft compartments. Steel bulkheads separate both the cabin and the chain locker from the hold, which has a close-boarded floor ('the ceiling') made of wooden boards 6 in. wide and 1½ in. thick supported about 6 in. above the bilges. The boards running along each side of the ceiling are known as limber boards and may readily be removed to allow access to the bilges. The boards generally fit well enough to prevent grain and other small particles getting into the bilges. The gas-tightness of the ceiling depends on the state of the boards and on how well the joints have been caulked.

The two steel bulkheads which separate the cabin and chain locker from the hold are not reliably gas-tight because there are often narrow gaps at the joints between these bulkheads and the outer hull of the barge. Moreover, the bulkheads, at their lower corners below the level of the ceiling, are cut away leaving triangular holes about 6 in. across to allow free circulation of the bilge water, and there are often similar triangular holes in the top corners of the bulkheads, leading into the hold. The bilge water can be pumped out through four pipes, two each in the cabin and chain locker, situated in the corners formed by the junction of the bulkheads with the hull. After the barge has been loaded, wooden hatch covers are placed over the hold and covered by a double layer of tarpaulin sheets. These sheets are stretched and firmly battened down at the edges thus forming a satisfactorily gas-tight seal with the coamings.

Fumigation procedure

Most Australian dried fruit consists of raisins, currants and sultanas which are shipped in wooden boxes, 56 or 60 lb. in weight and about $1\frac{1}{4}$ cu. ft. in size. The minimum period for fumigation is 14 hours, but in practice barges are often left under gas for 20 hours or even longer.

Gaseous methyl bromide is introduced into the hold through a T-piece attached to a rubber hose. The tarpaulins are pulled back at one side of the hold halfway along the barge and one or two hatch covers removed so that the T-piece may be placed on top of the boxes with the shorter arms pointing fore and aft along the barge. Like the hose, the T-piece has an internal diameter of 1 in., but the open ends are reduced to $\frac{1}{2}$ in. diameter. This arrangement causes a small pressure to be built up in the T-piece during the introduction of the gas which is consequently liberated under a pressure that should be sufficient to direct it towards the end of the hold, and to ensure that the fumigant is evenly distributed along the length of the barge.

There are several advantages in vaporising the fumigant before leading it into the barge over the more usual procedure of liberating the fumigant as a spray within the hold. Firstly, local cooling of the air by evaporation of the spray and thermal layering of the fumigant is avoided. Secondly, safety in application is increased. Long lengths of delivery hose are easily joined by using short lengths of $\frac{3}{4}$ -in. barrel as push-fit connectors since the gas pressure is only a few lb./sq. in. The corresponding joints, if fumigant is delivered as a liquid, must be liquid-tight against a pressure of 60–80 lb./sq. in., since if liquid fumigant leaks from a joint, and wets an operator's skin, pronounced blistering is likely.

Since a barge may be five bottoms out from the quay and may have to be fumigated at night the importance of ease and safety of operation will be apparent.

Experimental

Loss of methyl bromide during fumigation

An examination of the distribution of methyl bromide was made during the fumigation of a barge loaded with cases of fruit (Table I).

The concentrations of gas were estimated using a thermal conductivity apparatus.² This apparatus was calibrated by comparing readings with concentrations determined by the catalytic

Table I

Concentrations of methyl bromide (oz./1000 cu. ft.) in the barge 'Two'

Barge capacity 8200 cu. ft.; contents 4844 cases of fruit; initial dose 18 lb. of methyl bromide *

Position of sampling point (Figs. 1 and 2)	Time after start of fumigation		
	00.30 h.	02.30 h.	03.55 h.
N	0	6	3
C2	47	12	51
D4	12	3	13
G3	20	9	31
P	65	27	36
Q	60	25	13

* A second dose of 10 lb. of methyl bromide was added at 03.30 h.

combustion method (Lubatti & Harrison;³ Lubatti & Blackith⁴). Agreement within ± 2 oz. per 1000 cu. ft. was obtained over the whole scale of the instrument. 'Telcothene' tubing, 5.5 mm. internal bore, was used in taking the samples.

The cases were stowed so that the top surface of the stack was fairly flat. There were spaces about 4–5 ft. wide between the cargo and the bulkheads at each end of the hold and the T-piece was laid on top of the boxes. Some of the concentrations of methyl bromide in the cabin were higher than in the hold, and the rate of fall in concentration was rapid (Table I).

While a second dose of gas was being introduced, the deck of the barge was examined with a halide detector lamp for leakage of methyl bromide. There was no detectable loss from the tarpaulin cover, except where the hose carrying the gas passed into the hold. There were, however, substantial leaks from the cabin and chain locker, particularly from the cabin chimney and the chain hole in the deck over the chain locker.

