The Stability and Assay of Contact Insecticides

A Thesis submitted for the Degree of Doctor of Philosophy in the Faculty of Science, London University.

by

R.E. Blackith

B.Sc., A.R.C.S., D.I.C.

With 14 Text-Figures

and 54 Text-Tables,

together with an appendix containing 100 auxiliary Tables .

Author's Note.

The greater part of this work was carried out between October 1948 and October 1950 at the Field Station of the Imperial College of Science and Technology, in the Department of Zoology and Applied Entomology. The work described in section 1 was undertaken in the laboraties of the Department at South Kensington, during the period 1945 -46.

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"The Stability and Assay of contact Insecticides".

Summary

The stability of three contact insecticides has been investigated, with particular reference to the action of ultra-violet light. The relative importance of atmospheric oxidation and ultra-violet radiation in causing deterioration of exposed pyrethrum-in-oil films has been assessed; the latter effect was found to preponderate, at least with pyrethrum preparations containing anti-oxidants. Within a practically important range, the storage temperature does not appear materially to influence decomposition.

Two methods of inhibiting the photolytic decomposition of the pyrethrins have been investigated, depending respectively on the ability of certain dyes to absorb ultra-violet radiation, and the alleged stabilization afforded by certain pyrethrum synergists, notably piperonyl butoxide. In the first case, only one of the many dyes examined was successful, but this substance, benzene azo-β-naphthol roughly doubled the effective life of pyrethrum-in-oil films both in mercury discharge radiation in the laboratory and in direct sunlight. Pyrethrum synergists were found not to confer any detectable increase of stability on the pyrethrins; reports of such an increase have probably been caused by confusion with the synergist effect itself.

Several interesting features concerning the mode of action of pyrethrum synergists were revealed in this investigation, for the biological assay of which a new experimental design, the "Twin Crossover" method, was adapted.

An improved method of applying insecticide to filter papers on which insects are subsequently allowed to crawl was also developed. The test insects used were adult grain weevils, Calandra granaria L.

The sensitivity of films of DDT in oil to ultra-violet radiation was found to be comparable to that of the pyrethrins; DDT can also be partially protected against ultra-violet photolysis by benzene-azo-β - naphthol. Benzene hexachloride appears to be extremely stable to light of the frequency used in these experiments.

List of symbols used in the Tables of this Thesis.

Unless otherwise stated, all figures given in the tables are the angular transforms of the percentage responses of batches of 30 weevils, all drawn from a homogenious population.

n = number of insects per batch.

r = number of insects responding to treatment.

p^1 = percentage response of batch.

p = percentage response corrected for responses in the control batches.

c = percentage response in the control batches.

x = logarithm of dosage, usually to base e.

y = working response metamer, usually in probit or angular units.

w = weighting coefficient.

M = log.ratio of Potency.

ρ = relative potency, linear scale of units.

S^2 = Mean square for criterion of departure from parallelism between regression lines.

s^2 = Mean square for criterion of log. interval between regression lines.

T = sums of differences (all positive) of observed responses.

U = sums of differences (1st. and 4th. negative) of observed responses.

D.F. = degrees of freedom.

F = variance ratio.

P = Probability of correctness of null hypothesis.

Introduction.

For a considerable period of time after the introduction of the chlorinated hydrocarbon insecticides, the widespread impression persisted that such substances had as their chief advantage over the older organic insecticides a prolonged residual effect under a wide variety of circumstances.

As a broad generalization, there is of course, much truth in this assertion, but its limitations have in recent years become increasingly apparent. The recent development of interest in the stability of contact insecticides is reflected in the references appended to this thesis, nearly half of which have appeared within the two years over which the greater part of this work was carried out.

Two contact insecticides, pyrethrum and DDT, have been chosen for special study in this thesis, representative of the two main groups previously mentioned. It appeared to the author that the residual toxicity of the former was underrated, that of the latter overestimated, under certain conditions.

The many conditions which have been held to influence the decomposition of the pyrethrins, have been discussed in detail by Gnadinger (1936, 1945), in a critical review. For this reason the older work on this insecticide will not be reviewed at length, particularly as with much of it difficulties associated with the development of the chemical and

biological assays of this insecticide appear to be inextricably entangled, and many of the investigations reported by Gnadinger appear to be somewhat superficial.

Potter (1935,1938) has shown that heavy oil solutions of the pyrethrins exert a prolonged residual effect when sprayed on interior surfaces under warehouse conditions, where direct illuminations of the films of insecticide by sunlight is not an important factor in producing breakdown of the active constituents. This work has been developed into a widely used control practice in Great Britain, but appears neither to have received the attention it deserves in other parts of the world, nor have its implications when conditions are changed been adequately considered. Similar results have been obtained by O'Farrell et al (1940) and also by the present author for pyrethrum-in-oil films stored in the dark, and it is necessary to consider the reasons for the widespread reputation for instability which such treatments have acquired.

The factors which affect the apparent residual toxicity of insecticide deposits are undoubtedly complex, but sufficient progress has been made with their study to enable most of the more important factors to be isolated, and their relative importance under various conditions estimated. The major categories into which such factors may be grouped are:-

(1) Those which are artefacts introduced by the method of assaying the toxicity of the insecticide by chemical, or, more particularly, biological methods.

(2). The chemical or physical interactions of the insecticide with its solvent, or diluent, and of these with the substrate to which the material is applied.

(3). The chemical and physical changes brought about by the temperature, humidity, incident radiation, and the constituents, particularly oxygen, of the atmosphere in which the insecticide is deposited.

It is the factors of Group 3 to which particular attention is paid in this thesis, the methods of biological assay developed in Sections 1 and 2 having been adopted primarily with a view to reducing the number of relevant factors of Groups 1 and 2 above, and of ensuring that the essential minimum of such factors were under the sufficient control of the experimenter.

With these considerations in mind, it became clear that a considerable part of any investigation into the stability of contact insecticides would involve the critical examination of the biological assay systems used to determine the toxicity of the deposits. In particular, a simple reproducible and rapid method of applying the insecticides was required in order that all measurements of toxicity might readily be made in terms of relative potency assays, since with most experimental designs intended to estimate relative potency the majority of the factors of Group 1 can be eliminated. For example, tolerance fluctuations of the insects between the occasions on which the toxicity of a deposit is examined are either eliminated, as in successive "four-point" and similar assays,

or utilized as the error variation, as in the twin cross-over design. (see Section 3).

A number of factors affect the responses of the test insects to the insecticide, both during and after treatment, and these factors have been given a rather detailed investigation by Pradhan (1949a, 1949b, [illegible]), who has also reviewed the literature. Such factors as temperature and humidity during and after treatment, though important in control work, should be irrelevant to a properly designed assay.

It is not always easy to seperate the factors of Groups 1 and 2 unless the actual weight of the insecticide acquired by the test insects can be measured. When this process has been carried out, considerable progress has been made in unravelling the complexities of bioassay systems.

For flying insect assays with the pyrethrins, it has been shown that the relationship between the dose applied in the form of a spray and that received by the insects is very complicated. For this type of assay system, and for several which involve fungicides, a detailed but incomplete analysis of the various type (1) factors involved has been made. (Blackith 1950a)

For an assay system in which insects are dipped into suspensions of DDT crystals, Mackintosh (1947) has investigated some of the factors which influence the relation between the applied and received doses. Some of the difficulties which Mackintosh encountered in interpreting the data have

been resolved by Stringer (1948), using a more comprehensive method of analysing the data, in the course of which the importance of the "availability" of the insecticide to the insects was emphasised.

Among the factors of Group 2 are those which arise from the penetration of the insecticides into the substrate, where they may be removed by translocation, or by chemical incompatability with the substances composing it. The virtually complete restriction of the use of the pyrethrins to the control of insect infesting stored products and those causing annoyance to man has led to a gap in the literature on the interaction between pyrethrins and various types of foliage.

Fogg (1948) has shown that DNOC is absorbed and to some extent translocated on living leaves, whilst Hadaway and Barlow (1949) have discussed the penetration of DDT and BHC into mud. Howlett and Parkin (1947) have investigated the penetration of the former insecticide into building materials, and suggested that penetration could be reduced by the pre-spraying application of such materials as size.

Barnes (1945) and O'Farrell et al(1949) among many other authors have shown that the comparative effectiveness of insecticides may be altered according to the nature of the solvent and substrate on which they are tested. The findings of these two workers on the persistence of the pyrethrum films deposited from oil on wood, relative to those of the chlorinated hydrocarbons are very divergent, and it is not

possible to offer any useful suggestions to account for it, except possibly that the extent of the illumination of the deposit was greater in the former case. Barnes found that pyrethrum deteriorated after only 3 weeks, O'Farrell et al, that comparable films remained toxic for several months. Potter (1938) found that these films remained toxic for at least a week and possibly a good deal longer.

In the case of this insecticide, as Parkin and Howlett (1946) point out, interaction with the substrate is likely to include chemical decomposition by any alkalies present as well as the important absorption effect common to all insecticides applied in oily solution. Walker (1931) and Pattersfield and Hobson(1931) have shown that the pyrethrins become relatively unstable at the pH values associated with surfaces containing cement.

The importance of the absorption of the solutions of insecticide into porous substrates has been emphasised by many authors, and as a good example may be noted the observations of O'Farrell et al that on concrete, without pre-treatment, DDT, BHC and the pyrethrins were all rendered innocuous within a few hours by this process. This important factor has been extensively studied by various workers at the Post Infestation Laboratory(D.S.I.R.) in papers already quoted, and a partial preventive in the form of non-porous sealing pre-treatments has been offered by them.

Interaction with the solvent, another of the group 2 factors, may be exemplified by Martin's (1943) observations

of the greater activity of the pyrethrin I Group in emulsions, whereas in oil solutions pyrethrins I and II had roughly equal potency. A further example has been given by Merritt and Mitchell (1948). DDT reacts at 900°F (attained momentarily in a Fog Generator with kerosene) so that up to 33% of the insecticide can be lost. With heavy oil no such loss occurs, and the pyrethrins do not react detectably under these conditions with either solvent.

Some of the factors in Group 3 are conveniently summarised by J.T.Martin (1949) at least so far as the pyrethrins are concerned. The observations of Potter previously quoted themselves followed earlier work by Abbott (1919) and Tattersfield, and co-workers (1931, 1932, 1934), which indicated that under certain conditions, pyrethrum flowers, both ground and whole, are more stable than is commonly believed. The action of sunlight, even without its ultra-violet component, in decomposing pyrethrum powder was clearly shown, and it was found that blue light was slightly more active than red in promoting the breakdown of the active substances present. This indication that light of shorter wavelengths was more active than visible light was followed by the absorptiometric studies of Gillam and West (1942, 1944) which showed that the pyrethrins had a very strong absorption maximum at about 3,200 Å.U., in the middle ultra-violet.

These workers did not consider ultra-violet absorptiometry to be very promising, but a rapid method based on this

principle has been evolved by Beckley, who has also demonstrated the stability of ground pyrethrum flowers when protected from the light. These topics have been reviewed recently by Page (1948a) and Page and Blackith(1948b)

Busvine and Barnes (1947) have shown that whereas dry DDT films are fairly stable in diffuse daylight, those of BHC and the pyrethrins are not. In the former case, the volatility of the insecticide was shown to be the major factor, since deposits which in the open air lost their toxicity rapidly could be preserved in the active condition by storage in an atmosphere saturated with the vapour of the insecticide. With the pyrethrins, however, light, by which one infers diffuse daylight, was shown to accelerate decomposition, but it is not possible to make quantitative deductions from the data given, nor is it known whether antioxidants were present in the films of insecticide.

Martin(1948) has indicated the possibility of reducing the photolysis of the pyrethrins by adding substances which absorb light over as wide a range of wavelengths as possible, and particularly over the critical ultra-violet range mentioned previously. The experiments reported in Martin's note were inconclusive but it was stated that such investigations were worth further study. A patent has been issued to Domenico and Domenico (1948) for the use of Chloramine Fast G. for this purpose and further work on these lines is reported in section B of this thesis.

Dove (1947) has suggested that piperonyl butoxide stabilized the pyrethrins as well as synergising them. A possible mechanism for this process was suggested by Page and Blackith (1940a), and its potential importance, if the effect were verified, has been stressed by Page (1948). Although a considerable amount of work was necessary to establish the fact that the alleged effect, if it occurs at all, is of negligeable importance, some interesting side-lines have arisen from it which are discussed, together with the main point at issue, in section 6 of this thesis.

The work of Dove, which appears to have been made the basis of claims of an alleged stabilization effect, not only does not appear to have been the subject of much critical thought by its author, but has become additionally misleading in the course of repeated transcriptions through technical journals. The work originally quoted (Dove 1947) has been rewritten in an article in "Pest Control and Sanitation" and thence has been abstracted by the Editor of Pyrethrum Post (Vol.1, No.1, Page 14). An example is quoted as offering "striking evidence of the synergistic effect of piperonyl butoxide on the pyrethrins". The example quoted may be written in the form of a table:-

Insecticide.	Concentration, mg: sprayed.	Knock-down of Musca domestical. %
Pyrethrins	40	82
Piperonyl butoxide.	400	82
Mixture	Both together	98.

Examination of the above, the only data quoted in the abstract, shows that the χ^2 value for the discrepancy between the data and the figures expected on the hypothesis of independent action, with no synergist effect, is only 6.10, for a single degree of freedom. The corresponding probability of the null hypothesis being correct lies between the 5% and 1% levels, but, as Finney (1947b) indicates, a χ^2 test, which is the only method available under the circumstances, is liable materially to overestimate any discrepancies from the null hypothesis of independent action. In this instance, therefore, it would be rash to deduce even the well-known synergistic effect of the two substances from what is described as a "striking example of synergism". The example, though in itself trivial and not meriting detailed consideration of this kind, has been quoted to emphasise the need for restraint in accepting statements of the type quoted, at their face value, more particularly in the case of the alleged stabilization effect.

The special features of DDT and BHC, wherein they differ in stability from the pyrethrins, are fully discussed in Section 7 of this thesis. Although the photolytic effect of ultra-violet light is undoubtedly the most important factor with DDT, the effect of volatalization must also be considered. In the case of BHC, this effect is extremely well-known, and its influence on the persistence of sparse films of the substance already noted. With DDT this effect

is less marked, but has been detected by Wichmann et al (1946). These authors found, however, that the losses of weight from heated films of crude DDT, were substantially caused by the greater volatility of the impurities, and that the heat treatment served mainly to "clean up" the film. With pure *p-p'-* DDT, only some 2% had volatalized after 30 days heat treatment at 150° F.

These observations are consistent with some unpublished ones made by the author, when by steam distilling pure DDT and determining the ratio of DDT to water in the distillate, it was shown that the vapour presence of DDT at the boiling point of water was only 11 mm. The DDT determination was carried out partly by direct weighing of the crystals deposited on cooling, partly by biological assay of that remaining in solution. The biological assay was conducted by A.Stringer, against the fourth instar larvae of Aëdes aegypti.L

Goldenson et al (1940) have found that, when heated much above its melting point, DDT was rather unstable. This decomposition occurred even with the very brief thermal activity of solutions in an aerosol generator, where the temperature attained ranges from 600° F to 700° F. The DDT decomposition increases from 5% to 18% over this range.

It was also observed that the higher reaction temperature increased the number of chlorine atoms split off from the DDT molecules. The important effect of the solvent interaction, previously noted in this section, was overlooked by the above authors, so that in discussing the mechanism of the thermal

decomposition, reaction with the solvent may be more important than thermal instability.

Humidity is a factor which might, by the catalytic effect of water, influence the rates of one of the mechanisms by which the pyrethrins and other insecticides are decomposed. Insofar as it controls the moisture content of the stored flowers, and catalyses the oxidation of the insecticides, this topic was investigated by Tattersfield, its effect on the photolytic action of the ultra-violet light is examined in Section 4. of this thesis. Staudinger (1958) states that moisture "is known to facilitate pyrethrins decomposition", but the evidence on which this statement is based has not been traced by the present writer. Extremes of heat (near freezing and near the boiling points of water) were found by Staudinger and co-workers to inhibit and accelerate decomposition respectively. Over the narrower ranges likely to be of practical importance, an increase of storage temperature of the bulk solutions stabilized with an antioxidant from 26 °C to 35 °C resulted in only a small increase in decomposition after some 8 months.

The effect of antioxidants as detailed in the literature is confusing, and it may well be that many claims for these compounds, as for alleged synergists, have been made without adequate evidence, for patent and other purposes. It appears that hydroquinone is the most universally recommended substance, and is more effective with long storage periods

than with short. [Tattersfield (1928), Tattersfield and Martin (1934), West (1942), Buchland et al (1946).] A concensus of opinion, expressed by Gnadinger (loc.cit) is that bulk solutions of the pyrethrins are more easily stabilized by this means than are films, and that although many dilute solutions such as fly-sprays are sufficiently stable without such protection, the addition of antioxidants to concentrated extracts is of value. The former conclusion is fully consistent with, and is indeed partially based on, the work of Martin (1943).

Development of bioassay system.

There are two main types of method available for the assay of contact insecticides by biological methods. One of these methods is the type of assay involving the flight of test insects through a mist of the insecticide dispensed in droplets of an oil or water base. This type of assay has the advantage that the actual weight of insecticide accumulated by the test insect can be determined directly. In the other type of assay, which involves insects crawling on a surface impregnated with the toxic substance, it is necessary to make various assumptions about the relationship between the applied and received dosages. Perhaps the most important of these assumptions is that the independent variate, the measure of the dosage applied to the system, is linearly related to the mean weight of insecticide picked up by the insects. This assumption has been examined for the flying insect system, and for various fungicide assays, and has been found unreliable. (Blackith 1948). In the former case the relationship which has been established is complex and depends very substantially on the level of flight activity of the insects. (Page et al 1946, 1949). It does not lend itself readily to determinations of relative potency, such as are frequently employed throughout this thesis. In addition the manipulation of spray systems is cumbersome, and the insects used usually involve more difficulties in their mass rearing than is necessary with many crawling insects.

For the purposes of the assays subsequently described, it was decided to use grain weevils, adult Calandra granaria L which were allowed to crawl on filter papers impregnated with an oily solution of the insecticide. These insects are easily bred in quantity, and the technique used for this work has been used in several laboratories for some years. It has been described, inter alia by Stringer (1949b). The economic importance of these insects, their life-history and some of the reasons for their selection as test insects have been recently reviewed by the writer.(1949) The test papers are laid on glass sheets, and labelled in hard pencil with a code letter. The test insects are confined on the papers by 6.5 cm rings with vertical sides. Originally, glass rings were used, since Calandra granaria, once its tarsi are contaminated with oil, are unable to climb vertical glass surfaces. The difficulty of replacing these fragile rings, however, led to their replacement by brass rings, which, after plating with nickel and buffing on the inside were found to be acceptable substitutes.

The filter papers used throughout the work were 7cm.No.1 Whatman discs. In view of the considerable number of such papers used, and the fact that no particular advantage seems to be associated with the use of any other type of paper, cost and availability indicated the use of this particular type. There is also the advantage of being able to relate results obtained with it, to those obtained by Stringer (1948 -1949 a&b)

The oil solvent used for the insecticides, to avoid the unnecessary complications inherent in the assay of dry deposits, was Pool 31. This oil is a white, refined oil consisting almost entirely of unsaponifiable straight-chain hydrocarbons of approximately the composition of medicinal paraffin, boiling range $\approx$ 360°C. A specification of this oil has been published by Robinson (1948).

Various methods in the past have been used to secure a uniform deposit on the filter papers. For example, later modifications of the Potter tower method ensure even distribution of oil when this is dispersed as a mist above the filter papers, and then allowed to settle.(Potter 1941) This method, however, is slow compared with direct application from a pipette, and in the case of a large scale experiment involving the application of many different solutions might be prohibitively so.

Small aliquots of the oil have been applied at random to or in the centre of the surface of the paper, which was afterwards allowed to stand for some hours to allow the diffusion gradient to disappear. (Stringer 1949b) Aliquots have also been applied from a micro-pipette spirally across filter papers mounted on pins, to avoid any excess spreading on to the glass sheets on which the papers are conveniently laid.(Busvine and Barnes 1947)

The advantages of applying the oil-based insecticide solution diluted with a volatile solvent are considerable. They include increased accuracy, on account of the larger

volumes used, and the reduced viscosity and hence drainage errors, and the more even distribution of the insecticide over the filter papers. This technique appears to have been devised independently, and roughly simultaneously, by R.G.Davies at East Malling Research Station (private communication) and by the present author. In the former case benzene was used as the diluent, but in this work petroleum ether (Boiling range 100° - 120° C) was preferred. "Analar" grade petroleum ether "freed from aromatics", is known to volatalize completely, but not too rapidly, at normal room temperatures. The lower boiling ranges evaporate too quickly, and, particularly under the conditions of a constant temperature and humidity cabinet, are liable to cause condensation of water on the papers, despite their low latent heats.

The laterally even distribution of insecticide obtained with excess volatile solvent has been discussed by Busvine and Barnes (1947), but these authors did not employ a residual solvent as well. They considered it possible that vertically the distribution might not be so satisfactory, but in cases where a residual solvent is used any vertical diffusion gradient would even out.

The slope of probit-log concentration regression lines obtained from bioassays with crawling insects has been held by a variety of authors to be a measure of the standard deviation of the distribution of tolerances among the test insects. (Finney 1947) The inadequacy of this view has been

~~has been~~ stressed by the writer (1940b) and the many factors which can affect the slope have been analysed. Although one can say with Finney that the slope of a regression line is less under the control of the experimenter than some other parameters, Stringer (1948) has related the ratio of slopes of pairs of like assays to the relative availabilities of the insecticide in each of the pairs. One way of increasing the availability of the insecticide is to increase the volume of residual oil applied to the filter papers in the system under discussion. The limiting factor which prevents indefinite increase in this quantity is the "control" mortality of weevils caused by the action of the oil alone. In the case of assays with pyrethrum films, 12hr. exposures have been used, and control responses caused by oil only are naturally less than in the case of longer assays extending over several days, such have often been used with dry deposits of some halogenated hydrocarbons. It is also convenient for computational purposes to keep the expected control mortality to less than 1 insect per batch, i.e. less than three per cent for batches of 30 insects such as are used here. Under these conditions there is little point in adjusting the response rates, since the very small adjustments will be very inadequately estimated in any ordinary case. The special case of control responses in assays involving the "twin cross-over" design has been fully discussed in a previous paper.(Blackith 1950b)

A preliminary experiment was designed to examine the

TABLE. I.I

Effect of solvent composition on sensitivity of biological assay.

Composition of Solvent.	60.0% 40.0%	80.0% 20.0%	90.0% I0.0%	99.0% I.0%	Petroleum Ether. P.3I.Oil.
Concn: of Pyrethrins. (Residual) %	Response. Percentage. Probit.	Response. Percentage. Probit.	Response. Percentage. Probit.	Response. Percentage. Probit.	
0.80	- -	- -	70.0 5.52	57 5.I8	
0.70	- -	- -	- -	47 4.92	
0.60	- -	- -	70 5.52	28 4.42	
0.50	- -	- -	69 5.50	33 4.56	
0.40	- -	- -	6I 5.28	I9 4.I2	
0.30	I00	- -	58 5.20	26 4.36	
0.20	I00	46 4.95	2I 4.I9	8 3.60	
0.I8	- -	27 4.39	- -	- -	
0.I6	- -	29 4.45	- -	- -	
0.I5	- -	- -	2I 4.I9	7 3.52	
0.I4	97 6.88	26 4.36	- -	- -	
0.I2	- -	I4 3.92	- -	- -	

Continued on next page under similar column heads.

TABLE 1.1 (continued.)

0.10	73	5.61	6	3.45	19	4.12	-
0.08	72	5.58	9	3.66	-	-	-
0.06	52	5.05	4	3.25	-	-	-
0.04	22	4.23	-	-	-	-	-
0.02	3	3.12	-	-	-	-	-
0.00	3	-	0	-	0	-	-

Computed Probit regression lines for the above data.

60%/40% solvent mixture :- $Y = 4.25x + 1.63$.

Standard error of slope is ± 3.26, X^2 for 6 d.f. is 0.25, not sig. @ 5% level of P.

80%/20% solvent mixture :- $Y = 3.16x + 0.64$.

Standard error of slope is ± 3.53, X^2 for 6 d.f. is 0.11, not sig. @5% level of P.

90%/10% solvent mixture :- $Y = 1.96x + 2.03$.

Standard error of slope is ± 1.76, X^2 for 6 d.f. is 0.19, not sig. @ 5% level of P.

99%/ 1% solvent mixture :- $Y = 2.13x + 0.96$.

Standard error of slope is ± 2.38, X^2 for 6 d.f. is 0.06, not sig. @ 5% level of P.

optimum ratio of residual oil to volatile petroleum ether in the solvent mixture. The optimum total volume of liquid which can be applied to 7cm. No.1 Whatman filter paper is 0.5ml.(Stringer 1949b) Greater volumes creep beyond the boundaries, lesser volumes may not carry the insecticide to the edges of the paper.

Four solvent ratios were included in an experiment which could not be made fully factorial on account of the very different availabilities of pyrethrum films made up with differing volumes of residual oil. The layout and experimental data are shown in Table I.1 and so are the regression lines fitted to the four sets of data. It will be observed that the slope of the regression lines increases with the amount of residual oil present, at least over the range of compositions studied; although these slopes are determined with but low precision. Part of the heterogeneity of the data is doubtless caused by the abnormally low exposure period used, namely 6 hrs. This short exposure time was chosen to investigate the slopes of the curves for oil concentrations at which control responses become rather high. For example, after 24hrs exposure, Stringer (1949b) has shown that 0.16ml P31 per filter paper or approximately 0.5ml of a 30:70 mixture on the basis adopted here, causes severe "control" mortality, but that this level is a critical one, the responses to oil alone being negligable below this dosage even at 96 hrs. exposure. As a result of these investigations, the mixture with 20% P31 and 80% Petroleum ether was standardized as the solvent

in all the work described subsequently. In two cases, however, that of the pyrethrum synergists sesamin and n-propyl isome, the solubilities of the substances in the mixture were lower than that required, so that part of the petroleum ether was replaced by the volatile solvent methyl ethyl ketone, small quantities of which (5%) greatly improve the solvent powers of the mixture for most organic compounds. There are indications that the relation between applied and received dose in some crawling insect tests may depend to some extent on the activity of the insects, (Busvine and Barnes, 1947) though probably not to the same overriding extent as the flying insect assays.

It was thought desirable to follow the relation between the applied dose and that actually received by the insect. Such an investigation implies that some substance which is included in the oil applied to the filter papers is to be determined from the insects after their responses to the insecticide have been estimated. Two obvious difficulties immediately appear, that of recovering the substance quantitatively from the insect in the minute amount present, and the likelihood that the tracer substance and the insecticide may be acquired by the insect at different rates from the substrate.

Both these difficulties can be overcome by using as the tracer substance one which is both itself insecticidally active and contains a readily detectable metal forming part of the molecule, so that this may be determined after the complete

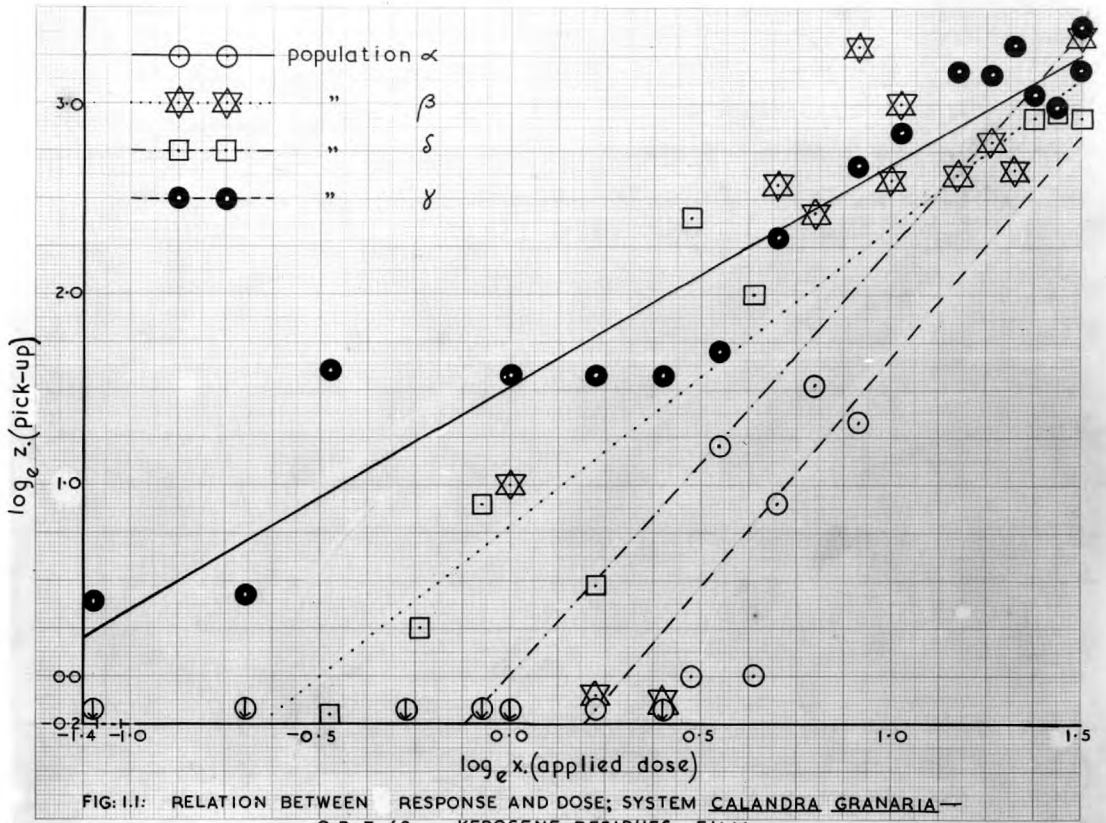
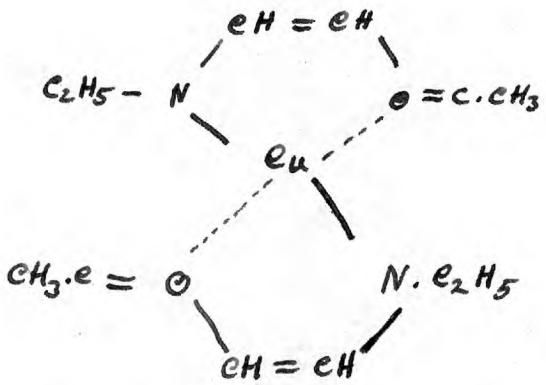


FIG: I.1: RELATION BETWEEN RESPONSE AND DOSE; SYSTEM CALANDRA GRANARIA — O.R.T. 69. — KEROSENE RESIDUES FILM

incineration of the organic material of the insect by wet or dry methods. A substance with these properties was, rather fortuitously, discovered during an examination of potential tracer substances. This material, known as "OMT 6p", consists of the chelated copper derivative of ethyl aminomethylene acetone, and was supplied as a solution in the kerosene by the Royal Dutch Petroleum Co., throught the courtesy of Professor E.R.H.Jones of Manchester University. Originally, it was prepared as part of an investigation into compounds intended to inhibit the formation of "gum" round piston rings in internal combustion engines, and has the following structure:-



The pure compound is said to be a liquid miscible in all proportions with oils. Many of the properties of the kerosene solution have been given a preliminary investigation by Morcos(1947). The weight of copper determined in an insect in excess of that naturally contained by them is a direct measure of the amount of insecticide accumulated in the course of the assay. Similarly, the quantity and distribution of insecticide in the substrate can be investigated directly, whereas investigations based on direct weighing of solvents,

etc., have to assume that the diffusion of the solvent parallels that of the insecticide, without chromatographic seperation.

Unpublished investigations by the author in 1945 -46, prior to those of Morcos, developed methods for determining the copper both from insect and substrate. The chemical aspects of the determination depend on the complete oxidation of the organic matter by a mixture of fuming nitric acid and hydrogen peroxide (100vols.) as described by Page et al (1949). The residual ash, after solution in dilute acid, is extracted with a chloroform solution of dithizone (diphenyl-thiocarbazone), with which reagent the copper develops a real colour which may be compared with a set of standards. Details of the procedure are described by Morcos (loc. cit)

By pipetting a 5% solution of copper ethylaminomethylene acetone in Kerosene on to the centre of a 7cm.No.1 Whatman filter paper, in progressively increasing doses, it is possible to determine the spread of the insecticide through the filter paper, by estimating its concentration in annuli of increasing radius. It will be observed, from Fig.1.2 which shows the results of a series of such experiments, that the concentration of insecticide falls off rapidly from the centre of the paper when applied in the manner described. The importance of applying the insecticide in a manner which distributes it over the filter paper evenly is thus emphasised, since the weevils do not distribute themselves at random over the substrate, but concentrate near the perimeter.

TABLE I 2 (1)

Dosage response data for four populations of Calandra granaria exposed on Kerosene films of copper ethylaminomethylene acetone (O.R.T. 69) 0.5 ml. of Kerosene per 7 cm. No.1. Whatman filter paper. Exposure time 12 hours.

Population	% O.R.T. 69 in Kerosene	% Dead 12 hour	% K.D. 12 hour	No. Used	Total Cu: Micrograms	Blank Cu: Micrograms	Net wt. Cu: Micrograms	Pick up of O.R.T. 69/ insect, micrograms.
	0.00	4.35	4.35	69	22	25	-3	-
	"	13	13	30	12	11	1	0.15
	"	14.8	14.8	27	8.5	9.5	-1	-
	"	3.06	3.06	33	13	12	1	0.15
	0.25	92	96	76	20	24	-4	-
	"	23	25	51	12	11	1	0.15
	0.50	27	36	70	21	22	-1	-
	"	16	16	32	12	11	1	0.14
	0.62	12.5	51	56	21	20	1	0.08
	0.75	39	54	83	19	26	-7	-
	"	40	40	27	13	10	3	0.50
	0.80	46	46	52	20	18.5	1.5	0.13
	0.94	59	59	72	15	23	-8	-
	"	81	82	43	20	17.5	2.5	0.25
	1.00	78	88	74	24	24	0	zero
	"	88	99	51	21	16	5	0.27
	"	57	57	30	14	10.8	3.2	0.48
	1.25	57	57	49	19	18	1	0.09
	"	100	100	26	10	9.5	.5	0.06
	"	98	98	30	14	10.8	3.2	0.48
	"	71.4	71.4	56	22	20	2	0.16
	1.50	98	100	71	11	23	-12	-
	"	95	100	56	21	20	1	0.08
	"	100	100	30	14	10.8	3.2	0.48
	1.62	100	100	70	29	22	7	0.10
	"	100	100	34	20	12	8	1.1
	1.75	94	99	73	26	21	5	0.34
	"	100	100	56	20	20	0	zero.
	"	100	100	28	13	10.1	3	0.49
	1.88	87	95	77	29	25	4	0.1
	"	100	100	31	16	11	5	0.75
	2.0	100	100	67	27	23	4	0.25
	"	100	100	17	11	6	5	1.33
	"	100	100	29	17.5	10.5	6.5	1.02
	2.25	100	100	58	24	18	6	0.48
	"	100	100	30	18	11	7	1.06
	2.50	100	100	11	10	4	6	2.46
	"	100	100	28	19	10.1	9	1.46
	"	100	100	78	31	25	6	0.38

TABLE I 2 (a)

Population	O.R.T.69 in Kerosene	% Dead	% K.D.	No. used	Total Cu. Micro-grams	Blank Cu. Micro-grams	Net wt, Cu. Micro-grams	Pick up of O.R.T. 69/ insect micrograms
CCR	2.75	100	100	31	19	11	9	1.56
	3.00	100	100	25	20	9	11	2.02
	"	100	100	31	23	11.2	12	1.76
Resistant strain	3.25	100	100	30	20	11	9	1.36
	"	100	100	30	27	10.8	16	2.42
	3.5	100	100	30	22	11	11	1.66
	"	100	100	31	27	11.2	16	2.35
	3.75	100	100	32	21	11	10	1.42
	"	100	100	30	29	10.8	18	2.73
	"	100	100	30	25	10.8	14	2.14
	"	100	100	29	24	11	12.5	1.9
	4.25	100	100	32	13	11	2	0.23
	"	100	100	22	22	10.1	12	1.95
	"	100	100	29	27	12	15	2.0
	4.5	100	100	30	30	11	19	2.94
	"	100	100	30	30	10.5	19.5	3.06
	"	100	100	30	23.5	11	12.5	1.90
	"	100	100	28	25	10.1	15	2.43

TABLE. 13.

Regression coefficients : system C. granaria - O.R.T. 69 - Kerosene.

y = probit response

x = $\log_e$ (pick up of O.R.T. 69)

z = " (applied concentration)

Simple regression coefficients

Population.	y on x	y on z	z on x (availabilty coeff:)
Sprs	2·90 - 0·64	-0·05 - 0·33	2·72 - 1·54
	1·29 - 3·14	-0·56 - 0·83	2·27 - 0·45
	3·84 - 0·50	1·79 - 2·13	1·13 - 0·09
	2·99 - 0·94	1·53 - 0·17	1·65 - 0·18

Partial regression coefficients

Sprs	0·49 - 2·93	-0·14 - 0·86
	0·89 - 1·51	-0·01 - 0·24
	4·02 - 0·62	-0·58 - 0·84
	1·00 - 0·88	1·19 - 0·40.

The effect noted above is so marked that if the volume of liquid applied is reduced so that it does not diffuse to the perimeter of the enclosed area the toxicity falls to a negligeable value. The relationship between the volume of kerosene solution applied and the mortality of grain weevils confined to the substrate after 24 hours exposure is shown in Fig. 1. 8. It is clear that when the insecticide does not diffuse to the annulus next to the confining ring it is not acquired by the insects. This situation may be regarded as an extreme case of the influence of the solvent on the availability of the insecticides. Robinson(1943) has solved this problem by incorporating a stimulant (4-chloro-2-methyl-phenol) in his solutions, thus compelling the insects to keep moving. Such an irritant might, however, be undesirable when primary responses are to be used as the criterion of insecticidal effect.

The relation between the applied and received doses of copper ethylaminomethylene acetone has also received attention. Batches of about 50 insects were confined on various concentrations of copper ethylaminomethylene acetone, and the responses of the insects after 18hrs estimated. The insects were then incinerated, and the amount of copper picked up determined for each batch by the techniques previously outlined.

It was found that the relationship between pick-up and applied dose was of the exponential type discussed by Stringer (1948) that is, the plot of the log pick-up against log.

concentration applied was linear. The slope of the lines representing the regression of log.pick-up on log. dose is the availability coefficient for this system, and this quantity was calculated by the usual regression techniques for each of the four populations of insects used (Fisher 1946). The data on which the calculations were based are applied concentration demonstrated graphically in Fig. 1.1. A striking and unexpected feature of this figure is that the availability coefficient for population δ is significantly lower than those for both β and δ, though all are consistent with the slope of the regression relating to population α, which was determined with only low precision. This situation suggests that for those insecticides which have to penetrate into the ~~cuticle~~ cuticle before reaching their site of action, the availability coefficient in any particular assay system may itself be a complicated quantity, dependent at least in part on the factors which control penetration through the insect cuticle.

As will be shown in the next section, for the pyrethrins which it is thought do not penetrate the cuticle before inducing primary responses, the slopes of dosage-response regression lines remain constant for insects of differing populations and generations.

The increase of pick-up with applied concentration can be followed by chemical means over a much wider range of doses than is possible utilizing biological responses. (Page et al 1949) For this reason the mutual variation of the response and dose can only be determined within

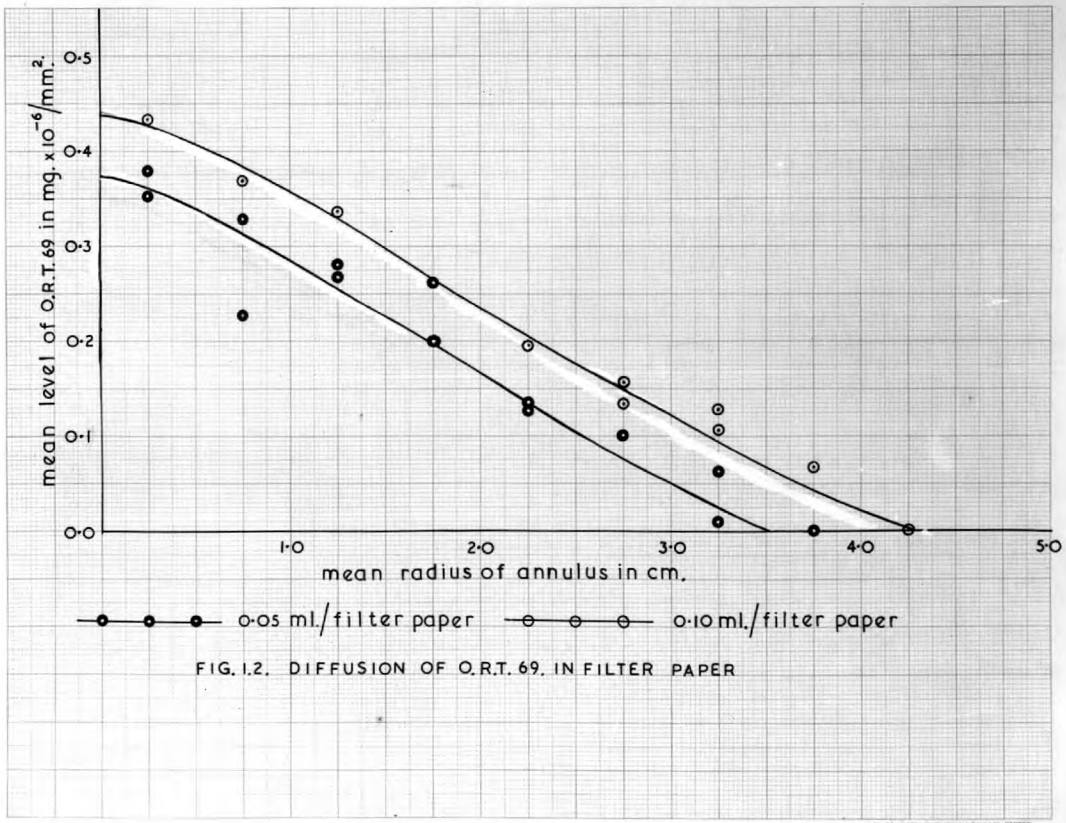


FIG. 1.2. DIFFUSION OF O.R.T. 69. IN FILTER PAPER

narrower limits and with lower precision than the relationship just discussed. For the first time for any crawling insect assay, it has proved possible to partition the simple regression of probit response on log.dose into its components, the partial regressions of probit response on log. pick-up and log.applied dosage, respectively. The partial regressions were calculated according to the method outlined by Fisher (1946) with the modifications introduced by Finney (1947) to deal with quantal response data.

An attempt was made to allow for the error, in determining the pick-up of insecticide on the insects, which is quite comparable to that of the biological measurements. A recently introduced modification of Wald's technique for determining regression coefficients when both variables are subject to error [Bartlett (1940)] was adopted for this purpose.

Although this was the only method available, it suffers from two serious disadvantages, namely that it does not at present lend itself to multiple regression analysis, and, being an approximate method, rejects the information from such a large fraction of the data that reliable results can only be obtained from fairly large numbers of points. In the case under discussion the method was tried out on the simple regression of y on z for population 8 , with the following results:-

Error assumed in y only, $b = 1.855 \pm 0.175$ *

" " " y and z, $b = 1.580 \pm 0.824$

* significant at the 5% level of P.

Thus the effect of allowing for the joint error of both variables is to reduce the precision of the estimate of the slope, b, by $\beta\beta/\alpha\alpha$, and since some such factor as this probably applies to the other regression lines, no useful purpose would be served by attempting to draw conclusions from the present body of data. It would, however, be interesting when time permits, to follow up the conclusions drawn from the availability coefficients, which are not vitiated by the double source of error, when the improved method of applying insecticide to the filter papers described in the earlier part of this section could be adopted.

Copper ethylaminomethylene acetone is an insecticide of comparable toxicity to DDT. The median lethal dose for C.granaria is of the order of 2 µgm./mgm. body-wt. The substance suffers fairly rapid decomposition in air with the formation of free copper ions, and deposits from kerosene solutions lose all their toxicity after some 48 hours. The substance is, however, interesting in that it does not appear to be a nerve poison like the pyrethrins and DDT, and produces no detectable primary responses. The death of the insects, which takes place at a rate much faster than that attained with most halogenated hydrocarbon insecticides, though not quite as rapidly as with pyrethrins, is preceded by a very short period of lethargic behaviour, as the similarity between the columns headed "Knocked down" and "Dead" in Table 1.2. indicate. It seems possible that its similarity to the

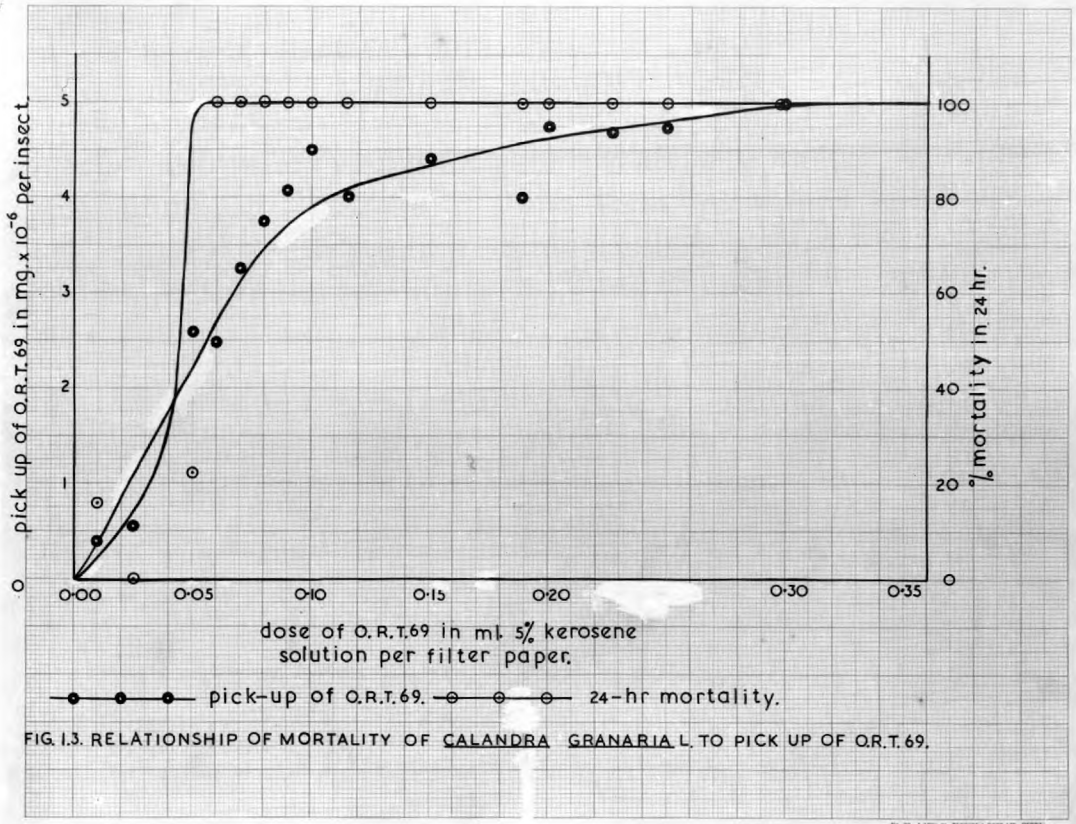


FIG. 1.3. RELATIONSHIP OF MORTALITY OF CALANDRA GRANARIA L. TO PICK UP OF O.R.T.69.

copper derivative of glycine (amino-acetic acid) may be important; the blocking of an enzyme essential to the insects metabolism may be indicated.

Experimental designs for the estimation of pyrethrins stability.

The special features of pyrethrum assays with crawling insects enable the residual concentrations, after various treatments have been applied to the test surface, to be assessed by means of experimental designs not necessarily suitable for other types of assay.

Two features which differentiate the assays used here from many others are (1), the use of the angular, rather than the probit transformation as a measure of the dependent variate, and (2), the use of a reference calibration curve, with its implication that the slopes, though not necessarily the positions, of the regression lines derived from this type of assay are effectively constant within the limits of sampling error from one experiment to another. It is the latter property of the pyrethrum assays used here which is particularly convenient, and which, after[a] stringent investigation, has been made use of throughout much of the work reported in this thesis.

The use of the angular transformation is convenient in the manipulation of the data, since the sampling variance of the observations is thereby, as a matter of definition, equalised. If equal numbers of test insects are used in each batch, no weighting coefficients are required unless goodness of fit is being tested, when the weighting coefficient need not be introduced until the end of the computations, since it is a

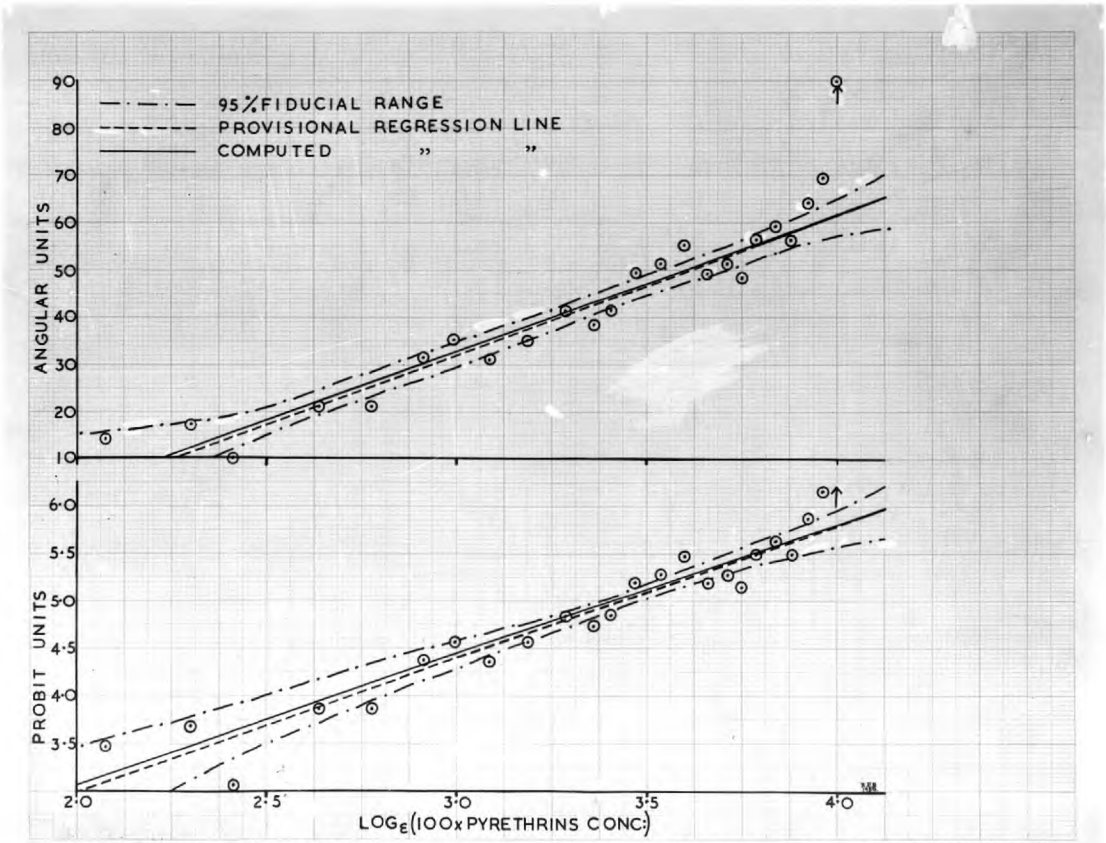


FIG.2.1: RELATION BETWEEN RESPONSE AND DOSE: SYSTEM C. GRANARIA—PYRETHRINS—OIL FILM

constant for this transformation, independent of the value of dependent variate.

The equalisation of the sampling variance enables the transformed data to be subjected to an analysis of variance and thereby full advantage can be taken of such shorter methods of computation as the method of orthogonal polynomials (Fisher and Yates, 1948,) as adapted to bioassay calculations by Emmens (1948), in terms of his "factorial coefficients".

It is not necessary for the valid use of the angular transformation that the data should be held theoretically to conform to the distribution function $\phi = \sin^{-2} p$. but that the residual dispersion about a fitted angular unit-log. dose regression line should be at least as small as when probit units are fitted. The statistical aspects of the use of alternate transformations has been discussed more fully by Finney (1947a and 1949) and by the present author (1950a).

The biological assay system used in this work has been examined by constructing the calibration curve later used as a reference standard. Twenty-four batches, of 20 weevils each, were drawn from a single homogeneous population of the insects, and subjected, after the usual standardisation procedure, to the graded series of doses of the pyrethrins shown in Table S. 1.

The proportion of the weevils paralysed after the standard 12 hours exposure period was estimated as described earlier, and the percentage responses converted into both the probit and angular transformations. The provisional and computed regression

TABLE 2.1.

Computation of the dosage-response regression line for the paralysis of Calandra granaria L, by the pyrethrins.

Part I.

Response values forced into equal variates.

Pyrethrins concn: λ	$x = \log_e(100\lambda)$	Number of insects n.	Number responding	Percent. Response	Angular transform.
0.54	3.99	32	32	100.0	90.0
0.52	3.95	33	29	88	69.7
0.50	3.91	32	26	81	64.2
0.48	3.87	32	22	69	56.2
0.46	3.83	31	23	74	59.5
0.44	3.78	32	22	69	56.2
0.42	3.74	32	18	56	48.4
0.40	3.69	31	19	61	51.4
0.38	3.64	31	18	58	49.6
0.36	3.58	32	22	69	56.2
0.34	3.53	31	19	61	51.4
0.32	3.47	32	30	58	49.6
0.30	3.40	32	14	44	41.6
0.28	3.33	33	13	39	38.6
0.26	3.26	32	14	44	41.6
0.24	3.18	34	11	33	35.1
0.22	3.09	31	8	26	30.7
0.20	2.99	30	10	33	35.1
0.18	2.89	30	8	27	31.3
0.16	2.77	30	4	13	21.1
0.14	2.64	31	4	13	21.1
0.12	2.48	31	1	3.2	10.3
0.10	2.30	31	3	9.7	17.5
0.08	2.07	31	2	6.5	14.2

$\Sigma nw^a x^2$ 10.6671 $\Sigma nw^a x . nw^a y^a$ 144.6605 $\Sigma nw^a y^{a2}$ 2,055.6867

$(\Sigma nw^a x)^2$ 10.4212 $\Sigma nw^a x . \Sigma nw^a y^a$ 137.0651 $(\Sigma nw^a y^a)^2$ 1,802.3491

S_{xx}. 0.2459 S_{xy}. 7.5954 $S_{y^a y^a}$ 253.3491

$(\Sigma xy^a)^2 / S_{xx}$ 216.0626

χ^2_{22} d.f. = 36.3865

P = 0.026.

All quantities in above and succeeding "sums of squares" table understood to be divided by the appropriate sum of weights.

Part II.

Probit transform. E	weight. w^p	$n.w^p$.	$n.w^a$	Expected angle. y^a	Expected probit. y^p	Working angle. y^a
$+\infty$	0.5026	16.12	0.0390	61.5	5.75	77.2
6.17	0.5316	15.45	0.0401	60.5	5.70	68.9
5.88	0.5316	17.00	0.0390	59.1	5.65	65.9
5.50	0.5580	17.85	0.0390	57.7	5.59	56.2
5.64	0.5810	18.10	0.0378	56.5	5.52	59.5
5.50	0.5810	18.61	0.0390	55.0	5.46	56.2
5.15	0.6005	19.29	0.0390	54.0	5.41	48.3
5.28	0.6005	18.61	0.0378	52.9	5.36	51.1
5.20	0.6161	19.12	0.0378	51.8	5.30	49.6
5.49	0.6274	20.10	0.0390	49.5	5.20	55.9
5.28	0.6343	19.61	0.0378	47.7	5.12	51.3
5.20	0.6364	34.05	0.0634	45.8	5.02	49.6
4.85	0.6343	20.25	0.0390	43.8	4.94	41.4
4.72	0.6343	20.91	0.0401	42.3	4.86	37.7
4.85	0.6274	20.02	0.0390	40.1	4.76	41.4
4.56	0.6005	20.42	0.0414	37.3	4.62	35.1
4.36	0.5810	18.10	0.0378	34.3	4.50	30.8
4.56	0.5810	17.44	0.0366	31.6	4.37	35.1
4.38	0.5026	15.09	0.0366	29.2	4.25	31.4
3.87	0.4714	14.14	0.0366	25.4	4.06	21.3
3.87	0.4047	12.51	0.0378	21.1	3.87	21.1
3.15	0.3020	9.06	0.0366	14.5	3.56	10.8
3.70	0.2375	7.35	0.0378	11.2	3.41	20.3
3.49	0.1544	4.78	0.0378	5.0	3.10	83.9
		413.98	0.9485			

$\Sigma nw^p x^2$ 4,895.5473 $\Sigma nw^p xy^p$ 7,212.2767 $\Sigma nw^p y^{p2}$ 10,630.567

$\Sigma(nw^p x)^2/\Sigma nw^p$ 4,816.858 $\Sigma nw^p x \Sigma nw^p y^p / \Sigma nw^p$ 7,095.461 $(\Sigma nw^p y^p)^2/\Sigma nw^p$ 10,451.952

Σx^2 78.689 $\Sigma x.y^p$ 116.130 Σy^{p2} 178.615

$(\Sigma x.y^p)^2/\Sigma x^2$ 140.454

χ^2 22 d.f. 38.161

P 0.017.

Weighted means:- $\bar{y}^p$ 5.0245 $\bar{y}^a$ 41.583

$\bar{x}^p$ 3.435 $\bar{x}^a$ 5.321

TABLE 2.1 (Contd.).

Part III.

Working probit. y^p	$nw^a x$	$nw^p x$	$nw^a y^a$	$nw^p y^p$
6.53.	0.1568	64.32	3.0108	105.26
6.09	0.1584	61.03	2.7629	94.09
5.86	0.1525	69.42	2.4921	99.62
5.49	0.1509	69.08	2.1918	97.99
5.64	0.1448	69.32	2.2415	102.08
5.50	0.1474	70.35	2.1918	102.36
5.14	0.1459	72.14	1.8837	99.15
5.28	0.1395	68.67	1.9316	98.26
5.20	0.1376	69.59	1.8749	99.42
5.48	0.1396	71.96	2.1801	110.15
5.28	0.1334	69.22	1.9591	103.54
5.20	0.2199	118.15	3.1446	177.06
4.85	0.1326	68.85	1.6146	98.21
4.72	0.1335	69.63	1.5118	98.69
4.85	0.1271	65.27	1.6263	97.10
4.56	0.1317	64.93	1.4531	93.11
4.36	0.1168	55.93	1.1642	78.92
4.57	0.1094	52.14	1.2847	79.70
4.39	0.1058	43.61	1.1492	66.25
5.89	0.1014	39.17	0.7796	55.00
3.87	0.0998	35.03	0.7976	48.41
3.31	0.0908	22.47	0.3953	29.99
3.80	0.0869	16.90	0.7673	227.93
3.73	0.0782	9.89	0.9034	17.83.
	3.1395/1412.22		41.2920	2,080.06

Computed Fiducial Limits.

x.	$V(y^a)$.	95% Limits.
2.000	7.620	10.533
		0.000
2.500	1.751	20.819
		15.365
3.000	1.446	34.867
		29.881
3.521	1.057	43.675
		39.411
3.800	1.885	58.072
		52.380

x.	$V(y^p)$	95% Limits.
2.000	0.0286	3.4591
		2.6720
2.500	0.0135	4.0165
		3.5341
3.000	0.0048	4.5970
		4.3090
3.435	0.0024	5.1263
		4.9226
3.900	0.005165	5.7946
		5.44968

Computed regression lines.

a) In angular units:- $y^a = -53.321 + 28.565.x$

Log.L.D_{50} is 3.448 with 95% fiducial limits 3.515
3.569

Abs.E.D_{50} " 0.313 " " " " 0.336
0.291

b) In probit units:- $y^p = 0.4353 + 1.536.x$

Log.E.D_{50} is 3.417 with 95% fiducial limits 3.490
3.344

Abs.E.D_{50} " 0.305 " " " " 0.330
0.284.

lines fitted to the two sets of data, with a common logarithmic normalising transformation applied to the dosage, are shown in Fig. 2.1.

As is often the case with assays for which fairly extensive data are available, the agreement between the eye-fitted provisional and the first-cycle computed lines is quite good, so that no further cycles of computation were undertaken.

The computed calibration curve in terms of angular units is $Y = -53.381 + 38.565\,x.$; $\chi^2_{22} = 36.36.$ and in terms of probit units

$$Y_p = 0.4585 + 1.356\,x. \quad ; \quad \chi^2_{22} = 38.161.$$

Inspection of Fig. 2.1 or examination of the values of the residual dispersion given above, or in the model computation of these data shown in Table 2.1 , shows that the data are fitted very slightly better by the angular than by the probit transformation, thereby justifying the use of the former in calculations of this kind.

As Finney (1947b) and Bliss and Cattell (1948), have pointed out, the slopes of many biological assay systems show much less variation than their positions, and the uniformity of the slopes of regression lines from the pyrethrins-oil film-*S. granaria* system have been established by the examination of the responses of a number of generations of weevils bred under the conditions standardised for this work. The comparison between different generations of the weevils is a critical test

of the hypothesis that the slopes do not vary, since it takes into account components of variation due to sampling error, population differences, and environmental and genetical differences reflected in the variation of the tolerance distribution from one generation to another. In particular, the generations examined in this instance were those from which all the insects used in the investigations described in sections 3 to 8 were drawn. It is these sections which contain experiments dependent on the common slope of their regression lines for the validity of their interpretation.

For the examination of slopes, four samples, subsequently referred to as populations, were drawn from the four cultures maintained to supply experimental insects. Each population consisted of four batches of 50 weevils, and was drawn two weeks after the emergence of the cultures in question.

Four doses of the pyrethrins, equally spaced on a logarithmic scale, were used to evaluate the slope of the regression of response on log. dose. The responses were subjected to the angular transformation, and lines of common slope were fitted by eye to all the 24 sets of four points. From these lines provisional angular units were derived, from which, as in a corresponding probit analysis, the adjusted scores were calculated. The data in question are shown in Table 2. 5.

These adjusted scores were then subjected to an analysis using the appropriate orthogonal polynomial coefficients (Fisher and Yates, loc. cit.) to represent the log. doses. This device,

TABLE 2.2

χ^2 residues for the 24 regression lines examined for slope.

Population.	Generation:- F_1.	F_2.	F_3.	F_4.	F_5.	F_6.
1.	3.266	0.191	10.217^x	3.367	3.062	2.697
2.	2.473	0.957	3.699	2.239	3.868	13.474^x
3.	0.133	3.712	0.065^x	2.462	8.469^x	16.377^x
4.	19.377^x	3.269	7.257^x	1.758	1.391	2.602

Upper limit for χ^2 based on 2 d.f. 5.991 at 5% level of P.

Lower limit for χ^2 based on 2 d.f. 0.103 at 5% level of P.

Residues marked ^{x} fall outside these limits.

Partitioned χ^2 Analysis of the data for the Homogeneity of slope of the 24 regression lines, four from each generation.

Departure from Parallelism.	D.F.	χ^2	Mean square.	Variance Ratio.
Populations	3	18.538	6.1793	3.3449^x
Generations	5	14.900	2.9800	1.5901 n.s.
Residual	63	116.387	1.8474	
Total variation, excepting tolerance fluctuations.	71	149.826		

TABLE 2.3.

Adjusted scores of responses of C. granaria form successive generations to the pyrethrins. (Homogeneity of slopes expt:).

Generation.	Population.	Log. dose in factorial coefficients. -3	-I	I	3.
F I.	I.	28.6	42.2	56.3	56.7
	2.	I6.5	30.7	43.7	45.9
	3.	24.5	33.0	44.7	53.0
	4.	2I.7	57.7	6I.0	64.5
F 2.	I.	I4.2	22.5	35.0	45.5
	2.	I8.I	22.3	32.0	44.0
	3.	I7.5	2I.7	44.I	50.0
	4.	I9.3	20.7	35.0	50.0
F 3.	I.	22.5	30.I	59.8	57.8
	2.	20.9	22.9	43.8	50.I
	3.	22.8	33.8	43.4	55.5
	4.	23.0	25.6	52.9	56.I
F 4.	I.	I6.5	36.0	37.3	50.2
	2.	I0.4	24.3	24.4	29.6
	3.	I0.3	I7.7	I7.5	32.5
	4.	I8.3	23.8	37.I	53.I
F 5.	I.	42.4	53.3	62.3	59.9
	2.	43.0	50.0	63.0	57.0
	3.	29.I	54.7	53.3	62.0
	4.	35.I	42.0	49.I	64.7
F 6.	I.	57.I	58.I	62.6	76.5
	2.	26.6	38.I	36.8	65.4
	3.	45.6	3I.2	56.9	66.6
	4.	39.2	56.6	60.6	67.0.

which materially shortens the calculations, was not employed exactly as suggested by Emmens (loc. cit.) but was introduced into a multiple regression type of calculation similar to that used by Finney (1947b), since an estimate of the χ^2 residual values was required, as well as an estimate of the common slope of the lines.

The result of partitioning the χ^2 value for the whole set of 96 observations, tolerance differences having been eliminated by the fitting of separate regression lines to each set of four observations, is shown in Table 8.8.

It will be seen from this table that the data taken as a whole are markedly heterogeneous. Using the quantity $(2\chi^2)^{\frac{1}{2}} - (2n - 1)^{\frac{1}{2}}$ as a normal deviate, the total heterogeneity corresponds to 5.8 normal deviates from the expected value, and is thus definitely significant.

Partition of the total heterogeneity into residual component and one due to the differences between generations shows that no excessive contribution to the dispersion is attributable to this latter cause, but that in fact most of the sets of four observations are homogeneous, only six having residuals significantly greater than, and one having the residual significantly less than, the expected value for two degrees of freedom. With twenty-four sets of four observations, one excessively high and one excessively low χ^2 residual would be expected at the 5% level of significance adopted.

The unexpectedly high dispersion encountered in the sets of observations whose residuals are marked with an asterisk in

Table 2.2 is the more puzzling since the calibration curve, which is based on 24 observations, has a χ for residual dispersion which is only slightly higher than expectation.

Notwithstanding the heterogeneity of the data, the component of variation isolated for the test of departure from parallelism between generations is not significantly greater than the residual variance. There is some slight evidence that the four populations used have developed separate slopes during the course of the six generations studied here. It must be remembered, however, that this experiment is much more sensitive than any of those which are reported in later sections of this thesis and which depend on the results obtained here for their validity. There is, therefore, evidence to support the null hypothesis that the 24 regression lines examined have a common slope, and from this statement it may be deduced that the slopes of this type of assay system do not vary from test to test, outside the limits of sampling error.

Miller and Simanton (1938) have carried out what appears on superficial examination to be a similar examination of the slopes of mortality-concentration curves with the pyrethrins as insecticide and houseflies as test insect. The marked variation in slope obtained is in contrast not only to the work reported above, but to that of Murray, which has been conveniently summarised by Bliss (1939). Miller and Simanton interpreted the differences of slope in successive assays to the difference in sex-ratio among samples drawn from a continuously emerging fly population. Their conclusions, which weredrawn

from the untransformed response data without statistical examination, do not take into account the special properties of the flying insect-pyrethrins assay system, and the extent to which they are justified is rather uncertain.

The greater part of the work described in this thesis refers to the stability of films in which the pyrethrins are the active constituents. For the assay of this substance it is practicable to use the fully reversible responses of activation and paralysis. The division of responses in biological assays into two categories, the reversible primary responses such as those mentioned above, and the usually irreversible secondary responses such as that of mortality, has been discussed by Blackith (1946). The advantages of selecting the primary responses where these are available has been outlined by Page et al (1949).

The extent to which paralysis of grain weevils by the pyrethrins is reversible has been examined in detail, since very few similar investigations are known to the writer. Tenhet(1947) found that sub-lethal doses of the pyrethrins reduced the subsequent oviposition rate of tobacco beetle females, Lasioderma serricorne Fab.

It is suspected also that mercury vapour, which is relatively non-toxic to adult S. granaria does however reduce the fertility, and possibly also the fecundity of gravid females.(E.S.Corringe, private communication).

With the pyrethrins, however, although oviposition rates have not been specifically studied, the primary responses of

grain weevils are completely reversible in that sub-lethal doses do not influence the weevil's responses to a second dose, when they have been allowed to recover from the first. This fact has been made the basis of an experimental design in which the test insects are used on more than one occasion.

The design, termed the twin cross-over design by its originators (Smith et al .1944) was originally intended for the assay of insulin in rabbits, using as a graded response the fall in blood sugar consequent on the administration of the drug. By using the quantal response of paralysis, with batches of 30 weevils, it has proved possible to adapt this form of assay to the evaluation of the relative potencies of pyrethrum preparations. (Blackith 1950 b).

A feature of this design is that the variation in the tolerances of the test insects between the occasions of testing can be used as the error variance instead of the sampling variation. Since the tolerance level of the insects bred under highly standardized conditions, is fairly constant for periods of a few days, this innovation results in a considerable increase in the precision with which information can be recovered from an assay, an increase ranging from 3 to 100 times that obtainable from the same body of data analysed by the ordinary methods using the sampling variation as an error variance.

This design was used extensively for the lengthy series of assays which were found necessary to investigate the alleged stabilizing action of piperonyl butoxide, and other synergists, on the pyrethrins.

Stability of Pyrethrum-in-oil films stored in the dark.

As noted in the introductory section, Potter (1938) and several other workers have observed that pyrethrum-in-oil films applied within warehouses and elsewhere, exhibited residual effects for some weeks, and that the stability of such films was considerably greater than had been previously suspected.

The stability of the pyrethrins in the absence of light has been confirmed by a series of experiments in which two sets, of five filter papers each, were treated with 0.5 ml. of the diluted insecticide solution so that the residual pyrethrins concentration was 1.0% in 0.1 ml. of the heavy white oil solvent used.

One set of five of these papers was stored in complete darkness in the constant temperature cabinet used to contain the papers undergoing assay. The temperature at which the cabinet was maintained was 30°C, and the relative humidity was usually in the neighbourhood of 40 -60%. The other set of papers was stored, alsoin darkness, in an insulated safe, the temperature of which was not controlled. Readings of a thermometer taken at intervals throughout the storage period in this safe indicated that the temperature did not rise above 17°C nor fall below 14°C.

Initially, the two sets of filter papers were assayed at fortnightly intervals, with batches of 20 weevils, all drawn from a homogeneous population from which five samples of 20

weevils were also drawn for the purpose of calibration against a known concentration of the pyrethrins. In order to ascertain the earliest time at which a significant diminution of the activity of the insecticide could be detected, this reference concentration was fixed at 1%, so that direct comparisons with either of the other two sets of papers could be made. Responses by C.granaria to this concentration however, are rather high, so that a certain loss of sensitivity was thereby incurred.

Making use of the results established in the preceding section, that the regression of response on Log. dose is linear when the response is measured in angular units, and that the slope of this type of regression line does not alter materially from assay to assay, a direct comparison may be made between assays conducted at different times, and therefore with samples of insects drawn from populations with different tolerance levels.

The mean difference, in terms of the angular transforms of the responses to the standard 1% reference concentration is calculated between any pair of assays conducted at different times, and specifically, between the original calibratory assay and each of those used in this investigation. Such a difference, may then be applied as an adjustment to the responses of a further set of samples from these subsequent populations, and thereby used to eliminate the tolerance difference between these and the population used to construct the calibration curve. The adjusted response may then be referred to the corresponding concentration.

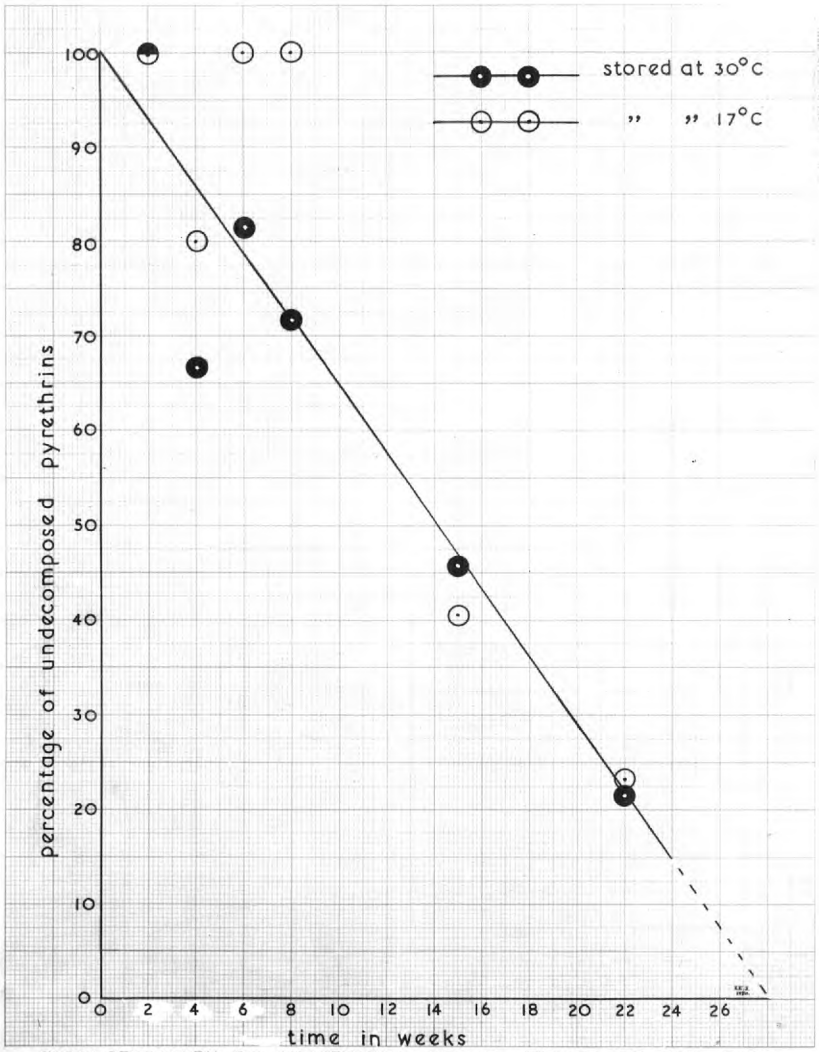


FIG.3.1. STABILITY OF PYRETHRUM FILMS STORED IN THE DARK.

This method is a very rapid one for constructing the best estimate of a concentration relative to some other standard and is particularly suitable for the routine measurements described in this and some subsequent sections of this thesis. Its extension to the elimination of a second variable involves little additional complication, and is described in section 8.

It would be possible, though cumbersome, to calculate the fiducial limits of such an estimate, by the application of a theorem of Fieller (1944) on the fiducial limits of a ratio. However, the experimental design adopted here was selected for those experiments in which the calculation of fiducial limits is not required, since only those effects which were large in comparison with the errors attached to them were of interest. In those cases where fiducial limits or tests of significance were required, a rather different set of designs have been selected, embodying internal estimates of the slopes and linearity of the regression lines used.

The experimental data obtained from the several assays are given in Tables 3.I to 3.VI. The figures given are the angular transforms of the responses of the batches of 50 weevils used. These angular transforms have been adjusted, as shown in the tables mentioned, so as to be directly referable to the calibration curves shown in Fig. 2.1. These corrected responses and the concentrations corresponding to them obtained from the calibration curve are shown graphically in Fig.3.1.

For the first four assays, covering at equally spaced

intervals the eight initial weeks of storage, no significant differences were observed between the fresh and stored 1% pyrethrins-in-oil films.

As table S.II indicates, the differences were on the borderline of significance at the 5% level after 4 weeks storage of the films, but the likelihood of obtaining a border-line "false positive" out of four such assays is quite appreciable, and the interpretation of the data in this manner is supported by the fact that at 6 and 8 weeks, no such significant differences were obtained.

The two sets of treated filter papers were then stored in the dark for longer periods of time, under the same conditions as before, and were again assayed against freshly prepared films at 15 and 22 weeks after the commencement of the experiment.

After 15 weeks, the residual concentration of the pyrethrins had fallen to 0.405% and 0.467% in the cases of the papers stored at 17°C and 30°C respectively. When the exposure period had increased to 22 weeks, the corresponding figures were 0.231% and 0.215% respectively. Inspection of Fig S.1. shows that there is no reason to expect from the somewhat limited evidence of this experiment, that the decomposition of the pyrethrins had been materially hastened by the higher storage temperature, at least within the range examined, which covers that normally encountered in light-free installations where infestations might be expected to exist. (K. S. Salmond; Priv. Comm.).

Inspection of Fig. 3.1. shows that a straight line has been fitted to the experimental points. The implication of such a line is that the decomposition of the pyrethrins is proportional to the time for which the insecticide is stored, and the slope of the line would indicate that complete decomposition would have occurred after 28 weeks storage. A mode of decomposition of this type is most unlikely on chemical grounds; it would be expected rather that the concentration of the insecticide would fall off exponentially with time. Possibly some such curve would be found by a more extensive examination, but there is obviously little justification for fitting such a curve to the available data.

The outstanding conclusion to be drawn from Fig. 3. 1 is that in the absence of light, pyrethrum-in-oil films exert a very considerable residual effect, retaining a useful degree of activity for periods of time up to six months after application. It must, of course, be borne in mind that the pyrethrins solutions used were made up from a concentrate containing an anti-oxidant in the form of a small concentration of hydroquinone.

The results obtained with solutions not containing an anti-oxidant might be less striking, but the experiments reported here confirm and amplify those of Potter (loc. cit), and suggest that some cause other than simple oxidation must be looked for to explain the rapid deterioration of pyrethrum films exposed to light.

The most obvious such cause is the photolysis of the insecticide, a term which as used in this thesis is intended to cover any or all of those light-induced reactions which may lead

to the loss of activity of the pyrethrins. Among such reactions oxidation and polymerisation appear to be most likely to be prominent.

A general investigation of the photolysis of the pyrethrins is given in subsequent sections of this thesis.

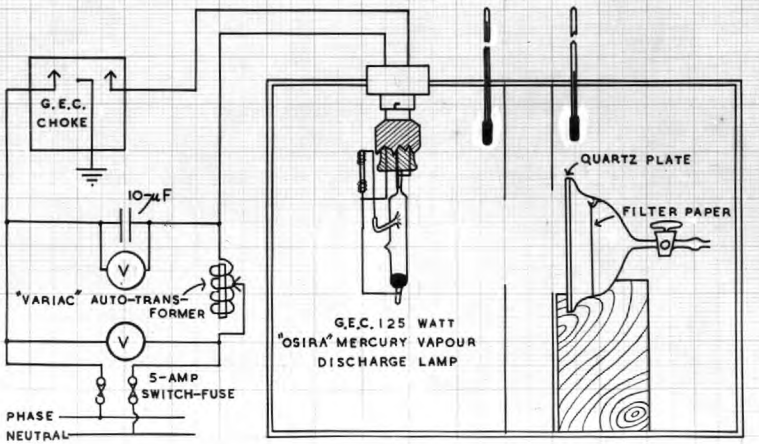
Photolytic decomposition of the pyrethrins

(1) The Irradiation Apparatus.

The majority of the experiments described in this section were carried out in visible and ultra-violet light, so that in investigating the decomposition of the pyrethrins and other insecticides by this medium, a number of experiments were required in order to establish the properties of the light system used.

The source of ultra-violet light was an "Osira" G.E.C. 125 watt high pressure mercury vapour discharge lamp. This light was unfiltered, as the outer evacuated glass envelope of the lamp was cut away, and the quartz internal envelope is sufficiently transparent to all but the shortest of wave-lengths. The lamp as used thus yields a substantially continuous spectrum from about 3000 Å.U. up to the visible region.

The filter papers to be irradiated were suspended in an apparatus specially designed for the purpose, and consisting of a large quartz window, constructed of 2 mm sheet rolled transparent quartz, seated by means of a ground flange to the filter paper container, which was constructed in hard "Pyrex" glass. The window of the apparatus was approx. 9 cm. diameter so that the whole of the filter papers suspended in the



VOLTAGE CONTROL CIRCUIT. IRRADIATION CHAMBER

APPARATUS FOR THE IRRADIATION OF INSECTICIDE FILMS IN ULTRA-VIOLET LIGHT.

FIG. 4.1:

container were irradiated with unfiltered ultra-violet light. A tap was provided in the apparatus to permit of the irradiation being carried out under vacuum, or in atmospheres other than air. The general plan of the apparatus is shown in Fig. 4.1.

The walls of the irradiation apparatus were constructed of asbestos board, painted a matt black to reduce reflection of light, and framed with wooden slats to provide a foundation to which was attached the electrical equipment indicated in the diagram. The voltmeters and "Variac" autotransformer were added to the circuit in order to measure and control the excessive voltage fluctuations met with throughout the work. The early experiments were carried out when the only A.C. supply was that from a small 8amp D.C. - A.C. convertor, whose output voltage was 60° out of phase. It was found that no constant voltage transformer available would operate satisfactorily from this source of supply, so that the manually operated controls were introduced. (Fig. 4.2.)

When a mains A.C. supply became available, it was found that the voltage fluctuations were of as great a magnitude(± 15v) though not of so frequent occurrence, and in consequence the voltage control circuit was retained in the apparatus.

With the aid of this manually operated control, the potential applied to the tapping of the choke actuating the lamp, which was set for a nominal voltage of 235, was stabilised to within a few volts.

An experiment was designed to establish whether such

fluctuations would effect the decomposition of the pyrethrins to a detectable extent.

24,7 cm. No.1 Whatman filter papers, were each impregnated with 0.5 ml.of the mixed solvent previously described, so that a residual concentration of the pyrethrins of 2% was obtained. These papers were divided into four equal batches, which were irradiated for 6 min. each at 210, 220, 230, and 240 volts respectively. This range could not conveniently be exceeded. Below it the lamp was liable to extinction, and above it the temperature to which the lamp is raised after 30 min. operation is liable to burn out the connections, which in the lamp used in this work, deprived of its outer evacuated envelope, are exposed to atmospheric oxygen.

Prior to the irradiation of the filter papers, the lamp was allowed to run for 30 min. at its nominal tapping rating of 235 volts to "warm up" and establish something approaching a steady state in its emission spectrum. During irradiation, the filter papers were suspended from the glass hook in the chamber, so that the light from the lamp was normally incident upon them. After the fixed period of exposure, the papers were withdrawn and used as the substrates for 24 batches of weevils each, all drawn from a single homogeneous population.

The data of this assay are shown in Table 4.0 where the responses obtained after the standard 12 hours exposure period are given in terms of their corresponding angular transforms. It will be observed from the analysis of variance of the data

TABLE. 4. 0

Influence of mains input voltage on ultra-violet emission of G. E. C. "Osira" Lamp, with nominal tapping set at 235 volts.

All papers irradiated with lamp for 10 mins. after impregnation with 2% Pyrethrins soln.

Angular transforms of batches of 50 weevils confined on the papers for 18 hrs.

Mains voltage (volts)	Replicate 1	2	3	4	5	6	Mean
210	64·2	54·3	68·0	64·2	57·4	58·7	61·13
220	74·7	68·0	58·7	68·9	47·9	53·1	61·88
230	61·3	56·2	54·9	61·3	62·7	50·8	57·97
240	54·9	47·3	53·7	74·7	53·7	28·7	52·16

Analysis of Variance.

Item.	D.F.	Sums of Squares.	Mean Square	F.
Treatments	3	348·62	116·21	1·241
Residual	20	1, 872·38	93·62	
Total	23	2, 221·00		

Treatment effects are thus not significant at 5% level of P.

that there is no significant treatment effect, so that it cannot be said that the voltage differences influence the output of ultra-violet radiation from the lamp, although that of heat and visible light is markedly affected by this factor. For the latter reason, the applied potential was standardised in subsequent experiments at 235 volts.

(2) Effect of irradiation with visible light compared with ultra-violet.

It has often been considered that visible light accelerates the decomposition of the pyrethrins, so that a brief set of experiments was carried out to investigate the relative deleterious effects of visible and ultra-violet light. Each of fourteen 7 cm. Whatman No.1 filter papers was impregnated with 1% solution of the pyrethrins in heavy oil by the usual technique, and irradiated by the ultra-violet lamp for periods of up to 15 min.

The pyrethrins contents of the irradiated papers were assayed by the usual batches of 50 weevils, drawn from a homogeneous population, and the responses obtained, subjected to the angular transformation, are shown in Table 4. 2. From this table it will be seen that there is a significant regression of responses on time of exposure to the ultra-violet light, and that in consequence the data suggest that the pyrethrins were being steadily decomposed by this agency.

To estimate the contribution of the visible fraction of the

TABLE 4. 1

Effect of irradiating pyrethrum films with visible light. Source of light:- 60 watt pearl incandescent lamp, placed 3 ins: from filter paper to give equal visible light intensity to ultra-violet lamp.

Time of irradiation (mins.)	n	r	p	Angular transform	Mean.	χ^2_4
0	29	9	31·0	33·8		
"	29	9	31·0	33·8		
"	30	10	33·3	35·4	36·82	1·46
"	28	11	39·3	38·8		
"	31	14	45·2	42·3		
Controls.	32	0	0			
5	27	9	33·3	35·4		
10	29	14	48·3	44·0		
15	30	12	40·0	39·2	34·30	7·24
20	29	5	17·3	24·7		
25	27	6	22·2	28·2		

$t = 0·6478$, not significant at 5% level of P.

light emitted from the discharge lamp, a similar experiment was carried out in which the discharge lamp was replaced by a 60 watt pearl tungsten filament lamp, giving only visible light and heat.

Two sets of six filter papers, each impregnated with a 0.2% solution of the pyrethrins, were used, one being left unirradiated, the other irradiated with the incandescent filament bulb for periods of up to 85 min., the bulb being held at such a distance that its visible emission was approximately as intense as that from the discharge lamp in the irradiation apparatus. This approximate equalisation was obtained by using one filter paper with a single drop of P.51 oil at the centre as a grease spot photometer within the apparatus, though the differing colours of the light from the two lamps made an accurate comparison difficult by this means.

The responses obtained as the result of assaying these and the unirradiated papers in the usual manner are given in angular units in Table 4. 1. Since there is manifestly no significant regression of response on time of irradiation, the two sets of papers may be compared by means of a t-test on the data given. The value of t obtained, (0.848, n = 10) showed that no significant decomposition had been effected by the treatment given, during the exposure periods used. (t, for 10 d.f. and P = 5% is 2.306.)

Comparison of the results quoted above indicates that the wavelengths concerned in the decomposition of the insecticide

TABLE 4. 2

Action of ultra-violet light on pyrethrum films.

Time of irradiation (mins.)	n	r	p
0·0	30	30	100·0
1·0	29	29	100·0
2·0	27	24	89·0
3·0	29	26	89·5
4·0	27	25	92·5
5·0	30	26	87·0
6·0	27	23	85·2
7·0	30	24	80·0
8·0	28	20	71·2
9·0	31	25	80·5
10·0	29	18	62·0
11·0	30	14	46·8
12·0	30	5	16·7
15·0	27	6	22·2
Control.	28	0	0·0

lie in the ultra-violet. This result is not unexpected, since the absorption spectrum of the pyrethrins shows a maximum at about 2,300 Å.U.(Gillam and West, 1942, 1943)

It is noteworthy that although the papers irradiated with visible light only had retained their toxicity apparently unimpaired, the colour of the films rapidly disappeared. In many cases, it has been observed that the pyrethrins and the associated colouring materials disappeared together, (c.f.Martin 1942) but although a widespread impression exists that colourless films are useless, this impression is not necessarily valid. Gnadinger (1936) has pointed out that the colouring matter is much more readily oxidized than the pyrethrins themselves.

(3) Rate of decomposition in ultra-violet light.

It is desirable to know whether the decomposition of the pyrethrins is proportional to the time of irradiation since the presence of a number of different active constituents in the extract with differing rates of decomposition would tend to cause this quantity to diminish with time.

An obvious way of carrying out the experiment would be to irradiate the pyrethrins solutions on the filter papers, and to relate the responses obtained, via a calibration curve to the concentrations remaining undecomposed, as discussed in the previous section.

Such a method would be less sensitive, however, for the present purposes than a null method, among other reasons since it requires a test

TABLE 43.

Rate of decomposition of the pyrethrins in ultraviolet light.

Treatment.	Replicate.				
	I.	2.	3.	4.	5.
A.	43.0	39.8	43.2	4I.0	46.8
B.	45.6	48.4	40.I	34.8	53.I
C.	46.4	40.5	44.4	38.9	37.8
D.	46.I	45.9	4I.6	36.3	39.9

Treatment.	Pyrethrins conon. (w/v %.)	Duration of Irradiation. (minutes).	Treatment Total,	Mean.
A.	2.0	8.0	2I5.8	42.8
B.	I.5	6.0	222.0	44.4
C.	I.0	4.0	208.0	4I.6
D.	0.5	2.0	207.8	4I.5

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	F.
Treatments.	3	22.8I	7.60	0.35I
Error.	I6	346.46	2I.65	
Total.	I9	369.27		

of linearity of the regression line which will always be based on fewer degrees of freedom than an estimate of the probability of a null hypothesis that the treatments given do not lead to different responses.

A suitable null-point method consists in irradiating the series of pyrethrins solutions on filter papers for periods of time such that the product of the initial concentration and the reciprocal of the time of irradiation remains constant. If the rate of decomposition of the total active ingredients of the pyrethrum extract remains proportional to the period of exposure to ultra-violet light, the residual concentration of the insecticide after irradiation will then be the same, within the limits of the sampling error of the method of assay, in all cases.

The data from an experiment designed on these lines are given in Table 4.5, where the figures represent the transformed responses of batches of 40 weevils, after the usual 18 hr. exposure period on the irradiated filter papers. Reference to Table 4.5 shows that there is no significant treatment effect, and indicates that the rate of decomposition of the pyrethrins is independent, within the limited ranges studied, of the concentration initially present, and is directly proportional to the time of irradiation by ultra-violet light.

(4) Effects of absorption coefficient, texture, etc: of filter paper substrates.

It was thought desirable to carry out some experiments on any possible variations in the toxicity of filter papers impregnated with pyrethrum-in-oil solutions and presented to test insects either on the irradiated or on the unirradiated sides. Since filter papers differ slightly in texture on the rough and smooth sides, according to the way in which they are cut, the experiment was also designed to include any effects of this factor. A further effect studied was the differential amount of heat and light radiation absorbed on the surface of white and black papers, since the reflection coefficients of papers treated with the various dyes examined in later sections of this thesis for protectant action would be expected to be intermediate between these two extremes.

Two aspects of each of the three factors mentioned were thus tested, giving $2^3 = 8$ treatments. A complete replication was provided to enlarge the experiment and to provide an independent estimate of the experimental error should any of the main factors give rise to significant treatment effects.

The white filter papers used were the 7 cm. No.1.Whatman papers normally used in this work, and the black papers were represented by 7 cm. Whatman No. 89 circles the vat dye of which is insoluble in either oil or water.

As the analysis of variance of Table 4.4. indicates/none of the three factors examined gave rise to any significant treatment effect, although in view of the relatively high variance ratio attributable to the contrast between smooth and rough sides it was decided to standardise all subsequent

TABLE 4. 4

Effect of filter papers on ultra-violet photolytis of 2% pyrethrins solutions. Three factor experiment; each factor at two levels.

Colour of 7 cm. Filter paper — (W = White, B = Black)

Side of filter paper irradiated — (S = Smooth, R = Rough)

Side of filter paper assayed — (σ = Smooth, ρ = Rough)

Angular transforms of percentage responses of batches of 45 *C. granaria* confined for 12 hrs. on irradiated papers. Time of irradiation:- 10 mins.

Code letters.	Responses.	Code letters.	Responses
W. S. σ	23·6	B. S. σ	31·1
	30·0		30·2
W. S. ρ	19·6	B. S. ρ	28·0
	24·1		29·0
W. R. σ	38·5	B. R. σ	30·6
	25·2		37·8
W. R. ρ	20·5	B. R. ρ	29·4
	31·5		27·3

Code letters.	B.	W	R	S	σ	ρ	Grand
Totals	243·0	213·4	240·8	215·6	247·0	209·4	456·4
Means.	30·375	26·67	30·10	26·95	30·875	26·175	28·525.