It seemed likely that, immediately after introducing the fumigant, a large proportion fell to the bottom of the hold between the end bulkheads and the cargo and thereafter extensive penetration into the cabin and chain locker occurred, either through the ceiling or through the bulkheads. Because of the high rate of leakage from these compartments into the open air much of the gas was lost.

Alterations of fumigation procedure

In subsequent fumigations, a number of alterations in procedure were made in an attempt to reduce these very considerable losses of fumigant.

(1) Spaces left between the cargo and bulkheads were originally intended to facilitate airing of the barge prior to unloading. In view of the rapid escape of methyl bromide from fumigated goods during normal airing, especially from a barge where cargo can be so thoroughly uncovered, this precaution was felt to be unnecessary. In subsequent treatments the cases of fruit were stacked close to the bulkheads as shown in the figures so that the cases, acting as a barrier to gravitational layering, would prevent the methyl bromide from falling straight down to the floor of the hold and passing thence into the cabin and chain locker.

(2) The loss of methyl bromide from the hold could be reduced either by preventing its reaching the chain locker and cabin or by allowing the gas to penetrate to these two end compartments but preventing its escape into the open air through hatches and vents. Of these two methods, the latter is the more readily developed. Prevention of loss from the hold to the cabin and chain locker would involve sealing off the cracks and gaps around the bulkheads and possibly caulking the ceiling. It is much easier to prevent loss of methyl bromide by sealing the hatch covers, chain port, cabin chimney and any other vents, and this was done by tying polythene sheeting over the various points of leakage. If a chain was in position in the port leading from the chain locker, the port was blocked by damp cotton waste.

(3) In order to improve the distribution and circulation of methyl bromide in the free space over the cargo, the T-piece from which the gas issues was raised as high as possible either by tying it to the centre of the beam of the barge or securing it on top of cases raised above the general level.

The distribution of methyl bromide in four barges fumigated under these conditions has been determined and the results shown in Tables II and III.

Discussion

The results shown in Tables II and III indicate that the dosage of methyl bromide was sufficient to kill all insect pests associated with dried fruit with a considerable safety margin. The localised high concentrations of gas at the bottom of the barge disappeared after 2 to 3 hours. Immediately under the beam, the concentrations were lower than elsewhere in the hold. After 22 hours, however, the lowest concentration obtained beneath the beam was 4 oz./1000 cu. ft., which indicates a concentration-time product of 180 oz. h./1000 cu. ft., which would be lethal to any insect which had wandered towards the beam from the cases. Such wandering occurs mainly in hot weather when the precaution is taken of spraying the tops of the cases and the undersides of the hatch covers with pyrethrums in oil before fumigating the fruit.

The differences between the new procedure now recommended for fumigation with methyl

Table II

Concentrations of methyl bromide (oz./1000 cu. ft.) in the four barges fumigated after the alterations of procedure mentioned in the text had been made

Barge	Goa	Longburn	Wanganui	Stratosphere							
Capacity of hold (cu. ft.)	7500	8500	8900	12000							
No. of cases	4078	5212	5256	7114							
Dose of fumigant	20 lb.	20 lb.	20 lb.	30 lb.							
	Time after start of fumigation (h.)										
	00.10	02.10	03.40	00.20	03.00	00.30	01.30	17.30	00.20	02.55	22.00
Sampling point see Figs. 1 and 2)											
N	0	4	8	0	26						
M				55	26						
A1	94	6	13	33	10	28	20	12			
B1				38	22				35	26	11
B2	86	25	20								
D4						34	27	13	15	17	9
E4	>100	31	23	40	20	35	27	12	41	27	10
E5									43	28	10
F3						34	26	14			
G3	>100	31	22	43	21						
H3									71	26	9
H5									68	28	10
J6						36	26	14			
J7						36	28	14			
K8											
L8	72	35	24			44	29	14	15	16	4
P	52	38	29						33	24	8
Q	0	4	9								
R				38	23						

Table III

Approximate concentration time products (oz.h./1000 cu. ft.) after 14 h. in barges Wanganui and Stratosphere

Position in barge	Wanganui	Stratosphere
Under centre beam	240 (A1)	180 (K8)
On top of cases	315 (F3)	320 (E4)
Bottom of hold	—	390 (H5)

bromide and that described by Burns Brown¹ for fumigation with ethylene oxide are doubtless occasioned by the difference in properties between the two fumigants, in particular the differences in density and in sorption by dried fruit and by timber. It seems likely that the fumigation of many commodities in barges would follow more or less the pattern described here for the fumigation of dried fruit, but there may be exceptions; for example whale meat meal has been shown to be highly sorptive for methyl bromide (Burns Brown & Heuser⁵) and might behave more as dried fruit behaves with ethylene oxide, where the importance of gravitational layering and of leakage from the hold is reduced by the action of the commodity in holding reversibly a large proportion of the fumigant in the sorbed state.

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