Analysis of Variance of above data

Item.	D.F.	Sums of Squares.	Mean Square	F.
B v W	1	53.695	53·695	2·681
S v R	1	39·690	39·690	1·982
σ v ρ	1	86·360	86·360	4·413
Residue	12	240·267	20.022	
Total	15	422.010	-	

$F_{1,12}$ for P = 0.05 is 4.75

assays with the smooth side uppermost.

(5) Effect of humidity on the photolytic decomposition of the pyrethrins.

A further possible factor influencing the photolysis of the pyrethrins is the humidity of the air in which irradiation takes place. There is certainly a fairly widespread belief that moisture accelerates the decomposition of pyrethrum flowers, and of pyrethrinised dusts, (Martin 1949) and Tattersfield (1932) found that humidity increased the rate of oxidation of pyrethrum powder.

An experiment was designed to examine this effect, by irradiating films of solutions of the pyrethrins on filter paper in wet and dry atmospheres. The wet atmosphere was obtained by placing a wad of wet cotton wool between the tap of the irradiation chamber and the paper, but pressed into the back of the chamber so that no direct transference of water took place between the wad and the paper. The dry atmosphere was secured by replacing the cotton wool by coarse grade, self indicating silica gel. In each case the chamber, sealed at the tap and the quartz plate, was allowed to stand for 15 min. at 30° C before irradiation, to allow the moisture content of the air inside at least to approach equilibrium.

After irradiation for 10 min. the two sets of papers were assayed as usual and the transformed data are shown in Table 4.5. Reference to this Table indicates that although the humidity

TABLE 4.5.

Effect of Atmospheric Humidity on the Decomposition of the Pyrethrins in Ultra - violet Light.

Experiment with six replicates.
2% Pyrethrins solution in oil irradiated in Whatman No:.I filter papers for I0 min.

	Humid Atmosphere, (I00 % R.H.)	Dry Atmosphere , (0 % R.H.)
	43.3	54.3
	48.4	45.0
	33.8	44.4
	40.4	56.2
	5I.4	50.8
	48.4	47.3
Totals.	265.7	298.0
Means.	44.3	49.7

Grand Total 563.7
" Mean 46.9

Analysis of Variance.

Items.	Sums of squares.	D.F.	Variance.	Variance Ratio.
Treatment.	I43.58	I.	I43.58	4.534.
Residue.	3I6.68	I0.	3I.67	(5% level of F = 4.96)
Total.	460.26	II.		

TABLE 4.5A.

Effect of Atmospheric Humidity On the Photolysis of the Pyrethrins.

(Experiment with I2 replicates.)

	2% Pyrethrins irradiated in dry atmosphere, (0% R.H.)	2% Pyrethrins irradiated in wet atmosphere, (I00% R.H.)
Replicate.		
I.	50.8	52.5
2.	53.I	49.0
3.	35.I	45.0
4.	45.9	43.3
5.	36.3	46.7
6.	3I.3	43.3
7.	33.2	46.7
8.	3I.3	39.2
9.	42.I	35.I
I0.	36.3	37.5
II.	33.2	46.7
I2.	4I.0	32.6
Totals.	467.6	5I7.6
Means.	38.9	43.I

Grand Total :- 985.2, Grand Mean :- 4I.05.

Analysis of Variance.

Items.	Sums of squares,	D.F.	Variance.	Variance Ratio
Treatment.	55.74	I.	55.74	I.I95.
Residue.	I026.28	22.	46.65	
Total.	I082.02	23.		

difference does not produce a significant effect on the decomposition of the pyrethrins, the value of the variance ratio obtained for this comparison is on the borderline of significance at the 5% level of probability so that it was decided to replicate the experiment on double the scale.

The transformed responses obtained during the assay of the irradiated papers from the replicated experiment are given in Table 4.5A from which it will be seen that the variance ratio for the comparison between atmospheres does not approach significance at the 5% level of P.

The combined probability of the truth of the null hypothesis that the atmospheric humidity does not influence the rate of decomposition of the pyrethrins is obtained from both these experiments by the method of Fisher (1948), in which use is made of the property that $-\log_e P$ is distributed as is $\frac{1}{2}\chi^2$, with 2 degrees of freedom.

F	P	$-\log_e P = \frac{1}{2}\chi^2$ Table 4.6	D.F.	Experiment.
4.034	0.08	1.2628	2.	as for Table 4.5
1.195	0.32	0.5697	2.	" " " 4.5A.
	0.12	1.8325 $\chi^2 = 3.66$	4	Combined data.

χ^2 for 4 degrees of freedom is 7.5700, at 5% level of P.

The evidence from the combined data of the two experiments cannot therefore be held to contradict the null hypothesis, and atmospheric humidity cannot be held, on this evidence, to influence the rate of decomposition of the pyrethrins. Doubtless

the decomposition of damp flowers is accelerated indirectly through the agency of micro-organisms, and that of dusts is due to an oxidative mechanism catalysed by light and moisture.

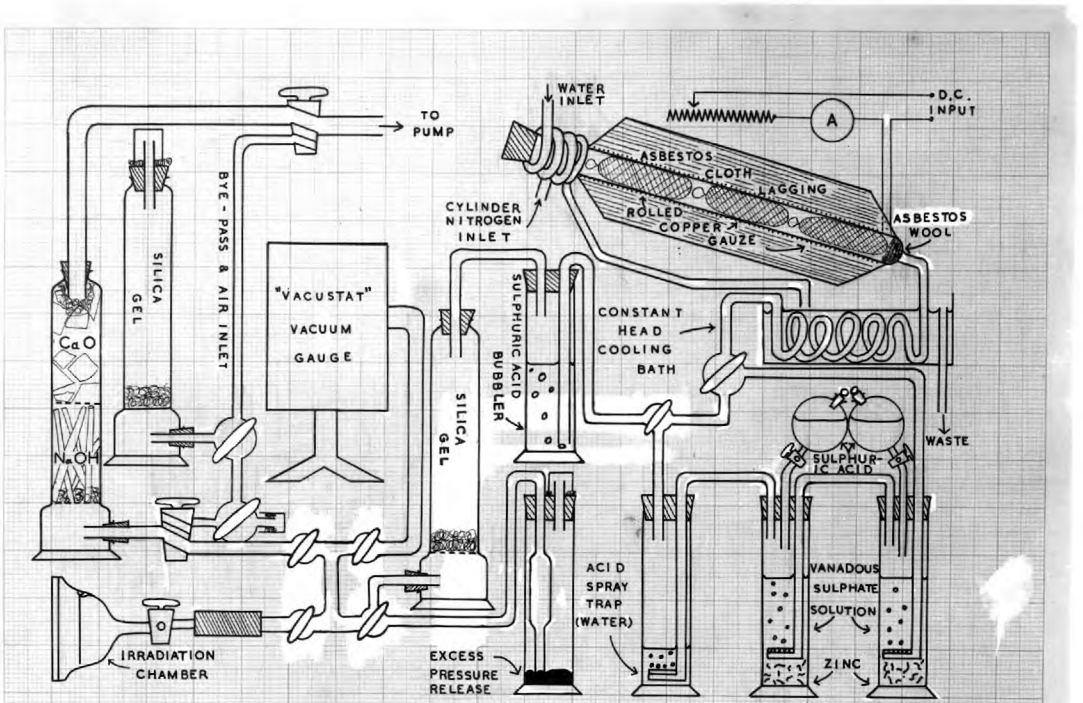
(6) Influence of available oxygen on the photolysis of the pyrethrins.

As Martin (1949) has pointed out, the inactivation of pyrethrum preparations is frequently attributed to oxidation, and certainly the addition of antioxidants improves the persistence of the active constituents of the extracts and flowers. Inactivation without oxidation is also possible, however, and the mechanism suggested by Harper (1949), namely a Diels-Alder condensation along the side-chains of the alcoholic component of the insecticide molecules, would be of this type. A further possibility which requires consideration is the type of reaction, induced by high-frequency radiation, in which activated molecules are produced, capable of reacting with any free oxygen if this is present, but becoming deactivated to the ground-state by collision with other molecules if free oxygen is absent.

Experiments were therefore carried out to ascertain whether the decomposition of the pyrethrins under ultra-violet irradiation could occur at all in the absence of free oxygen, and whether, if such decomposition took place, it was in any way inhibited by a reduction of pressure of the oxygen supply.

The atmospheres chosen for investigation were as follows:-

Oxygen. Obtained directly from a cylinder without further



APPARATUS FOR THE PRODUCTION OF NITROGEN FREE FROM OXYGEN. FIG.4.2.

purification. The flange of the irradiation chamber was degreased, and the quartz plate held against the filter paper container by two rubber bands, thus forming in effect a rough non-return valve. Oxygen gas was passed into the chamber through the tap under sufficient pressure to force a way out through the flange joint, and the passage of the gas was continued for a few minutes to ensure the removal of the air, and its replacement by oxygen.

Air. No special treatment.

Nitrogen. Ordinary cylinder nitrogen contains some 1 - 3% of oxygen, so that further purification was necessary. Of the available methods recorded in literature, passage of the nitrogen over red-hot copper turnings, or bubbling the gas through vanadous sulphate solutions, have been recently recommended. (L.Meites and T.Meites, 1948). These authors suggest that both the methods mentioned are of approximately equal efficiency, but were only able to express this efficiency in arbitrary units. Indeed, the determination of oxygen in very small traces in an inert gas is a matter of considerable difficulty, and the only efficient recent apparatus described in the literature was found to be so complicated that its construction was not considered practicable with the facilities available at the time.

Instead, it was thought preferable to rely on the complete removal of oxygen by the two methods quoted above, and the apparatus was set up with the two oxygen removing units, one

dependent on each method, arranged in series, the nitrogen first being circulated over the heated copper gauze, and then passed through two bubblers containing vanadous sulphate solutions prepared according to the directions of L. and T. Meites, (Loc. cit.)

The design of apparatus finally adopted is shown diagrammatically in Fig. 4.2 . As no furnace of suitable design was available, a suitable container for the copper was prepared from a quartz "Vitreosil" tube 25cm. long. This tube was heated electrically by winding "chromalloy" (Nichrome) wire, 20 Standard wire gauge, round the tube, the wire being of such a length that about 600 watts were dissipated. The wire was held in place on the tube by winding it on to a layer of fireproof aluminous cement laid directly on to the tube, and externally the heating element was thermally insulated by binding with several successive layers of asbestos cloth and tape. It was found necessary, in order to maintain the internal temperature of the tube furnace at about 600°C, to limit the current to 1.5amp. by means of a resistance. When nitrogen was allowed to flow through the tube, and over the tightly rolled copper gauze with which it was packed, the heat lost in this way was compensated for by raising the current to 2amp, by cutting out the external resistance.

The copper filling in the quartz tube consisted of fine mesh copper gauze rolled into solid cylinders some 7 cm. long. Three such cylinders were inserted through the open end of the tube by means of a copper wire hooked into copper rings sewn

on to the ends of the cylinders. These rings were also used for removing the cylinders for regeneration when exhausted. Regeneration was accomplished by withdrawing the red-hot cylinders and plunging them into methanol, when the copper oxide coating was reduced to copper again.

After passing through a cooling coil, the nitrogen from the furnace was bubbled through two successive traps containing about 250 ml. of 0.1M V_2SO_4 solutions made 0.1 N with respect to sulphuric acid. The lilac coloured vanadous salt is regenerated, after oxidation to the green vanadic sulphate, by reduction with zinc. The metallic zinc is kept immersed in the solutions in the bubblers, and regeneration of the solutions is then easily effected by admitting a little of the conc. sulphuric acid stored in the seperating funnels shown in the diagram. The vanadium was originally introduced as the yellow hydrated pentoxide, but oxidation by the free oxygen in the gas stream does not carry the reaction further than the vanadic salt. Since some regeneration of the vanadic salt is always occurring on a small scale, the stream of purified nitrogen issuing from the apparatus is contaminated with traces of hydrogen, but the presence of this latter gas is not thought to be undesirable. Traces of sulphuric acid spray from the bubblers were removed in the aqueous spray trap, and the gas stream was then dried by passage through a concentrated sulphuric acid scrubber and then through a silica gel drying tower. The rate of flow of the gas could

TABLE 4. 7.

Approximate weights of oxygen in irradiation chamber filled with various atmospheres.

Atmosphere.	Wt: of O_2 in mgm.
Oxygen (cylinder)	102
Vacuum (0·5 mm.Hg) (After filling with Nitrogen)	0·0001
Nitrogen (purified, after repeated flushings).	0·01
Air.	20.

be approximately estimated by the passage of bubbles through the scrubber.

Vacuum system. The vacuum system shown in the diagram (Fig.4. 2) was introduced to allow the irradiation of the filter papers in vacuo and also to facilitate filling the irradiation chamber with purified nitrogen. The nitrogen was allowed to bubble out through the escape excess pressure release, until the chamber had been evacuated, and then admitted slowly into the chamber. This process was repeated several times to ensure the complete absence of residual oxygen, except for that remaining on the papers in an adsorbed state, and the traces not removed from the cylinder nitrogen.

The weight of oxygen remaining in the irradiation chamber after evacuation down to 0.5 mm. the limit of the system used, is still large compared with the quantity of oxygen required to react with the amount of pyrethrins present. (see Table 4. 7.) This difficulty is overcome by evacuation after filling the chamber with the purified nitrogen. The quantity of oxygen then present is very small, though not easily estimated, but it is not likely to exceed that adsorbed on the filter papers.

Irradiation of 8% solutions of the pyrethrins on the usual filter papers was carried out in each of the four atmospheres mentioned above, five replicates being used for each atmosphere. After 10 min. irradiation with ultra-violet light, the papers were withdrawn and assayed in the manner

previously adopted in this work, except that the data given in Table 4.5 represent the transformed responses of batches of 45 weevils, all the 20 batches used being drawn from a single population.

It will be seen from Table 4.8 that when the individual means in the analysis of variance are compared by the method of Tukey (1949), none prove to be significant, though that for irradiation in vacuo, representing the smallest obtainable residue of oxygen, approaches significance at the 5% level of probability. Actually, when the interval between the mean for irradiation in vacuo and the grand mean is converted into a normal deviate, its value is 1.4408, and the 5% level of P, being represented, as is well known, by the value 1.96.

It may be concluded therefore that even with this treatment, no significant diminution of the loss of toxicity of the pyrethrins is secured, and since a rough estimate, such as that of Table 4.7, of the residual weights of oxygen for each treatment, indicates that the weight of oxygen left after evacuation from the purified nitrogen is less than one mol. for each mol. of the pyrethrins present on the filter paper, it may tentatively be deduced that the decomposition of the insecticide in ultra-violet light is not necessarily accompanied by oxidation.

It would appear that there are at least two quite distinct processes involving loss of toxicity during storage

TABLE 4.8.

Effect of different atmospheres on photolytic decomposition of the pyrethrins. All filter papers initially impregnated with 2% pyrethrins solution, and irradiated for 10 mins.

Angular transform of percentage responses of batches of 45 *O. granaria* confined for 18 hrs on filter papers.

Atmosphere. (For details see Text)	Replicate.					Means.
	1	2	3	4	5	
Oxygen	40·4	50·8	53·1	47·9	43·3	47·1
Vacuum	47·9	50·2	49·6	58·1	60·0	53·1
Nitrogen	45·6	41·0	51·9	45·6	50·2	46·9
Air.	37·5	35·1	47·3	43·3	50·8	42·8
					Grand Mean	47·5

Analysis of Variance

Item	D.F.	Sums of Squares.	Mean Square.	F.
Treatment	3	247·90	82·65	2·70
Residue	16	482·42	30·15	
Total	19	730·38.		

Thus the treatment effects are not significant at the 5% level of P, though they do approach it, due mainly to the contribution of the "Vacuum" atmosphere.

of the pyrethrins, one a slow atmospheric oxidation, greatly inhibited by anti-oxidants, the other a much more rapid process involving the action of light, particularly of high-frequencies, and not dependent on the presence of free oxygen, and therefor not inhibited by the presence of anti-oxidants. These two mechanisms appear to be sufficient to account for the observed facts, except that in the case of unextracted pyrethrum flowers, the action of micro-organisms may play an overriding part in destroying the active principles, if the flowers are allowed to become damp.

Martin (1940) has shown, however, that the accelerated decomposition of pyrethrum flowers in glass vessels can be inhibited by replacing the oxygenated atmosphere by one of, say, nitrogen. Thus the light-catalysed oxidation differs fundamentally from the direct ultra-violet photolysis.

Stabilisation of the pyrethrins by the addition of dyestuffs.

The major conclusion to be drawn from the previously reported experiments was that pyrethrum-in-oil films deteriorate much more readily in the presence of light than in darkness, and this much more so if the light concerned is in the ultra-violet region of the spectrum. It appeared logical, therefore, to concentrate on attempts to minimise the effect of ultra-violet light on the insecticide rather than to carry out further work on anti-oxidants.

Two main lines of work appeared to offer promise, one the chromatic protection of pyrethrum films by the addition of dyestuffs absorbing in the ultra-violet region, the other a method dependent on the stabilising effect of certain pyrethrum synergists, an effect suggested to exist by a number of workers in the United States, particularly Dove (1947).

Many substances, whether coloured or not, absorb radiation on various regions of the ultra-violet. In the main, the coloured substances which have only subsidiary absorption bands in the ultra-violet are stable on irradiation whereas such colourless substances as the pyrethrins, which have their main absorption band in the ultra-violet, are often unstable. It was thought that certain dyestuffs,

mixed in solution with the pyrethrins might serve to absorb sufficient of the incident radiation to prolong the length of life of the insecticide.

The pyrethrins are decomposed by radiation most rapidly when spread in a thin film, and it is in this form that the insecticide is applied where a residual effect is desired. Since the insecticide is soluble in oils but not in water, the protective dye selected must be soluble in the former type of solvent, and since in the thin film its absorption must be intense to be effective, considerable solubility in the medium is desirable.

Dyestuffs generally have, as a consequence of their complex resonance structure, considerable stability or "light-fastness", and cover a wide range of the ultra-violet region in their absorption spectra. On the other hand, direct dyes, particularly those having a strong affinity for cellulose, are obviously unsuitable as they will become seperated from the insecticide by chromatographic adsorption during the spreading of the solvent.

Dyes insoluble in oil can be used as pastes, and indeed Lissamine Fast Yellow S.G.S., kindly supplied by the dyestuffs division of Imperial Chemical Industries, Ltd., gave substantial protection when spread over the test films at 0.9mg. per sq. cm., a density corresponding to a 40% solution in the amount of oil present. It is uncertain how much of this protectant action is mechanical, i.e. due to reflection

rather than absorption, and as in any case the method is inconvenient and probably uneconomical in practice, the use of solid protectant substances was not pursued further.

A much more promising substance is benzene azo-β-napthol also known as "B.D.H.Oil-Soluble Yellow" Oil Orange E and Sudan I, which was first shown to be effective by Miss A. Taylor, B.Sc., working with the present author in 1947. This dye has now been subjected to fairly extensive tests.

Solutions of the dye in the standard test solution adopted for this work, namely a 1% pyrethrins (residual) solution in 80% petroleum ether and 20% heavy white oil, were irradiated in ultra-violet light, and their pyrethrins content compared, by the bioassay technique previously described, with a similarly treated solution containing the pyrethrins alone. The responses obtained on papers treated with the dye and insecticide were converted, by means of the angular transformation, into dependent variates linearly related, through the calibration curve, to the log. dosage. Each point, corresponding to the addition of a known proportion of the dye, was determined by means of six replicates, so that the homogeneity of the test insects could be checked in each case.

Corresponding to each set of determinations on the protected pyrethrins, a similar set of six replicate determinations was carried out on the pyrethrins irradiated alone. A t-test, or analysis of variance, could then be carried out on the two sets of data. This form of experimental design

was in the first place intended to detect whether the dyestuff, at the concentration applied, produced any significant protectant effect, and secondly, to provide a measure of that effect. Its use was adopted primarily because of the relative ease with which it lent itself to routine comparison of a number of dyes, or different concentrations of one dye, against a common standard.

In addition, the chosen design enables not only tolerance fluctuations between different occasions of testing to be estimated and eliminated in constructing comparisons between protective materials, but also the fluctuations in the emission spectrum of the discharge lamp to be eliminated. This latter point was secured by comparing each set of responses from papers irradiated in the absence of the dye with a similar set of irradiated papers assayed with samples from the same homogeneous population used in determining the calibration curve. By working back through these two intermediate standards, an adjustment to be applied to the responses to the protected papers could rapidly be calculated as the mean difference between the angular transformed responses to the two intermediate standards and, when this adjustment had been applied, the concentrations of pyrethrins remaining after irradiation could be read off directly from the calibration curve. This method of adjustment is essentially similar to the mean probit difference method of Finney(1947b), suggested for use in another context, but

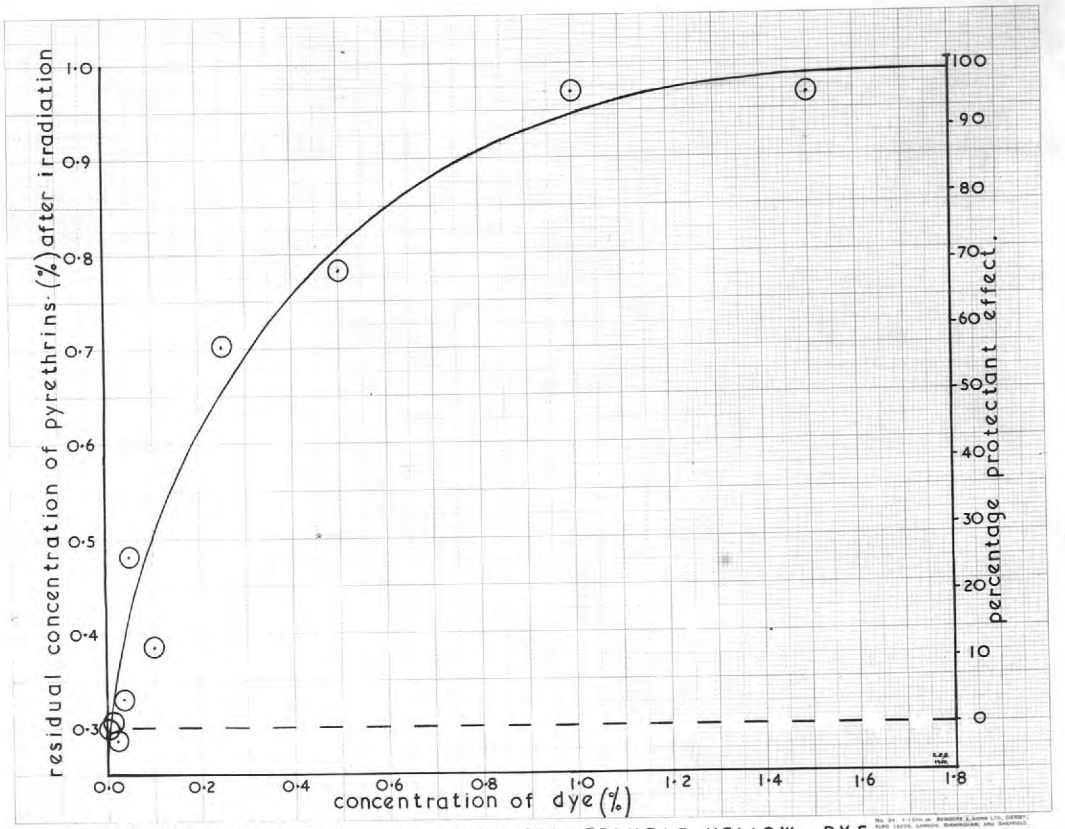


FIG.5.1: PROTECTANT EFFECT OF B.D.H. OIL-SOLUBLE YELLOW DYE.

refers to the angular, rather than the probit, transformations.

Having established a method for assessing the concentrations remaining in irradiated papers, it was clearly desirable to express the degree of protectant action exerted by any added substance. This measure may be conveniently estimated as follows:-

If W = the initial deposit of pyrethrins.

W_1 = deposit of pyrethrins remaining after irradiation of the protected film.

and W_2 = deposit of pyrethrins remaining after irradiation of unprotected film.

then the "percentage protectant effect" may be defined thus

$$\frac{(W_1 - W_2).\ 100}{(W - W_2)}$$

Since the areas of the test papers used are virtually indentical, the quantities represented in the above expression can be conveniently refer to concentrations, rather than directly to weight deposits, since it is concentrations which are estimated as the result of the biological assays.

The stabilising effect of B.D.H. Oil-Soluble Yellow dye on irradiated pyrethrum films is shown in Fig. 5.1 and the data on which this graph is based are given in the appendix in Tables 5.I -5V, summarised for convenience in Table 5.1.

The dye is soluble to about 1.5% in heavy mineral oil at room temperatures, and from this concentration down to approximately 0.5% the protection afforded is very considerable.

TABLE 5.1.

Protectant effect of B.D.H. Oil - soluble yellow dye on Pyrethrum in oil films.

I % Pyrethrins irradiated alone.	Reference response level.	Adjustment.	% Dye.	Mean Response Level.	Corrected Response level.	χ^2, n = 5.	Percentage protectant effect.
43.42	43.42	0.00	0.00	-	-	I.25	-
27.92	43.42	I5.50	0.0I	29.05	44.55	5.09	not sig.
27.92	43.42	I5.50	0.02	30.00	45.50	2.56	" "
38.I6	43.42	5.26	0.03	4I.96	47.2I	4.33	" "
38.I6	43.42	5.26	0.05	5I.78	57.04	I.8I	28
40.88	43.42	2.54	0.I0	50.I6	52.70	5.29	I2
37.63	43.42	5.79	0.25	6I.54	67.3I	I.72	58
35.06	43.42	8.36	0.50	76.58	84.59	4.53	68
35.I7	43.42	8.25	I.00	74.30	82.55	7.I0	97
40.88	43.42	2.54	I.50.	79.95	82.49	4.79	96

Saturation Concentration of B.D.H. Oil - soluble yellow in heavy white oil is approx: I.50.%.

below this lower concentration the effectiveness of the dye falls off rapidly, until the three observations made below 0.05% give protectant effects which do not differ significantly from zero.

The minimum concentration of the dye which gives protection to irradiated pyrethrins might be associated with the quantity of dye which is itself decomposed by the ultra-violet light during the exposure period. This form of decomposition is not likely to be so noticeable with the higher concentrations, as the decompositionsof the dye is likely to be much slower than that of the insecticide.

A cylindrical quartz cell was prepared of the same diameter as the filter papers normally used in these experiments but approximately ½ in. thick. The cell was filled with 85 ml. of a solution of the dye in heavy white oil, of concentration 0.005%. The visible light absorption of the cell was determined in a Spekker photoelectric colorimeter, using a yellow filter, and the cell was then inserted into the irradiation apparatus in the same position as the irradiation chamber normally occupied. The cell was irradiated for 5 hours, being withdrawn at intervals to permit of colorimeter readings being taken. The deviations of the colorimeter are shown in Table 5.9., from which it will be seen that only an unsystematic and very small set of deviations from the initial position were obtained, these being well within the expected "drift" limits of the

colorimeter. The instrument was a particularly sensitive one, and would have been capable of detecting with certainty a loss of as little as 0.5% of the weight of dye actually present in the cell.

Since the diameters of the cell and the filter papers were the same, an identical amount of light fell on each, in a given period. The low concentration and longer period of irradiation of the quartz cell produced a comparable ratio of incident ultra-violet light to that relating to the filter weight of dye paper, so that is any appreciable fraction of the falling off of effectiveness with concentration were due to decomposition, it should be detectable by the experiments reported above. In view of the strong convection currents in the cell when irradiated for five hours, it seems unlikely that the greater absorption of the solvent in that case would vitiate the comparison. The loss of protectant effect may therefore be attributed mainly to lack of covering power of the dye, which in turn is probably due to absorption on the filter paper fibres. The roughly logarithmic curve obtained, as recorded in Fig. 5.1., is suggestive of a simple physical effect of this kind.

Although there was no evidence that B.D.H. Oil-soluble yellow dye in any way influenced the toxicity of the pyrethrins, the practical possibilities of the protectant action of this substance indicated that this point should be investigated further.

Accordingly, the toxicity of sets of six filter papers

impregnated with the pyrethrins alone, with the pyrethrins and the dye, and with the dye alone, were determined. The data obtained are given in terms of their angular transforms, in Table 5. 10. A t-test on the first two columns shows that t= 0.516, for 10 degrees of freedom, a value which is less than unity, but not significantly so. The dye itself is completely without effect on S.granaria within the 12 hours exposure periods used in this type of assay.

The health hazards of this dye are rather hard to assess. For many years it was used as a colouring agent for oily foodstuffs, particularly butter, but its use for this purpose has been discontinued in view of the quantity production of diacetyl, the natural colouring agent.

On the other hand, Kirby and Peacock (1940) have shown that the dye is suspected of being a weak carcinogenic agent. Kirby(private communication) has suggested that the effect may be caused by an impurity, and the matter is being investigated. The dye is readily available in quantity, and, being the simplest of the azo-dyes is not very expensive to manufacture.

Protectant effect of B.D.H. Oil-Soluble Yellow in direct sunlight.

In view of the promising properties of B.D.H.Oil soluble Yellow dye in inhibiting the decomposition of the pyrethrins in the laboratory, it was decided to carry out semi-field scale trials in the open air in direct sunlight.

Two parallel sets of six filter papers were prepared in the usual way, the one impregnated with 1% pyrethrins alone, the other with a similar solution containing an equal concentration of the dye. The glass plate on which the papers were laid was exposed for 30 min. to strong sunlight (Mean total Radiation 60 cal./ sq. cm./ hr.) in the open air, with a very light breeze blowing. The relative humidity was 43% and the shade temperature was 81° F. (27.2° C).

The papers were then removed to the laboratory and assayed in the usual manner. As it became evident that a considerable measure of protection had been afforded to those papers with the dye added the assay was terminated at 6 hr. exposure to the weevils, as otherwise the responses would have been too high for reasonable sensitivity. The following untransformed percentage responses were obtained and are shown in Table 5. 11. indicating clearly the effect of the dye.

On the following day at the same time (3 p.m. B.S.T) the same papers were again exposed for 1½ hr. to direct sunlight under similar conditions to those on the previous day. (Mean Total Radiation 62 cal./sq. cm./hr. R.H. 38%, shade Temperature 89.0° F. 31.7° C). The actual temperature of the papers during the exposure period, as far as could be ascertained by wrapping one of them round the bulb of a thermometer, was in the neighbourhood of 40° C. After removal to the laboratory, the papers were again assayed, the concentrations on the protected papers having fallen to the level required for a sensitive response range to be obtained after 18 hours exposure to the weevils.

In order to determine the residual concentrations on the protected films, the responses of the weevils were calibrated with a set of six papers containing 0.4% pyrethrins. The results of this assay, again in untransformed percentages, are given in Table 5.11.B. It will be observed that the unprotected papers had been virtually completely decomposed, giving rise to responses not significantly different from zero. The protected material, on the other hand, gave responses which, when adjusted, transformed and referred to the calibration curve indicated that 0.3% of the pyrethrins remained undecomposed. In terms of the percentage protectant effect the rating of the dye at 1% concentration under these conditions is 84. This figure, however, is clearly an underestimate, since the virtually complete decomposition of the pyrethrins in the films without the dye would probably have occurred prior to the end of the irradiation period.

The experiment was therefore repeated under the same conditions with a fresh set of impregnated filter papers, exposed for 1 hr. and 10 min. only. A slightly fresher breeze was blowing, and although the total radiation was greater than on the previous occasions, the amount of ultra-violet light in this radiation was probably smaller, owing to the occurrance of a thin layer of light cloud. The meteorological data were as follows:- Mean Total Radiation, .65 cal./sq.cm./hr., Relative Humidity 55%, Shade Temperature 74.5° F. The data obtained by the usual assay with C. granaria

in terms of the angular transforms of their responses, are given in Table S. 12.

The concentration of insecticide remaining in the unprotected papers is obtained by reference to the calibration curve as 0.21%, and that remaining in the protected papers as 0.70%. The percentage protectant effect is therefore

$$\frac{0.701 - 0.215}{1.00 - 0.215} = \frac{0.486}{0.785} = 62\%$$

This value is appreciably lower than the corresponding figure of 90% obtained by inspection of Fig. S.1. and referring to the laboratory experiments with ultra-violet light. The difference indicated that a small part of the decomposition in the open air is caused by a mechanism not operative in the case of the laboratory experiments. Nevertheless, the life of a pyrethrins-in-oil film is more than doubled by protection with an equal weight of the dye. It may also be reasonably inferred that the decomposition of the pyrethrins in strong sunlight takes place mainly, though not wholly, as the result of photolysis by ultra-violet light.

General examination of dyestuffs for protectant action.

In view of the promising properties of S.D.M. Oil-soluble Yellow dye, it was decided to test various other dyes for protectant action against ultra-violet light.

For sorting out ineffective dyes from those worthy

of further investigation, the most important point for initial investigation is whether there is any significante protectant action when the dye is present at half-saturation concentration, if this is low, or at a practicable economic strength of, say, 1%. In most cases, dyes are only soluble in the heavy white oil used in these experiments to the extent of 2% or less. From the point of view of the sorting-out investigation, it is not of much importance whether a dye fails the test because its absorption band is weak over the critical range active in decomposing the pyrethrins, or because the dye is insufficiently soluble in the solvent.

Testing of the dyes proceeded in a similar manner to that adopted on the case of the different concentrations of B.D.H. Oil-Soluble Yellow dye, except that the means of the several responses to different dyes compared at the same time with a common standard consisting of unprotected pyrethrins, were compared by the method recently outlined by Tukey(1949). In the case of dyes showing significant protectant action, this was rated in terms of the percentage effect by similar means to those already described for the B.D.H.Oil-Soluble Yellow.

It might at first sight appear rather arbitrary to examine the various dyes at "half-saturation" concentration, but in practice the determination of the equilibrium saturation concentration is troublesome, and the amount of

Table S. 2.

Dyes showing significant protectant effect against ultra-violet light.

All dyes tested at 0.5% concentration.

Dye.	Percentage protectant effect.
B.D.H. Oil-soluble Yellow	84
B.D.H. Oil-soluble Green.	81
B.D.H. Oil-soluble Black.	10

Dyes showing no significant protectant effect.

B.D.H. Oil-soluble Orange.

B.D.H.Oil -soluble Brown.

B.D.H. Oil-soluble Blue.

B.D.H. Oil-soluble Violet.

B.D.H. Oil-soluble Red.

Sudan III.

Sudan IV

Auramine B.D.H.Paste.

Anthracite Black B.

Nitrazol C.F.

Durrol Yellow Brown P.

Diamine Yellow N.Powder.

Immedial Indone Violet R.Conc.

- - - - - - - - - - - - - - - - - - - -

TABLE 5. 2

First comparision of dyes for protectent action on pyrethrins.

Test Code No.	Name of dye.	Concentration.
A	Waxoline G.S. Paste	½ Saturation.
B	B.D.H. Oil soluble Blue	"
C	" " Green	"
D	" " Brown	"
E	Pyrethrins only	1%
F	Oil only	-

Angular transforms of response after 12 hrs. exposure to films irradiated for 10 mins.

Replicate Code No.	1	2	3	4	Total	Mean.
A	35·21	30·66	43·45	35·24	142·56	35·64
B	30·92	26·56	33·83	28·25	119·56	29·89
C	42·46	45·00	44·03	40·05	171·54	42·88
D	41·78	29·20	32·33	26·13	129·44	32·36
E	34·45	29·60	25·70	26·35	116·60	29·15
F	0·00	0·00	0·00	0·00	0·00	0·00

C 42·9
A 35·6
D 32·4
B 29·8
E 29·1

Preliminary Analysis of Variance.

Item	D.F.	Sum of Squares	Mean Square	F
Treatment	4	431·50	107·87	4·15
Residue	15	390·10	26·01	
Total	19	821·60		

Standard error of mean is 2·55.

Least significant interval between means arranged in order of magnitude 7·68
Only treatment C splits off as a distinct group, and the percentage protectant effect of this dye (B.D.H. oil-soluble green) is estimated as 21. The interval between the most straggling of the remaining means, and the general mean for the population of treatments not already split off is 1·54, in terms of unit normal deviates, and this quantity is less than 1·96, the 5% point of the normal deviate.

TABLE 5. 3.

Second Comparision of dyes for protectant action on the pyrethrins.

Test Code No.	Name of dye	Concentration.
A	B.D.H. oil-soluble red	1%
B	" " "	0·5%
C	" " violet	½ satr:
D	Sudan IV	"
E	Pyrethrins only	1%
F	oil only	-

Angular transforms of response after 12 hrs: exposure to films irradiated for 10 mins.

Replicate Code No.	1	2	3	4	Total	Mean
A	41·55	46·85	42·99	46·38	177·75	44·44
B	50·77	52·48	51·35	40·28	194·88	48·72
C	40·69	40·11	32·53	41·09	154·22	38·55
D	42·02	38·76	45·86	42·53	169·17	42·29
E	57·76	36·06	34·08	35·79	145·69	36·42
F	0·00	0·00	0·00	0·00	0·00	0·00

Preliminary Analysis of Variance.

Item.	D.F.	Sum of Squares	Mean Square	F.
Treatment	4	352·08	88·02	4·95
Residue	15	266·99	17·80	
Total	19	619·07		

Standard error of mean is 2·11

Least significant interval between means arranged in order of magnitude is 6·36. No group separates off distinctly. The interval between the most struggling mean (B) is 8·63, which may be compared as a normal deviate by the method of Tukey (1949). Since it only attains 1·71 compared with the 5% level of 1·96, none of the dyes tested can be said to give significant protection to the insecticide when the latter is irradiated.

TABLE 6. 4

Third comparision of dyes for protectant action on the pyrethrins.

Test Code No.	Name of dye	Concentration.
A	B.D.H. Oil-soluble black	1%
B	" " "	0·5%
C	Sudan III	0·5%
D	Pyrethrins alone	1·0%
E	Oil carrier only	-

Angular transforms of response after 12 hrs. exposure to films irradiated for 10 mins.

Replicate Code No.	1	2	3	4	Total	Mean
A	48·4	42·7	51·9	46·1	189·1	47·3
B	46·1	52·5	54·9	43·3	196·8	49·2
C	41·6	38·1	36·3	30·7	146·7	36·7
D	36·3	45·6	45·3	44·4	169·6	42·4
E	3·0%	0·0	0·0	0·0	0·88	-

Preliminary Analysis of Variance.

Item.	D.F.	Sums of Squares	Mean Square	F.
Treatment	3	389·38	129·79	6·53
Residue	12	238·76	19·89	
Total	15	628·14		

Standard error of mean is 2·23.

Least significant interval between means arranged in order of magnitude is 6·86. Thus only treatment B splits off, by a bare margin, as a group and the contrast between the two levels of B.D.H. oil-soluble black and the other two dyes contributes most to the variance ratio.

TABLE 5.5

Examination of B.D.H. oil-soluble orange for protectant action on the Pyrethrins.

Replicate	1	2	3	4	5	6	Total	Mean
Dye and Pyrethrins (0·5%+1%)	38·1	36·9	31·3	46·7	38·1	43·3	234·4	39·1
Pyrethrins alone (1%)	38·6	41·6	42·1	35·7	41·6	30·0	229·6	38·3

Analysis of Variance.

Item.	D.F.	Sums of Squares.	Variance	Variance Ratio.
Treatment	1	1·82	1·82	< 1
Residue	10	239·66	23·97	
Total	11	241·48		

There is no evidence that the dye exerts any protectant action whatever.

TABLE 5.6

Fifth examination of dyes for protectant action on the pyrethrins.

Dye and Concentration.	Replicates	Means →
Anthiracite Black B (half-satn.)	25·1, 31·3, 28·7, 66·4, 27·3, 26·6.	34·23
Nitrazol C.F. "	59·3, 42·1, 28·7, 39·2, 21·1, 56·9.	41·21
Pyrethrins alone "	27·3, 27·3, 22·0, 35·1, 40·4, 39·2.	31·88

Analysis of Variance.

Item	D.F.	Sums of Squares.	Mean Square.	F.
Treatment	2	278·94	139·47	< 1
Residue	15	2,677·80	178·52	
Total	17	2,956·74		

Since the variance ratio for treatment does not exceed unity, neither of the dyes tested has any useful effect.

TABLE 5. 7

Sixth examination of dyes for protectant action on the pyrethrins.

Test Code No.	Name of dye.	Concentration.
A	Furrol Yellow Brown D.	½ satn.
B	Diamine Yellow N. Powder	"
C	Immedial Indone VioletB. Conc:	"
D	Pyrethrins alone	1%
E	Oil only	-

Angular transforms of responses after 12 hrs: exposure to films irradiated for 10 mins:

Replicate Code No.	1	2	3	4	5	6	Means
A	51·9	50·8	33·1	47·9	90·0	45·0	53·45
B	42·1	56·2	42·1	22·0	49·0	58·1	41·58
C	55·7	51·5	32·6	56·2	30·7	20·0	34·42
D	32·6	38·1	50·8	18·4	35·1	33·2	34·70
E	0·0	0·0	0·0	0·0	0·0	0·0	-

Preliminary Analysis of Variance.

Item.	D.F.	Sums of Squares.	Mean Square	F.
Treatments	5	1,427·60	475·87	2·57
Residue	20	3,703·34	185·16	
Total	23	5,130·94		

None of the above dyes has any significant protectant action, as indicated by the non-significant variance ratio for treatments.

TABLE 5. 9.

Effect of ultra violet light on the stability of benzene azo - β -naphthol.

Time from start of irradiation. (hours)	Colorimeter Reading	
	Standard. solution.	Test solution.
0·00	19·9	19·9
0·75	19·2	19·8
1·67	19·8	20·5
3·25	19·9	20·4
5·50	19·7	19·9

TABLE. 5. 10.

Action of benzene azo - β-naphthol on unirradiated pyrethrum films.

0·3% Pyrethrins alone (not irradiated)	0·3% Pyrethrins 1% dye (not irradiated)	Controls (Oil only)	1% Dye alone (not irradiated)
63·9	62·1	0·0	0·0
48·6	62·5	0·0	0·0
56·6	47·6	0·0	0·0
46·8	45·0	0·0	0·0
56·0	54·7	0·0	0·0
44·2	52·2	0·0	0·0
Totals			
316·1	324·1	0·0	0·0
Means			
53·2	54·0	0·0	0·0

The above figures are the angular transforms of the percentage responses of batches of 30 weevils, all drawn from the same population. For the first two columns, $t = 0{\cdot}316$, not significant at the 5% level of P. the dye alone has no action on the weevils, over the exposure periods used.

TABLE 5. 11A

Untransformed percentage responses of batches of 30 *C. granaria* confined on filter papers exposed to direct sunlight.

(First Experiment 11/7/49 - 12/7/49).
assay Terminated at 6 hrs. see text.

1% Pyrethrins (irradiated)	1% Pyrethrins+1% dyo (irradiated)
25·0	80·0
15·6	78·1
9·7	83·5
10·7	67·8
12·5	55·6
17·1	81·4

TABLE. 5. 11 B.

Untransformed percentage responses of batches of 30 *C. granaria* confined on filter papers exposed to direct sunlight.

(First Experiment 11/7/49 - 12/7/49 continued).

Controls (Oil only)	1% Pyrethrins (irradiated)	1% Pyrethrins+1% dye (irradiated)	0·4% Pyrethrins not (irradiated)
0·0	0·0	34·5	37·5
0·0	3·2	33·3	45·2
0·0	0·0	28·6	31·0
0·0	0·0	21·5	41·4
0·0	0·0	32·4	20·0
0·0	0·0	20·6	35·5
Total 0·0	3·2	170·9	210·5
Means 0·0	0·5	28·5	35·1

TABLE. 5. 12

Angular transforms of percentage responses of batches of 30 weevils, confined for 12 hrs. on filter papers exposed to direct sunlight.

(Second Experiment 28/7/49)

Controls Oil Only	1% Pyrethrins (irradiated)	1% Pyrethrins+1% dye (irradiated)	0·4% Pyrethrins (not irradiated)
0·0	38·7	60·7	39·2
0·0	19·4	64·9	50·8
0·0	23·6	59·3	35·1
0·0	25·8	52·5	39·2
0·0	16·4	50·2	41·0
Total 0·0	113·9	287·6	205·3
Means 0·0	22·8	57·5	41·1

work involved out of proportion to the utility of the information. It is more convenient to prepare a saturated solution at an elevated temperature, and to allow the solution to stand in contact with excess solid at room temperature for some time. The solution is then diluted with an equal volume of the 2% stock solution of the pyrethrins before use, thus giving rise to the required 1% pyrethrins solution, together with the dye at half-saturation.

The data obtained in five different sets of comparisons, involving sixteen dyes, with the pyrethrins alone, are given in Tables 5.3 - 5.7. For convenience, the results of these Tables are collected together in Table 5. 8.

In all of the Tables 5.3 to 5.7, the comparisons of the means of the replicates of the treated pyrethrins are made by splitting the means into groups, and then comparing each mean against that for the group in which it occurs. The group splitting as based on the criterion of the least significant interval, calculated in the usual way, and the comparisons within groups are carried out by converting the differences into approximately normal deviates, by the methods summarised by Tukey, (loc. cit.) and examined as such.

It will be observed that in the cases where a dye has proved sufficiently soluble in the heavy white oil solvent to be tested at both 0.5% and 1.0%, the lower concentration is at least as effective as the higher. This result holds

for B.D.H. Oil-soluble Red, (Table 5.3.), B.D.H. Oil- soluble Black, (Table 5.4) and B.D.H.Oil-soluble Yellow (Fig 5.1). The effect is probably due to the levelling off of the protectant action of the dyes with concentration shown in Fig. 5.1 .

A selection of some 150 dyes was then examined. These dyestuffs and intermediates were originally samples from Leopold Cassella and Co., and we are indebted to Dr. S.H. Huston of Imperial College Botany Dept. for their transfer. It was thought that the probability of a dye having appreciable solubility in both oil and water was negligable, so that the dyes were first examined for water solubility, and only those sparingly soluble in water were further examined for oil-solubility .

About 0.1g. of the dyes were covered in a boiling tube with 5 ml. of heavy white oil and maintained at 100°C for 18 hr. In most cases little or no solution had occurred, and the supernatant liquid remained colourless. In such instances the dyes were rejected. Five of the 150 dyes, however, gave signs of some appreciable solubility in oil, though not approaching the 5% which complete solution in the amount of solvent added would have indicated. These dyes were tested for protectant action as previously described, at half-saturation concentration, and the results of the examination are reported in Tables 5.6 and 5.7. Only Durrol Yellow Brown approaches significance in its protectant action, and none of the dyes examined compares

with B.D.H. oil-soluble Yellow.

Stability of Allylrethrin I

Preliminary experiments have been carried out to examine the stability to ultra-violet light of the allyl homologue of Cinerin I, a synthetic pyrethroid also known by the abbreviation "Allethrin" (in U.S.A.) and "Allylrethrin I" (in Great Britain).

A brief survey of the literature indicated that little work of any importance had been done on this subject but that there was probably not much difference between the stability of the synthetic and natural pyrethroids. It was therefore decided to use a null point method to examine this small difference; i.e. a method in which, after ultra-violet irradiation, all the preparations under assay should have the same toxicity according to the null hypothesis in question.

The first step in such a method is a preliminary determination of the relative potencies of the natural and synthetic products.

The layout of this preliminary investigation is given in Table 5.13 from which it will be observed that the experimental design is the simple "4 - point" assay, the use of which is dealt with elsewhere in this Thesis. The natural pyrethrins solution was diluted eight times relative to the synthetic

TABLE. 5. 13.

Relative potency of natural pyrethrum and Allylrethrin to *C. granaria* L.

Allylrethrin.		Concentration	Pyrethrum.	
3·2%	1·6%		0·4%	0·2%
51·4	38·6		50·8	17·5
48.7	35.1		35.1	11.5
48.4	33.8		43.3	22.0
46.1	31.9		25.8	28.7
42.1	37.5		28.7	14.2
41.6	34.4		22.8	15.3
41.0	30.7		30.7	38.1
46.7	30.7		50.2	19.4
43.9	36.9		46.1	14.2
43.3	37.5		46.7	23.6
46.1	34.4		38.1	25.8
42.7	36.9		28.7	12.9
Mean.	Mean.		Mean.	Mean.
45.0	34.87		37.25	20.27

Analysis of Variance of above data.

Items.	D.F.	Sums of Squares.	Mean Square.	Standard Error.
Insecticides	1	1,146.15	1,146.15	33.853
Linear Regn:	1	2,205.94	2,205.94	46.958
	2		3,325.09	57·896
Departure from Parellelism.	1	140·77	140·77	Variance Ratio 3·18
Error.	44	1,947.93	44.27	
Total.	47	5,440.79		

TABLE. 5.13. Con:

Log: Relative potency (as diluted) $= 0^{\cdot}3010 \times \frac{33^{\cdot}853}{46^{\cdot}958} = 0^{\cdot}2170$

Log: Dilution factor. $= 0^{\cdot}9031$

Log: Relative potency. $= \overline{0^{\cdot}6861}$

Relative potency; (Pyrethrins Allylrethrin) $= 4^{\cdot}86$

Standard error, of M $= 44^{\cdot}27 \times 0^{\cdot}3010 \times 57^{\cdot}896$

$2,205^{\cdot}94$

$= 0^{\cdot}3497$

Approximate 95% fiducial limits for M (1·3855
(T·8670

corresponding relative potencies and (24·3

(0·737

derivative in view of the generally much lower toxicity of the latter to stored products insects. As Table 5.13 indicates, this was rather fortunate assessment, the natural pyrethrins proving to be 4·26 times as toxic to C. granaria as the synthetic material. Details of the assay procedure are identical with those described in sections 2 - 5. The next stage in the experiment was to prepare filter papers to which both types of pyrethroid were separately applied, in the presence or absence of benzene azo - β - naphthol dye. A 1% solution of the dye was used, and under these circumstances the experiments described earlier in this section had shown that the rate of decomposition of the natural pyrethrins was almost exactly half that occuring by photolysis in the absence of the dye. The Allylrethrin I was therefore present at 4·26 times the natural pyrethrins concentration, and the concentrations of both active materials in the dyed papers were halved re ative to the undyed comparison. Four replicate papers were prepared for each of the four classes of insecticide specified by the experimental design.

The null hypotheses under examination can thus be broken down into two constituents, the hypothesis that the two types of pyrethroid photolyse:

TABLE. 5. 14.

Relative protective action of benzene azo - β - naphthol on irradiated pyrethrum and allylrethrin films.
All films irradiated for 8 mins.

2% Pyrethrins alone.		1% Pyrethrins + 1% dye.	
68·9		56·8	
58·1	Mean.	56·8	Mean.
59·3	60·15	66·4	60·32
54·3		61·3	

9·72% Allylrethrin alone.		4·85% Allylrethrin + 1% dye.	
90·0		64·2	
80·0	Mean.	63·4	Mean.
80·0	82·12	68·0	64·95
78·5		64·2	

Analysis of Variance of above data.

Items.	D.F.	Sums of Squares.	Mean Square	F.
Insecticides	1	712·91	712·91	30·74***
Protectant effect.	1	294·35	294·35	12·69**
Interaction	1	290·15	290·15	12·51**
Residue	12	278·28	23·19	
Total.	15	1,575·69		

at the same rates and the hypothesis that the activity of the dyed papers decreases at half the rate of the undyed ones.

All the papers were irradiated in balanced order for 8 mins. the details of the irradiation being as described in section 4 and the assay procedure was the same as in sections 2 - 5. The results of the assay are given in Table 5.14. This table and the analysis of variance which follows it, discloses that the synthetic allylrethrin I is definitely more stable to ultra violet light than the natural pyrethrins complex. The second null hypothesis is upheld for the natural product, but combined reference to the significant interaction term in the analysis and the mean responses of Table 5.14 demonstrates that the allylrethrin I is significantly less well protected than the natural extract by the added dye. Presumably the critical wavelengths for the synthetic product are different.

It may be concluded that although allylrethrin I is more stable to ultra violet light than the natural complex, much of this advantage is lost when benzene azo - β - naphthol is added as a protectant dye, since it protects the synthetic derivative less well than the natural compounds.

The actual magnitudes of the effects noted briefly in this preliminary investigation of allylrethrin I remain however, to be determined by more detailed work, as do its reactions to oxygen etc:

The Stabilization of the Pyrethrins by Synergists.

The augmentation of the toxicity of the insecticides by other substances requires careful investigation, as three seperate and independent quantities contribute to it. These quantities are the toxicity of the synergist alone in the particular assay system used, the synergist effect proper, and the possible influence of the synergist on the persistence or availability of the insecticide. All three quantities may in themselves be complex, and the various components of synergistic action have been reviewed by Page and Blackith (1949).

Dove (1947) suggested that, of the pyrethrins synergists, piperonyl butoxide not only increased the toxicity of the insecticide, but prolonged its action by stabilising the pyrethrins against destruction from light or oxidation in the air. Page and Blackith (loc. cit) pointed out that this claim could only be properly investigated of the maghitude of the synergistic effect, and its variation with different molecular proportions of synergist, were established with sufficient accuracy.

The twin cross-over design was used for a series of relative potency assays, from the data of which Table 6.3 is compiled. This table is a list of the relative potencies of the mixtures of the pyrethrins and piperonyl butoxide, compared with the pyrethrins alone. These figures are summarised for convenience in Fig. 6.1. It will be observed that the toxicity of the mixtures increases with addition of the synergist

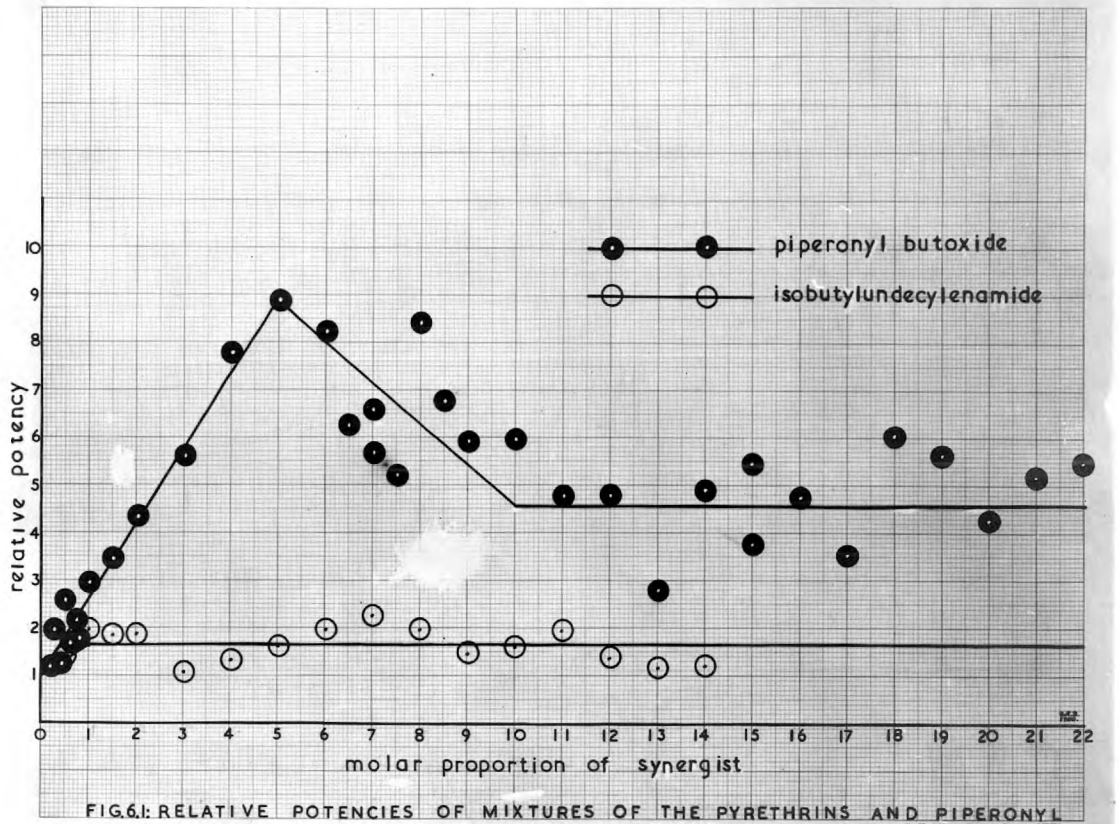


FIG.6.1: RELATIVE POTENCIES OF MIXTURES OF THE PYRETHRINS AND PIPERONYL BUTOXIDE AND ISOBUTYLUNDECYLENAMIDE

until a 5: 1 ratio in favour of the latter is reached, when the relative potency is approximately 9 times that of the insecticide alone.

The increase of potency over this range is nearly proportional to the mol. fraction of synergist present. This fact is associated with two important difficulties. In estimating a stabilizing action of the synergist, the rates of decomposition of the insecticide and synergist are not likely to be the same, when both are, for example, irradiated with ultra-violet light. The molecular ratio of the two compounds will therefore be different at the end of the exposure to light from that of the mixture as originally made up, and for this reason it is clearly desirable that the molecular ratios used in such experiments should lie within a range over which the relative potency is independent of the proportions of synergist and pyrethrins. Such a range was found for sesamin, and also for isobutylundecylenamide, by Page and Blackith (loc.cit)and the range of synergist compositions for piperonyl butoxide and the pyrethrins was extended with the hope that such a range might be found for this synergist also.

Further addition of synergist, beyond the 5:1 ratio, depresses the relative potency of the mixture compared with the maximum previously noted, until at the 10:1 ratio the relative potency has dropped to 4.5, at which level it continues independent of further additions of the synergist. This depressant action is not a dilution effect, since the

synergist merely replaces an equivalent volume of the solvent. A somewhat similar situation has been reported with the synergist "Van Dyk 264" (N- (2-ethylhexyl) bicyclo- [2.2; 1] -5 heptene 2:3 dicarboximide) where antagonistic action, or rather a diminished synergism, is stated to occur when the proportion of "Van Dyk 264" to the pyrethrins exceeds 65:1 (Hartzell 1949).

This range fulfils the requirement noted above for assay of the protective effect of synergists, but an unexpected development prevented its exploitation for this purpose. Fig 6.1 indicates that the variation between the results of successive assays of the relative potencies of mixtures with more than 5:1 molecular proportions of the synergist is considerably in excess of that expected not only for comparable assay of other synergists discussed in this section but also of those conducted on similar mixtures with lower proportions of this synergist. This very considerable increase in variation is difficult to account for, but is of sufficient magnitude to render experiments within the range considered insensitive, and also, perhaps, misleading.

The assay of the protectant effect had therefore to be carried out on that part of the curve shown in Fig 6.1 for which the relation between potency and mol: fraction of synergist is approximately linear. At this stage the second of the two difficulties associated with this part of the curve may be considered briefly. If the hypothesis concerning the mode of action of pyrethrum synergists outlined by Page

TABLE 6.3.

Relative Potencies of Mixtures of the Pyrethrins with Piperonyl Butoxide.

Molecular Proportion of Synergist.	Dilution Factor for Test.	Log: Ratio of Potency.	Relative Potency.
0.00	-	0.0000	I.00
0.20	I/I	0.0683	I.I7
0.25	I/I	0.29I4	I.96
0.40	I/I	0.I903	I.26
0.50	I/I	0.35II	2.25
0.60	I/2	0.2I24	I.64
0.75	I/I	0.33I8	2.I5
0.80	I/2	0.2425	I.75
I.00	I/I	0.4695	2.95
I.50	I/I	0.54I2	3.48
2.00	I/I	0.6345	4.3I
3.00	I/I	0.7462	5.58
4.00	I/2	0.89I9	7.79
5.00	I/2	0.9448	8.8I
6.00	I/I0	0.9I46	8.22
6.50	I/I0	0.7948	6.23
7.00	I/2	0.9485	8.8I
7.00	I/I0	0.8I80	6.58
7.50	I/I0	0.7I04	5.I4
8.00	I/I0	0.9250	8.4I
8.50	I/I0	0.7803	6.03
9.00	I/I0	0.7698	5.89
I0.00	I/I0	0.773I	5.93
II.00	I/I0	0.6780	4.76
I2.00	I/I0	0.6762	4.75
I3.00	I/I0	0.4400	2.78
I4.00	I/4	0.6965	4.96
I5.00	I/3	0.7355	5.44
I5.00	I/I	0.5785	3.79
I6.00	I/4	0.6696	4.67
I7.00	I/3	0.5455	3.5I
I8.00	I/3	0.778I	6.00
I9.00	I/3	0.7487	5.6I
20.00	I/3	0.6246	4.2I
2I.00	I/5	0.7III	5.I5
22.00	I/5	0.7350	5.44.

TABLE 6. 4

Relative Potencies of mixtures of the pyrethrins with Piperonyl Cyclonene.

Molecular Proportion of Synergist.	Dilution Factor for Test.	Log: Ratio of Potency	Relative Potency.
0.00	-	0.0000	1.00
0.25	1/2	0.2773	1.89
0.50	1/3	0.4104	2.57
0.75	1/4	0.4246	2.65
1.00	-	0.6602	4.58
1.00	1/3	0.5697	3.89
2.00	1/4	0.5338	3.41
2.50	1/4	0.4951	3.13
3.00	1/4	0.5609	3.64
4.00	1/4	0.6661	4.63
5.00	1/4	0.5477	3.53
5.00	1/4	0.8807	7.60
6.00	1/4	0.6923	4.95
8.00	1/4	0.6385	4.35
12.00	1/4	0.6360	4.33
16.00	1/4	0.6495	4.46.

TABLE 6.5

Relative Potencies of mixtures of the pyrethrins with Sesamin.

Molecular Proportion of Synergist.	Dilution Factor for Test.	Log: Ratio of Potency	Relative Potency.
0·00	-	0·0000	1·00
0·50	-	0·1781	1·52
1·00	1	0·3980	2·50
2·00	1/3	0·5057	3·20
3·00	1/3	0·4740	2·98
4·00	1/3	0·4819	3·03
6·00	1/2	0·4737	2·98
10·00	1/3	0·4549	2·85
12·00	1/3	0·4548	2·85

TABLE 6. 6

Weighted mean estimate of limiting relative potency of the pyrethrins in the presence of *isobutylundecylenamide*, (I. N. 930).

95% Fid. Range.	ρ	V	V^{-1}	ρ	$\rho.V^{-1}$
6·02	1·50	2·25	0·44	1·99	0·87
1·40	0·35	0·12	8·25	1·84	15·18
2·47	0·62	-0·39	2·60	1·88	4·88
186·00	46·52	2,160·00	0·01	1·83	0·01
8·48	2·12	4·49	0·22	1·51	0·29
12·20	3·05	9·30	0·11	1·63	0·18
4·56	1·14	1·30	0·77	1·98	1·52
2·54	0·64	0·413	$2{\cdot}44_{-4}$	2·26	5·51
315·00	79·00	$6\times10_{4}$	$2\times10_{-5}$	1·93	0·00
4,860·00	121·50	$1{\cdot}5\times10$	$1{\cdot}5\times10$	1·49	0000
3·30	0·82	0·67	1·52	1·61	2·45
2·18	0·54	0·29	3·44	1·94	6·67
4·39	1·10	1·21	0·83	1·37	1·14
7·40	1·85	3·41	0·29	1·18	0·34
14·10	14·10	12·44	0·08	1·20	0·09
			21·00		39·13

$$\bar{\rho} = \frac{\Sigma \rho . V^{-1}}{\Sigma V^{-1}} = 1{\cdot}86$$

TABLE 6.7

Weighted mean estimate of limiting Relative Potency of the pyrethrins in the presence of Sesamin.

95% Fiducial Range	σ	V	V^{-1}	ρ	$V^{-1} \cdot \rho$
1·05	0·26	0·0676	14·8	2·50	37·00
1·01	0·25	0·0625	16·0	3·20	51·20
0·46	0·11	0·1121	82·6	2·98	246·15
0·08	0·02	0·0004	2500	3·037	7,575·00
0·86	0·22	0·0484	20·7	2·98	61·69
0·74	0·19	0·0361	27·8	2·85	79·23
0·63	0·16	0·0256	39·1	2·85	111·44
			2,701·0		8,161·71

$$\bar{\rho} = \frac{\Sigma \rho . V^{-1}}{\Sigma V^{-1}} = 3{\cdot}02.$$

TABLE 6.8

Weighted mean estimate of the limiting relative potency of the pyrethrins in the presence of piperonyl cyclonene.

95% Fiducial Range.	ρ	V	V^{-1}	ρ	$V^{-1}.\rho$
2·48	0·62	0·3844	2·60	4·58	11·91
1·96	0·49	0·2401	4·16	3·89	16·18
1·08	0·27	0·0729	13·72	3·41	46·79
1·98	0·49	0·2401	4·16	3·13	13·02
1·57	0·39	0·1521	6·57	5·64	23·92
0·77	0·19	0·0361	27·70	4·63	128·25
1·73	0·43	0·1849	5·41	3·53	19·09
8·60	2·15	4·6225	0·22	7·60	1·67
2·69	0·67	0·4489	2·23	4·33	9·66
1·68	0·42	0·1764	5·67	4·46	25·29
			72·44		295·78

$$\bar{\rho} = \frac{\Sigma V^{-1}.\rho}{\Sigma V^{-1}} = \underline{4·08}$$

TABLE 6.2

Weighted mean estimate of the limiting relative potency of the pyrethrins in the presence of piperonyl butoxide.

95% Fiducial Range.	σ	V	V^{-1}	ρ	$V^{-1}.\rho$
0·68	0·19	0·0361	27·66	5·93	164·02
2·31	0·59	0·3481	2·88	4·73	13·62
2·32	0·59	0·3481	2·88	4·75	13·68
2·05	0·51	0·2601	3·84	4·86	18·66
36·09	6·52	42·5104	0·02	5·44	0·11
26·15	6·55	42·6400	0·02	3·79	0·10
2·07	0·51	0·2601	3·84	4·67	17·93
0·09	0·02	0·0004	2,500·00	3·51	8,775·00
1·44	0·36	0·1292	7·73	5·61	43·37
2·03	0·51	0·2601	3·84	4·21	16·17
1·60	0·40	0·1600	6·25	5·15	32·19
1·23	0·51	0·0961	10·40	5·44	56·58
			2,569·36		9,151·43

$$\bar{\rho} = \frac{\Sigma \rho . V^{-1}}{\Sigma V^{-1}} = 3{\cdot}56$$

TABLE 6.10.

Stabilisation of the Pyrethrins by Sesamin.

Replicate.	0.65 % pyrethrins & I.33 % sesamin.	2.0 % pyrethrins alone.
I.	46.I	58.I
2.	56.8	57.4
3.	53.7	64.2
4.	54.9	5I.4
5.	56.8	6I.3
6.	5I.4	43.3
Totals.	3I9.7	335.7
Means.	53.3	55.9

Grand Total 655.4 , Grand Mean 54.6

Analysis of Variance.

Items.	Sums Of Squares.	D.F.	Variance .	Variance Ratio.
Treatment.	20.80	I.	20.80	0.5498
Residual.	378.26	I0.	37.83	
Total.	399.06	II.		

Treatment effect not significant at 5 % level of P.

TABLE 6.11.

Stabilisation of the pyrethrins by Isobutylundecylenamide.

Replicate.	I.I8 % pyrethrins & I.I8 % I.N.930.	2.0 % pyrethrins alone.
I.	39.2	59.3
2.	46.I	53.I
3.	37.5	39.2
4.	39.8	60.0
5.	4966	43.3
6.	43.9	35.1
7.	33.8	33.2
8.	35.I	40.4
Totals.	326.0	363.6
Means.	4I.0	45.4

Grand Total 689.6 , Grand Mean 38.3I

Analysis of Variance.

Items.	Sums of squares.	D.F.	Variance.	Variance Ratio.
Treatment.	I5I.68	I.	I5I.68	2.485
Residual.	854.56	I4.	6I.04	
Totals.	I,006.24	I5.		

Treatment effect not significant at 5 % level of P.

TABLE 6. 12

Stabilization of the pyrethrins by Piperonyl butoxide.

0·66% Pyrethrins and 0·66% Piperonyl butoxide (8 mins. irradiation).

n	r	p	Angular transform.
35	28	80·0	63·4
43	41	95·4	78·0
33	23	69·7	56·6
40	35	87·5	69·3
45	40	88·9	70·5
35	26	74·3	59·5
32	24	75·0	60·0
36	23	63·9	53·0
			510·3

2% Pyrethrins alone (8 mins. irradiation).

n	r	p	Angular transform.
32	15	46·9	43·2
41	32	78·0	62·0
37	22	59·5	50·5
36	26	72·2	58·3
31	20	64·5	53·4
37	23	62·2	52·0
35	23	65·7	54·1
35	24	68·6	55·9
			429·4

0·2% Pyrethrins alone, not irradiated.

n	r	p	Angular transform.
37	8	21·6	27·8
34	8	23·5	29·0
34	9	26·5	31·0
35	6	17·1	24·5
42	15	35·7	36·7
33	7	12·1	20·4
36	18	50·0	45·0
33	16	48·5	44·1
			258·5

TABLE 6. 12 (2).

Analysis of Variance for comparison of irradiation treatment.

Item	D.F.	Sums of Squares.	Mean Square.	F.
Treatments	1	413·65	413·65	*7·76
Residue	13	693·44	55·34	
Total	15	1,107·09		

* Treatment effect significant at 5% level of F, but not at 1% level.

Mean angular difference for 0·2% Pyrethrins, compared with calibration curve (fig. 2.1.) = 32·9 - 32·3 = 0·6 units. Corrected mean responses, to mixture 64·4 units, to pyrethrins alone 54·3 units. Equivalent pyrethrins concentrations are 0·618% and 0·425%. Percentage protectant effect = $\frac{0·618 - 0·425}{2·000 - 0·425} \times 100 = 12·25$.

TABLE 6. 15

Stabilization of the pyrethrins by Piperonyl cyclonene.

0.66% Pyrethrins and 10% Piperonyl cyclonene.

n	r	p	Angular transform.
30	22	73	58°7
30	20	67	54°9
30	27	90	71°6
36	32	89	70°6
31	29	94	75°6
30	24	80	63°4
30	30	100	90°0
30	27	90	71°6
30	26	87	68°9
29	28	97	80°0
30	26	87	68°9
29	29	100	90°0
		Totals	864°2

2% Pyrethrins alone.

n	r	p	Angular transform.
32	31	94	75°6
32	31	97	80°0
29	29	100	90°0
30	23	77	61°3
30	20	67	54°9
31	20	65	53°7
50	29	97	80°0
29	19	66	54°3
30	29	97	80°0
29	27	93	74°7
30	26	87	68°9
28	18	64	53°1
			826°5

Grand Total = 1,6907.

Analysis of Variance.

Items.	D.F.	Sums of Squares.	Mean Square.	F.
Treatments	1	58°5625	58°5625	< 1
Residue	22	3,131°2555	142°3290	
Total	23	3,189°618		

Treatment effect not significant at 5% level of P.

TABLE 6. 14.

Toxicities of pyrethrum synergists *per se* to *C. cynaria*
All 2% solutions in 80/20 solvent (0·5 ml).

Synergist	Replicate	n	r	p
Sesamin.	1	29	0	
	2	30	0	
	3	31	0	
	4	30	0	
	5	31	1	
		151	1	0·66
I.N. 930.	1	34	0	
	2	30	0	
	3	32	0	
	4	32	1	
	5	30	0	
		158	1	0·63
Piperonyl butoxide.	1	27	0	
	2	29	0	
	3	51	0	
	4	27	0	
	5	30	1	
		144	1	0·69
Piperonyl Cyclonene.	1	30	3	
	2	30	0	
	3	34	0	
	4	32	0	
	5	32	3	
		158	6	3·80

TABLE 6. 14 (2).

Synergist	Replicate	n	r	p
D.H.S. Activator.	1	27	0	
	2	32	0	
	3	30	1	
	4	30	0	
	5	30	1	
		149	1	1·34
Van Dyk 264.	1	30	0	
	2	31	0	
	3	30	0	
	4	30	0	
	5	29	0	
		150	0	0·00
n-propylisome.	1	30	0	
	2	34	0	
	3	28	0	
	4	31	0	
	5	30	0	
		153	0	0·00
Compound "469".	1	31	0	
	2	31	1	
	3	31	0	
	4	30	0	
	5	27	0	
		150	1	0·67
"Controls Oil only".	1	60	0	
	2	62	0	
	3	61	0	
	4	62	0	
	5	59	0	
	6	58	0	
		362	0	0·00

TABLE 6.I5.

Test of linearity of curve representing the increase of relative potency of a pyrethrins - piperonyl butoxide mixture as the molecular ratio of the synergist is increased to a I:I proportion.

0.2 % Prethrins & 0.I% Piperonyl butoxide.	0.I % Pyrethrins & 0.I % Piperonyl butoxide.
I5.3	I0.0
I5.3	I5.3
I8.4	I5.3
I8.4	2I.I
43.3	00.0
I0.0	I0.0
28.0	I5.3
43.9	00.0
4I.0	I5.3
I0.0	I5.3
I8.4	00.0
I0.0	20.3
I5.3	I0.0
46.7	I8.4
26.6	I0.0
I8.4	22.0
I8.4	00.0
27.3	I0.0
Mean. 23.59	Mean. II.57

Analysis of variance of above data.

Items.	D.F.	Sums of squares.	Mean square.
Treatments.	I.	I,34I.I3	I,34I.I3
Residue.	34.	3,475.05	I02.2I
Total.	35.	4,8I6.I8	

$F_{I,34}$ is I3.I2, significant at 0.I % level of P.

TABLE 6.16

Molecular weights and parachors of the active constituents of the Pyrethrins and of their synergists.

Insecticides.

	M. W.	Parachors.
Pyrethrin I	328	738
Pyrethrin II	372	798
Cinerin I	340	730
Cinerin II	384	790

Synergists.

Piperonyl butoxide	399	774
Piperonyl Cyclonene	230	530
Sesamin	354	727
N-isobutyl undecylenamide	211	602
'n-propyl isome'	522	800
'Van Dyk 264'	275	644
Sulfoxide Compound 469	315	

and Blackith is correct, the implication of strict linearity would be that for mixtures with an excess of pyrethrins molecules, only those of the latter for which synergist molecules were available would be exerting toxic action. That this situation does not occur has been shown by the experiment whose data are reported in Table 6.15 from which it will be seen that additional pyrethrins molecules, in excess of a 1:1 ratio with the synergist, do exert an independent action. Strict linearity, therefore, is probably not a feature of the curve in question, and indeed it would be expected to be concave towards the independent variate axis up to a value of unity for this quantity.

Taken altogether, it might be predicted from the effects of the two complicating factors just considered that even in the absence of any stabilisation of the pyrethrins by piperonyl butoxide, a mixture of the two substances having initially the same toxicity as a given solution of the insecticide alone would, after equal irradiation of both films, be slightly more toxic than the latter.

In fact, this situation did arise when a 1:1 molecular ratio of the two substances, (0.66% each) was assayed against a 2% solution of pyrethrins alone, after both had been irradiated as films on filter papers, exactly as if the protectant action of a dyestuff were being evaluated. Fig. 6.1 shows that the two solutions used were initially of identical toxicity, and the analysis of Table 6.12 indicates that after irradiation the synergist mixture was superior. By comparing the resistance

of the test insects on an unirradiated set of filter papers impregnated with 0.2% pyrethrins, and reference to the calibration curve for this system as indicated in the section on the protectant action of dyestuffs, an estimate of the percentage protectant effect of piperonyl butoxide could be made. The value obtained for this quantity 12.1, does not, in the author's opinion, justify a claim that piperonyl butoxide exerts any protectant action on pyrethrum-in-oil films, when the factors outlined above are taken into account.

No simple experiment can be designed to examine this difficulty, since the appropriate null hypothesis would be that there is no difference between the increased toxicity caused by the alleged stabilization effect and that caused by the other factors discussed above. Since the magnitude of the latter effects cannot readily be determined, the null hypothesis cannot be examined.

It was thought desirable to extend this investigation to other pyrethrum synergists for two reasons. Firstly, no record of such investigations has been published though the possibility of stabilization has been discussed, and secondly, the increase in relative potency found with piperonyl butoxide are not consistent with those established for sesamin and iso-butyl undecylenamide in the flying insect-pyrethrins system. (Page and Blackith 1949). In the latter cases, the toxicity rises to three times that of the pyrethrins alone, when a 1:1 mixture of synergist and pyrethrin is present, and remains constant at this level when higher

proportions of the synergists are present.

The distinction between the behaviour of the three synergists in the two different assay systems might be due to any one of the three following reasons:-

(a) Change of synergist.

(b) Substitution of oil-base for water-base insecticide.

(c) Use of C. granaria as test insect in place of A. aegypti.

Conditions (b) and (c) could be jointly investigated by introducing sesamin and isobutyl-undecylenamide into the system in which piperonyl butoxide was tested. As for the latter synergist, the necessary first step in such an investigation is to determine the way in which the relative potency varies as the synergist content of the mixtures increases. With sesamin, the twin-cross-over design was used to evaluate the relative potencies, for the corresponding assays, whilst with isobutylundecylenamide a simple "four-point" design was adopted, on account of a temporary curtailment of laboratory facilities. The variation between these latter assays is of course not comparable with those conducted by the twin cross-over design.

Table 6.5 and Fig. 6.2 show that the behaviour of sesamin in the new system is identical with that obtained with flying insects and water-base pyrethrum emulsions. The limiting toxicity occurs at the 1:1 synergist-pyrethrins ratios and remains at the value there obtained, which is three times that of the pyrethrins alone.

It would appear from this similarity that the effects

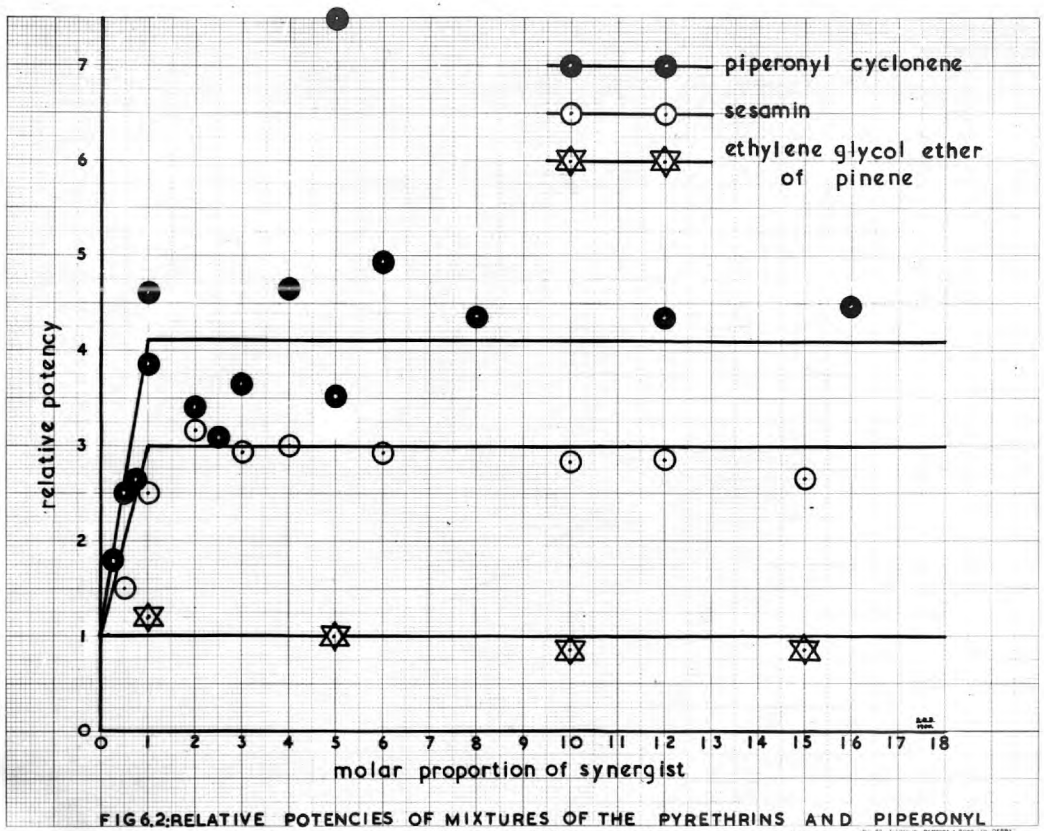


FIG 6.2: RELATIVE POTENCIES OF MIXTURES OF THE PYRETHRINS AND PIPERONYL CYCLONENE, SESAMIN, AND ETHYLENE GLYCOL ETHER OF PINENE.

obtained with piperonyl butoxide are attributable to this synergist rather than to the assay system involved. On the other hand, when a similar set of observations was made using isobutylundecylenamide as synergist, the general features of the curve obtained in the flying insect-water base spray type of assay are maintained, as Table 6.1 and Fig. 6.1 indicate, but the limiting toxicity is much less than the value of three times that of the pyrethrins alone which would have been expected.(see Table 6.6) The cause of the change of this quantity from a 3 to about 1.8 is probably physical in origin, though in what way the change of assay system induces the change in limiting relative potency it is not easy to discover.

Since for both the latter synergist the relative potency is independent of the ratio they bear to the pyrethrins, provided that they are present in excess, the assay of their possible protectant actions is relatively uncomplicated. These assays were conducted in the same way as that described for piperonyl butoxide, and the data from them are set out in Tables 6.10 and 6.11. From these tables it will be observed that no significant protective action is exerted by either synergist.

Stabilization of the pyrethrins is thus unlikely to be a general property conferred by the addition of pyrethrum synergists, but the investigation was extended to one further member of this class of compounds 'piperonyl cyclonene' which is a trivial name for a group of alkylated piperonyl cyclo-

hexenones, related to piperonyl butoxide.

From the point of view of quantitative work "piperonyl-cyclonene" suffers from the disadvantages that it is both sparingly and incompletely soluble in non-aromatic oils, and is a mixture of indeterminate composition, the two preponderant constituents being a complex alkyl methylene dioxyphenyl cyclohexenone derivative, and the other a similar derivative of carbethoxy cyclohexene(Wachs,1947).

However, as Table 6.4 and Fig 6.2 show, the variation of the relative potencies of mixtures of these substances is of the same form as that obtained with sesamin and isobutylundecylenamide, i.e. the relative potency reaches an upper limit with a 1:1 mixture with the insecticide. The upper limit has a mean value of about 4.2, which, it may be noted, is approximately the same as the limiting value for piperonyl butoxide,4.6. These limiting relative potencies have been taken from the graphs, rather from the mean estimates shown in Tables 6.6, 6.7, 6.8, and 6.9. It is possible that these latter estimates might be biassed by an extreme mean (in view of the heterogeneous nature of the data) with exceptionally narrow fiducial limits.

There is, however, no evidence for the rise and fall of the curve with mixtures having rather higher synergist ratios than 1:1, although the variation between successive assays is very marked with piperonyl cyclonene, as with piperonyl butoxide.

The twin cross-over design, which was used in most of

these relative potency determinations, considerally reduces the fiducial limits of the estimates made of this quantity. At first sight, therefore, the appearance of such excessive variation between the assays might be attributed to some flaw in the experimental design, such as occurs with, for example, systematic plot designs in which the intra-replicate variance is under-estimated with the result that the inter-replicate variance is inflated. However, perfectly satisfactory results have been obtained with this design when sesamin was the synergist under examination, and when the possible synergistic action of DHS Activator (ethylene glycol ether of pinene) was studied. This latter compound produced no synergist action in the assay system used. (See Table 6.2 and Fig. 6.2)

With the piperonyl derivatives too, the variation is quite low for mixtures whose relative potencies lie on the range over which this quantity increases with the proportion of synergist present. It appears that this excessive variation is restricted to those portions of the curves under discussion for which, according to the hypothesis put forward by Page and Blackith(1949), the orientation of the pyrethrins molecules at the peripheral nerve-sheath is determined by the presence of the piperonyl compounds. Such orientation might be critically dependent on an as yet unknown factor brought into action when piperonyl synergists are present.

Such a factor might operate through the instability of the postulated film on the nerve sheaths. For example, Jody (1946) has given evidence for the formation of

intermolecular complexes by penetration into interfaces. These complexes follow stoichiometric ratios, but the ratios can be quite heavily in favour of one or other of the components. Thus it may be postulated, admittedly without any adequate experimental evidence, that a 5:1 complex of the pyrethrins and piperonyl butoxide is possible, but on the borderline of stability, and that when piperonyl butoxide in excess of this ratio is present, the complexes breaks down to a series of local aggregations of 1:n complex, where n, to judge from the graphs, is 1,2, or 3., and of inert excess synergist.

Such conditions would be extremely labile, as they would represent two ordered assemblies of molecules with an extreme of low energy of transition between the different states of order. If this hypothesis were correct, however, it would not only account for the fall in toxicity from the extremely toxic 5:1 complex to the less toxic 1:*n* association, but would suggest that the relative potencies of mixtures with more than 5:1 molecular proportions of the synergist would be unstable, as is found to be the case. Also it would suggest that the limiting relative potencies of the 1:*n* complexes of pyrethrins with piperonyl butoxide and piperonyl cyclonene, which have similar [redacted] molecular volumes, should be about the same value, and possibly of similar instability.

It may be of interest to note here, in connection with

the suggestion that pyrethrum synergists may act by replacing the insecticide in a film on the nerve surfaces, that most such compounds have molecular weights, and even more so molecular volumes, which are within the same range as that occupied by the pyrethrins themselves. The figures are given in Table 6.16.

It must be emphasised that, in the writer's opinion, the experimental correctness of these suggestions does not provide critical evidence for the hypothetical situation outlined, since this was framed to account for them. On the other hand, the hypotheses outlined by Page and Blackith (1949a) and elaborated in this thesis do account for nearly all the observed facts, whilst retaining self-consistency and a measure of simplicity. It is interesting to note that Welsh and Gordon (1947) have put forward a very similar theory to account for modes of action of the pyrethrins and DDT, independently of, and prior to, those of the present writer.

Welsh and Gordon conclude that the pyrethrins and DDT molecules are sorbed on to the surfaces of the nerve axons in crustacea, producing a discharge of the resting potential of the nerve surfaces. This conclusion is arrived at from direct investigation of the impulses arising from treated nerves, but is manifestly similar to that put forward by Page and Blackith (1949a)

In considering the spread of the pyrethrins along nerve axon/sheath interfaces, it is interesting to notice that David (1946) has obtained striking evidence of the surface

surface activity of the pyrethrins on an aqueous protein/ kerosene interface, and Crisp (1946) has given an interesting discussion of the transport of molecules along interfaces. Kulkarni (1949) has determined the dielectric constants, and hence the dipole moments, of a series of DDT analogues, and has demonstrated that the toxicity of such compounds increases regularly with the dipole moment. This work lends added support to the theoretical interpretation of the mode of action of these nerve poisons outlined above.

The Stability of DDT and BHC.

Whilst DDT and BHC are notable for their stability to oxidation and in the first case to volatalization, evidence is accumulating that the former insecticide is liable to severe decomposition when exposed to direct sunlight. (Fleck and Haller, 1945). In the solid, crystalline state, DDT deposits are not rapidly inactivated, and if such irradiated deposits are sprayed with a solvent not containing DDT, any lost activity is restored. It appears that a protective coating of a decomposition product, not itself insecticidally active, is formed on the crystals. (Lindquist et al 1946) It has been suggested that the incorporation of a volatile solid, such as sulphur, would help mechanically to disperse the superficial layer of decomposition products. (Lindquist et al, 1945)

When the insecticide is dissolved in oil, however, no such protective layer is possible, and the inactivation can be carried out to completion. Lindquist et al (1946) have shown that DDT films, made up with a variety of solvents, decomposed in ultra-violet light at a rate comparable with that found for synergised pyrethrum preparations.

Plates of glass covered with DDT as a 5% solution in kerosene were almost completely inactivated after 48hrs exposure to sunlight, (Hadaway and Barlow, 1949), and it was shown that in these and similar deposits, the ratio of hydrolysable chlorine to total chlorine had increased, contrary to the result which

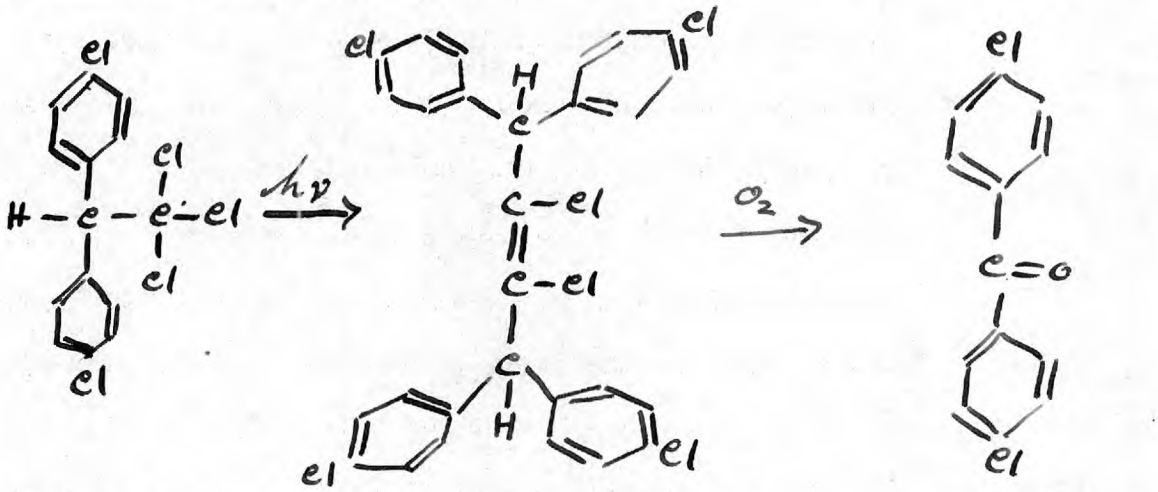
would be expected if the insecticide had been dehydrochlorinated to the ethylenic derivative. Part of the loss of toxicity of these deposits was attributed to the setting of the insecticide to a hard resin, with a resulting reduction of availability to insects alighting upon it, but a loss due to decomposition was also noted.

Sazenov and Andreev (1949) have shown that the decomposition of DDT by sunlight, as measured by its residual toxicity to houseflies, is almost entirely caused by the ultra-violet constituents of the sunlight.

The chemical aspects of the photolysis of DDT have been extensively investigated by Fleck (1949). This author found that two types of photolysis were possible, according as the DDT solution was irradiated with ultra-violet light in the presence or absence of air. In the former case, which will be that to which most practical conditions with approximate 4:4' dichlorbenzophenone is the main reaction product as was also found by (Wichmann et al (1946) although in the absence of oxygen an intermediate stage in the reaction can be detected, in which 2:3 dichloro- 1;1';4;4'; tetrakis (p-chlorophenyl)-2-butene is formed. This latter substance interferes withthe most useful colourimetric method for DDT estimation, that proposed by Schechter et al (1945), by producing a red, rather than blue, colouration.

It is noteworthy that this substance, which might accumulate outside irradiated crystals of DDT, was found by Fleck to exert a marked protectant action on the intact

insecticide and, by precipitation on the walls of the irradiation chamber, when solutions of the latter were being irradiated with ultra-violet light. The sequence of reactions when DDT is decomposed by ultra-violet light may be summarised as follows:-



p-p-DDT. 2:3dichlor 1:1;4:4 tetrakis (p-chlorphenyl)-2-butene 4:4dichlor-benzophenone.

Fleck makes the important observation that irradiation of DDT solutions in alcohol results in the formation of acetaldelyde by oxidation, presumably by chlorine liberated from the insecticide, and goes on to suggest that the presence of a chlorine acceptor, such as a hydrocarbon oil, would accelerate the decomposition process. Thus part of the greater reactivity of DDT in solution may be due to the chemical nature of the solvent rather than the greater mobility of solutes in the film, as compared with crystalline or resinous material. It would not, however, be at all easy to examine this hypothesis experimentally as there can be few, if any, liquids which are adequate solvents for DDT

and yet are unaffected by chlorine formed in the free radicle (nascent) conditions.

It is difficult to follow the decomposition of DDT in surface films by determining the amount of hydrolysable chlorine. Even if the complete absence of the intermediate butene derivative can be assumed, the change in the ratio of hydrolysable to total chlorine is not a very sensitive measure of the decomposition. However, Hadaway and Barlow have shown that DDT in kerosene has a higher ratio when irradiated by sunlight than when fresh. Thus it appears that the photolytic reaction may be partially anaerobic, probably on account of the low solubility of oxygen in the solvent.

In view of these uncertainties of the chemical methods of evaluating irradiated DDT deposits, it was decided to use biological assay methods for the work subsequently discussed, in which the rate of decomposition of DDT is measured, and the effect of BDH Oil-soluble yellow dye, (benzene azo-β naphthol) as a stabilising agent is determined. As Stringer (1948) has pointed out, the biological assay of dry deposits of DDT presents a number of difficulties probably the most important of which is the very low regression of probit response on log-dose, which renders the assay insensitive

Even when the availability of the DDT is increased by the addition of a residual oil deposit, the regression remains low, (Stringer 1949a), and in this case fixed deposits must be applied, and not varied throughout an experiment. (Stringer 1949b)

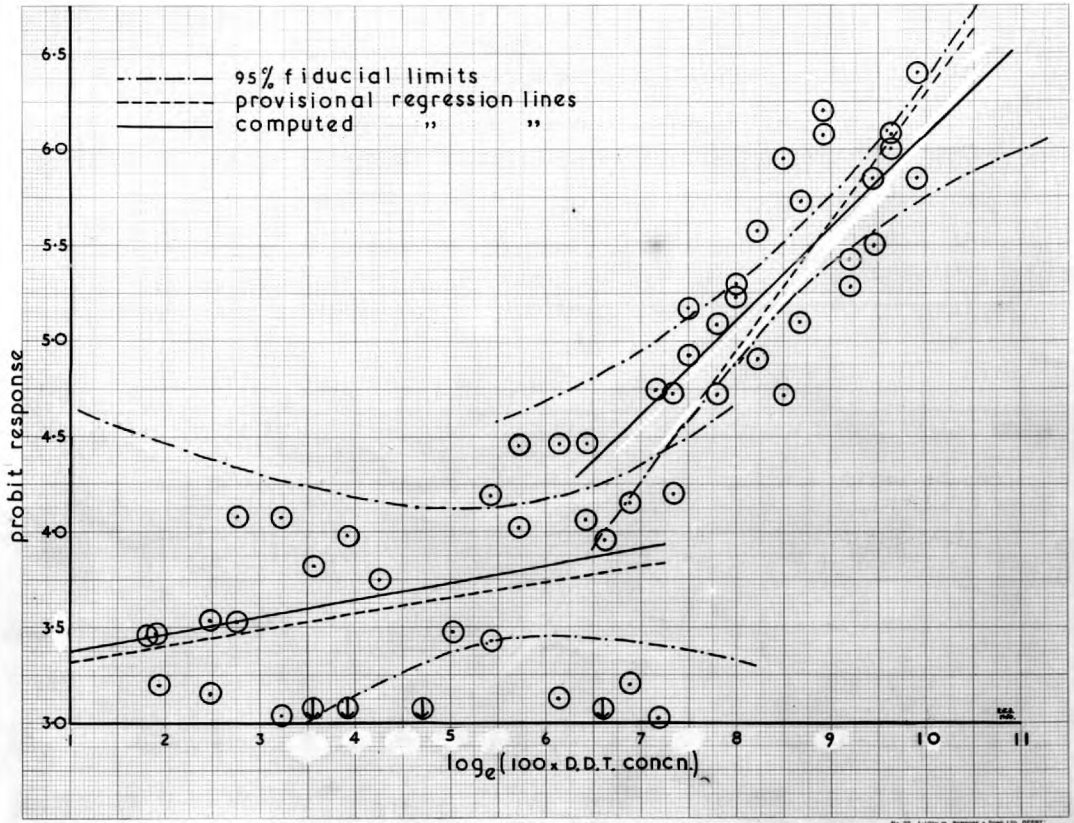


FIG.7.1: RELATION BETWEEN RESPONSE AND DOSE; SYSTEM C. GRANARIA – D.D.T. – O.I.L. FILM

The DDT was therefore applied to 7cm: Whatman No.1 filter papers in solution in a mixed solvent of volatile and non-volatile oils, in exactly the same way as the pyrethrins were applied in earlier sections. The sample used was pure p-p-dichlordiphenyl trichlorethane, m.p.106° C, recrystallized three times from alcohol. Primary responses could not, however, so conveniently be used to assess the toxic effect, so that mortality was employed as the criterion. In view of the very wide range of DDT concentrations necessary to cover the range of incomplete responses, it was decided to carry out a detailed examination of the dosage response relationship with this type of assay system. The author has observed that in a number of assay systems the slope of the regression of transformed responses on log.dose is not constant over a wide range of concentrations, as first one factor and then another predominate in determining its magnitude. (Page, Stringer and Blackith, 1946,1949., Blackith 1948, 1950a).

This situation obtains with the assay system now under consideration, as the data of Table 7.1 and Fig 7.1 show. This calibratory assay was conducted with a considerable number of batches of insects, 64 batches of 30 each all told, all drawn from the same culture jar. The insects were kept in continuous contact, at 25° C, with the treated papers, and estimation of the mortality was made after inspection under the 15 watt lamp as previously described. Experience had shown that marked heterogeneity was to be expected in this system much more so than was the case with the pyrethrum assays previously discussed. It was not known a priori how the

TABLE 7.1.

Relation between response and dose, system.

x.	n	r	p'	p	Empirical Probit
9·904	30	24	80	76·4	5·72
"	26	24	95	92·0	6·41
9·616	28	24	86	84·1	6·00
"	30	26	87	85·5	6·06
9·393	28	26	93	80·4	5·86
"	31	22	71	69·0	5·50
9·210	27	18	67	61·0	5·28
"	29	20	69	66·6	5·43
8·923	29	26	90	89·0	6·23
"	30	26	87	86·0	6·08
8·700	30	24	80	76·4	5·72
"	31	18	58	54·5	5·11
8·517	27	23	86	83·0	5·95
"	32	14	43	38·4	4·71
8·230	29	22	76	71·4	5·57
"	30	15	50	46·0	4·90
8·006	30	20	67	61·8	5·30
"	29	18	62	59·0	5·23
7·824	25	12	48	39·2	4·73
"	30	17	57	53·5	5·09
7·523	28	15	54	46·9	4·92
"	29	17	59	56·3	5·16
7·313	29	14	48	39·2	4·73
"	29	8	27	21·6	4·21
7·131	28	14	50	41·1	4·76
"	31	3	10	2·4	3·02
6·824	29	10	34	23·8	4·29
"	28	3	11	3·6	3·20

TABLE 7.1.

D.D.T. -oil film - Calandra granaria. (24 hrs. exposure).

Y	nw	y	n.y	nwx
6.25	9.2	5.484	50.2608	91.11
"	"	5.398	58.8616	"
6.05	9.8	5.988	58.6824	94.25
"	"	5.080	59.5840	"
5.90	10.8	5.840	62.4880	100.53
"	"	5.472	58.0689	"
5.75	11.0	5.185	57.0350	101.31
"	"	5.392	59.3120	"
5.60	12.8	6.093	77.9904	114.21
"	"	6.005	76.8384	"
5.40	14.0	5.684	79.5760	121.79
"	"	5.114	71.5960	"
5.25	12.7	5.856	74.3712	108.17
"	"	4.676	59.3852	"
5.10	15.8	5.529	76.3002	113.57
"	"	4.900	67.6200	"
4.90	15.6	5.583	72.1206	108.89
"	"	5.227	71.6872	"
4.80	12.9	4.721	60.9009	100.93
"	"	5.105	65.8545	"
4.55	11.6	4.941	57.3156	87 .27
"	"	5.185	60.1460	"
4.45	10.7	4.731	50.6217	78 .25
"	"	4.248	45.4536	"
4.35	10.0	4.807	48.0700	71 .31
"	"	3.557	35.5700	"
4.20	7.9	4.897	38.9463	53 .92
"	"	3.607	28.4953	"

x.	n	r	p'	p.	Empirical Probit
6·820	29	8	28	14·8	3·96
"	30	2	7	0·0	-∞
6·446	27	8	20	17·8	4·08
"	31	10	33	27·6	4·41
6·131	28	11	39	28·5	4·43
"	29	3	10	30·0	3·12
5·737	28	11	39	28·5	4·43
"	28	6	22	16·1	4·01
5·438	25	5	20	5·9	3·44
"	30	8	27	20·6	4·18
5·011	30	4	13	6·3	3·47
4·701	28	3	10	0·0	-·∞
"	31	3	10	2·4	3·02
4·249	29	5	17	10·5	3·75
3·912	28	8	28	14·8	3·96
"	29	2	7	0·0	-∞
3·555	28	7	25	11·8	3·82
"	30	1	3	0·0	-∞
3·219	26	8	30	17·8	4·08
"	30	3	10	2·7	3·07
2·708	30	9	30	17·8	4·08
"	29	4	14	6·8	3·51
2·485	28	5	18	3·2	3·15
"	29	4	14	6·8	3·51
1·946	27	5	19	3·6	3·20
"	30	4	13	6·3	3·47
1·792	31	4	13	6·1	3·45

Y.	nw	y	nwy	nwx
5:95	6:0	3:964	23:7840	39:72
"	"	5:344	20:0640	"
5:80	4:2	4:154	17:3628	27:07
"	"	4:649	19:5258	"
5:75	4:2	4:701	19:7442	25:75
"	"	4:031	16:9302	"
5:70	3:5	3:810	11:5850	19:03
"	"	4:361	15:2635	"
5:65	3:5	3:458	12:1975	17:54
"	"	3:135	10:9725	16:45
"	"	3:252	11:3820	"
3:60	2:7	3:795	10:2465	11:47
"	"	4:062	10:9674	10:56
"	"	3:061	8:2647	"
3:55	"	3:862	10:4274	9:60
"	"	3:061	8:2647	"
3:50	2:1	4:374	9:1854	6:76
"	"	3:216	6:7536	"
3:45	"	4:374	9:1854	5:69
"	"	3:525	7:4025	"
"	"	3:216	6:7536	5:22
"	"	3:525	7:4025	"
5:40	1:6	3:267	5:2272	3:11
"	"	3:447	5:5152	"
"	"	3:447	5:5152	2:87

	$\bar{x}$	$\bar{y}$
(Controls)	32	4
	36	4
	28	5
	27	5
	28	0
	30	3
	31	3
	29	0
	29	5

Upper Regression Line.

$nwx = 2,440 \cdot 46$

$nwy = 1,551 \cdot 48$

$nw = 285 \cdot 60$

$\bar{x} = 8 \cdot 5450$

$\bar{y} = 5 \cdot 5625$

nwx^2	$nwxy$	nwy^2
21,020·5539	13,166·8039	8,287·2072
20,853·7991	13,086·5858	8,212·2762
166·7548	80·2781	74·9310
		38·6470
		χ^2_{22} 36·2840

$b = 0 \cdot 4814$

$V(b) = 0 \cdot 00989$

$Y = 1 \cdot 2487 - 0 \cdot 4814_x$

Lower Regression Line.

$nwx = 685 \cdot 65$

$nwy = 461 \cdot 87$

$nw = 121 \cdot 40$

$\bar{x} = 5 \cdot 6478$

$\bar{y} = 3 \cdot 8045$

nwx^2	$nwxy$	nwy^2
4,085·5565	2,628·2218	1,855·7389
3,872·4541	2,608·5700	1,757·1909
213·1024	19·6518	98·5480
		1·8139
		χ^2_{22} 96·7341

$b = 0 \cdot 0923$

$V(b) = 0 \cdot 0157$

$Y = 5 \cdot 2852 - 0 \cdot 0923x.$

"breaks" in the line would be distributed, if indeed they occurred at all, and this point could scarcely be investiggted by small scale experiments, as only the most marked effects would then prove significant.

Three important conclusions can be drawn from Fig. 7.1, together with the statistical analysis of the data of Table 7.1. The first conclusions is that a marked change of slope occurs, before which the regression of response on dose is not significant, although responses considerably higher than the controls occur down to the lowest concentrations of the insecticide tested. Secondly, the regression of response on the higher concentrations is effectively linear, although of a magnitude many times less than that of the analogous pyrethrum system. It is unlikely that the fitting of two straight lines properly represents the dosage-response relationship. However, with a considerable body of data, the computational difficulties are thereby considerably reduced compared with the fitting of a continuous curve of, say, quadratic form. Provided that the limitations of this form of representaion are borne in mind, it is unlikely to be misleading.

The third conclusion to be drawn is that over the lower range of doses, the responses are markedly heterogeneous. Over this lower range where the regression is non-significant, the heterogeneity, and therefore the 95% fiducial limits shown in Fig. 7.1, are probably greatly exaggerated by the small expectations corresponding to the very low levels of response involved, but it is apparent that there is a very real

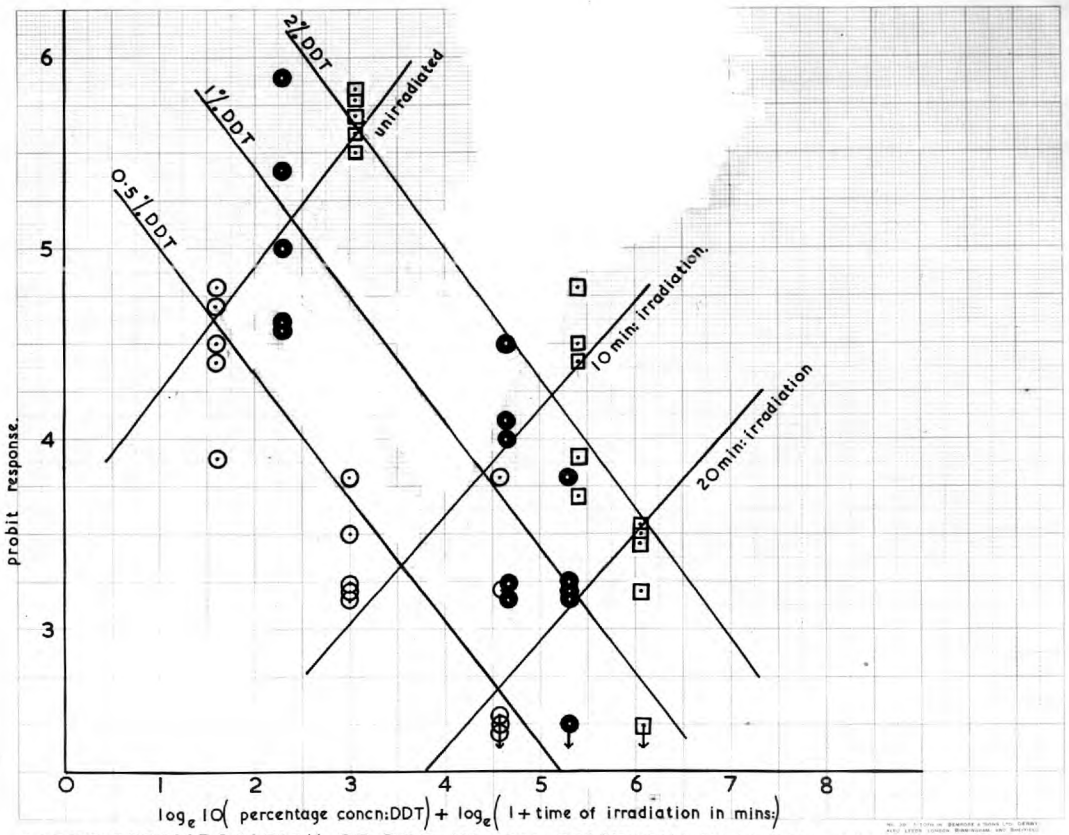


FIG. 7.2: DECOMPOSITION OF DDT-IN-OIL SOLUTIONS IN ULTRA-VIOLET LIGHT.

departure from homogeneity. On the other hand, in the case of the regression line over the higher range, the heterogeneity is scarcely significant, (χ^2_{22} = 36.2840, χ^2_{22} for 5% level of P is 33.924.) In view of the reduction which pooling the points with low expectations would produce in this quantity, the heterogeneity is quite reasonable.

Action of light on DDT.

In order to cover the possible effect of DDT concentration on the rate of decomposition, it was decided to conduct the experiment in a form suitable for analysis by means of the probit plane. (Finney 1947b). Five replicates of each of the combinations of three concentrations and three times of irradiation were used. The three concentrations were equally spaced on a logarithmic scale, and although the times of irradiation were equally spaced on a linear scale, it was thought more appropriate to use the logarithms of these quantities in the analysis, since the action of the ultraviolet light is essentially to decompose the DDT, and thereby reduce the effective dosage.

The results obtained, together with the provisional regression lines fitted, are shown diagrammatically in Fig. 7.2 the data on which this figure is based, together with the details of the computation of the probit plane, are given in Table 7.2.

The main conclusion to be drawn is that irradiation under the conditions employed decomposes the DDT at a rate which

TABLE 7. 2

Decomposition of D.D.T. -in-oil solution by ultra violet light.

Computation for fitting Probit plane.

x_1	x_2	n	r	p'	p(c=3)	Empirical Probit
1·61	3·00					
		30	1	3	0	-
"	"	30	4	15	12	5·8
"	"	29	2	7	4	5·2
"	"	32	1	3	0	-
"	"	30	1	3	0	-
2·30	"					
		30	2	7	4	5·2
"	"	30	1	3	0	-
"	"	30	2	7	4	5·2
"	"	31	4	15	12	5·8
"	"	30	2	7	4	5·2
2·00	"					
		29	2	7	4	5·2
"	"	32	3	10	7	5·5
"	"	31	1	3	0	-
"	"	30	3	10	7	5·5
"	"	30	3	10	7	5·5
1·61	2·30					
		27	4	15	12	5·8
"	"	29	2	7	4	5·2
"	"	29	2	7	4	5·2
"	"	30	3	10	7	5·5
"	"	29	2	7	4	5·2

X	w	nw	y	nwx_1	nwx_2	nwy
2·7	0·0138					
		0·38	2·321	0·612	1·140	0·852
"	"	0·38	6·558	0·612	1·140	2·492
"	"	0·37	3·755	0·596	1·110	1·381
"	"	0·41	2·321	0·660	1·230	0·952
"	"	0·38	2·321	0·612	1·140	0·882
3·2	0·730					
		2·19	3·252	5·037	6·570	7·122
"	"	2·19	2·745	5·037	6·570	6·012
"	"	2·19	3·252	5·037	6·570	7·122
"	"	2·26	4·265	5·198	6·780	9·639
		2·19	3·252	5·037	6·570	7·122
		11·02		25·348	33·060	37·017
3·6	0·1828					
		5·30	3·328	15·900	15·900	17·638
"	"	5·85	3·528	17·550	17·550	20·639
"	"	5·67	3·061	17·010	17·010	17·356
"	"	5·48	3·528	16·440	16·440	19·333
		5·48	3·528	16·440	16·440	19·335
		40·78		83·340	83·340	94·299
		40·78		111·778	122·100	137·905
3·3	0·953					
		2·57	4·102	4·138	5·911	10·542
"	"	2·76	3·251	4·444	6·348	8·973
"	"	2·76	3·251	4·444	6·348	8·973
"	"	2·86	3·570	4·605	6·578	10 ·216
"	"	2·76	3·251	4·444	6·348	8·973
		13·71		22·075	31·533	47·671

TABLE 7. 2.

Decomposition of D.D.T. -in-oil solution by ultra violet light.

Computation for fitting Probit plane.

x_1	x_2	n	r	p'	p(e-3)	Empirical Probit
2·50	2·30					
		29	9	31	29	4·5
"	"	34	8	23	19	4·1
"	"	31	6	19	16	4·0
"	"	30	3	10	7	3·2
"	"	31	3	10	7	3·2
3·00	2·30	29	12	42	40	4·8
"	"	30	9	30	28	4·4
"	"	32	4	13	10	3·7
"	"	30	10	33	30	4·5
"	"	31	5	16	13	3·9
1·61	0·00	31	14	45	43	4·8
"	"	30	9	30	28	4·4
"	"	32	11	34	32	4·5
"	"	30	5	17	14	3·9
"	"	30	13	43	38	4·7
2·30	0·00	29	24	83	82	5·9
"	"	29	11	38	36	4·6
"	"	29	19	66	65	5·4
"	"	31	12	39	37	4·6
"	"	30	16	53	51	5·0

Y	w	nw	y	nwx_1	nwx_2	nwy
3•8	0•2919	8•46	4•701	19•458	19•458	39•770
"	"	9•92	4•186	22•816	22•816	41•525
"	"	9•05	4•031	20•815	20•815	36•481
"	"	8•46	3•568	19•458	19•458	30•185
"	"	9•05	3•568	20•815	20•815	32•290
		44•94		103 •562	103•562	180•251
4•2	0•4586	12•72	4•849	38•160	29•256	61•679
"	"	13•16	4•435	39•480	30•268	58•364
"	"	14•04	3•814	42•120	32•292	53•548
"	"	13•16	4•504	39•480	30•268	59•272
"	"	13•60	5•917	40•800	31•280	55•271
"	"	66•68		200•040	153•364	286•137
		125•33		325•477	288•359	514•059
4•6	0•5510					
		17•08	4•832	27•498	0•000	82•531
"	"	16•53	4•425	26•613	"	73•145
"	"	17•63	4•533	28•384	"	79•917
"	"	16•53	4•044	26•613	"	66•847
"	"	16•53	4 696	26•613	"	77•625
		84•30		135•71	0•000	380•065
5•2	0•5956	17•27	5•816	39•721	0•000	100•448
"	"	17•27	4•690	39•721	"	80•996
"	"	17•27	5•381	39•721	"	92•930
"	"	18•46	4•716	42•458	"	87•057
"	"	17•87	6•023	41•101	"	89•761
		88•14		202•752	0•000	451•186

x_1	x_2	n	r	p'	p(c-5)	Empirical Probit
5·00						
"	0·00	28	22	79	78	5·8
"	"	28	20	71	70	5·5
"	"	28	21	75	74	5·6
"	"	28	22	79	78	5·8
"	"	30	23	77	76	5·7

X	w	nw	y	nwx_1	nwx_2	nwy
5·6						
	0·5351	14·98	5·765	44·940	0·000	86·330
"	"	14·98	5·585	44·940	"	83·735
"	"	14·98	5·043	44·940	"	84·552
"	"	14·98	5·763	44·940	"	86·330
"	"	16·05	5·705	48·150	"	91·553
		75·97		227·910	0·000	431·460
		248·41		766·523	0·000	1,368·711
		414·46		1003·608	10·416	1,924·675

x_1	x_2	n	r	p'
0%	0 Mins.			
"	"	29	1	
"	"	30	0	
"	"	29	5	
"	"	32	1	
"	"	30	1	
"	"	31	0	
"	"	30	0	
"	"	29	1	
"	"	29	0	
"	"	25	1	
"	"	30	0	
(Controls)		354	10	3·08

$\bar{x}_1 = 2{\cdot}4215$

$\bar{x}_2 = 0{\cdot}9903$

$\bar{y} = 4{\cdot}6436$

	nwx_1^2	nwx_1x_2	nwx_2^2
Between replicates	4·9781	0·2736	17·2800
	4·9799	0·2736	17·2800
	0·0000	0·0000	0·0000
	58·2958	76·0360	99·1800
	58·2958	76·0368	99·1800
	0·0000	0·0000	0·0000
	250·0200	250·0200	250·0200
	250·0200	250·0260	250·0200
	0·0000	0·0000	0·0000
	55·5407	50·7725	72·5259
	55·5439	50·7725	72·5259
	0·0000	0·0000	0·0000
	257·7526	257·7526	257·7526
	257·7526	257·7526	257·7526
	0·0000	0·0000	0·0000
	600·1200	460·0920	385·7372
	600·1200	460·0920	386·7372
	0·0000	0·0000	0·0000
	218·5198	0·0000	0·0000
	218·5076	0·0000	0·0000
	0·0032	0·0000	0·0000
	466·2606	0·0000	0·0000
	466·2606	0·0000	0·0000
	0·0000	0·0000	0·0000
	883·7300	0·0000	0·0000
	883·7300	0·0000	0·0000
Totals	0·0000	0·0000	0·0000
	8,558·1936	1,083·9267	1,029·4757
	8,430·2801	993·8208	406·4174
	124·9685	90·1059	625·0583

$\bar{x}_1 = 2{\cdot}4215$

$\bar{x}_2 = 0{\cdot}9903$

$\bar{y} = 4{\cdot}6438$

	nwx_1y	nwx_2y	nwy^2
Between replicates	10·6065	19·7665	27·8016
	10·6110	19·7670	22·6119
	0·0000	0·0000	5·1897
	85·1370	111·0495	127·0958
	85·1370	111·0519	110·9066
	0·0000	0·0000	16·1892
	282·8970	282·8970	321·0540
	282·8970	282·8970	320·0972
	0·0000	0·0000	0·9568
	76·7563	100·6434	167·2067
	76·7569	100·6455	165·7567
	0·0000	0·0000	1·4500
	414·5773	414·5773	7,309·4811
	414·5773	414·5773	7,229·7336
	0·0000	0·0000	79·7475
	856·2020	856·1062	1,237·7815
	856·4110	856·1157	1,327·8792
	0·0000	0·0000	0·9113
	611·8951	0·0000	1,719·5764
	612·8974	0·0000	1,713·5161
	0·0000	0·0000	6·0603
	1,037·7398	0·0000	2,325·5286
	1,037·7378	0·0000	2,309·8075
	0·0020	0·0000	15·7211
	1,294·3770	0·0000	2,451·0118
	1,294·3660	0·0000	2,450·4111
	0·0110	0·0000	0·6007
Totals.	4,072·3800	1,596·0397	9,107·8042
	4,418·4258	1,806·8044	8,033·1971
	+ 253·9001	- 210·8447	+1,074·6071

$$124{\cdot}9685\ b_1 - 90{\cdot}1059\ b_2 = 255{\cdot}9604$$
$$90{\cdot}1059\ b_1 - 623{\cdot}058\ b_2 = -210{\cdot}8447$$

$$124{\cdot}969\ b_1 - 90{\cdot}106\ b_2 = (1,0)$$
$$90{\cdot}106\ b_1 - 623{\cdot}058\ b_2 = (0,1)$$

c - covariance matrix $= \begin{pmatrix} +40{\cdot}008954, & -0{\cdot}001298 \\ -0{\cdot}001298, & +0{\cdot}001752 \end{pmatrix}$

$$b_1 = +2{\cdot}5412$$
$$b_2 = -0{\cdot}7059$$

Residual Heterogeneity between doses. $\chi^2_{42} = 1,074{\cdot}6071 - 799{\cdot}8466 = 274{\cdot}7605$

Components of Heterogeneity

Item	D.F.	Sums of Squares.	Mean Square.
Multiple Regression	2	799·8466	399·9233
Between Doses.	6	210·7072	35·1179
Between Replicates	36	64·0533	1·7793
Total Heterogeneity	44	1,074.6071	

$\sqrt{2\chi^2} = 11·34$

$\sqrt{2n-1} = 8·42$

normal dev. 2·92 $\quad P \approx 0·0035$

Significant Heterogeneity between replicates.
" " " doses.

Heterogeneity factor = 6·5419

$V(b_1) = 0·0524$ $\quad V(b_2) = 0·0105$

$\sigma b_1 = 0·229$ $\quad \sigma b_2 = 0·102$

Regression of response on concentration, in natural log. units
= 0·2386 ± 0·0214.

may be estimated from the relative magnitude of the regression coefficients for response on log. concentrations and log. time, and corresponds to a reduction in the DDT concentration of about 1.5% in 15 mins, or 75% of the original DDT present. This rate of decomposition is almost identical with that found for the pyrethrins under the same conditions, as reported earlier. It must be admitted that the conditions employed are perhaps the least favourable to the DDT, but the comparison is at least a striking one.

The inclusion of replicate for each point of this probit plan allows the total heterogeneity of the data to be partitioned in the way shown in Table 7.2. From this table we see that the variation between replicates, although significantly greater than expectation, is not excessively large, and that most of the discrepancies arise from comparisons between doses. Whilst a further cycle of computation would probably improve matters, there does seem some evidence that the points are inadequately fitted by a probit plane of this type, and some curvature of the plane, concave to the time axis, might be shown by more detailed experiments. If such an effect existed, it might indicate that the rate of decomposition of the DDT was being slowed down by accumulated breakdown products.

Protectant action of Benzene azo- β - naphthol.

In view of the severe decomposition suffered by DDT in ultra-violet light, it was thought that benzene azo- β - naphthol, the most successful of the dyes tested for their

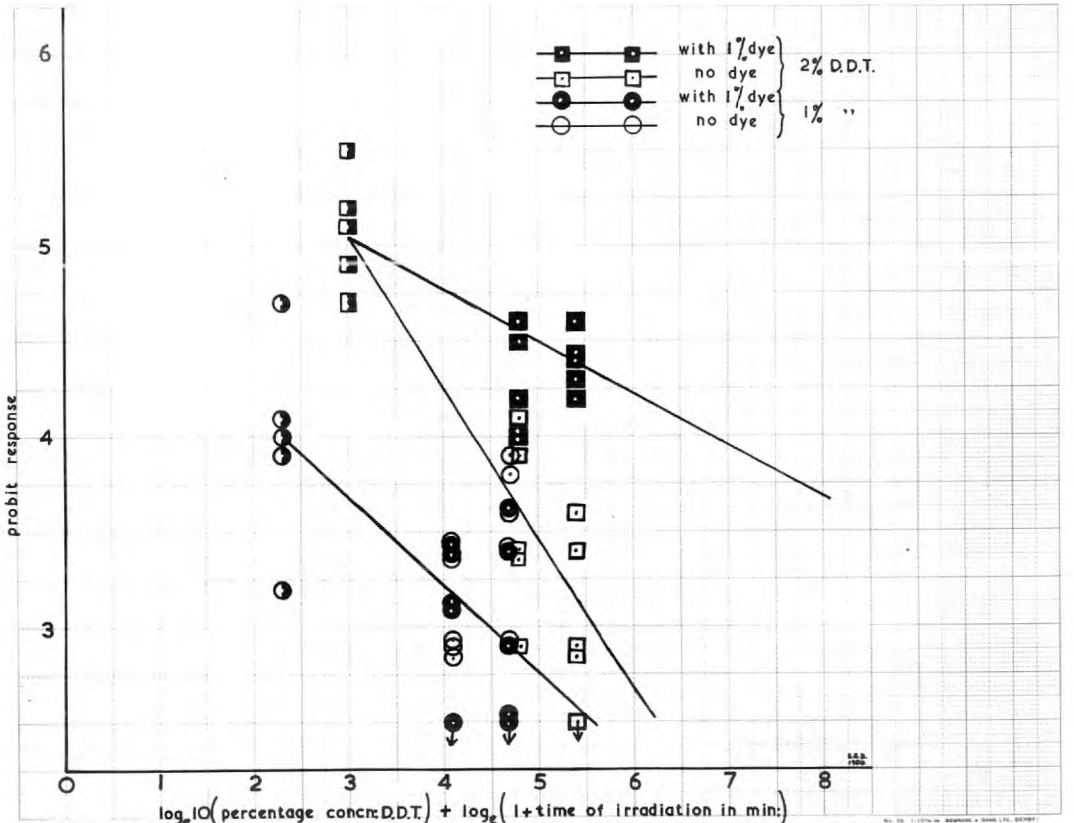


FIG. 7.3: PROTECTANT ACTION OF B.D.H. OIL SOLUBLE YELLOW ON D.D.T.

protectant action on the pyrethrins, might exert a similar effect on DDT. This situation would obviously arise if the wavebands absorbed by the dye were responsible for much of the photolysis of both insecticides. An experiment was therefore designed analogous to the preceding one, one probit plane composed of results from papers irradiated with 1% of the dye, the other without such protectants. As relatively low responses were obtained with the papers impregnated with 0.5% DDT solutions or irradiated for 20 min. it was decided to omit the former concentration and to irradiate the filter papers for 5, 10, and 15 min. only.

In spite of these precautions, a considerable increase in the resistance of the weevils between the occasions of testing caused low responses to be obtained with the lower concentration higher time combinations. As a result of the peculiar property of the assay system revealed by Fig. 7.1, namely that for lower concentration ranges there is no significant regression of probit response on log. dose, there is no seperation of the points representing papers irradiated with and without protection, when these had initially a 1% residual solution of the insecticide. It is perfectly obvious from Fig. 7.3 that no significant seperation exists, statistical proof is superfluous.

On the other hand, for papers impregnated with 3% DDT residual solution, the protectant action of the dye becomes clear, and the seperation of the lines in Fig. 7.3 becomes well marked. The negative slope of these lines represents

TABLE. 7. 3

Decomposition of D.D.T.-in-oil solutions by ultra violet light, in presence and absence of B.D.H. oil soluble yellow dyestuff. (Benzene azo beta-naphthol).

Computation for fitting ofProbit Regression Lines.

x_1	x_2	n	r	p'	p(c= 5)	Empirical Probit
		no dyestuff present				
2·30	2·40					
		51	6	19	15	3·9
"	"	29	3	10	5	3·4
"	"	33	5	15	11	3·8
"	"	30	4	13	8	3·6
"	"	29	2	7	2	2·9
2·30	1·79					
		30	2	7	2	2·9
"	"	31	3	10	5	3·4
"	"	35	2	7	2	2·9
"	"	32	3	10	5	3·4
"	"	34	2	7	2	2·9
2·30	0					
		35	8	23	19	4·1
"	"	30	12	40	37	4·7
"	"	29	5	17	13	3·9
"	"	32	3	9	4	3·2
"	"	30	6	20	16	4·0
3·00	2·40					
		29	2	7	2	2·9
"	"	29	1	3	0	$-\infty$
"	"	31	2	7	2	2·9
"	"	30	4	13	8	3·6
"	"	29	3	10	5	3·4

TABLE 7. A (Contd).

Decomposition of D.D.T. -in-oil solutions by ultra violet light, in presence of D.D.H. oil soluble yellow dye-stuff. (Benzene azo-beta-naphthol).

Computation for fitting of Probit Regressions/ Lines.

x_1	x_2	n	r	p'	p(c. 5)	Empirical Probit
		no dyestuff present				
3·00	1·79					
		30	3	10	5	3·4
"	"	30	3	10	5	3·4
"	"	30	3	17	12	3·8
"	"	52	7	23	18	4·1
"	"	31	2	7	2	2·9
3·00	0					
		29	12	41	37	4·7
"	"	30	17	57	55	5·1
"	"	31	15	48	45	4·9
"	"	33	23	70	69	5·5
"	"	31	18	58	56	5·2
		1% dyestuff present				
2·3	2·40					
		30	4	13	8	3·6
"	"	32	3	10	5	3·4
"	"	29	1	3	0	$-\infty$
"	"	32	1	3	0	$-\infty$
"	"	29	2	7	2	2·9
2·3	1·79					
		30	1	3	0	$-\infty$
"	"	30	3	10	5	3·4
"	"	30	3	10	5	3·4
"	"	29	2	7	3	3·1
"	"	29	2	7	3	3·1

TABLE 7. 3. (cont'd)

Decomposition of D.D.T.-in-oil solutions by ultra violet light, in presence and absence of B.D.H. oil soluble yellow dyestuff. (Benzene azo-beta-naphthol).

Computation for fitting of Probit Regression Lines.

x_1	x_2	n	r	p'	p(c=5)	Empirical Probit
3·00	2·4					
		27	8	30	26	4·4
"	"	29	9	31	27	4·4
"	"	30	7	25	19	4·1
"	"	30	11	37	34	4·6
"	"	29	8	28	24	4·3
3·00	1·79					
		30	10	35	29	4·5
"	"	33	7	21	17	4·0
"	"	30	11	37	34	4·6
"	"	32	8	25	21	4·2
"	"	29	6	21	17	4·0
0%	0					
		30	1			
"	"	30	1			
"	"	34	0			
"	"	31	2			
"	"	30	3			
"	"	25	1			
"	"	30	4			
"	"	30	1			
"	"	32	1			
(Controls)		272	14	5·1		

the rate of decomposition of the insecticide, so that the equation to each line was calculated. Since the points representing the responses on the papers not irradiated at all are common to both lines, the latter will obviously intersect at that level, and any attempt to constrain them to parallelism would be artificial. Seperate equations were therefore fitted to the data of Table 7.3.

The slope of the regression of response on log.(1 + time of irradiation) in the absence of dye is -0.793, and this set of data is homogeneous, since after fitting the straight line χ^2_{13} =12.2785. The similar curve for protected DDT has a slope of -0.402, but this set of points exhibits marked heterogeneity, χ^2_{13} =301.0188. Details of this calculation follow those given in Tables 3.1, 7.1, and 7.4.

Thus, it will be noted, the effect of 1% benzene azo-β - naphthol is roughly to double the life of the DDT films in ultra-violet light, since the initial concentration of the two sets of papers was the same, and a regression coefficient are in the ratio 2:1 almost exactly. The somewhat unexpected similarity of DDT to the pyrethrins, therefore, extends not only to their rates of decomposition when oily solutions are irradiated in ultra-violet light, but the addition of benzene azo-β - naphthol approximately doubles the effective life of both substances under such conditions. The widely different stability of the two compounds in daylight not containing an appreciable ultra- violet component, which is a generally noted fact of experience (Martin, 1949),

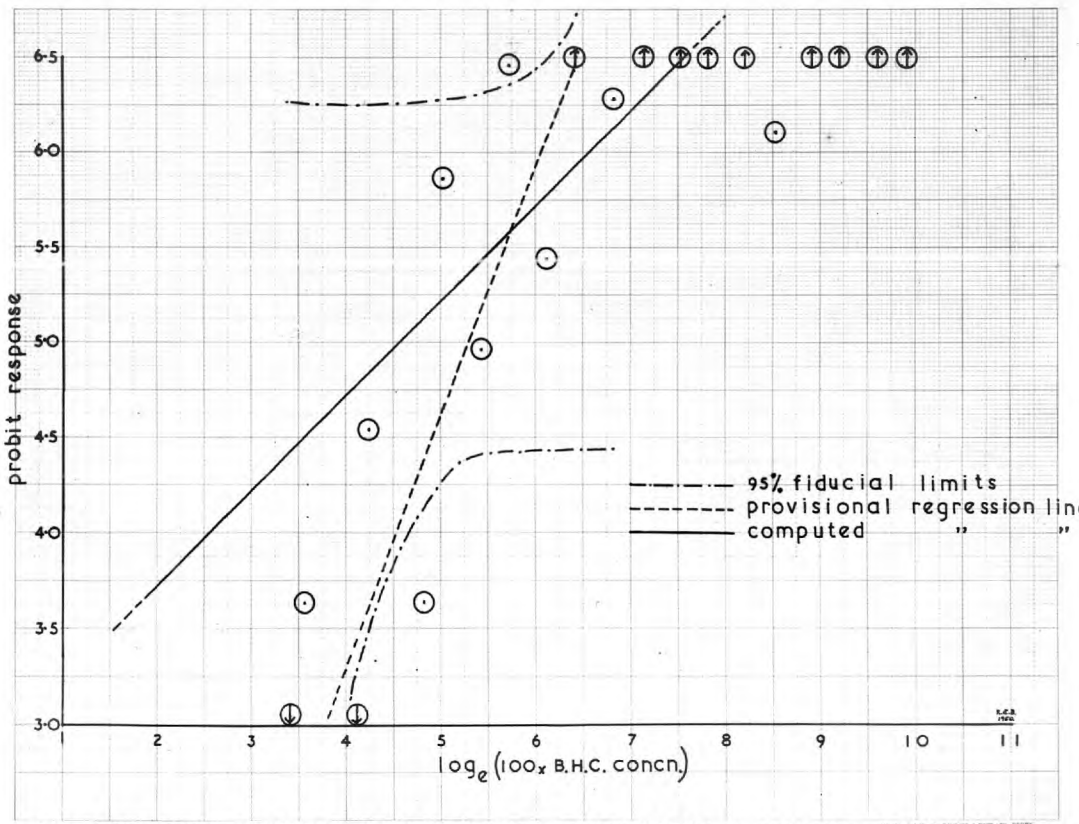


FIG.7.4: RELATION BETWEEN RESPONSE AND DOSE; SYSTEM C. GRANARIA — B.H.C. — OIL FILM

would appear explicable on the assumption that this range of wavelengths, whilst being much less active against the pyrethrins than ultra-violet, is at least slightly active, though not causing any decomposition of DDT. That is to say, that the loss of photolytic activity of light with increasing wavelength is much less rapid when pyrethrins are the insecticide than is the case with DDT films. There is, of course, no guarantee that the waveband of the lamp used was equally strong in the regions most active in catalysing the breakdown of the two different insecticides, which do not necessarily have similar absorption spectra in the ultra-violet.

The action of ultra-violet light on B.H.C.

In view of the fact that benzene hexachloride is formed by irradiating a mixture of chlorine and benzene, the substance would scarcely be expected to decompose in ultra-violet light with much rapidity. Nevertheless, a slow reaction is possible in which the most toxic "γ"— isomer could acquire sufficient energy to undergo re-arrangement to the other less toxic isomers, and this possibility was thought worthy of investigation.

As with the other insecticides tested, a "calibratory" assay was conducted to determine whether the form of the dosage-response curve was suitable for further experiments, when the pure "γ" - isomer of BHC was substituted for DDT, all the other conditions remaining exactly as for the DDT assay previously described. The 24 batches of 30 C.granaria weevils

TABLE 7.4

Relation between response and dose, system B.H.C.

x	n	r	p'	p(c=2)	Empirical Probit.
9·904	29	29	100	100·0	
9·616	30	30	100	100·0	
9·210	30	30	100	100·0	
8·923	30	30	100	100·0	
8·577	31	27	87	86·8	
8·230	29	29	100	100·0	
7·824	30	30	100	100·0	
7·523	30	30	100	100·0	
7·131	31	31	100	100·0	
6·824	31	28	92	91·9	6·40
6·446	30	29	97	97·0	6·88
6·131	27	19	68	67·3	5·45
5·737	29	27	93	92·8	6·46
5·438	30	15	50	49·0	4·97
5·011	31	25	81	80·5	5·86
4·828	30	3	10	8·1	5·60
4·249	30	10	33	31·6	4·52
4·155	30	1	3	1·0	2·67
5·555	30	3	10	8·1	5·60
3·434	31	1	3	1·0	2·67
Controls	24	0			
"	30	2			
"	30	0			
"	28	0			
"					
	112	2	1·8		

$\bar{y} = 5·3255$

$\bar{x} = 5·2107$

$y = 0·4907x + 2·7686$

Oil film - Galandra granaria

Y	nw	y	nwy	nwx
weights attached to points in this region negligable.				
8·2	0·2433			
7·8	0·7227	8·125	5·8705	5·4568
7·5	1·5125	7·854	11·8792	10·7856
6·9	4·6866	6·118	28·6726	13·2350
6·5	7·8993	6·784	53·5888	50·9189
6·1	10·6758	5·208	55·5996	65·4553
5·6	16·2786	6·213	101·1389	93·3903
5·2	18·1821	4·972	90·4014	98·8742
4·6	17·5751	5·864	103·0604	88·0688
4·3	14·7072	3·845	56·5492	71·0064
3·6	7·2321	5·265	38·0770	30·7292
3·4	5·1924	2·996	15·5564	21·4705
2·9	1·5441	4·767	7·3607	5·4893
2·7	0·8076	2·674	2·1595	2·7733
	107·0161		569·9142	557·6516

Σ nwx^2

3,131·8405
3,035·0779
96·7626

Σ nwxy

3,017·1492
2,969·6669
47·4823

Σ nwy^2

3,157·0884
2,905·6656
251·4128
23·2996
x^2_{11} = 228·1132

b = 0·4907

were all derived from a common culture, and kept in continuous contact with the impregnated filter papers for 24 hours, after which period the proportionate mortality of each batch was determined by inspection with the 25 watt lamp as previously described. The metal rings imprisoning the insects were not covered for these assays involving BHC, so as to avoid building up concentrations of the vapour which could exert a fumigating effect independent of the contact action. Normally stored products insects are rather sensitive to draughts so that the rings are covered with a second untreated filter paper before insertion into the constant temperature cabinet.

The results obtained are shown in Fig. 7.4, from which it will be observed that there is no evidence for any departure from linearity of this regression line, and that satisfactory assays are possible with this system. It is noteworthy that the slope of the fitted regression line is almost identical with that for the corresponding DDT assay, over the sensitive range of the latter, but that the BHC is much the more toxic to C.granaria. A precise comparison is not possible, since in these experiments the relative potencies are confounded with tolerance fluctuations, the experiments not being designed to include such comparisons. Lord (1948) has also found the slopes relating to oily solutions of DDT and BHC to be very similar, using a crawling insect assay with Oryzaephilus surinamensis as test insect. The similarity of slope for oil-base solutions of the two insecticides has also been found by Gersdoff and McGovran(1945), using

TABLE 7.5.

Action of ultra-violet light on B.H.C solutions in Heavy white oil. All irradiated filter papers impregnated with 0·02% w/v pure γ- isomer of B.H.C in 0·1 ml. "White oil 70".

Time of irradiation (minutes)	Replicate	n.	r.	p. %
0	1	30	10	33
	2	29	16	55
	3	31	18	58
5	1	30	10	33
	2	30	12	40
	3	31	20	65
10	1	32	11	34
	2	31	10	32
	3	29	18	62
15	1	30	12	40
	2	30	6	20
	3	30	8	27
Controls		90	0	-

Analysis of variance of angular transforms.

Item	D.F.	Sums of squares	Variance	F.
Times	3	250·3825	83·4608	1·136
Residue	8	587·8800	73·4850	
Total	11	838·2625		

M.domestica as test insect. The much lower slope obtained by these workers with the pyrethrins is probably an artefact of the flying insect system used for the assay, for which simple probit analysis is not appropriate. The difference in the relative potencies of the two compounds for the assays with flying and crawling insects, 9 and 25 respectively is much greater than could reasonably be attributed to a tolerance fluctuation among the insects, and is probably caused by the change of test system.

In view of the similarity between the test systems, the examination of the action of ultra-violet light on BHC could have proceeded on the same lines as that for DDT. However, since it was expected that the effects would be less marked, an alternative design was adopted. A set of 12 filter papers were impregnated with 0.02% $^{w}/_{v}$ residual concentration of BHC, and divided into four sets of three, each trio being irradiated for one of the four periods 0,5,10,15 mins. Afterwards the filter papers were assayed with 12 batches of 30 weevils, all drawn from the same culture, and the analysis of variance, shown in Table 7.5, was carried out on the angular transforms of the percentage responses obtained. As the mean square for "Times of irradiation" is only slightly greater than that for error, there is no evidence that the insecticide is affected by ultra-violet light.

Discussion.

The work reported in this thesis has shown clearly the relative rates at which the pyrethrins and DDT become inactivated under the various environmental conditions to which they are subjected in practice. It is interesting to compare these rates for the different insecticides under various conditions and to relate the results obtained in this thesis to those of some other workers.

Qualitatively, there is no material discrepancy between the results reported here and those of earlier and contemporary workers. Quantitatively, on the other hand, differences do appear, although most of these are accountable for in terms of the differences in the experimental techniques used.

For example, Tattersfield and Hobson (1931) report that pyrethrum powder deteriorates at roughly logarithmic rates when exposed to both air and sunlight in open trays. Also, it was found that the pyrethrin II constituents appeared to decompose at a slightly lower rate than the pyrethrin I complex. On the other hand, no such evidence is available from the work reported, in Section 4 of this thesis, where the pyrethrins were found to decompose linearly with time in the presence of ultra-violet light. Apart from the fact that chemical estimation, particularly of pyrethrin II, is not necessarily a good index of the biologically active compounds present (Mitchell 1949), a certain amount of "cellular protection" is afforded the constituents most shaded within the particles

of dust. (Martin 1949). The externally situated molecules would then be expected to decompose according to the rate of the light-catalysed reaction, those in theprotected parts of the grains of powder according to the rate of decomposition in the dark. The graded transition between the two rates would account for the observations quoted. It does not appear likely that the natural colouring materials, chorophylls, xanthophylls, etc., in the flowers and extracts would materially reduce the photolysis, since they are decomposed so much more rapidly than the pyrethrins that their protectant action, if any, would soon be eliminated.

The work surveyed by Martin clearly demonstrated the activity of sunlight in decomposing the pyrethrins in thin layers althought the ultra-violet constituents were filtered out by exposing thelayers of dust in glass-houses. Shielding the layers from direct illumination reduced the rate of decomposition by a factor of about 10. Although this factor is very much less than that between the irradiated filter papers and those stored in the dark, (vide section 4) which is of the order of 10^4, the difference between the conditions is much less extreme.

It is possible, as a result of the experimental work outlined above, to form a fairly clear picture of the ways in which pyrethrum insecticides decompose. There appear to be four quite distict chemical processes which can be isolated by varying the experimental conditions so that one or other of them is predominant, and forms the effective rate-determining

process.

Reaction Mechanism I.

Tattersfield (1928) showed that damp whole pyrethrum flowers, stored in the dark, lost activity through the development of moulds and fermenting micr̊organisms. With dried material, or extracts, this mode of decomposition is not likely to be important.

Reaction Mechanism II.

Secondly, with flowers or extracts stored in the dark, there is a direct oxidation mechanism which can be inhibited by the presence of anti-oxidants, or by storage of the material in an atmosphere deficient in oxygen.

Reaction Mechanism III.

In addition, there is the light-catalysed oxidation, which is subject to the same methods of inhibition and also acceleration by moisture. These two reactions may involve the same or seperate parts of the pyrethrins molecules, and it would only be possible to settle this point by chemical investigation of the breakdown products, an investigation which would itself be dependent on the availability of the various pure compounds involved in sufficient quantity for chemical analysis.

Reaction Mechanism IV.

Fourthly, there can occur a distinct photolytic reaction of the pyrethrins in ultra-violet light, extending perhaps to a limited extent in visible light, which appears to take

place in the absence of oxygen. This photolysis is many times more rapid than any of the other processes, and is not detectably inhibited by removing moisture or oxygen from the surrounding atmosphere, nor by the presence of anti-oxidants; it may however, be materially modified by adding to the pyrethrum preparation a dye or other substance which absorbs strongly at similar wavebands to those of the insecticide. The most efficient such substance which has been investigated here is benzene azo-β-naphthol, which reduces the rate of photolysis by a factor of two, when present at about the same concentration as that of the insecticides used in this work.

These conclusions confirm and amplify the tentative suggestions by Martin(1949) For example, the greater effectiveness of blue rather than red light reflects the transition from a type III reaction mechanism to one of Type IV. Exposure of dusts to sunlight involves reaction mechanisms III and IV, so that anti-oxidants would, underthese conditions, be expected to show a slight beneficial effect such as Martin found. On the other hand, with pyrethrum-in-oil spray deposits in the open air, anti-oxidants were not found effective. It has now been demonstrated that under these conditions about 70% of the decomposition is by reaction mechanism IV, when the sun is shining.

Anti-oxidants, such as hydroquinone, are also well-known to be inhibitors of polymerisation reactions such as the Diels-Alder side-chain condensation suggested by Harper(1948). This reaction is very similar to that put forward earlier

by West (1943) in which the formation of insoluble substances of no insecticidal activity but assaying chemically as "pyrethrins" was ascribed to polymerisation of molecules across the pentadienyl side-chain. Again, the differentiation between these two possible reaction mechanisms will have to await the availabiltiy of pure compounds, and their reaction products,(see, for example, Bushland et al 1945).

This list of possible reaction mechanisms and their inhibitors is not exhaustive. The alleged protective action of piperonyl butoxide certainly does not exist to any material extent when mechanism IV is the rate-controlling reaction. Although it is possible that the other reactions might be inhibited by a reduction of the free energy of pyrethrum in films brought about by the synergist, it does not seem very likely that this effect, should it exist, could be of much importance.(Page and Blackith 1949a). Another point which has not received attention is the possibility that the presence of the resinoid materials associated with the pyrethrins might influence their stability. This type of investigation is of even greater importance with extracts from derris and the other rotenone-containing plants, where the apparent toxicity has been recognised to be greatly influenced by the associated resins. Since the stability of the rotenone and its associated compounds is being investigated by another worker, the problem has not been included in the scope of this thesis.

With DDT, the situation is considerably simpler. The

persistance of DDT and BHC in soil indicates that microbiological decomposition is not an important factor in their stability. Fleck(1944) has shown that reaction mechanisms II and III are not of any importance with DDT, and it is well known that BHC is thus not affected. Reaction mechanism IV, however, appears to be as important with DDT as it is with the pyrethrins. Also, it may be modified by ultra-violet absorbing substances to a similar extent for both insecticides. It appears, therefore, that where DDT is superior in stability to the pyrethrins, the conditions are such that reaction mechanisms II and III are of importance. In bright sunlight, there is not much to choose between the oil-base solutions of the two insecticides.

This thesis contains the first published analysis of the various mechanisms by which pyrethrins and DDT can be decomposed. The relative roles of oxidation and ultra-violet light are substantially disentangled, and the various methods which have been suggested for preventing or reducing such decomposition have then been assessed, according to their capacity for reducing oxidation, light-catalysed oxidation, or ultra-violet photolysis of the insecticides. DDT has been shown to be as easily decomposed by ultra-violet light as the pyrethrins, but only when the formation of protective films of decomposition products is prevented. In this thesis, it is demonstrated that the effective life of oil films of both insecticides can be doubled by incorporating benzene, azo-β-naphthol in the solution. With both insecticides, the main

benefit from such dyes derives from their ultra-violet absorption, as DDT decomposes almost entirely by ultra-violet photolysis and it is shown in Section 5 that about 70% of the deterioration of the pyrethrins in direct sunlight is brought about by this means.

On the other hand, a dye which absorbs light in the near ultra-violet and visible regions would be beneficial for the pyrethrins, for which insecticide the light-catalysed oxidation is important. As the absorption spectrum of benzene azo- (β -naphthol is not yet known to the author, it is not possible to say how far this substance may be effective in this way. It is doubtless this light-catalysed reaction which accounts for the discrepancy between the widely held opinion of the relative instability of the pyrethrins compared with DDT, and the marked stability of the former insecticide in the dark, noted by earlier workers and confirmed in this thesis.

These problems concerning the stability of the two insecticides chiefly studied which have been given in detailed examination have yielded quite definite results. In much of the earlier work reviewed in the introduction, the experimental work has yielded results which are ill-defined, and in many cases the conclusions based on them are of uncertain validity and unknown generality. It seems to the author that much of this lack of definition resulted from the uncertainties associated with the chemical analysis of the breakdown products of DDT and the pyrethrins, and in

this thesis the conclusions have been drawn entirely from biological assay. Particular attention has been paid to the design of these assays, to ensure that ambiguities arising from such situations as the confounding of different factors, the neglect of interactions, and the biological variability of the test insects did not arise.

By breaking down the types of reaction by which the insecticides can be decomposed the degree of generality, as well as the validity, of many of the conclusions arrived at is made plain. For example, one would not expect an antioxidant to be effective in reducing the photolytic reactions, which appear not to involve the oxidation of the insecticides.

It must be emphasised, however, that many details of the reactions which have been enumerated in this thesis remain inadequately investigated. For example, the relative importance of oxidation and polymerization, and the chemical nature of these and other reactions has only been hinted at. Such investigations are relatively unsuitable for the methods which have been used here, and their solution doubtless will await the availability of pure pyrethrum constituents in sufficient quantity for the more orthodox chemical methods to be applied.

Particular attention has been paid to the photolytic reaction since this mechanism appears to have been the least adequately investigated. Although not of importance for longer terms storage of pyrethrum preparations, ultra-violet

photolysis is probably the most important factor limiting the use of pyrethrum in horticultural and agricultural control. This mechanism is also the only chemical one of any practical consequence by which DDT is inactivated. Of the two methods of inhibiting this reaction which have been investigated, one, the use of pyrethrum synergists, has proved completely unsuccessful, but the work involved was undoubtedly necessary, and many interesting problems have arisen, which, when time permits, it is hoped to investigate further.

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TABLE 3. I.

Toxicity of Pyrethrum - kin - oil films stored in the dark.
Assay conducted after 2 weeks storage.

Replicate.	Films stored at 17° C.	Freshly prepared films.	Films stored at 30° C.
I.	80.0	72.5	90.0
2.	80.0	7I.6	7I.6
3.	90.0	80.0	90.0
4.	80.0	80.0	75.8
5.	7I.6	90.0	80.0
Total.	40I.6	394.I	407.4
Mean .	80.3	78.8	8I,5
Adjusted mean.	79.5	-	80.7
Concentration.	I.I6 %		I.29 %

Grand total I,203.I
" mean 80.2066.

Analysis of variance of above data.

Items.	D.F.	Sums of squares.	Variance.	F.
Treatments.	2.	I0.03	5.0I	< I n.s.
Residue.	I2.	675.9I	56.33	
Total.	I4.	685.94		

TABLE 3, II.

Toxicity of Pyrethrum - in - oil films stored in the dark.

Assay conducted after 4 weeks storage.

Replicate.	Films stored at 17°C.	Freshly prepared films.	Films stored at 30°C.
I.	80.0	80.0	65.6
2.	68.9	90.0	68.0
3.	71.6	80.0	80.0
4.	90.0	90.0	68.9
5.	75.8	75.8	66.4
Total.	386.3	415.8	348.9
Mean .	77.2	83.1	69.8
Adjusted mean.	72.1	-	64.7
Concentration.	0.799%		0.667 %

Grand total I,151.0
" mean 76.73.

Analysis of variance of above data.

Items.	D.F.	Sums of squares.	Variance.	F.
Treatments.	2.	412.33	206.16	3.990
Residue.	12.	620.02	51.666	just sig.
Total.	14.	1,032.35		@ 5 % P.

TABLE 3. III.

Toxicity of Pyrethrum - in - oil films stored in the dark.

Assay conducted after 6 weeks storage.

Replicates.	Films stored at I7°C.	Freshly prepared films.	Films stored at 30°C.
I.	80.0	70.6	64.9
2.	80.0	80.0	6I.3
3.	70.6	68.0	66.4
4.	70.6	72.5	68.9
5.	60.7	65.6	69.7
Total.	36I.9	356.7	33I.2
Mean .	72.4	7I.3	66.2
Adjusted mean.	79.I	-	72.9
Concentration.	I.I0 %		0.7I6 %

Grand total I,049.8
" mean 69.986.

Analysis of variance of the above data.

Items.	D.F.	Sums of squares.	Variance.	F.
Treatments.	2.	88.4I	44.205	I.I9I
Residue.	I2.	445.43	37.II9	n.s.
Total.	I4.	533.84		

TABLE 3. IV.

Toxicity of Pyrethrum - in - oil films stored in the dark.

Assay conducted after 8 weeks storage.

Replicates.	Films stored at I7°C.	Freshly prepared films.	Films stored at 30°C.
I.	75.8	65.6	55.7
2.	58.I	60.7	62.0
3.	53.4	69.7	56.8
4.	66.4	69.7	65.6
5.	57.4	52.5	38.6
Totals.	36I.I	3I8.2	276.7
Means.	64.2	-	55.3
Adjusted means.	53.6		56.0
Concentrations.	0.405 %		0.457 %

Grand Total. 9I6.0
Grand Mean, 6I.066

Analysis of variance of above data.

Items.	D.F.	Sums of squares.	Variance.	F.
Treatments.	2.	2I6.59	I08.295	I.445
Residue.	I2.	898.4I	74.95I	n.s.
Total.	I4.	I,II5.00		

TABLE 3. V.

Toxicity of Pyrethrum - in - oil films stored in the dark.

Assay conducted after I5 weeks storage.

Replicates.	Films stored at I7°C.	Freshly prepared films.	Films stored at 30°C.
I.	5444	6I.3	32.6
2.	37.5	56.2	4I.0
3.	35.8	58.I	40.4
4.	30.0	64.9	40.4
5.	4I.6	59.3	35.I
Total.	I77.5	299.8	I89.5
Mean.	35.5	59.9	37.9
Adjusted mean.	53.6	56.0	56.0
Concentrations.	0.405 %	.	0.457.%

Grand total. 666.6
" mean. 44.444

Analysis of variance of above data.

Items.	D.F.	Sums of squares.	Variances.	F.
Treatments.	2.	I,8I0.52	905.26	57.56
Residue.	I2.	I88.72	I5.7266	sig. @ 0.00I % level of P.
Total.	I4.	I,999.24		

TABLE 3. VI.

Toxicity of Pyrethrum - in - oil films stored in the dark.

Assay conducted after 22 weeks storage.

Replicate.	Films stored at 17°C.	Freshly prepared films.	Films stored at 30°C.
I.	45.6	90.0	39.2
2.	56.8	90.0	33.2
3.	38.6	90.0	33.8
4.	39.2	68.0	38.6
5.	47.3	90.0	62.0
Total.	226.9	428.0	206.8
Mean.	45.4	85.6	41.3
Adjusted mean,	37.8		33.7
Concentrations.	0.25I %		0.2I5 %

Grand total 86I.2
" mean 57.4I3.

Analysis of variance of the above data.

Items.	D.F.	Sums of squares.	Variances.	F.
Treatments.	2.	6,054.64	3,0I7.27	30.80
Residue.	I2.	I,I75.4I	97.9508	sig. @ 0.00 level of P.
Total.	I4.	7,2I0.05		

TABLE. 5. I

Protectant effect of benzene azo-β-naphthol on irradiated pyrethrum films.

1% Pyrethrins.

n	r	p	Angular transform	Mean	χ^2_5
32	6	18·7	25·6		
33	7	21·2	27·5		
33	10	30·3	33·4	27·92	2·21
32	7	21·8	27·8	Δ = 15·50	
32	8	25·0	30·0		
32	5	15·6	23·2		

1% Pyrethrins + 0·01% Dye stuff

n	r	p	Angular transform	Mean	χ^2_5
32	9	28·1	32·0		
31	10	32·3	24·6		
31	5	16·1	23·7	29·05	5·09
31	8	25·8	30·6		
32	12	37·5	37·8		
32	6	18·7	25·6		
31	0	0·0			

↖ Controls

Corrected mean level = 29·05+15·50 = 44·55

TABLE 5. II

Protectant action of benzene azo-β-naphthol on irradiated pyrethrin films.

1% Pyrethrins alone.

As for Table 5. I.

1% Pyrethrins + 0·02% Dye stuff.

n	r	p	Angular transform	Mean	χ^2_5
32	6	18·7	25·6		
32	7	21·8	27·8		
30	7	23·3	29·0	30·0	2·56
32	9	28·1	32·0		
32	8	25·0	30·0		
32	11	34·4	36·0		

Corrected mean level = 30·0+15·5 = 45·0

TABLE 5. III

Protectant effect of benzene azo-ρ- naphthol on irradiated pyrethrin films.

1% Pyrethrins alone.

n	r	p	Angular transform	Mean	χ^2_5
30	11	36·7	37·5		
33	17	52·5	56·3		
32	10	31·2	33·9	38·16	
29	13	44·9	42·0	(= 5·26)	14·12
32	10	31·3	33·9		
32	6	18·8	25·6		
28	0	0·0	← Controls		

1% Pyrethrins + 0·025% Dye stuff

n	r	p	Angular transform	Mean	χ^2_5
30	20	66·6	54·6		
32	18	56·2	48·8		
32	23	72·0	58·1	51·78	1·81
32	17	53·1	46·8		
32	20	62·5	52·2		
30	19	59·4	50·4		

Corrected mean level = 51·78 + 5·26 = 57·04

TABLE 5. IV

Protectant effect of benzene azo-β-naphthol on irradiated pyrethrins films.

1% Pyrethrins alone.

As for Table III.

1% Pyrethrins + 0·03% Dye stuff.

n	r	p	Angular transform	Mean	χ^2_5
32	15	46·9	43·2		
32	13	40·6	42·7		
32	15	46·9	43·2	41·95	4·53
30	17	56·6	48·8		
31	8	25·8	30·6		
32	15	46·9	43·2		

Corrected mean level = 41·95 + 5·26 = 47·21.

TABLE 5. V

Protectmt effect of benzene azo β-naphthol on irradiated pyrethrin films.

1% Pyrethrins alone.

n	r	p	Angular transform	Mean	χ^2_5
32	10	31·3	33·9		
32	15	46·9	43·0		
30	17	56·6	48·8	46·983	
33	12	36·4	37·2	(Δ 2·54)	4·77
31	11	35·3	36·4		
30	16	53·3	46·9		

1% Pyrethrins + 0·1% Dyestuff

n	r	p	Angular transform	Mean	χ^2_5
32	20	62·5	52·3		
32	17	53·1	46·8		
31	25	80·6	63·8	50·16	5.29
30	14	46·7	43·1		
32	13	40·0	45·0		
33	19	57·6	49·7		

↖ Controls

Corrected mean level = 50·16 + 2·54 = 52·70

TABLE 5. VI

Protectant effect of benzene azo-β-naphthol on irradiated pyrethrin films.

1% Pyrethrins alone.

n	r	p	Angular transform	Mean	χ^2_3
33	11	33·4	35·3		
30	12	40·0	39·2	37·65	0·61
33	11	33·4	35·3	(Δ = 5·79)	
33	14	42·6	40·7		

1% Pyrethrins + 0·25% Dye stuff.

n	r	p	Angular transform	Mean	χ^2_3
31	22	71·1	57·5		
30	25	83·4	65·9		
32	23	72·0	58·1		
30	25	83·4	65·9	61·52	1·72
30	21	70·0	56·8		
33	27	82·0	64·9		
32	0	0·0			

Controls.

Corrected mean level = 61·52 + 5·79 = 67·31

TABLE 5. VII

Protectant effect of benzene azo-β naphthol on irradiated pyrethrins films.

1% Pyrethrin Alone.

n	r	p	Angular Transform	Mean	χ^2_8
30	11	36·7	37·2		
31	13	41·9	40·3		
32	11	34·4	35·9		
30	14	46·6	45·1	35·06	4·85
32	10	31·3	34,0	$(\Delta = 8·36$	
31	7	22·6	28·4		
30	8	26·7	31·1		
31	7	22·6	28·4		
31	11	35·4	36·6		

1% Pyrethrins + 0·50% Dye stuff.

n	r	p	Angular transform	Mean	χ^2_5
31	30	96·8	79·8		
32	28	87·5	69·4		
29	29	100·0	90·0		
29	27	94·1	75·9	76·23	4·53
31	26	84·0	66·4		
29	27	94·1	75·9		
29	1	3·0			

↖ Controls

Corrected mean level = 76·23 + 8·36 = 84·59

TABLE 8. VIII

Protectant effect of benzene azo-β-naphthol on irradiated pyrethrin films.

1% Pyrethrins alone.

n	r	p	Angular transform	Mean	χ^2_5
29	11	37·9	38·0		
32	12	37·6	37·9		
30	9	30·0	33·2	35·17	1·82
30	12	40·0	39·2	(b = 8·25)	
30	6	27·6	31·7		
30	8	26·6	31·0		

1% Pyrethrins + 1% Dye stuff.

n	r	p	Angular transform	Mean	χ^2_5
29	29	100·0	90·0		
28	23	82·5	65·1		
30	29	96·9	79·9	74·3	7·10
29	24	82·9	65·5		
29	28	96·5	79·1		
30	25	83·5	66·0		
29	1	3·0			

← Controls

Corrected mean level = 74·3 + 8·25 = 82·55

TABLE 5. IX

Protectant effect of benzene azo-β- naphthol on irradiated pyrethrin films.

1% Pyrethrins alone.

As for Table 5. V.

1% Pyrethrins + 2·0% Dye stuff.

n	r	p	Angular transform	Mean	χ^2_5
31	29	93·5	75·2		
30	30	100·0	90·0		
32	32	100·0	90·0	79·95	4·80
32	28	87·5	69·4		
32	30	93·8	75·5		
32	31	96·8	79·6		

Corrected mean level = 79·95 + 2·54 = 82·49

TABLE 6. I.

Ratio of piperonyl butoxide to the pyrethrins :- 0.2 : I.
Test solutions not diluted relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
	I.	51.9	18.4	70.3	-33.5
I.	2.	50.2	27.3	77.5	-22.9
	3.	52.5	22.8	75.3	-29.7
	Sums.			223.1	-86.1
Reduced sums of squares.				48.10	57.68
	I.	11.5	36.3	47.8	24.8
2.	2.	00.0	36.9	36.9	36.9
	3.	22.8	50.2	73.0	26.4
	Sums.			157.7	90.1
Reduced sums of squares.				653.12	80.21
	I.	15.3	63.4	78.7	48.1
3.	2.	15.3	55.6	70.9	40.3
	3.	33.8	68.0	101.8	34.2
	Sums.			251.4	122.6
Reduced sums of squares.				516.42	105.26
	I.	47.3	31.3	78.6	+16.0
4.	2.	45.0	31.3	76.3	-13.7
	3.	39.2	21.1	60.3	-15.9
	Sums.			215.2	-47.6
Reduced sums of squares.				205.90	11.28
Sums of reduced sums of squares.				1,397.54	254.43

s^2/w 232.86
s^2/w 42.39.

Criterion of parallelism 9.3
Standard error of criterion 15.25
Departure from parallelism is not significant at 5% level of P.

T 26.2
U 115.4

U^2 13,317.16
$t^2 s^2/w$ 182.84
U'^2 13,144.32

M' 0.0683
M 0.0683.

C 1.0132
CM' 0.0692
CM'^2 0.0047.

Log. relative potency is 0.0683 with 95% fiducial limits 0.1043
0.0323

Abs. " " " 1.17 " " " " 1.27
1.08.

TABLE 6. II.

Ratio of piperonyl butoxide to the pyrethrins :- 0.25 : I.
Test solutions not diluted relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	54.7	53.I	I07.8	-0I.6
I.	2.	58.9	52.7	III.6	-06.2
	3.	59.3	49.I	I08.4	-I0.2
	Sums.			327.8	-I8.0
Reduced sums of squares.				8.38	37.04
	I.	26.6	65.9	92.5	39.3
2.	2.	26.6	65.9	92.5	39.3
	3.	23.I	68.6	96.7	40.3
	Sums.			28I.7	II9.I
Reduced sums of squares.				II.76	0.96
	I.	3I.I	70.5	I0I.6	39.4
3.	2.	29.4	79.5	I08.9	50.I
	3.	30.0	64.8	94.8	34.8
	Sums.			305.3	I24.3
Reduced sums of squares.				99.45	I27.39
	I.	6I.I	56.I	II7.2	-05.0
4.	2.	54.0	6I.I	II5.I	06.I
	3.	55.2	65.9	II9.I	I2.7
	Sums.			35I.4	I3.8
Reduced sums of squares.				8.0I	I60.02.
Sums of reduced sums of squares.				I27.57	325.4I

B^2/w 2I.36
D^2/w 54.23

Criterion of parallelism 30.7
Standard error of criterion 4.60
t 6.67, significant at the 5% level of P.

T	79.6	M	0.29I4
U	82.2	M	0.29I4
U^2	6,756.84	C	I.0446
t^2s^2/w	208.28	CM'	0.3044
U'^2	6,469.39	CM'^2	0.0849

Log. relative potency is 0.29I4 with 95% fiducial limits 0.498
0.085
Abs. " " " I.96 " " " " " 2.47
I.64.

TABLE 6. III.

Ratio of piperonyl butoxide to the pyrethrins :- 0.4 : I.
Test solutions not diluted relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
	I.	37.5	26.6	64.I	-I0.9
I.	2.	4I.0	2I.I	62.I	-I9.9
	3.	35.I	2I.I	56.2	-I4.0
	Sums.			I82.4	-44.8
Reduced sums of squares.				33.74	43.30
	I.	I5.3	49.0	64.3	33.7
2.	2.	I5.3	45.0	60.3	29.7
	3.	I9.4	64.9	84.3	45.5
	Sums.			208.9	I08.9
Reduced sums of squares.				337.63	I34.96
	I.	I5.3	37.5	52.8	22.2
3.	2.	I5.3	4844	63.7	33.2
	3.	35.8	68.0	I0I.8	34.2
	Sums.			2I8.3	89.6
Reduced sums of squares.				I,3I6.53	83.68
	I.	46.7	I8.4	65.I	-28.3
4.	2.	45.0	28.7	73.7	-I6.3
	3.	39.2	3I.3	70.5	-07.9
	Sums.			209.3	-52.5
Reduced sums of squares.				30.8I	24I.05
Sums of reduced sums of squares.				I,2I8.7I	504.99

S^2/w 286.38
s^2/w 84.I4

Criterion of parallelism -II.8
Standard error of criterion I8.92
Departure from parallelism is not significant at the 5% level of probability.

T	35.8	M'	0.I003
U	98.6	M	0.I003
U^2	9721.96	C	I.0344
t^2s^2/w	325.22	CM'	0.I058
U'^2	9398.74	CM'^2	0.0I04

Log.relative potency is 0.I003 with 95% fiducial limits 0.I592
0.04I4
Abs. " " " I.26 " " " " I.44
I.I0.

TABLE 6. IV.

Ratio of piperonyl butoxide to the pyrethrins :- 0.5 : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
	I.	56.II	62.94	II9.05	06.83
I.	2.	57.35	63.87	I2I.22	6.52
	3.	53.43	6I.07	II4.50	07.64
	Sums.			354.77	20.99
Reduced sums of squares.				23.53	00.66
	I.	3I.63	90.00	I2I.63	58.37
2.	2.	33.2I	90.00	I23.3I.	56.79
	3.	37.23	79.22	II6.45	4I.99
	Sums.			36I.39	I57.I5
Reduced sums of squares.				25.0I	05.08
	I.	35.9I	90.00	I25.9I	55.09
3.	2.	44.03	90.00	I34.03	45.97
	3.	54.03	90.00	I44.03	35.97
	Sums.			403.97	I47.03
Reduced sums of squares.				I64.76	I6I.36
	I.	48.79	50.77	999.56	0I.98
4.	2.	45.97	52.7I	98.68	06.74
	3.	46.89	54.70	I0I.59	07.9I
	Sums.			299.83	I6.63
Reduced sums of squares.				04.46	I9.73
Sums of reduced sums of squares.				2I7.76	I86.53.

S^2/w 36.28
s^2/w 3I.I4

Criterion of parallelism 86.89
Standard error of criterion 6.05
t,(8 d.f.) is 6.II, significant at I% level of P.

T I9.8
U I6.8

U^2 4,462.24
t^2s^2/w I65.65
U'^2 4,296.59

M' 0.5500
M 0.5500

C I.0386
CM' 0.4294
CM'^2 0.I776

Log. relative potency is 0.5500 with 95% fiducial limits 0.5364
0.3224.
Abs. " " " 3.59 " " " " I0.63
2.93

Table 6. V.

Ratio of piperonyl butoxide to the pyrethrins is :$\frac{2}{3}$ 0.6 : 1
Test solutions diluted twice relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	29.3	10.0	39.3	-19.3
I.	2.	33.7	10.0	43.7	-23.7
	3.	31.9	14.2	46.1	-17.7
	Sums.			149.1	-80.7
Reduced sums of squares.				317.12	424.64
	I.	18.4	31.3	49.7	12.9
2.	2.	14.2	33.2	47.4	19.0
	3.	22.0	33.8	55.8	11.8
	Sums.			152.9	43.7
Reduced sums of squares.				47.68	33.00
	I.	10.0	32.5	62.5	42.5
3.	2.	20.3	36.3	56.6	16.0
	3.	14.2	35.7	49.9	21.5
	Sums.			169.0	79.0
Reduced sums of squares.				85.12	446.80
	I.	36.8	15.3	78.1	-41.5
4.	2.	32.5	10.0	62.5	-42.5
	3.	49.0	10.0	59.0	-39.0
	Sums.			193.6	-123.0
Reduced sums of squares.				98.46	6.50
Sums of reduced sums of squares.				548.58	910.94

S_{ω}^2/w 62.57
s^2/w

Criterion of parallelism 7.0
Standard error of criterion 3.28
Departures from parallelism is not significant at the 5% level of P

T	-30.4	M'	0.0860
U	105.4	M	0.2142.
U^2	11,109.16	C	1.0410
t^2s^2/w	437.41	CM'	0.0904
U'^2	10,671.75	CM'^2	0.0078

Log. relative potency is 0.2142 with 95% fiducial limits 0.2774
0.1540
Abs. " " " E. 64 " " " " 1.90
1.43.

TABLE 6. VI.

Ratio of piperonyl butoxide to the pyrethrins :- 0.75 : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Responses to Test.	Sums.	Differences.
	I.	55.3	6I.I	II6.4	5.8
I.	2.	65.4	5I.7	II5.I	-II.7
	3.	60.6	54.0	II4.6	-06.6
	Sums.			346.I	-I2.5
Reduced sums of squares.				I.70	I6I.59
	I.	28.9	85.I	II4.0	56.2
2.	2.	57.3	85.I	I22.4	47.8
	3.	37.5	85.I	I22.6	47.6
	Sums.			359.0	I5I.6
Reduced sums of squares.				48.I9	62.76
	I.	28.6	90.0	II6.6	63.4
3.	2.	25.0	90.0	II5.0	65.0
	3.	25.0	90.0	II5.0	65.0
	Sums.			346.6	I93.4
Reduced sums of squares.				I.72	I4.60.
	I.	80.6	65.9	I26.5	5.3
4.	2.	58.9	75.0	I33.9	I6.I
	3.	60.6	68.6	I29.2	8.0
	Sums.			389.6	29.4
Reduced sums of squares.				28.05	65.I8
Sums of reduced sums of squares.				79.66	302.I3.

S^2/w I3.28
s^2/w 50.34

Criterion of parallelism I0.03.
Standard error of criterion 3.64
t 2.65, significant at the 5% level of P.

T I20.6
U I09.4

U^2 II,968.36
t^2s^2/w I93.38
U'^2 II,774.98

M' 0.33I8
M 0.33I8.

C I.0I64
CM' 0.3372
CM'^2 0.III9

Log. relative potency is 0.33I8 with 95% fiducial limits 0.4064
0.2690
Abs. " " " 2.I5 " " " " 2.56
I.87.

TABLE 6. VII.

Ratio of piperonyl butoxide to the pyrethrins :- 0.8 : I.
Test solutions diluted twice relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Standards.	Test.	Sums.	Differences.
I	I.	43.3	I5.3	58.6	-28.0
I.	2.	46.7	I5.3	62.0	-3I.4
	3.	53.7	I8.4	72.I	-35.3
	Sums.			I92.7	-94.7
Reduced sums of squares,				I05.03	25.53
	I.	33.8	40.4	74.2	06.6
2.	2.	I9.4	58.I	77.5	38.7
	3.	23.6	38.6	62.2	I5.0
	Sums.			2I3.9	60.3
Reduced sums of squares.				I43.92	554.22
	I.	24.4	44.4	68.8	20.0
3.	2.	2I.I	66.4	87.5	45.3
	3.	28.7	54.9	83.6	26.2
	Sums.			239.7	9I.5
Reduced sums of squares.				226.62	347.78
	I.	7I.6	I5.3	86.9	-56.3
4.	2.	68.9	I5.3	84.2	-53.6
	3.	75.8	I8.4	94.2	-57.4
	Sums.			I65.3	-I67.3
Reduced sums of squares.				62.57	2.07
Sums of reduced sums of squares.				537.94	927.60

S^2/w 67.24
s^2/w II5.95
Criterion of parallelism. I.4
Standard error of criterion. 8.20.
Departure from parallelism is not significant at 5% level of P.

T	-26.8	M'	-0.0580
U	I38.0	M	0.2425
U^2	I9,044.00	C	I.0239
t^2s^2/w	443.4I	CM'	0.0599.
U'^2	I8,598.59	CM'^2	0.0036

Log. relative potency is 0.2425 with 95% fiducial limits 0.2695
0.0470
Abs. " " " I.75 " " " " I.95
I.57.

TABLE 6. VIII.

Ratio of piperonyl butoxide to the pyrethrins:- I.0 : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Responses to Test.	Sums.	Differences.
I.	I.	45.92	60.53	I06,45	I4.6I
I.	2.	48.79	56.79	I05.58	08.00
	3.	54.I5	68.6I	I22.76	I4.46
	Sums.			334.79	36.07.
Reduced sums of squares.				I87,30	28.48.
	I.	24.04	79.37	I03.4I	55.33
2.	2.	24.50	90.00	II4.50	65.50
	3.	2I.47	90.00	III.47	68.73
	Sums.			329.38	I89.56
Reduced sums of squares.				65.7I	97.8I.
	I.	28.86	90.00	II8.86	6I.I4
3.	2.	3I.05	90.00	I2I.05	58.95
	3.	24.50	90.00	II4.50.	65.50
	Sums.			354.4I	I85.59
Reduced sums of squares.				22.24	22.24
	I.	39.23	68.6I	I07.84	29.38
4.	2.	52.7I	7I.56	I24.27	I9.85
	3.	55.37	6I.07	II6.44	05.70
	Sums.			348.55	54.93
Reduced sums of squares.				I38.56	I20.28
Sums of reduced sums of squares.				4I3.8I	338.8I

S^2/w
s^2/w

Criterion of parallelism 0.02
Standard error of criterion is
Departure from parallelism not significant at 5% level of P.

T	50.8	M'	0.4695
U	32.5	M	0.4695.
U^2	9,539.43	C	I.0I80
t^2s^2/w	225.3I	CM'	0.4779
U'^2	9,370.44	CM'^2	0.2244

Log. relative potency is 0.4695 with 95% fiducial limits

Abs. " " " 2.95 " " " "

TABLE 6. XI.

Ratio of piperonyl butoxide to the pyrethrins :- I.5 : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
	I.	63.44	7I.85	I35.29	08.4I
I.	2.	59.4I	79.06	I38.47	I9.65
	3.	56.79	63.44	I20.25	06.65
	Sums.			393.99	34.7I
Reduced sums of squares.				2II.52	99.59
	I.	4I.I5	75.00	II6.I5	33.85
2.	2.	44.03	90.00	I34.03	45.97
	3.	45.00	90.00	I35.00	45.00
	Sums.			385.I8	I24.82
Reduced sums of squares.				226.60	90.72
	I.	45.92	90.00	I35.92	44.08
3.	2.	42.02	90.00	I32.02	47.98
	3.	39.23	90.00	I29.23	50.77
	Sums.			397.I7	I42.83
Reduced sums of squares.				22.58	22.58
	I.	57.35	64.97	I22.32	07.62
4.	2.	53.25	75.23	I28.48	2I.98
	3.	65.44	75.46	I38.90	I2.02
	Sums.			389.70	4I.62
Reduced sums of squares.				I40.47	I08I26
Sum of reduced sums of squares.				60I.I7	32I.I5.

s^2/w I00.I7
s^2/w 53.5I

Criterion of parallelism 0.45
Standard error of criterion I0.0I
Departure from parallelism is not significant at 5% level of P.

T II4.65
U 65.77

U^2 4,066.6I
t^2s^2/w 284.74
U'^2 3,78I.87

M' 0.54I2
M 0.54I2

C I.0753
CM' 0.58I9
CM'2 0.II64.

Log. relative potency is 0.54I2 with 95% fiducial limits 0.857
0.307

Abs. relative potency is 3.48 with 95% fiducial limits 7.20
2.03.

TABLE 6. X.

Ratio of piperonyl butoxide to the pyrethrins :- 2.0 : I.
Test solutions not diluted relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Standards.	Test.	Sums.	Differences.
	I.	45.05	65.88	I08.93	22.83
I.	2.	54.70	88.6I	I83.3I	I3.9I
	3.	45.00	75.00	I20.00	30.00
	Sums.			352.24	66.74
Reduced sums of squares.				II3.43	I88.96
	I.	26.56	90.00	II6.56	63.44
2.	2.	24.50	90.00	II4.50	65.50
	3.	2I.72	90.00	III.72	68.28
	Sums.			342.78	I97.22
Reduced sums of squares.				II.80	II.80
	I.	28.32	90.00	II8.32	6I.68
3.	2.	24.50	90.00	II4.50	65.50
	3.	26.56	90.00	II6.56	63.44
	Sums.			349.38	I90.62
Reduced sums of squares.				7.32	7.3I
	I.	52.00	68.I9	I20.I9	I6.I9
4.	2.	47.93	65.42	II3.35	17.49
	3.	45.00	79.37	I24.37	34.37
	Sums.			357.9I	68.05
Reduced sums of squares.				6I.90	205.7I
Sums of reduced sums of squares.				I94.44	554.78

s^{B}/w 524.00
s^{D}/w

Criterion of parallelism I7.99
Standard error of criterion I8.00
Departure from parallelism is not significant at 5% level of P.

T	I74.20	M'	0.6345
U	84.36	M	0.6345
U^2	7,II8.6I	C	I.0462
t^2g^2/w	5I4.57	CM'2	0.6658
U'^2	6,602.05	CM'2	0.42I2

Log relative potency is 0.6345 with 95% fiducial limits 0.8I76
0.5I00
Abs. " " "4.3I " " " " 6.57
3.25.

TABLE 6. XI.

Ratio of piperonyl butoxide to the pyrethrins :-5.0 : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
I.	I.	46.89	73.68	I20.59	26.79
	2.	49.95	81.30	I31.75	31.35
	3.	64.30	82.20	I46.50	I7.90
	Sums.			398.84	76.54
Reduced sums of squares.				337.82	99.74
2.	I.	23.75	79.60	I03.35	55.87
	2.	24.I2	79.22	I03.34	55.I0
	3.	05.90	63.79	69.69	57.89
	Sums.			276.38	I68.86
Reduced sums of squares.				754.68	4.I6
3.	I.	27.20	6I.85	89.05	34.65
	2.	22.63	7I.56	94.29	49.02
	3.	I0.78	68.87	79.65	58.09
	Sums.			262.98	I4I.76
Reduced sums of squares.				I09.9I	279.39
4.	I.	49.60	60.00	I09.60	I0.40
	2.	46.78	63.44	II0.22	I6.66
	3.	46.66	75.II	I2I.77	28.45.
	Sums.			341.59	55.5I
Reduced sums of squares.				93.96	I67.99.
Sums of reduced sums of squares.				I,296.32	55I.28.

S^2/w 2I6.05
s^2/w 9I.86

Criterion of parallelism 67.03
Standard error of criterion I4.69
t,(8 d.f.) 4.56,significant at I% level of P.

T. II0.67
U 44.64

U^2 3,542.63
t^2s^2/w 352.87
U'^2 3,I89.76

M' 0.746I
M 0.746I

C I.II06
CM' 0.8287
CM'2 0.6I84.

Log. relative potency is 0.746I with 95% fiducial limits I.0262
0.4662

Abs. " " " 5.58 " " " " I0.63
2.93.

TABLE 6. XII.

Ratio of piperonyl butoxide to the pyrethrins :- 4.0 : I.
Test solutions diluted twice relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Responses to Test.	Sums.	Differences.
	I.	49.60	80.40	I30.00	30.80
I.	2.	50.07	74.77	I24.84	24.70
	3.	53.67	62.55	II6.22	08.88
	Sums.			371.06	64.38
Reduced sums of squares.				96.94	255.99
	I.	24.65	80.90	I05.55	56.25
2.	2.	30.79	85.I0	II5.89	54.3I
	3.	32.65	85.90	II8.55	53.25
	Sums.			339.99	I65.8I
Reduced sums of squares.				94.33	4.63
	I.	33.50	7I.95	I05.45	38.48
3.	2.	40.86	80.90	I2I.76	40.04
	3.	2I.56	8I.30	I02.86	59.74
	Sums.			330.07	I38.26
Reduced sums of squares.				209.98	280.84
	I.	43.68	56.73	I00.4I	I3.05
4.	2.	56.79	6I.27	II8.06	04.48
	3.	59.93	76.20	I36.I3	I6.27
	Sums.			354.60	35.80
Reduced sums of squares.				637.99	74.27
Sums of reduced suns of squares.				I,039.22	6I5.72

B^2/w 223.20
s^2/w I02.59

Criterion of parallelism I8.53
Standard error of criterion I4.94
t,(8 d.f.) I.240,not significant at 5% level of P.

T	I35.42	M'	0.5909
U	67.96	M	0.89I9
U^2	4,6I9.0I	G	I.I340
t^2s^2/w	545.93	GM'	0.670I
U'^2	4,073.08	GM'2	0.3960

Log. relative potency is 0.89I9 with 95% fiducial limits I.2264
0.7I58

Abs. " " " 7.79 " " " " I6.83
5.23.

TABLE 6. XIII.

Ratio of piperonyl butoxide to the pyrethrins :- 5.0 : I.
Test solutions diluted twice relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Standards.	Tests.	Sums..	Differences.
	I.	46.9	57.7	I04.6	I0.8
I.	2.	40.0	65.I	I05.I	25.I
	3.	42.I	75.8	II7.9	33.7
	Sums.			325.6	67.6
Reduced sums of squares,				I2I.88	3I.69
	I.	28.9	90.0	II8.9	6I.I
2.	2.	30.0	I20.0	I20.0	60.0
	3.	23.7	90.0	II3.7	66.3
	Sums.			352.6	I87.4
Reduced sums of squares.				22.65	22.65
	I.	30.0	75.2	I05.2	45.2
3.	2.	34.5	74.6	I09.I	40.I
	3.	54.6	82.7	II7.3	48.I
	Sums.			331.6	I33.4
Reduced sums of squares.				70.29	32.8I
	I.	58.I	63.0	I0I.I	24.9
4.	2.	46.9	63.8	II0.7	I6.9
	3.	53.5	60.3	II3.8	07.0
	Sums.			325.4	48.8
Reduced sums of squares.				85.6I	I60.8I
Sums of reduced sums of squares.				306.64	248.96

s^2/w 5I.057
s^2/w 4I.I50

Criterion of parallelism -II.I
Standard error of criterion 7.08.
Departure from parallelism is not significant at 5% level of P.

T 36.43 M' 0.6438
U I7.03 M 0.9448

U^2 4,642.I057 C I.0473
t^2s^2/w 2I8.9I26 CM' 0.9895
U'^2 4,425.9I26. CM'^{2} 0.9349

Log. relative potency is 0.9448 with 95% fiducial limits I.2097
0.7693.
Abs. " " " 8.85 " " " " I3.2I
5.88.

TABLE 6. XIV.

Ratio of piperonyl butoxide to the pyrethrins :- 6.0 : I.
Test solutions diluted IO times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
	I.	42.5	27.3	69.8	-I5.2
I.	2.	56.8	33.8	90.6	-23.0
	3.	48.7	20.3	69.0	-28.4
	Sums.			229.4	-66.6
Reduced sums of squares.				292.30	88.08
	I.	50.0	36.3	86.3	06.3
2.	2.	30.7	4I.6	72.3	I0.9
	3.	33.2	35.I	68.3	02.9
	Sums.			206.9	50.I
Reduced sums of squares.				52.46	52.34
	I.	28.7	43.3	72.0	I4.6
3.	2.	30.0	47.9	77.9	I7.9
	3.	30.0	46.7	76.7	I6.7
	Sums.			226.6	49.2
Reduced sums of squares.				27.00	5.58
	I.	44.4	3I.9	76.3	-I2.5
4.	2.	53.7	30.7	84.4	-23.0
	3.	5I.4	29.3	80.7	-22.I
	Sums.			241.4	-57.6
Reduced sums of squares.				24.84	67.74
Sums of reduced sums of squares.				376.60	I93.64

S^2/w 62.75
s^2/w 32.26

Criterion of parallelism I2.6
Standard error of criterion 7.92
t I.87, not significant at the 5% level of P.

T	-I8.3	M'	-0.0854
U	64.5	M	0.9I46
U^2	4,I60.25	G	I.0430
t^2s^2/w	I7I.62	GM'	-0.089I
U'^2	3,988.63	GM'^2	0.0076.

Log. relative potency is 0.9I46 with 95% fiducial limits 0.9786
0.8506
Abs. " " " 8.22 " " " " 9.52
7.09.

TABLE 6. XV.

Ratio of piperonyl butoxide to the pyrethrins :- 0.5 : I.
Test solutions diluted IO times relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	43.3	23.6	66.9	-19.7
I.	2.	41.6	20.3	61.9	-21.3
	3.	39.3	18.9	58.2	-16.9
	Sums.			186.5	-57.5
Reduced sums of squares.				1163.76	11.85
	I.	28.7	38.6	67.3	09.9
2.	2.	38.0	31.9	5969	03.9
	3.	24.4	33.2	57.6	08.8
	Sums.			178.8	19.6
Reduced sums of squares.				6.98	12.58
	I.	27.3	28.7	56.0	01.4
3.	2.	30.0	36.9	66.9	06.9
	3.	33.8	31.6	64.8	-01.6
	Sums.			187.7	06.7
Reduced sums of squares.				4.32	37.39
	I.	43.6	20.8	6444	-22.8
4.	2.	41.0	17.5	58.5	-23.5
	3.	39.8	22.0	61.8	-17.8
	Sums.			184.73	-64.1
Reduced sums of squares.				11.35	17.19
Sums of reduced sums of squares.				1186.39	82.99

$$\frac{S^2_w}{s^2/w}$$

Criterion of parallelism -0.4
Standard error of criterion 3.67
Departure from parallelism is not significant at the 5% level of P.

T	-33.0	M'	0.8052
U	48.4	M	0.7943

U^2	2,342.56	G	I.0384
t^2s^2/w	73.39	Gm'	0.3II9
U'^2	2,268.98	Gm'^2	0.0985

Log. relative potency is 0.7943 with 95% fiducial limits 0.6808
0.7868
Abs. relative potency is 6.23 with 95% fiducial limits 7.26
5.35.

TABLE 6. XVI.

Ratio of piperonyl butoxide to the pyrethrins :- 7.0 : I.
Test solutions diluted twice relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicate.	Response to Standard.	Response to Test.	Sums.	Differences.
I.	I.	48.4	56.2	I04.6	07.8
	2.	44.4	5I.9	96.3	09.8
	3.	43.9	53.7	97.6	09.8
	Sums.			298.5	25.I
Reduced sums of squares.				59.86	2.29
2.	I.	22.8	7I.6	94.4	48.8
	2.	29.3	77.I	I06.4	47.8
	3.	3I.9	75.6	I05.5	4I.7
	Sums.			306.3	I38.3
Reduced sums of squares.				69.54	29.54
3.	I.	34.4	73.6	I08.0	39.2
	2.	3I.9	7I.6	I03.5	39.7
	3.	30.7	63.4	94.I	32.7
	Sums.			305.6	III.6
Reduced sums of squares.				I20.98	30.50
4.	I.	39.2	5I.9	9I.I	I2.7
	2.	46.7	49.0	95.7	02.3
	3.	45.0	53.7	98.7	08.7
	Sums.			285.5	23.7
Reduced sums of squares.				I9.79	55.04
Sums of reduced sums of squares.				269.97	II7.37

s_1^2/w 44.983
s^2/w I9.556

Criterion of parallelism -9.2
Standard error of criterion 6.70
t I.373, not significant at the 5% level of P.

T	87.0	M'	0.4475
U	99.6	M	0.9485.
U^2	9,920.I6	C	I.00263
t^2s^2/w	75.II	CM'	0.4509
U'^2	9,845.05	CM'^2	0.2018

Log.relative potency is 0.9485 with 95% fiducial limits 0.7957
0.70I5.
Abs. " " " 5.6I " " " " 6.25
5.05.

TABLE 6. XVIII.

Ratio of piperonyl butoxide to the pyrethrins 7.0 : I.
Test solutions diluted I0 times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Response to Standard.	Response to Test.	Sums.	Differenc
I.	I.	37.5	I2.9	50.4	-24.6
	2.	45.6	20.5	66.9	-25.3
	3.	36.7	I9.4	54.I	-I7.3
	Sums.			I70.4	-67.2
Reduced sums of squares.				I48.I0	39.26
2.	I.	26.6	29.5	56.9	02.7
	2.	2I.I	5I.3	52.4	I0.2
	3.	24.4	36,3	60.7	II.9
	Sums.			I70.0	24.8
Reduced sums of squares.				23.62	47.I6
3.	I.	3I.3	3I.9	63.2	00.6
	2.	29.3	30.7	60.0	0I.4
	3.	3I.9	33.8	65.7	0I.7
	Sums.			I88.9	03.7
Reduced sums of squares.				28.92	0.77
4.	I.	39.8	25.I	64.9	-I4.7
	2.	37.5	22.8	60.3	-I4.7
	3.	39.8	22.8	62.6	-I7.0
	Sums.			I87.8	-46.4
Reduced sums of squares.				I0.58	I.02
Sums of reduced sums of squares.				2II.22	88.I5.

S^2/w 35.I9
s^2/w I4.69

Criterion of parallelism -0.8
Standard error of parallelism 5.93.
Departure from parallelism is not significant at 5% level of P.

T -88.6 M' 0.I820
U 47.2 M 0.8I80.

U^2 2,227.94 C I.0259
t^2s^2/w 56.45 CM' -0.I867
U'^2 2,I7I.4I CM'^2 0.0339.

Log.relative potency is 0.8I80 with 95% fiducial limits 0.2747
0.76I3.
Abs. " " " 6.56 " " " " 7.50
5.77.

TABLE 6. XVIII.

Ratio of piperonyl butoxide to the pyrethrins :- [illegible] : I.
Test solutions not diluted relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	40.2	32.9	55.1	-27.3
I.	2.	39.2	17.5	56.7	-21.7
	3.	41.6	16.4	58.0	-25.2
	Sums.			169.8	-74.2
Reduced sums of squares.				19.48	18.48
	I.	28.0	25.1	53.1	-02.9
2.	2.	30.5	28.0	58.5	-02.5
	3.	28.0	28.7	56.7	00.7
	Sums.			168.3	-04.7
Reduced sums of squares.				15.12	7.63
	I.	35.7	34.8	70.5	-00.9
3.	2.	37.5	34.8	72.3	-02.7
	3.	25.8	35.7	61.5	009.9
	Sums.			204.3	7.7
Reduced sums of squares.				66.96	66.09
	I.	35.1	22.8	75.9	-30.3
4.	2.	41.6	15.3	56.9	-26.3
	3.	42.2	16.4	60.5	-23.7
	Sums.			193.3	-80.3
Reduced sums of squares.				210.15	19.43
Sums of reduced sums of squares.				310.71	131.63.

$$S^2_B/w \quad 51.77$$
$$S^2_W/w$$

Criterion of parallelism -6.9
Standard error of criterion 7.19
Departure from parallelism not significant at 5% level of P.

T	-50.5	M'	0.2895
U	52.5	M	0.7104
U^2_2	2,756.25	G	1.0442
$t^2_2 S^2/w$	116.88	GM'	0.3023
U'^2	2,639.37	GM'^2	0.0876

Log. relative potency is 0.7104 with 95% fiducial limits 0.7991 0.6214
Abs. " " " 5.14 " " " " 6.30 4.18

TABLE 6. XIX.

Ratio of piperonyl butoxide to the pyrethrins :- 8.0 : I.
Test solutions diluted IO times relative to the standards.
Twin Cross-overddesign and analysis of relative potency.

Group.	Replicate.	Responses to Standard.	Test.	Sums.	Differences.
	I.	47.9	00.0	47.9	-47.9
I.	2.	53.7	2I.I	74.9	-32.6
	3.	49.6	00.0	49.6	-49.6
	Sums.			I72.4	-I30.I
Reduced sums of squares.				468.82	I84.00
	I.	22.0	22.0	44.0	00.0
2.	2.	I8.4	3I.3	49.7	I2.9
	3.	24.4	27.3	5I.7	02.9
	Sums.			I45.4	I5.8
Reduced sums of squares.				27.08	9I.08
	I.	I6.4	46.I	64.5	27.7
3.	2.	I8.4	65.6	84.0	47.2
	3.	3I.I	53.7	74.8	32.6
	Sums.			223.3	I07.5
Reduced sums of squares.				I97.77	209.39
	I.	3I.3	I8.4	49.7	-I2.9
4.	2.	4I.0	I8.4	59.4	-22.6
	3.	50.8	2I.I	7I.9	-29.7
	Sums.			I5I.0	-65.2
Reduced sums of squares.				253.76	I44.42
Sums of reduced sums of squares.				947.45	628.89.

s^2/w I57.87
s^2/w I04.79

Criterion of parallelism -5.2-.0.
Standard error of criterion. I2.56.
Departure from parallelism is not significant at 5% level of P.

T	-27.3	M'	-0.0750
U	I09.5	M	0.9250
U^2	II,990.25	C	I.0347
t^2s^2/w	402.58	CM'	0.0776
U'^2	II,587.67	CM'2	0.0058

Log. relative potency is 0.9250. with 95% fiducial limits 0.9828
0.8672
Abs. " " " 8.4I " " " " 9.62
7.37.

TABLE 6. XX.

Ratio of piperonyl butoxide to the pyrethrins.:- 8.5 : I.
Test solutions diluted I0 times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Responses to Test.	Sums.	Differences.
I.	I.	60.7	23.0	83.7	-37.7
	2.	51.4	21.1	72.5	-30.3
	3.	57.4	16.4	73.8	-41.0
	Sums.			229.0	-109.0
	Reduced sums of squares.			69.28	63.68
2.	I.	25.1	33.8	58.9	8.7
	2.	30.7	46.7	77.4	16.0
	3.	29.3	40.4	69.7	11.1
	Sums.			206.0	35.8
	Reduced sums of squares.			165.86	28.38
3.	I.	29.3	35.7	65.0	6.4
	2.	28.0	35.1	63.1	7.1
	3.	34.4	43.3	77.7	8.9
	Sums.			205.8	22.4
	Reduced sums of squares.			126.02	2.38
4.	I.	62.0	12.9	74.9	-49.1
	2.	49.0	17.5	66.5	-31.5
	3.	49.6	25.6	75.6	-26.0
	Sums.			214.6	-106.6
	Reduced sums of squares.			46.60	294.76
	Sums of reduced sums of squares.			407.76	389.90

s^2/w 67.96
s'^2/w 64.98

Criterion of parallelism I0.2
Standard error of criterion 5.I8
t I.3I5, not significant at the 5% level of P.

T	-52.43	M'	-0.2197
U	7I.83	M	0.7803
U^2	5,159.15	C	I.0727
t^2s^2/w	249.62	CM'	0.2357
U'^2	4,809.93	CM'^2	0.0518.

Log. relative potency is 0.7803 with 95% fiducial limits 0.8321 0.7285

Abs. " " " 6.03 " " " " 6.80 5.35.

TABLE 6. XXI.

Ratio of piperonyl butoxide to the pyrethrins :- 9.0 : I.
Test solutions diluted IO times relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	53.7	I4.8	67.9	-39.5
I.	2.	65.2	20.7	85.9	-44.5
	3.	55.7	I7.9	7I.6	-37.8
	Sums.			225.4	-II9.8
Reduced sums of squares.				I8I.47	38.52
	I.	32.0	54.I	66.I	02.I
2.	2.	32.6	35.I	67.7	02.5
	3.	30.5	33.6	63.I	02.I
	Sums.			I96.9	06.7
Reduced sums of squares.				II.56	00.I3
	I.	40.I	45.9	86.0	05.8
3.	2.	34.I	44.3	78.3	I0.I
	3.	36.2	44.2	80.I	08.3
	Sums.			244.4	24.2
Reduced sums of squares.				34.08	09.49
	I.	57.8	I6.8	74.6	-4I.0
4½	2.	53.7	20.I	73.8	-33.6
	3.	57.8	20.I	77.9	-37.7
	Sums.			226.3	-II2.3
Reduced sums of squares.				I0.20	27.86
Sums of reduced sums of squares.				257.30	76.00

s^2/w 39.54
s^2/w I2.66

Criterion of parallelism I0.4
Standard error of criterion. 6.29
t I.65, not significant at the 5% level of P.

T	-67.07	M'	-0.2302
U	87.65	M	0.7698
U^2	7,688.52	C	I.0088
t^2s^2/w	67.35	CM'	- 0.2323
U'^2	7,615.17	CM'^2	0.5347

Log. relative potency is 0.7698 with 95% fiducial limits 0.7933 0.7463
Abs. " " " 5.89 " " " " 6.22 5.58.

TABLE 6. XXII.

Ratio of piperonyl butoxide totthe pyrethrins :- I0.0 : I:
Test solutions diluted I0 times relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
I.	I.	47.6	I8.2	65.8	-29.4
	2.	6I.5	I8.2	79.7	-43.3
	3.	60.0	I4.3	74.3	-45.7
	Sums.			219.8	-I18.4
Reduced sums of squares.				99.67	I55.68.
2.	I.	29.I	36.7	65.8	07.6
	2.	32.0	37.4	69.4	05.4
	3.	27.9	36.7	64.6	08.8
	Sums.			I99.8	2I.8
Reduced sums of squares.				I2.48	46.09
3.	I.	3I.2	4383	74.5	I2.I
	2.	4I.3	35.7	77.0	-05.6
	3.	35.2	40.4	75.6	05.2
	Sums.			227.I	2I.7
Reduced sums of squares.				3.I4	I59.I8
4.	I.	54.3	I7.8	72.I	-36.5
	2.	60.3	20.0	80.3	-40.3
	3.	57.I	I3.6	70.7	-43.5
	Sums.			223.I	-I20.3
Reduced sums of squares.				55.27	24.56
Sums of reduced sums of squares.				I70.56	345.44

S^2/w 28.48
s^2/w 57.55

Criterion of parallelism 5.32
Standard error of criterion 5.33
Departure from parallelism is not significant at the 5% level of P.

T	-88.40	M'	-0.2269
U	90.72	M	0.775I
U^2	8,230.II	C	I.0386
t^2s^2/w	306.I6	CM'	0.2365
U'^2	7,923.95	CM'^2	0.0536

Log. relative potency is 0.775I with 95% fiducial limits 0.7967
0.7495

Abs. " " " 5.93 " " " " 6.26
5.58.

TABLE 6. XXIII.

Ratio of piperonyl butoxide to the pyrethrins :- II.0 : I.
Test solutions diluted ten times relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Standards.	Tests.	Sums.	Differences.
	I.	65.27	80.09	85.36	-45.18
I.	2.	63.44	84.43	87.87	-39.01
	3.	56.37	83.73	88.10	-34.34
	Sums.			260.33	-118.53
Reduced sums of squares.				42.54	56.38
	I.	59.29	40.69	78.90	02.40
2.	2.	42.48	38.83	75.31	-09.35
	3.	36.03	41.55	77.18	05.52
	Sums.			231.47	-01.73
Reduced sums of squares.				5.96	130.35
	I.	46.43	39.38	85.05	-06.91
3.	2.	39.52	41.09	80.61	01.57
	3.	40.34	39.76	80.10	-00.58
	Sums.			245.76	-05.92
Reduced sums of squares.				81.00	58.09.
	I.	61.07	33.80	94.96	-37.13
4.	2.	61.00	17.16	78.16	-43.84
	3.	49.02	21.89	70.91	-27.13
	Sums.			254.03	-108.15
Reduced sums of squares.				98.74	141.55
Sums of reduced sums of squares.				168.04	384.86

S^2/w 28.07
s^2/w 60.79

Criterion of parallelism 3.74
Standard error of criterion 5.29
Departure from parallelism is not significant at the 5% level of P.

4T	-78.21	M'	-0.3820
4U	75.11	M	0.6700
U^2	5,345.0721	C	1.0644
t^2s^2/w	385.4171	CM'^2	0.3427
U'^2	5,081.0649	CM'^2	0.1103.

Log. relative potency is 0.4780 with 95% fiducial limits 0.5899
0.2230
Abs. " " 4.76 " " " " 5.91
3.50

TABLE 6. XXIV.

Ratio of piperonyl butoxide to the pyrethrins :- II.0 : I.
Test solutions diluted ten times relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Standards.	Tests.	Sums.	Differences.
	I.	50.76	22.97	81.63	-55.89
I.	2.	60.00	19.75	79.75	-40.47
	3.	50.36	20.75	79.06	-27.66
	Sums.			240.42	-123.82
Reduced sums of squares.				3.55	9.71
	I.	53.89	33.58	87.47	-00.31
2.	2.	42.71	39.25	81.84	-02.48
	3.	56.30	35.67	70.97	00.37
	Sums.			230.38	-02.42.
Reduced sums of squares.				113.99	4.42
	I.	57.41	44.20	81.61	06.79
3.	2.	52.05	42.99	95.42	-09.64
	3.	55.55	44.20	89.75	06.85
	Sums.			207.18	03.00
Reduced sums of squares.				101.88	207.33
	I.	61.07	51.30	82.37	-30.77
4.	2.	55.34	48.98	78.19	-32.29
	3.	56.54	53.30	80.04	-23.04
	Sums.			240.60	-105.10
Reduced sums of squares.				7.95	34.22
Sums of reduced sums of squares.				227.19	255.96

S^2/n 57.05
s'^2/n 42.62

Criterion of parallelism is -2.13.
Standard error of criterion 6.15
Departure from parallelism is not significant at 5% level of P.

T	-75.64	M'	0.8250
U	70.50	M	0.0762.
U^2	4,962.09	C	1.0451
ts_e^2/w	826.80	CM'	0.3594
V'^2	4,715.89	CM'^{2}	0.1090.

Log. relative potency is 0.0762 with 95% fiducial limits 0.7740
0.5530
Abs. " " " 4.75 " " " " 5.95
3.55.

TABLE 6. XXIX

Ratio of piperonyl butoxide to the pyrethrins :- 13·0 ; I.
Test solutions diluted three times relative to the standard.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	1.	73·6	36·9	110·5	-36·5
1.	2.	74·7	68·0	142·7	-06·7
	3.	74·7	43·9	118·6	-30·8
	Sums.			371·8	-74·0
Reduced sums of squares.				573·48	505·38
	1.	48·4	90·0	138·4	41·6
2.	2.	46·1	80·0	126·1	33·9
	3.	51·4	80·0	131·4	28·6
	Sums.			395·9	104·1
Reduced sums of squares.				102·52	85·46
	1.	52·0	75·8	137·8	13·8
3.	2.	59·3	73·6	132·9	14·3
	3.	58·7	71·6	130·3	12·6
	Sums.			401·0	40·5
Reduced sums of squares.				55·74	6·94
	1.	90·0	53·1	145·1	-36·9
4.	2.	90·0	38·6	128·6	-59·4
	3.	90·0	45·0	135·0	-45·0
	Sums.			406·7	-141·3
Reduced sums of squares.				132·72	231·48
Sums of reduced sums of squares.				864·46	829·26

$S^2/w = 144·03$
$s^2/w = 138·17$

Criterion of parallelism = -6·1
Standard error of criterion = 12·0
Departure from parallelism is not significant at 5% level of P.

$T = -23·7$
$U = 120·1$

$M' = -0·0594$
$M = 0·4177$

$U^2 = 14,424·01$
$t^2 s^2/w = 530·77$
$U'^2 = 13,893·24$

$C = 1·0382$
$CM' = -0·0617$
$CM'^2 = 0·0037$

Log. relative potency is 0·4177 with 95% fiducial limits (0.4190 (0.4118

Abs. " " " 2.62 " " " " (2·63 (2·58.

TABLE 6. XXVI.

Ratio of piperonyl butoxide to the pyrethrins :- 14.0 : 1.
Test solutions diluted 4 times relative to the standards.
Twin Cross-overdesign and analysis of relative potency.

Group.	Replicate.	Response to Standard.	Response to Test.	Sums.	Differences.
1.	1.	68.0	39.8	107.8	-28.2
	2.	56.8	39.2	96.0	-17.6
	3.	56.2	43.9	100.1	-12.5
	Sums.			303.9	-58.1
Reduced sums of squares.				71.78	129.15
2.	1.	24.4	90.0	114.4	65.3
	2.	28.0	68.9	96.9	40.9
	3.	22.0	80.0	102.0	58.0
	Sums.			313.3	164.3
Reduced sums of squares.				172.45	525.37
3.	1.	64.8	56.2	120.4	-02.0
	2.	42.1	71.6	113.7	29.8
	3.	45.0	80.0	125.0	55.0
	Sums.			359.1	56.5
Reduced sums of squares.				64.58	1,097.05.
4.	1.	80.0	38.6	118.6	-41.4
	2.	80.0	31.3	111.3	-48.7
	3.	71.6	26.6	98.2	-45.0
	Sums.			328.1	-135.1
Reduced sums of squares.				202.75	301.35
Sums of reduced sums of squares.				511.56	1,853.12

s^2/w 85.24
s'^2/w 308.78.

Criterion of parallelism -84.1
Standard error of criterion 9.23
t 2.61, just significant at the 5% level of P, not at the 2% level

T	48.9	M'	0.0845
U	173.7	M	0.6865.
U^2	15,301.69	C	1.0840
t^2s^2/w	1,186.15	CM'	0.0916
U'^2	14,115.54	CM'^2	0.0077

Log. relative potency is 0.6865 with 95% fiducial limits 0.7773
0.5957
Abs. " " " 4.86 " " " " 5.99
3.94.

TABLE 6. ~~XXVII~~.

Ratio of piperonyl butoxide to the pyrethrins :- I5.0 : I.
Test solutions diluted three times relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Response to Standard. High.	Low.	Test. High.	Low.
I.	37.5	30.7	67.2	45.9
2.	38.I	20.3	68.0	28.0
3.	48.4	28.0	70.6	42.7
Totals.	I24.0	79.0	205.8	II4.6

Items.	Totals.	Means.
Samples.	203.0	33.8
320.	320.4	53.3
Doses.	329.8	54.9
	I93.6	32.2
Grand.	523.4	43.6

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error
Samples & doses.		2,638.I8	2,638.I8	5I.38
Samples.	I	I,II8.48	I,II8.48	33.47
Doses.	I	I,5I9.70	I,5I9.70	38.98
Error.	9	559.28	59.24	
Total.	II	5,I77.46 .		

M' 0.2564 ; M 0.7355.

Standard error of M' is 59.2 0.50I0 I.96 5I.38 / 2,5I9.70
or 0.6802.

Log. relative potency is 0.7355 with 95% fiducial limits I.4I57
0.0553

Abs. " " " 5.44 " " " " 26.20
0.II4.

TABLE 6. XXVIII.

Ratio of piperonyl butoxide to the pyrethrins :- 15.00 to1.
Test solutions not diluted relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicates.	Response to Standard.		Response to Test.	
	High.	Low.	High.	Low.
I.	39.8	36.0	70.6	55.7
2.	39.2	25.7	69.7	54.3
3.	38.1	30.0	71.6	47.9
Totals.	117.1	89.7	211.9	155.9

Items.	Totals.	Means.
Samples.	206.8	54.9
Doses.	245.6	40.9
Doses.	329.0	54.9
	245.6	40.9
Grand.	574.6	47.9

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		2,741.20	2,741.20	52.36
Doses.	I	583.80	583.80	24.16
Samples.	I	2,157.40	2,157.40	46.44
Error.	9	161.04	I 14.64	
Total.	II	2,902.84.		

M' 0.5786 M 0'5786

Standard error of M' is 14.64 0.5016 2.151 52.56 / 583.80
or 0.0421

Log. relative potency is 0.5786 with 95% fiducial limits $\frac{1.4247}{1.6650}$

Abs. relative potency is 5.79 with 95% fiducial limits $\frac{26.6}{0.45}$

TABLE 6. XXX.

Ratio of piperonyl butoxide to the pyrethrins :- 16.0 : 1.
Test solutions diluted 4 times relative to the standards. .
Twin Cross-over design and analysis of relative potency.

Group.	Replicate.	Response to Standard.	Response to Test.	Sums.	Differences.
	1.	50.6	29.5	80.1	-21.5
1.	2.	73.6	31.9	105.5	-41.7
	3.	56.2	22.8	78.0	-33.4
	Sums.			263.6	-96.6
Reduced sums of squares.				486.18	306.18
	1.	39.2	67.2	106.4	28.0
2.	2.	32.6	78.5	111.1	45.9
	3.	40.4	56.2	96.6	05.8
	Sums.			314.1	79.7
Reduced sums of squares.				109.46	541.42
	1.	41.6	69.7	111.3	28.1
3.	2.	33.8	67.2	101,0	33.4
	3.	46.1	63.4	109.5	17.3
	Sums.			321.8	78.8
Reduced sums of squares.				49.80	108.38
	1.	66.4	66.4	132.8	00.0
4.	2.	62.0	54.9	116.9	-07.1
	3.	47.9	66.6	114.5	18.5
	Sums.			363.0	11.4
Reduced sums of squares.				442.94	258.14
Sums of reduced sums of squares.				1,088.41	1,114.12.

s^2/w 181.35
s^2/w 190.15.

Criterion of parallelism -22.7
Satndard error of criterion. 13.46
t not significant at the 5% level of P.

T	20.7	M'	0.0676.
U	92.2	M	0.6696.
U^2	8,500.84	C	1.0940
t^2s^2/w	730.44	CM'	0.0739
U^2	7,770.40	CM'^2	0.0050

Log. relative potency is 0.6696 with 95% fiducial limits 0.7643
0.5749
Abs. " " " 4.67 " " " " 5.81
3.76.

TABLE 6. XXXI.

Ratio of piperonyl butoxide to the pyrethrins :- I7.0 : I.
Test solutions diluted three times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
	I.	58.I	35.2	93.8	-22.4
I.	2.	62.0	35.7	97.7	-26.3
	3.	64.2	39.2	I03.4	-25.0
	Sums.			294.7	-73.7
Reduced sum of squares.				95.75	I2.80
	I.	I8.4	53.7	72.I	35.3
2.	2.	3I.9	69.7	I0I.6	37.8
	3.	3I.9	69.7	I0I.6	37.8
	Sums.			275.3	II0.9
Reduced sums of squares.				570.99	II.56
	I.	59.3	56.2	II5.5	-03.I
3.	2.	59.3	75.8	II3.I	I6.5
	3.	66.4	75.8	I42.2	09.4
	Sums.			370.8	22.8
Reduced sums of squares.				52I.82	I96.96
	I.	68.9	53.I	I22.0	-I5.8
4.	2.	75.8	57.4	I33.2	-I8.4
	3.	7I.6	66.4	I38.0	-05.2
				393.2	-39.4
Reduced sums of squares.				I2I.72	99.I0
Sums of reduced sums of squares.				I,3I0.28	320.42

s^2/w 2I8.33
s^2/w 53.39

Criterion of parallelism I3.9
Standard error of criterion I4.77
Departure from parallelism not significant at 5% level of P.

T	22.I	M'	0.0684
U	97.3	M	0.5455
U^2	9,467.29	G	I.022I
t^2s^2/w	205.09	CM'2	0.0699
U'2	9,262.20	CM'^{2}	0.0048

Log. relative potency is 0.5455 with 95% fiducial limits 0.5525
0.5425

Abs. " " " 8.5I " " " " 3.57
3.48.

TABLE 6. XXXII.

Ratio of piperonyl butoxide to the pyrethrins :- I8.0 : I.
Test solutions diluted three times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
	I.	64.2	66.4	I30.6	02.2
I.	2.	66.4	66.4	I32.8	00.0
	3.	62.0	7I.6	I33.6	09.6
	Sums.			397.0	II.8
Reduced sums of squares.				439.06	54.52
	I.	43.3	90.0	I33.3	26.7
2.	2.	60.4	75.8	I36.5	I5.I
	3.	53.I	90.0	I43.I	36.9
	Sums.			4I2.9	78.7
Reduced sums of squares.				63.7I	240.57
	I.	90.0	63.4	I53.4	-26.6
3.	2.	75.8	75.8	I5I.6	00.0
	3.	90.0	80.0	I70.0	-I0.0
	Sums.			475.0	-36.6
Reduced sums of squares.				22I.62	36I.04
	I.	54.9	47.9	I02.8	-07.0
4.	2.	6I.3	64.2	I25.5	02.9
	3.	68.9	62.0	I30.9	-06.9
	Sums.			359.26	-I2.00
Reduced sums of squares.				456.66	65.42
Sums of reduced sums of squares.				700.05	72I.55

S^2/w I26.64
s^2/w I20.23

Criterion of parallelism -45.9
Standard error of criterion II.25
t,(8 d.f.) is 4.02, significant at 5 % level of P.

T I4.0
U I4.0

M' 0.30I0
M 0.778I

U^2 I96.00
$\frac{t^2 s^2}{w}$ 462.I2
U'^2 -266.I2

C is negative quantity.

Log.relative potency is 0.778I
For fiducial limits see text.
Abs. " " " 6.00

TABLE 6. XXXIII.

Ratio of piperonyl butoxide to the pyrethrins:- I9.0 :I.
Test solutions diluted three times relative to the ptandards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Response to Standards.	Test.	Sums.	Differences.
	I.	65.5	7I.6	I37.2	06.0
I.	2.	56.2	75.8	I32.0	I9.6
	3.	65.6	7I.6	I37.2	06.0
	Sums.			405.4	3I.6
Reduced sums of squares.				4.48	I24,36
	I.	3I.3	80.0	III.3	48.7
2.	2.	3I.3	90.0	I2I.3	58.7
	3.	39.3	80.0	I09.3	50.7
	Sums.			341.9	I58.I
Reduced sums of squares.				208.03	56.00
	I.	68.0	90.0	I58.0	22.0
3.	2.	68.9	90.0	I58.9	2I.I
	3.	68.9	90.0	I58.9	2I.I
	Sums.			475.8	64.2
Reduced sums of squares.				0.54	0.54
	I.	80.0	63.4	I43.4	-I6.6
4.	2.	80.0	65.6	I45.6	-I4.4
	3.	74.7	62.7	I37.4	-I2.0
	Sums.			426.4	-43.0
Reduced sums of squares.				50.24	I2.02
Sums of reduced sums of squares.				263.29	I92.92

S^2/w 43.87
s^2/w 32.I4

Criterion of parallelism 5.4
Standard error of criterion 6.63
Departure from parallelism is not significant at 5% level of P.

T 70.3
U 77.9

U^2 6,068.4I
t^2s^2/w I23.46
U'^2 5,944.95

M' 0.27I6
M 0.7487

C I.0208
CM' 0.2772
CM'2 0.0753

Log. relative potency is 0.7487 with 95% fiducial limits 0.8095
0.699I

Abs. " " " 5.6I " " " " 6.45
5.0I.

TABLE 6. XXXIV.

Ratio of piperonyl butoxide to the pyrethrins :- 20.0 : I.
Test solutions diluted three times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	60.7	33.9	94.5	-26.9
I.	2.	6I.3	54.9	II6.2	-06.4
	3.	46.7	29.3	76.0	-I6.7
	Sums.			286.7	-50.0
Reduced sums of squares.				800.I7	2I3.46
	I.	29.3	84.3	II3.6	55.0
2½	2.	25.8	75.8	I0I.6	50.0
	3.	33.8	64.2	98.0	30.4
	Sums.			3I3.2	I35.4
Reduced sums of squares.				I35.44	542.62
	I.	65.6	75.8	I4I.4	I0.2
3.	2.	67.2	80.0	I47.2	I2.8
	3.	58.I	90.0	I48.I	3I.9
	Sums.			436.7	54.9
Reduced sums of squares.				II.89	280.82
	I.	80.0	75.8	I55.8	-04.2
4.	2.	90.0	80.0	I70.0	-I0.0
	3.	80.0	68.9	I48.9	-0I.I
	Sums.			474.7	-I5.3
Reduced sums of squares.				247.3I	40.82
Sums of reduced sums of squares.				I,I92.8I	877.72

s^2/w I98.75
s^2/w I46.26

Criterion of parallelism 3.8
Standard error of criterion I4.I0
Departure from parallelism not significant at 5% level of P.

T	4I.7	M'	0.I475
U	85.I	M	0.6246.
U^2	7,242.0I	CM'	0.I599
t^2s^2/w	56I.80	CM'2	0.0236
U'2	6,680.2I	C	I.084I

Log. relative potency is 0.6246 with 95% fiducial limits 0.7348
0.5392.

Abs. " " " 4.8I " " " " 5.43
5.46.

TABLE 6. ~~XXXV~~.

Ratio of piperonyl butoxide to the pyrethrins :- 2I.0 : I.
Test solutions diluted five times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
I.	I.	80.0	28.7	I08.7	-5I.3
	2.	43.9	28.0	7I.9	-I5.9
	3.	67.2	25.I	92.3	-42.I
	Sums.			272.9	-I09.3
Reduced sums of squares.				697.98	678.39
2.	I.	33.2	74.7	I07.9	4I.5
	2.	52.6	75.8	I08.4	43.2
	3.	2I.I	7I.6	92.7	50.5
	Sums.			309.0	I35.2
Reduced sums of squares.				I59.26	54.74
3.	I.	60.7	7I.6	I32.3	I0.9
	2.	4I.0	60.0	I0I.0	I9.0
	3.	4I.0	62.0	I03.0	2I.0
	Sums.			338.3	50.9
Reduced sums of squares.				546.80	70.78
4.	I.	53.7	37.5	9I.2	-I6.2
	2.	67.2	34.4	I0I.6	-32.8
	3.	74.7	62.0	I36.7	-I2.7
	Sums.			329.5	-6I.7
Reduced sums of squares.				I,906.79	234.72
Sums of reduced sums of squares.				3,2I0.83	I,038.53

S^2/w 535.00
s^2/w I75.05

Criterion of parallelism -I6.6
Standard error of criterion 23.I3
Departure from parallelism is not significant at 5% level of P.

T 4.8
U II8.6

U^2 I4,065.96
t^2s^2/w 664.75
U'^2 I3,40I.2I

M' 0.0I22
M 0.7III

C I.0496
CM'_2 0.0I28
CM'^2 0.0002

Log. relative potency is 0.7III with 95 % fiducial limits 0.7787
0.6447

Abs. " " " 5.I5 " " " " " 6.0I
4.4I.

TABLE 16. XXXVI

Ratio of piperonyl butoxide to the pyrethrins :- 22.0 : 1
Test solutions diluted five times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Tests.	Sums.	Differences.
	I.	67.2	23.6	90.8	-43.6
I.	2.	56.8	26.6	83.4	-30.2
	3.	61.5	33.8	95.1	-27.5
	Sums.			269.3	-101.3
Reduced sums of squares.				61.07	145.31
	I.	24.4	80.0	104.4	55.6
2.	2.	24.4	72.5	96.9	47.1
	3.	22.8	80.0	102.8	57.2
	Sums.			304.1	152.9
Reduced sums of squares.				51.52	52.94
	I.	24.4	63.4	87.8	39.0
3.	2.	56.2	71.6	127.8	15.4
	3.	60.7	67.2	127.9	06.5
	Sums.			343.5	65.9
Reduced sums of squares.				1069.34	357.20
	I.	62.0	37.5	99.5	-24.5
4.	2.	54.9	37.5	92.4	-17.4
	3.	68.0	33.8	101.8	-34.2
	Sums.			293.7	-76.1
Reduced sums of squares.				48.02	147.32
Sums of reduced sums of squares.				1,229.95	708.77

S^2/w 204.94
s^2/w 118.99.

Criterion of parallelism -28.1
Standard error of criterion 14.32
t,(8 d.f.) is 1.962,not significant at 5 % level of P.

T	16.1	M'	0.0361
U	134.3	M	0.7350
U^2	18,036.49	C	1.0263
t^2s^2/w	453.63	CM'	0.0371
U'^2	17,582.86	CM'^2	0.0013

Log. relative potency is 0.7350 with 95 % fiducial limits 0.7850
0.6870

Abs. " " " 5.44 " " " " " 6.10
4.87.

TABLE 6. XXXVI(a).

Ratio of isobutylundecylenamide to the pyrethrins :- 0.5 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Response to Standard.		Test.	
	High.	Low.	High.	Low.
I.	43.9	28.7	35.I	22.0
2.	4I.6	28.0	39.2	20.3
3.	39.8	24.4	35.7	I9.4
4.	40.4	28.0	38.I	2I.I
Totals.	I65.7	I09.I	I48.I	82.8

Items.	Totals.	Means.
Samples.	274.8	34.3
	230.9	28.9
Doses.	3I3.8	39.2
	I6I.9	24.0
Grand.	505.7	3I.6

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples and doses.		I,044.97	I,044.97	32.40
Samples.	I	II8.53	II8.53	I0.88
Doses.	I	926.44	926.44	30.45.
Error.	I3	48.34	48.34	
Totals.	I5	I,I93.3I		

M' 0.I076 M 0.I937.

Standard error of M' is 0.30I0 2.I5I 3.72 32.40 / 926.44
or 0.0835.

Log. relative potency is 0.I934 with 95% fiducial limits 0.2769
0.I089

Abs. " " " I.56 " " " " I.89
I.29

TABLE 6. XXXVI(G).

Ratio of isobutylundecylenamide to the pyrethrins, I : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard. High.	Low.	Test. High.	Low.
I.	43.9	28.7	5I.9	29.3
2.	4I.6	28.0	44.4	I9.4
3.	39.8	24.4	5I.9	I9.4
4.	40.4	28.0	38.I	2I.I
Totals.	I65.7.	I09.I	I65.3	89.2

Items.	Totals.	Means.
Samples.	274.8	34.3
	255.5	3I.9
Doses.	332.0	4I.5
	I98.3	24.8
Grand.	530.3	33.2

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		I,I25.65	I,I25.65	33.52
Samples.	I	I,I2I.70	I,I2I.70	33.48
Doses.	I	I.95	I.95	I,39
Error..	I3	355.84	27.22	
Total.	I5.	I,477.49.		

M' 0.00I2 M 0.2968.

Standard error of M' is 27.22 33.52 0.30I0 2.I3I / I,I2I.70
or 0.52I8.

Log. relative potency is 0.2968 with 95% fiducial limits 0.8266
I.7770.
Abs. " " " I.99 " " " " 6.68
0.60.

TABLE 6. XXXVI(c).

Ratio of isobutylundecylenamide to the pyrethrins :- I.5 : I
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Response to Standard.		Response to Test.	
	High.	Low.	High.	Low.
I.	49.6	20.3	39.2	26.6
2.	46.7	29.3	46.I	20.3
3.	49.0	25.8	50.2	20.3
4.	47.9	3I.9	43.0	I8.4
Totals.	I94.2	I07.3	I78.5	85.6

Items.	Totals.	Means.
Samples.	30I.5	37.6
	264.I	33.0
Doses.	372.7	46.6
	I92.9	24.I
Grand.	565.6	35.4

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		2,023.93	2,023.93	44.99
Samples.	I.	29.46	29.46	5.43
Doses.	I.	I,994.47	I,994.47	44.69
Error.	I3.	I45.3I	II.I8	
Total.	I5.	2,I69.24.		

M' 0.0366 ; M 0.2644

Standard error of M' is II.I8 44.99 0.3020 2.I3I / I,994.47
or 0.0752.

Log. relative potency is 0.2644 with 95% fiducial limits

Abs. relative potency is I.84 with 95 fiducial limits

TABLE 6. XXXVII.

Ratio of isobutylundecylenamide to the pyrethrins : 2:I.
Test solutions diluted twice relative to the standards.
"Four-point assay"design and analysis of relative potency.

Replicate.	Response to Standard.		Response to Westl.	
	High.	Low.	High.	Low.
I.	49.6	20.3	45.6	24.4
2.	46.7	29.3	37.5	33.2
3.	49.9	25.9	4I.6	25.6
4.	47.2	3I.2	4I.0	24.4
Totals.	I94.2	I07.5	I65.7	I07.6

Items.	Totals.	Means.
Samples.	30I.5	37.6
	27I.5	33.9
Doses.	357.9	44.7
	2I5.I	26.9
Grand.	573.0	35.8

Analysis of Variance.

Items.	D.F.	Sums of Squares.	Mean square.	Standard error
Samples and doses.		I,299.77	I,297.77	36.00
Samples.	I	28.85	28.85	5.I8
Doses.	I	I,270.92	I,270.92	35.64
Error.	I3	I98.09	I5.24	
Total.	I5	I,495.86.		

M' 0.0457 M 0.2573.

Standard error of M' is I5.24 36.00 0.3010 2.I3I / I,270.92 or 0.

Log, relative potency is 0.2573 with 95% fiducial limits

Abs. " " " " " " "

TABLE 6. XXXVIII.

Ratio of isobutylundecylenamide totthe pyrethrins :- 3.0 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Response to Standard.		Response to Test.	
	High.	Low.	High.	Low.
I.	49.3	22.8	55.I	2I.I
2.	58.7	35.I	56.6	3I.3
3.	50.8	43.6	40.4	35.I
4.	56.8	24.4	37.3	20.9
Totals.	215.6	115.9	151.9	108.4

Items.	Totals.	Means.
Samples,	331.5	42.8
	260.3	32.5
Doses.	367.5	44.7
	224.3	28.0
Grand.	591.8	37.0

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		I,562.40	I,562.40	39.50
Samples.	I	751.35	751.35	27.42
Doses.	I	8II.05	8II.05	28.48
Error..	I3	698.I2	53.70	
Total.	I5	2,260.52		

M' 0.2898 ; M 0.0II2.

Standard error of M' is 53.70 39.50 0.50I0 2.I5I / 8II.05
or 2.2546

Log. relative potency is 0.0II2 with 95% fiducial limits 2.2458
2.2234.

Abs. " " " " " " "

TABLE 6. XXXIX.

Ratio of isobutylundecylenamide to the pyrethrins :- 4.0 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

	Responses to			
Replicate.	Standard.		Test.	
	High.	Low.	High.	Low.
I.	74.7	45.0	52.6	39.2
2.	65.6	42.I	49.6	39.2
3.	63.0	43.0	47.9	4I.2
4.	80.0	40.I	48.8	4I.2
Totals.	283.3	I70.2	I98.9	I60.8

Items.	Totals.	Means.
Doses.	482.2	60.3
Samples.	33I.0	4I.4
Samples.	453.5	56.7
	359.7	44.9
Grand.	8I3.2	50.8

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples and doses.		2,022.92	2,022.92	44.94
Doses.	I	I,469.50	I,469.50	38.34
Samples.	I	553.42	553.42	23.52
Error.	I3	544.32	4I.87	
Total.	I5	2,567.24		

M' 0.I847 M 0.II33.

Standard error of M' is 4I.87 2.I3I 0.30I0 44.94 / I,469.50
or 0.83I3.

Log. relative potency is 0.II63 with 95% fiducial limits 0.9376
I.2950
Abs. " " " I.3I " " " " 8.68
0.I97.

TABLE 6. XX XX

Ratio of isobutylundecylenamide to the pyrethrins:- 5.0 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard. High.	Standard. Low.	Test. High.	Test. Low.
I.	74.7	45.0	64.0	32.4
2.	65.6	42.I	5I.7	4I.I
3.	65.0	43.0	47.0	45.I
4.	80.0	40.I	74.0	39.4
Totals.	285.3	I70.2	236.7	I58.0.

Items.	Totals.	Means.
Samples.	453.5	56.7
	394.7	49.3
Doses.	520.0	65.0
	328.2	4I.0
Grand.	848.2	55.0I.

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		2,502.20	2,502.20	50.02
Samples.	I	209.08	209.08	I4.45
Doses.	I	2,293.I2	2,293.I2	47.88
Error.	I5	630.82	65.I4	
Total.	I5	3,323.02.		

M' 0.0908; M 0.2I02.

Standard error of M' is 65.I4 50.02 0.30I0 2.I5I / 820.82
or 0.8234.

Log. relative potency is 0.2I02 with 95% fiducial limits I.0936
I.3268

Abs. " " " I.63 " " " " I2.4
0.2I.

TABLE 6. XXXXI.

Ratio of isobutylundecylenamide to the pyrethrins, q- 6.0 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Response to Standard. High.	Standard. Low.	Test. High.	Standar Low.
I.	74.7	45.0	74.0	29.4
2.	65.6	42.I	74.0	3I.6
3.	65.0	43.0	79.4	45.9
4.	80.0	40.I	65.3	4I.4
Totals.	285.3	I70.2	292.7	I48,3

Items.	Totals.	Means.
Samples.	453.5	56.7
	44I.0	55.I
Doses.	576.0	72.0
	3I8.5	39.8
Grand.	694.5	55.94.

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		4,I55.85	4,I55.85	64.46
Doses.	I	4,I45.85	4,I45.85	64.38
Samples.	I	I0.00	I0.00	3.I6
Error.	I5	552.6I	42.50	
Total.	I5	4,708.46.		

M' 0.0I48 ; M 0.2962.

Standard error of M' is 42.50 0.30I0 2.I5I 64.46 / 4,I45.85
or 0.4238.

Log.relative potency is 0.2962 with 95% fiducial limits 0.7200
I.7624.
Abs. " " " I.98 " " " " 5.26
0.56.

TABLE 6. XLII.

Ratio of isobutylundecylenamide to the pyrethrins :-7.0 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard. High.	Standard. Low.	Test. High.	Test. Low.
I.	74.7	45.0	82.4	5I.8
2.	65.6	42.I	74.0	45.8
3.	63.0	43.0	77.7	43.I
4.	80.0	40.I	77.7	42.2
Totals.	283.3	I70.2	3II.8	I80.9

Items.	Totals.	Means.
Samples.	453.5	56.7
Doses.	492.7	6I.6
Doses.	595.I	74.4
	35I.I	43.9
Grand.	946.2	59.I3.

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean squares.	Standard error.
Samples & doses.		3,854.88	3,854.88	62.08
Samples.	I	II4.96	II4.96	I0.70
Doses.	I	3,739.92	3,739.92	6I.I4
Error.	I3.	283.65	2I.8I9.	
Total.	I5.	4,I38.53.		

M' 0.0527. ; M 0.3537

Standard error of M' is 2I.8I9 62.08 0.0010 2.I3I / 3,739.92. or 0.2323.

Log. relative potency is 0.3537 with 95% fiducial limits 0.5860 0.I2I4

Abs. " " " 2.26 " " " " 3.86 I.32.

Table 6. XLIII.

Ratio of isobutylundecylenamide to the pyrethrins :- 8.0 ; I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Response to Standard. High.	Low.	Test. High.	Low.
I.	74.7	45.0	45.0	47.0
2.	65.6	42.I	6I.5	45.8
3.	63.0	45.0	64.9	44.0
4.	80.0	40.I	65.7	45.2
Totals.	283.3	I70.2	237.I	182.0

Items.	Totals.	Means.
Doses.	520.4	65.I
Samples.	352.8	44.I
Samples.	453.5	56.7
	419.7	52.5
Grand.	873.2	54.57.

Analysis of Variance.

Items.	D.F.	Sums of Squares.	Mean square.	Standard error.
Doses & samples.		I,883.I8	I,883.I8	43.40.
Samples.	I	97.I8	97.I8	9.86.
Doses.	I	I,786.00	I,786.00	42.25.
Error.	I3	I,947.20	I49.78	
Total.	I5	3,830.38.		

M' 0.0I64 ; M 0.2846.

Standard error of M' is I49.78 2.I5I 0.30I0 43.39 / I,786.00
or 2.8I42.

Log. relative potency is 0.2846 with 95 % fiducial limits 2.4987
2.0705.
Abs. " " " I.93 " " " " 3I5.0
0.0I2.

Table. 6. XLIV.

Ratio of isobutylundecylenamide to the pyrethrins :- 9.0 : I
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard. High.	Low.	Test. High.	Low.
I.	74.7	45.0	49.8	41.6
2.	65.6	42.I	58.5	4I.2
3.	63.0	43.0	57.6	45.8
4.	80.0	40.I	68.9	39.5
Totals.	283.3	I70.2	233.8	I86.I

Items.	Totals.	Means.
Doses.	5I6.I	64.5
	296.3	37.0
Samples.	453.5	56.7
	356.9	44.6
Grand.	8I0.4	50.6

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		5,556.42	3,536.42	59.46
Doses.	I	2,997.88	2,997.88	54.72
Samples.	I	558.54	558.54	23.20
Error.	I5	3,590.70	276.2I	
Total.	I7	7,I47.I2		

M' 0.I276 M 0.I934.

Standard error of M' is 276.2I 0.30I0 8.IBI 59.46 / 8,997.88
or 3.5I57.

Log. relative potency is 0.I734 with 95% fiducial limits 5.687I
I.6507.
Abs. " " " I.49 " " " " 4,860
0.00045.

TABLE 6. XLV.

Ratio of isobutylundecylenamide to the pyrethrins :- IO : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Response To Standard. High.	Standard. Low.	Test. High.	Low.
I.	74.7	45.0	54.5	34.5
2.	65.6	42.I	6I.8	40.0
3.	63.0	43.0	6I.0	33.2
4.	80.0	40.I	68.I	36.0
Totals.	283.3	I70.2	245.4	I43.7

Items.	Totals.	Means.
Samples.	453.5	56.7
	389.I	48.7
Doses.	528.7	66.I
	3I3.9	39.2
Grand.	842.6	52.66

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean squares.	Standard error.
Samples & doses.		3,I7I.93	3,I7I.93	56.32
Samples.	I	29I.30	29I.30	I7.06
Doses.	I	2,880.63	2,880.63	53.67
Error.	I3	500.2I	23.09	
Total.	I5	3,472.I4		

M' 0.0057 M 0.2053.

Standard error of M' is 23.09 0.30I0 2.I3I 56.32 / 2,880.63
or 0.2895.

Log. relative potency is 0.2053 with 95% fiducial limits 0.4948
I.9I58

Abs. relative potency is I.6I with 95% fiducial limits 3.I2
0.82

Table 6.X LVI.

Ratio of isobutylundecylenamide to the pyrethrins:- II.0 : I
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard. High.	Low.	Test. High.	Low.
I.	74.7	45.0	73.6	40.4
2.	65.6	42.I	7I.6	37.3
3.	63.0	43.0	63.9	36.0
4.	60.0	40.I	78.8	39.2
Totals.	283.3	I70.2	287.9	I52.9

Items.	Totals.	Means.
Doses.	57I.2	7I.5
	325.I	40.4
Samples.	453.5	56.7
	440.8	55.I
Grand.	894.3	55.89.

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		3,9I2.93	3,9I2.83	62.54
Samples.	I	I0.I6	I0.I6	3.I9
Doses.	I	3,902.67	3,902.67	62.46
Error.	I3	294.I3	22.69	
Total.	I5.	4,207.76.		

MB 0.0I54 : M 0.2856.

Standard error of M' is 22.69 0.50I0 2.I3I 62.54 / 3,902.67.
or 0.2352.

Log. relative potency is 0.2856 with 95 % fiducial limits 0.5I88
0.0524
Abs.re " " " I.94 " " " " 3.30
I.I3.

TABLE 6. XLVII.

Ratio of isobutylundecylenamide to the pyrethrins :-12.0 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard High.	Standard Low.	Test High.	Test Low.
I.	38.6	33.8	22.8	20.0
2.	36.3	30.5	33.5	18.0
3.	42.5	25.0	39.8	20.3
4.	36.9	21.5	39.0	10.0
Totals.	154.3.	110.8	135.1	68.3

Items.	Totals.	Means.;	Totals.	Means.
Doses.	289.4	36.2	179.1	22.4
Samples.	265.1	33.3	203.4	25.4
Grand.	468.5	29.1.		

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples doses.		1,215.61	1,345.61	34.86.
Samples.	1	360,84	360.84	19.00
Doses.	1	854.77	854.77	29.23
Error.	13	269.71	20.747.	
Total.	15	1,485.32		

M' 0.1640.;M 0.1370.

Standard error of M' is 20.747 2.151 0.3010 34.86 / 854.77
or 0.5427.

Log.relative potency is 0.1370 with 95% fiducial range 0.6797
1.5943.
Abs. " " " 1.37 " " " " 4.78
0.395.

TABLE 6. XLVIII.

Ratio of isobutylundecylenamide to the pyrethrins :- I3.0 : I.
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard High.	Standard Low.	Test High.	Test Low.
I.	38.6	33.8	37.0	25.I
2.	36.3	30.5	25.I	24.8
3.	42.5	25.0	30.0	22.I
4.	36.9	2I.5	3I.9	I4.2
Totals.	I54.3	II0.8	I23.0	86.2

Items.	Totals.	Means.
Doses.	277.3	34.7
Sample	I97.0	24.6
Samples.	265.I	33.3
	209.2	26.I
Grand.	474.3	29.6

Analysis of variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		677.90	677.90	26.03
Samples.	I	248.67	248.67	I5.76
Doses.	I	429.23	429.23	20.7I
Error.	I3.	269.99	20.768	
Total.	I5.	947.89.		

M' 0.2290 : M 0.0720.

Standard error of M is 2o.768 0.30I0 2.I3I 26.03 / 429.23.
or 0.8078.

Log.relative potency is 0.0720 with 95% fiducial range 0.8798
I.2642.
Abs, " " " I.I8 " " " " " 7.58
0.I84.

Table 6. XLIX.

Ratio of isobutylundecylenoamide to the pyrethrins :- I4.0 : I
Test solutions diluted twice relative to the standards.
"Four-point assay" design and analysis of relative potency.

Replicate.	Responses to Standard. High.	Low.	Test. High.	Low.
I.	38.6	33.8	23.0	I9.0
2.	36.3	30.5	35.I	I0.4
3.	42.5	25.0	27.0	23.0
4.	36.9	2I.5	39.0	24.4
Totals.	I54.3	II7.8	I24.I	75.8.

Items.	Totals.	Means.
Doses.	278.4	34.8
	I86.6	23.3
Samples	265.I	33.3
	I99.9	24.9
Grand.	465.0	29.I

Analysis of Variance.

Items.	D.F.	Sums of squares.	Mean square.	Standard error.
Samples & doses.		778.44	778.44	27.90
Doses.	I	504.60	504.60	22.46
Samples.	I	273.84	273.84	I6.54
Error.	I3.	393.04	30.233.	
Total.	I5.	I,I7I.48		

M' 0.22I6 ; M 0.0794.

Standard error of M'is 30.233 0.30I0 2.I3I 27.90 / 504.60.
or I.0722.

Log. relative potency is 0.0794 with 95% fiducial range $\frac{I.I5I6}{I.0072.}$

Abs. " " " I.20 " " " " $\frac{I4.20}{0.I02.}$

TABLE 6. XLX.

Ratio of Sesamin to Pyrethrins :- I : 2.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Dose applied

Group.	Replicate.	Responses of Standard 1st day.	Responses of Test 2nd day.	Sums.	Differences.
	I	38.1	23.6	61.7	-14.5
I	2	42.1	34.4	76.5	-7.7
	3	33.8	51.9	85.7	-1.9
	Sums.			203.9	24.1.
Reduced sums of squares.				130.82	80.35
	I	35.1	49.0	84.1	13.2
2	2	10.0	56.2	66.2	46.2
	3	24.4	53.1	77.5	28.7
	Sums.			227.8	88.8
Reduced sums of squares.				171.48	496.22
	I	19.4	60.7	80.1	41.3
3	2	10.0	46.1	56.1	36.1
	3	22.8	53.1	81.9	30.3
	Sums.			218.1	107.7
Reduced sums of squares.				414.96	60.56
	I	41.6	17.9	59.5	-23.7
4	2	39.8	27.5	67.1	-12.3
	3	35.1	45.0	80.0	9.9
	Sums.			206.7	26.3
Reduced sums of squares,				217.04	584.51
Sums of reduced sums of squares.				934.30	1,221.64

s^2/w 155.67
s^2/w 193.47

Criterion of parallelism -11.7.30
Standard error of criterion 12.7.88
Departure from parallelism is not significant, at 5% level of P.

T 146.1 M' 0.1781.
U 246.9 M 0.1781.

relative potency 1.51.

U^2 6773.29 C 1.1791
t^2s^2/w CM' 0.2099
U'^2 1029.26 / 5744.03 CM'^{2} 0.0374

Log. relative potency is 0.1781 with 95% fiducial limits 0.3613
0.0586

Abs. " " " 1.52 " " " " 2.30
1.14.

TABLE 6. XLXI.

Ratio of Sesamin to Pyrethrins :- I:I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Group.	Replicate.	Responses to Standard	Responses to Test	Sums	Differences.
	I	40.4	46.I	86.5	5.7
I	2	26.6	42.I	68.7	I5.5
	3	36.3	43.3	79.6	7.0
	Sums.			234.8	28.2
Reduced sums of squares.				I53.26	38.50
	I	II.5	59.3	70.8	47.8
2	2	2I.I	59.3	80.4	38.2
	3	I4.2	67.2	8I.4	53.0
	Sums.			I52.6	I39.0
Reduced sums of squares.				96.26	I27.38
	I	22.0	7I.6	93.6	49.6
3	2	2I.I	59.3	80.4	38.2
	3	34.4	75.8	II0.2	4I.4
	Sums.			284.2	I29.2
Reduced sums of squares.				435.42	64.84
	I	40.4	43.3	83.7	2.9
4	2	42.I	50.2	92.3	8.I
	3	34.4	3I.9	64.3	-2.5
	Sums.			242.3	8.5
Reduced sums of squares.				8I.65	56.47.
Sums of reduced sums of squares.				766.57	277.I9.

s^2/w I27.73
s^2/w 34.65

Criterion of parallelism -I3.2
Standard error of criterion II.88
t I.I7, not significant at 5% level of P.

4F 305.9
4U 230.5

U^2 5898.24
$\frac{t^2 s^2}{w}$ I84.34
U'^2 5713.90.

M' 0.3980
M 0.3960.

G I.0323
GM' 0.4I08
GM'^2 0.I635

Log relative potency is 0.3980 with 95% fiducial limits 0.4885
0 3075

Abs. " " "3.50 " " " " 3.08

Table 6. X2 X11.

Ratio of Sesamin to Pyrethrins :- 2:I.
Test solution diluted three times relative to standards.
Twin cross-over design and analysis of relative potency.

Group.	Replicate.	Responses to Standard.	Responses to Test.	Sums.	Differences
	I	39.2	I5.0	54.2	-24.2
I	2	50.8	26.6	77.4	-24.2
	3	55.3	I5.0	50.3	-20.3
	Sums			I8I.9	-68.7
Reduced sums of squares.				435.35	I0.I4
	I	24.I	33.2	57.3	09.I
2	2	I8.8	47.9	66.7	29.I
	3	3I.7	5I.9	83.6	20.2
	Sums			207.6	58.4
Reduced sums of squares.				355.22	204.70
	I	27.I	5I.9	79.0	24.8
3	2	28.0	45.0	73.0	I7.0
	3	28.7	49.6	78.3	20.9
	Sums			230.3	62.7
Reduced sums of squares.				36.88	30.42
	I	39.2	33.2	72.4	-6.0
4	2	40.9	25.0	65.9	-I5.9
	3	42.I	32.6	74.7	-9.5.
	Sums			2I3.0	-3I.4
Reduced sums of squares.				4I.66	49.36
Sums of reduced sums of squares.				869.II	294.62.

S^2/w I44.8I
s^2/w 49.04.

Criterion of parallelism. -I6.3
Standard error of criterion I2.03.
t I.35, not significant at the 5% level of P.

4T 2I.0
4U 22I.2

U^2 5,43I.69
$\frac{t^2 s^2}{w}$ 260.89
U'^2 5,I70,80

M' 0.0286
M 0.5057

C I.0304
CM' 0.0300
CM' 0.0009.

Log relative potency is 0.5057 with 95% fiducial limits 0.5735
0.4379.

Abs. " " " 3.20 " " " " 3.75
2.74

TABLE 6. XLXIII.

Ratio of Sesamin to pyrethrins :- 3:I.
Test solutions diluted three times relative to standards.
Twin Cross-over design and analysis of relative potency.

Group.	Replicate.	Responses to Standard.	Test.	Sums.	Differences.
	I	39.2	2I.I	60.3	-I8.I
I	2	45.0	I8.4	63.4	-26.6
	3	35.I	I8.4	53.5	-I6.7
	Sums.			I77.2	-6I.4
Reduced sums of squares.				45.38	55.36
	I	I8.4	39.2	57.6	20.8
2	2	I0.0	33.2	43.2	23.2
	3	I8.4	4I.0	59.4	22.6
	Sums.			I60.2	66.6
Reduced sums of squares.				I57.68	03.I2
	I	I5.3	33.2	48.5	I7.9
3	2	3I.3	49.0	80.3	I7.7
	3	24.4	50.8	75.2	26.4
R	Sums.			204.0	62.0
Reduced sums of squares.				583.38	53.46
	I	37.5	I5.3	52.8	-22.2
4	2	35.I	I5.3	50.4	-I9.8
	3	37.5	I0.0	47.5	-27.5
	Sums.			I50.7	-69.5
Reduced sums of squares.				I9.II	28.73.
Sums of reduced sums of squares.				805.55	I40.67.

s^2/w I34.2I97.
s^2/w 23.434I.

Criterion of parallelism I2.I
Standard error of criterion II.60
t I.04,not significant at the 5% level of P.

U 86.5;T -0.90; m M' 0.003I: M 0.4740.

U^2 74832.5
$\frac{t^2 s^2}{w}$ 93.7
U'^2 7388.52

C I.0I27
CM' 0.003I
CM'^2 0.000I

Log.relative potency is 0.4740 with 95% fiducial limits 0.5079
0.440I.
Abs. " " " 2.98 " " " " 3.22
2.76.

TABLE 6. XLIV.

Ratio of Sesamin to Pyrethrins :- 4:I.
Test solutions diluted three times relative to the standards.
Twin cross-over design and analysis of relative potency.

Groups.	Replicate.	Responses to Standard.	Responses to Test.	Sums.	Differences.
I	I	54.3	I8.4	72.7	-35.9
	2	40.9	27.6	68.5	-I3.3
	3	30.2	24.5	74.7	-25.7
Sums				215.9	74.9
Reduced sums of squares.				34.42	26I.I8.
2	I	25.8	49.6	75.4	23.8
	2	5I.3	45.6	76.9	I4.3
	3	22.8	36.3	59.I	I3.5
Sums				2II.4	5I.6
Reduced sums of squares.				I87.88	65.66.
3	I	27.3	5I.4	78.7	24.I
	2	32.3	55.6	87.9	23.3
	2	30.7	57.4	88.I	26.7
Sums				254.7	74.I
Reduced sums of squares.				57.68	6.32
4	I	46.7	24.I	70.8	-22.6
	2	3I.9	25.7	57.6	-06.2
	3	43.0	25.0	68.0	-I8.0
Sums				I96.4	-46.8
Reduced sums of squares.				I09.84	I43.I2.
Sums of reduced sums of squares.				589.82	476.28.

S^2/w 64.79
s^2/w 779.54.

Criterion of parallelism - I8.I
Standard error of criterion 8.06
t 2.24 not significant at the 5% level of P.

4T 4.0
4U 247.4

U^2 6,806.25
$t^2 s^2/w$ 422.09
U'^2 6,384.I6.

M' 0.0048
M 0.48I9

C I.066I
CM' 0.005I
CM'2 0.0002.

Log. relative potency is 0.48I9 with fiducial limits (95%) 0.4879
0.4759

Abs. relative potency is 3.03 with fiducial limits (95%) 3.07

TABLE 6. XLXV.

Ratio of Sesamin to pyrethrins :- 6:I.
Test solutions diluted twice relative to standards.
Twin cross-over design and analysis of relative potency.

G

Group.	Replicate.	Responses to Standard	Responses to Test	Sums	Differences.
	I	45.0	32.6	77.6	-I2.4
I	2	39.2	28.7	67.9	-I0.5
	3	4I.6	30.7	72.3	-I0.9
	Sums			2I7.8	-33.8
Reduced sums of squares.				47.I8	0.88
	I	29.3	5I.4	80.7	22.I
2	2	22.0	59.3	8I.3	37.3
	3	I7.5	55.6	73.I	38.I
	Sums			235.I	97.5
Reduced sums of squares.				53.95	I62.56
	I	26.6	63.4	90.0	36.8
3	2	I6.4	58.7	75.I	42.3
	3	27.3	60.7	88.0	33.4
	Sums			253.I	II2.5
Reduced sums of squares.				I32.37	40.34
	I	47.3	33.2	80.5	-I4.I
4	2	39.8	33.2	73.0	-06.6
	3	46.I	44.4	90.5	-0I.7
	Sums			244.0	-22.4
Reduced sums of squares.				I62.30	77.26
Sums of reduced sums of squares.				365.80	28I.04.

S^2/w 60.95
s^2/w 46.82

Criterion of parallelism -8.8
Standard error of criterion 7.8I.
Departure from parallelism is not significant at 5% level of P

4T I55.8
4U 266.2

M' 0.I789
M 0.4739

U^2 7,867.69
$\frac{t^2 s^2}{w}$ 249.08
U'^2 7,6I8.6I.

C I.0326
CM' 0.I785
CM'^2 0.0309

Log. relative potency is 0.4737 with 95% fiducial limits 0.53
0.4I60

Abs½. " " " 2.98 " " " " Io

5.44
2.58

TABLE 6. XLXVI.

Ratio of Sesamin to Pyrethrins :- Io;I
Test solutions diluted three times relative to standards.
Twin Cross-over design and analysis of relative potency.

Group.	Replicate.	Responses to Standard.	Responses to Test.	Sums.	Differences.
	I	4I.0	25.6	67.6	-I4.4
I	2	54.3	26.6	80.9	-I7.7
	3	50.8	24.4	75.2	-26.4
Sums.				223.7	-58.5
Reduced sums of squares.				8I.59	76.86
	I	24.4	39.2	63.6	I4.8
2	2	26.6	4I.0	67.6	I4.4
	3	26.6	58.7	85.3	32.I
Sums.				2I6.5	6I.5
Reduced sums of squares.				259.5I	206.29
	I	26.6	45.3	7I.9	I8.4
3	2	26.6	4I.0	67.6	I4.4
	3	33.2	46.7	79.9	I3.5
Sums.				2I9.4	46.6
Reduced sums of squares.				85.24	I7.00
	I	49.0	24.4	73.4	-24.6
4	2	49.0	26.6	75.6	-32,4
	3	49.0	26.6	75.6	-22.4
Sums.				224.6	-59.4
Reduced sums of squares.				I8.20	05.54
Sums of reduced sums of squares.				444.54	305.69.

S^2/w 74.04
s^2/w 50.94

Criterion of parallesism I4.I
Standard error of criterion 8.60
t I.639, not significant at the 5% level of P.

U 77.5; T -5.70. M' -0.0222, M 0.4549

U^2 6,063.250
$t^2 s^2/w$ I95.676
U'^2 5,866.574.

G I.0334
GM' 0.0229
GM'^2 0.005I

Log. relative potency is 0.4549 with 95% fiducial limits 0.5I07
0.3977
Abs. " " " 2.85 " " " " 3.24
2.50.

TABLE 6. XLXVII.

Ratio of Sesamin to the pyrethrins :- I2 : I.
Test solutions diluted three times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates..	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	75.8	57.4	I33.2	-I6.4
I.	2.	68.0	65.6	I33.6	-02.4
	3.	71.6	60.7	I32.3	-I0.8
	Sums.			399.1	-29.7
Reduced sums of squares.				I4.I9	99.50
	I.	32.6	74.7	I07.3	42.I
2.	2.	54.4	70.6	I05.0	I6.2
	3.	47.3	80.0	I27.3	32.7
	Sums.			339.6	III.0
Reduced sums of squares.				300.85	45.I4
	I.	45.0	62.0	I07.0	I7.0
3.	2.	40.4	6I.3	I0I.7	I7.9
	3.	57.4	55.6	II3.0	-02.2
	Sums.			32I.7	32.7
Reduced sums of squares.				74.65	248.82
	I.	90.6	3I.3	III.3	-48.7
4.	2.	80.0	35.7	II5.7	-44.3
	3.	62.7	I8.4	6I.3	-44.5
R	Sums.			308.3	-I37.5
Reduced sums of squares.				7ZI.46	82.06
Sums of reduced sums of squares.				I,II8.90	4I8.82.

s^2/w I86.44
s^2/w 69.24

Criterion of parallelism. I5.3
Standard error of criterion I8.65
t (3 d.f.) is I.I8, not significant at 5 % level of P.

T	-07.7	M'	-0.0283
U	I03.5	M	0.4543
U^2	I0,7I8.25	G	I.0254
$t^2 s^2/w$	865.98	Gm'	-0.0283
U'^2	I0,446.27	Gm'2	0.0005

Log. relative potency is 0.4543 with 95 % fiducial limits 0.5032
0.4064

Abs. " " " 2.65 " " " " 3.I8
2.55.

TABLE

Ratio of piperonyl butoxide to Sesamin to the pyrethrins :- I5.0 ; I.
Test solutions diluted three times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	I.	73.6	36.9	II0.5	-36.5
I.	2.	74.7	68.0	I42.7	-06.7
	3.	74.7	43.9	II8.6	-30.3
	Sums.			57I.8	-74.0
Reduced sums of squares.				575.48	505.38
	I.	48.4	90.0	I38.4	4I.6
2.	2.	46.I	80.0	I26.I	33.9
	3.	5I.4	80.0	I3I.4	28.6
	Sums.			395.9	I04.I
Reduced sums of squares.				I02.52	85.43
	I.	52.0	75.3	I37.8	I3.8
3.	2.	59.3	73.6	I32.9	I4.3
	3.	58.7	7I.6	I30.3	I2.6
	Sums.			40I.0	40.5
Reduced sums of squares.				55.74	6.94
	I.	90.0	53.I	I43.I	-36.9
4.	2.	90.0	38.6	I28.6	-59.4
	3.	90.0	45.0	I35.0	-45.0
	Sums.			406.7	-I4I.3
Reduced sums of squares.				I58.72	23I.48
Sums of reduced sums of squares.				864.46	829.26

S^2/w I44.03
s^2/w I38.I7

Criterion of parallelism -6.I
Standard error of criterion I2.0
Departure from parallelism is not significant at 5 % level of P.

T	-23.7	M'	-0.0594
U	I20.I	M	0.4I77
U^2	I4,424.0I	C	I.0582
t^2s^2/w	530.77	CM'	-0.06I7
U'^2	I3,893.24	CM'2	0.0037

Log. relative potency is 0.4I77 with 95% fiducial limits 0.4I90
0.4II8

Abs. " " " 2.62 " " " " 2.63
2.58.

TABLE 6. XLXVIII.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 0.25 : I.
Test solutions diluted twice relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
I.	I.	50.8	24.4	75.2	-26.4
	2.	58.7	3I.3	89.0	-27.4
	3.	49.0	2I.I	70.I	-27.9
	Sums.			234.3	-7I.7
Reduced sums of squares.				I9I.22	5I2.50
2.	I.	2I.I	6I.3	82.4	40.2
	2.	40.4	57.4	97.8	I7.0
	3.	22.8	5I.9	74.7	29.I
	Sums.			254.9	86.3
Reduced sums of squares.				293.68	266.4I
3.	I.	3I.3	54.9	86.2	23.6
	2.	3I.3	49.0	80.3	I7.7
	3.	I8.4	37.5	55.9	I9.I
	Sums.			222.4	50.4
Reduced sums of squares.				523.50	388.34
4.	I.	48.4	3I.3	79.7	-I7.I
	2.	56.8	25.I	8I.9	-3I.7
	3.	54.9	I5.3	70.2	-39.6
	Sums.			25I.8	-88.4
Reduced sums of squares.				69.60	257.66
Sums of reduced sums of squares.				I,078.00	I,424.9I

S^2/w I79.66
s^2/w 237.47

Criterion of parallelism - 3.6
Standard error of criterion I3.39
Departure from parallelism not significant at 5 % level of P.

T	- 7.8	M'	-0.0237
U	99.0	M	0.2773.
U^2	9,80I.00	C	I.I026
t^2s^2/w	9I2.24	CM'	-0.026I3
U'^2	8,888.76	CM'^2	0.0006.

Log. relative potency is 0.2773 with 95 % fiducial limits 0.3740
0.I806

Abs. " " " I.89 " " " " 2.37
I.52.

TABLE 6. XLIX.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 0.5 : I.
Test solutions diluted three times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
	I.	50.8	I8.4	69.2	-32.4
I.	2.	4I.6	24.4	65.4	-I6.6
	3.	38.6	22.0	60.6	-I6.6
	Sums.			I95.2	65.6
Reduced sums of squares,				30.64	I64.24
	I.	25.8	5I.4	77.2	25.6
2.	2.	I5.5	39.8	55.I	24.5
	3.	24.4	43.3	67.7	I8.9
	Sums.			200.0	69.0
Reduced sums of squares.				239.I4	25.82
	I.	28.7	30.7	59.4	02.0
3.	2.	33.2	35.I	68.3	0I.9
	3.	28.7	42.I	70.8	I3.4
	Sums.			I98.5	I7.3
Reduced sums of squares.				65.I9	86.83
	I.	45.0	I8.4	63.4	426.6
4.	2.	39.8	00.0	39.8	-39.8
	3.	52.5	49.0	I0I.5	-03.5
	Sums.			204.7	-69.9
Reduced sums of squares.				I,945.5I	675.I8
Sums of reduced sums of squares.				2,556.05	942.07

s^2/w 426.00
s^2/w I57.0I

Criterion of parallelism 0.40
Standard error of criterion 20.62
Departure from parallelism not significant at 5 % level of P.

T	-I6.4	M'	-0.0667
U	74.0	M	0.4I04
U^2	5,476.00	C	I.I237
t^2s^2/w	603.I5	CM'	-0.0750
U'^2	4,872.85	CM'^2	0.0050

Log. relative potency is 0.4I04 with 95 % fiducial limits 0.5I90
0.30I8

Abs. " " " 2.57 " " " " 3.3I
2.00.

TABLE 6. I.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 0.75 : I.
Test solutions diluted four times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
	I.	50.2	24.4	74.6	-25.8
I.	2.	55.7	23.6	77.3	-30.I
	3.	45.0	I7.5	62.5	-27.5
	Sums.			2I4.4	83.4
Reduced sums of squares.				II7.I0	93.80
	I.	33.8	48.4	82.2	I4.6
2.	2.	23.6	33.8	57.4	I0.2
	3.	2I.I	37.5	58.6	I8.4
	Sums.			I98.2	4I.2
Reduced sums of squares.				384.54	2I.72
	I.	35.I	35.I	70.2	00.0
3.	2.	34.4	30.7	65.I	-03.7
	3.	20.3	35.I	55.4	I4.8
	Sums.			I90.7	II.I
Reduced sums of squares.				I06.69	I9I.66
	I.	58.7	I7.5	76.2	-4I.2
4.	2.	56.2	I4.2	70.4	-42.0
	3.	50.8	I5.3	66.I	-35.5
	Sums.			2I2.7	II8.7
Reduced sums of squares.				5I.38	2I.I7
Sums of reduced sums of squares.				659.7I	243.93

S^2/w I09.94
s^2/w 40.65

Criterion of parallelism I2.7
Standard error of criterion I6.47
t I.2I3, not significant at 5 % level of P.

T - 50.0
U 84.8

U^2 7,I9I.04
t^2s^2/w I56.I6
U'^2 7,034.88

MB -0.I774
M 0.4246

C I.0823
CM' -0.I8I4
CM'2 0.0322

Log. relative potency is 0.4246 with 95 % fiducial limits 0.4765
0.3727

Abs. " " " 2.65 " " " " 2.99
2.36.

TABLE 6. LI.

Ratio of piperonyl cyclohexenone to the pyrethrins :- I : I.
Test solutions not diluted relative tothe standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differehces.
	I.	39.8	69.7	I09.5	-29.9
I.	2.	27.3	67.2	94.5	-39.9
	3.	28.7	69.7	98.4	-4I.0
	Sums.			302.4	II6.8
Reduced sums of squares.				I2I.I4	78.50
	I.	25.I	80.0	I05.I	54.9
2.	2.	23.6	90.0	II3.6	66.4
	3.	2I.I	80.0	I0I.I	58.9
	Sums.			319.8	I80.2
Reduced sums of squares.				8I.50	62.I6
	I.	25.8	90.0	II5.8	64.2
3.	2.	27.3	90.0	II7.3	62.7
	3.	I8.4	90.0	I08.4	7I.6
	Sums.			341.5	I98.5
Reduced sums of squares.				56.79	38.79
	I.	50.8	54.9	I05.7	-04.I
4.	2.	4I.6	66.4	I08.0	-I4.8
	3.	46.I	58.I	I04.2	-I2.0
	Sums.			3I7.9	-30.9
Reduced sums of squares.				28.52	6I.58
Sums.of reduced sums of squares.				287.95	24I.04

S^2/w 47.98
s^2/w 40.I7

Criterion of parallelism - I5.7
Standard error of criterion 6.927
t I977, not significant at 5 % level of P.

T I73.5 M' 0.6602
U 79.I M 0.6602

U^2 6,256.8I C I.0253
t^2s^2/w I54.3I CM' 0.6769
U'2 6,I02.50 CM'2 0.4469

Log. relative potency is 0.6602 with 95 % fiducial limits 0.7764
0.5440

Abs. " " " 4.58 " " " " 5.98
3.50.

Table 6. 211.

Ratio of piperonyl cyclohexenone to the pyrethrins :- I : I.
Test solutions diluted three times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
I.	I.	60.0	35.8	95.8	-26.2
	2.	58.I	45.0	I03.I	-I3.II
	3.	45.0	50.8	95.8	I5.8
	Sums.			292.7	-83.5
Reduced sums of squares.				38.I7	924.39
2.	I.	I8.4	58.I	76.5	39.7
	2.	2I.I	6I.5	82.4	40.2
	3.	30.0	74.7	I04.7	44.7
	Sums.			263.5	I24.6
Reduced sums of squares.				486.36	I9.32
3.	I.	2I.I	46.7	67.8	25.6
	2.	35.7	7I.6	I07.3	35.9
	3.	2I.I	7I.6	92.7	50.5
	Sums.			267.8	II2.0
Reduced sums of squares.				788.88	3I6.82
4.	I.	7I.6	2I.I	92.7	-50.5
	2.	5I.9	28.7	80.6	-23.2
	3.	62.7	50.2	II2.9	-I0.5
	Sums.			286.2	-84.2
Reduced sums of squares.				532.58	832.72
Sums of reduced sums of squares.				I,845.99	2,093.25

S^2/w 307.66
s^2/w 348.87

Criterion of parallelism I5.9
Standard error of criterion I7.54.
Departure from parallelism is not significant at 5 % level of P.

T 42.9		M'	0.II26
U II4.7		Mb	0.5897
U^2	I3,I56.09	G	I.II34
$t^2 s^2/w$	I,340.I8	GM'	0.I254
U'^2	II,8I5.9I	GM'^2	0.0I4I

Log. relative potency is 0.5897 with 95 % fiducial limits 0.6985
0.4809

Abs. " " " 3.89 " " " " 5.00
3.04.

TABLE 6. 2III.

Rationof piperonyl cyclohexenone to the pyrethrins :- 2 : I.
Test solutions diluted four times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
	I.	46.I	I8.4	64.5	-27.7
I.	2.	66.4	23.6	90.0	-32.8
	3.	53.I	27.3	80.4	-25.8
	Sums.			234.9	-86.3
Reduced sums of squares.				33I.74	23.33
	I.	33.2	39.2	72.4	06.0
2.	2.	3I.3	43.3	74.6	I2.0
	3.	26.6	56.3	83.4	36.2
	Sums.			230.4	54.2
Reduced sums of squares.				67.76	509.42
	I.	38.6	59.3	97.9	I0.7
3.	2.	38.6	6I.3	I00.9	22.7
	3.	34.4	59.3	93.7	24.9
	Sums.			292.5	58.3
Reduced sums of squares.				26.I6	II4.44
	I.	53.7	23.6	77.3	-30.I
4.	2.	55.6	2I.I	76.7	-3I.5
	3.	54.3	27.3	8I.6	-26.0
	Sums.			235.6	-90.6
Reduced sums of squares.				22.I4	36.I4
Sums ofreduced sums of squares.				437.80	683.33

S^2/w 72.93
s^2/w II3.90

Criterion of parallelism - I7.5
Standard error of criterion 8.559
t 2.049, not significant at 5 % level of P.

T - 2I.8 M' -0.0682
U 96.2 M 0.5338

U^2 9,254.44 C I.0496
t^2s^2/w 437.55 CM' -0.07I6
U'^2 8,8I6.89 CM'^2 0.0049.

Log. relative potency is 0.5338 with 95 % fiducial limits 0.6025
0.465I.

Abs. relative potency is 3.4I with 95 % fiducial limits 4.00
2.92 .

TABLE 6. XIV.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 2.5 : I.
Test solutions diluted four times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
	I.	68.9	45.0	II3.9	-23.9
I.	2.	68.0	54.3	I23.3	-I3.7
	3.	49.0	52.5	IOI.5	02.5
	Sums.			337.7	-35.I
Reduced sums of squares.				307.73	354.48
	I.	63.4	74.7	I38.I	II.3
2.	2.	43.9	59.3	I03.2	I5.4
	3.	4I.6	78.5	I20.I	36.9
	Sums.			36I.4	63.6
Reduced sums of squares.				597.I6	378.I4
	I.	62.7	65.6	I28.3	02.9
3.	2.	65.6	58.7	I24.3	-06.9
	3.	62.0	70.6	I32.6	08.6
	Sums.			385.2	04.6
Reduced sums of squares.				34.46	I23.08
	I.	73.6	49.6	I23.2	-24.0
4.	2.	90.0	54.9	I44.9	-35.I
	3.	74.7	25.8	I00.5	-48.9
	Sums.			388.6	-I08.0
Reduced sums of squares.				973.56	3II.22
Sums of reduced sums squares.				I,8I2.9I	I,I66.92

S^2/w 302.I44
s^2/w I94,482

Criterion of parallelism - I3.4
Standard error of criterion I7.38
Departure from parallelism not significant at 5 % level of P.

T	- 85.0	M'	-0.I069
U	70.4	M	0.495I
U^2	4,956.I6	C	I.I775
t^2s^2/w	747.I0	CM'	-0.I259
U'^2	4,956.I6	CM'^2	0.0I35

log. relative potency is 0.495I with 95 % fiducial limits 0.6309
0.3593.

Abs. relative potency is 3.I3 with 95 % fiducial limits 4.27
2.29.

TABLE 6.IV.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 3 : I.
Testnsolutions diluted four times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Standard.	Test.	Sums.	Differences.
	I.	7I.6	33.8	I05.4	-34.8
I.	2.	65.6	33.2	98.8	-32.4
	3.	45.0	29.3	74.3	-I5.7
	Sums.			278.5	-85.9
Reduced sums of squares.				546.29	268.35
	I.	28.0	54.9	82.9	26.9
2.	2.	33.2	58.7	9I.9	I4.5
	3.	3I.9	46.I	78.0	I4.2
	Sums.			252.8	55.6
Reduced sums of squares.				82.55	I06.90
	I.	33.2	52.5	85.7	I8.3
3.	2.	36.7	65.6	I0I.3	29.9
	3.	40.4	58.7	99.I	I8.3
	Sums.			286.I	56.5
Reduced sums of squares.				I33.05	50I.59
	I.	64.2	54.4	98.6	-29.8
4.	2.	59.3	54.4	93.7	-I4.9
	3.	63.4	46.7	II0.I	-I6.7
	Sums.			302.4	-6I.4
Reduced sums of squares.				I4I.74	I28.I9
Sums of reduced sums of squares.				905.63	I,005.03

S^2/w I50.59
s^2/w I67.52

Criterion of parallelism I3.I9
Standard error of criterion I2.49
t I.II3, not significant at the 5 % level of P.

T - II.8 M' -0.04II
U 86.4 M 0.5609

U^2 7,464.96 Cb I.0943
t^2s^2/w 643.53 CM' -0.0449
U'^2 6,82I.43 CM'^2 0.00I8

Log. relative potency is 0.560$ with 95 % fiducial limits 0.6542
0.4676.

Abs. " " " 3.64 " " " " 4.5I
2.94.

TABLE 6. XVI.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 4 : I.
Test solutions diluted four times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
I.	I.	5I.9	45.0	96.9	-06.9
I.	2.	57.4	45.0	I02.4	-I2.4
	3.	46.7	45.0	9I.7	-0I.7
	Sums.			29I.2	-2I.0
Reduced sums of squares.				9.74	57.26
	I.	24.4	68.0	92.4	4I.6
2.	2.	36.9	78.5	II5.4	4I.6
	3.	37.5	80.0	II7.5	42.5
	Sums.			325.3	I25.7
Reduced sums of squares.				398.65	0.54
	I.	5I.9	74.7	I26.6	22.8
3.	2.	5I.9	90.0	I4I.9	38.I
	3.	52.5	74.7	I27.2	22.2
	Sums.			395.7	83.I
Reduced sums of squares.				I50.I8	I87.35
	I.	74.7	33.2	I07.9	-4I.5
4.	2.	74.7	45.0	II9.7	-29.7
	3.	80.0	37.5	II7.5	-42.5
	Sums.			345I.I	-II3.7
Reduced sums of squares.				90.25	I0I.36
Sums of reduced sums of squares,				648.82	346.5I

S^2/w I08.I3
s^2/w 57.75

Criterion of parallelism -28.2
Standard error of criterion I0.397
t 2.7I3, significant at 5 % level of P.

T 24.4
U II4.2

U^2 I3,04I.64
t^2s^2/w 22I.85
U'^2 I2,8I9.79

M0 0.0640
M 0.666I

G I.0I73
CM' 0.065I
CM'2 0.0042

Log. relative potency is 0.666I with 95 % fiducial limits 0.7066
0.6256

Abs. " " " 4.63 " " " " 5.09
4.22.

TABLE 6. LVII.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 5 : I.
Test solutions diluted four times relative to the standards.
Twin Cross - over desigh and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Test.	Sums.	Differences.
	I.	49.0	33.2	82.2	-I5.8
I.	2.	47.9	45.9	9I.8	-04.0
	3.	65.4	45.0	I05.4	-I8.4
	Sums.			282.4	-38.2
Reduced sums of squares.				560.80	II9.06
	I.	64.2	72.5	I36.7	08.3
2.	2.	57.4	90.0	I47.4	32.6
	3.	I8.4	80.0	98.4	6I.6
	Sums.			382.5	I02.5
Reduced sums of squares.				I,327.46	I,420.7I
	I.	63.4	7I.6	I35.0	08.2
3.	2.	60.7	65.6	I26.3	04.9
	3.	58.7	7I.6	I30.3	I2.9
	Sums.			39I.6	26.0
Reduced sums of squares.				50.98	3I.46
	I.	7I.6	35.I	I06.7	-36.5
4.	2.	80.0	2I.I	I0I.I	-58.9
	3.	90.0	38.I	I28.I	-5I.9
	Sums.			335.9	-I47.3
Reduced sums of squares.				394.9I	262.64
Sums of reduced sums of squares.				2,I34.I5	I,833.87

S^2/w 355.684
s^2/w 305.652.

Criterion of parallelism - 5I.9
Standard error of criterion I8.84
t 2.755 , significant at 5 % level of P.

T	- I8.9	M'	-0.0543
U	I04.7	M	0.5477
U^2	I0,962,09	Q	I.I200
t^2s^2/w	I,I74.08	CM'	-0.0608
U'^2	9,788.0I	CM'^2	0.0033

Log. relative potency is 0.5477 with 95 % fiducial limits 0.6539
0.44I5

Abs. " " " 5.55 " " " " 4.50
2.77.

TABLE 6. XVIII.

Ratio of piperonyl cyclohexenone to the pyrethrins :- 5 : I.
Test solutions diluted four times relative tot the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences
	I.	6I.3	56.8	II8.I	-04.5
I.	2.	58.7	68.9	I27.6	-I0.2
	3.	6I.3	58.7	I20.0	-02.6
	Sums.			365.7	03.I
Reduced sums of squares.				50.54	I27.95
	I.	5I.4	78.5	I29.9	27.I
2.	2.	47.9	80.0	I27.9	32.I
	3.	23.6	74.7	98.3	5I.I
	Sums.			356.I	II0.3
Reduced sums of squares.				628.24	3I6.99
	I.	62.7	74.7	I37.4	I2.0
3.	2.	54.3	74.7	I29.0	20.4
	3.	5I.4	72.5	I23.9	2I.I
	Sums.			390.3	53.5
Reduced sums of squares.				2,9I3.56	53.07
	I.	63.4	53.I	II6.5	-I0.3
4.	2.	57.4	74.7	I32.I	I7.3
	Sums.	72.5	56.2	I28.7	-I6.3
	Sums.			377.3	-09.3
Reduced sums of squares.				I22.0I	670.93
Sums of reduced sums of squares.				3,7I2.35	I,I2I.I7

S^2/w 6I8.70
s^2/w I86.86

Criterion of parallelism - I.I
Standard error of criterion 84.88
Departure from parallelism is not significant at 5 % level of P.

T	52.5	M'	0.2787
U	56.7	M	0.8807
U^2	3,2I4.89	C	I.2875
t^2s^2/w	7I7.82	CM'	0.3568
U'^2	2,497.07	CM'^2	0.I999

Log. relative potency is 0.8807 with 95 % fiducial limits I.II48
0. 6466

Abs. " " " 7.60 " " " " I3.03
4.43.

TABLE 6. LIX

Ratio of piperonyl cyclonene to the pyrethrins :- 6·0 ; 1.
Test solutions diluted four times relative to standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
	1.	68·0	24·4	92·4	-43·6
1.	2.	58·1	34·4	92·5	-23·7
	3.	59·3	35·1	94·4	-24·2
	Sums.			279·3	- 91·5
Reduced sums of squares.				2·54	257·54
	1.	47·3	70 ·6	117·9	23·3
2.	2.	45·0	60 ·0	105·0	15·0
	3.	49·0	71 ·6	120·6	22·6
	Sums.			343·5	60·9
Reduced sums of squares.				139·02	42·38
	1.	52·5	73·6	126·1	21·1
3.	2.	46·1	90·0	136·1	43·9
	3.	50·8	90·0	140·8	19·2
	Sums.			403·0	84·2
Reduced sums of squares.				126·16	375·04
	1.	68·0	47·9	115·9	-20·1
4.	2.	62·7	74·7	137·4	12·0
	3.	71·6	54·9	126·5	-16·7
	Sums.			379·8	-24·8
Reduced sums of squares.				231·14	624·36
Sums of reduced sums of squares.				498·86	1,299·32

$s^2/w = 83·144$
$s^2/w = 216·541$

Criterion of parallelism = -29·3
Standard error of criterion = 9·135
t = 3·207, significant at 5% level of P.

T = 26·1
U = 87·1

U^2 = 7,586·410
$t^2 s^2/w$ -831·842
U'^2 = 6,754·568

M' = 0·0902
M = 0·6923

C = 1·1231
CM' = 0·1013
CM = 0·0091

log. relative potency is 0·6923 with 95% fiducial limits (0·8031 / 0·5815)

Abs. relative potency is 4.93 " " " (6·36 / 3·82)

TABLE 6. LX

Ratio of piperonyl cyclonene to the pyrethrins :- 8 ; 1.
Test solutions diluted four times relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Responses to Test.	Sums.	Differences.
1.	1.	61·3	61·3	122·6	00·0
	2.	61·3	33·2	94·5	-28·1
	3.	63·4	63·4	126·8	00·0
	Sums.			343·9	-28·1
Reduced sums of squares.				628·31	525·47
2.	1.	47·3	68·0	115·3	20·7
	2.	60·0	71·6	131·6	11·6
	3.	46·1	68·0	114·1	21·9
	Sums.			361·0	54·0
Reduced sums of squares.				203·16	61·64
3.	1.	65·6	90·0	155·6	24·4
	2.	56·8	80·0	136·8	-23·2
	3.	56·8	80·0	136·8	23·2
	Sums.			429·3	70·3
Reduced sums of squares.				235·00	00·96
4.	1.	73·6	52·5	126·1	-21·1
	2.	80·0	56·8	136·8	-23·2
	3.	74·7	49·0	123·7	-25·7
	Sums.			386·6	-70·0
Reduced sums of squares.				84·40	12·94
Sums of reduced sums of squares.				1,150·87	

s^2/w = 191·81
s^2/w = 100·17

Criterion of parallelism = -13·9
Standard error of criterion = 13·85
t = 1·457, not significant at 5% level of P.

T = 9·0
U = 74·4

U^2 = 5,535·36
$t^2 s^2/w$ 384·80
U'² = 5,150·56

M' = 0·0364
M = 0·6385

C = 1·0747
CM' = 0·0392
CM'Σ 0·0014

Log. relative potency is 0·6385 with 95% fiducial limits.

(0·7213
(0·5557

Abs. " " " 4·35 " " "

(5·27
(3·59

TABLE 6. 2X1.

Ratio of piperonyl cyclohexanone to the pyrethrins :- I2 : I.
Test solutions diluted four times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
I.	I.	68.0	27.3	95.3	-40.7
	2.	54.9	45.0	99.9	-09.9
	3.	48.4	35.I	83.5	-I3.3
	Sums.			278.7	-63.9
Reduced sums of squares,				I43.I2	570.32
2.	I.	39.8	70.6	II0.4	30.8
	2.	35.I	63.4	98.5	28.3
	3.	37.5	63.4	I00.9	25.9
	Sums.			309.8	85.0
Reduced sums of squares.				88.83	I4.84
3.	I.	63.4	80.0	I43.4	I6.6
	2.	53.I	62.7	II5.8	09.6
	3.	50.8	74.7	I25.5	23.9
	Sums.			384.7	50.I
Reduced sums of squares.				404.9I	I02.26
4.	I.	90.0	53.I	I43.I	-36.9
	2.	65.6	46.I	III.7	-I9.5
	3.	7I.6	54.3	I25.9	-I7.3
	Sums.			380.7	-73.7
Reduced sums of squares.				494.48	965.I3
Sums of reduced sums of squares.				I,III.84	I,658.54

S^2/w I85.22
s^2/w 275.42

Criterion of parallelism -II.7
Standard error of criterion I3.6I.
Departure from parallelism not significant at 5 % level of P.

T 9.I
U 80.9

M' 0.0339
M 0.6360

U^2 6,544.8I
t^2s^2/w I,058.03
U'^2 5,486.78

CM' 0.0404
C I.I928
CM'^{2} 0.00I4.

Log. relative potency is 0.6360 with 95 % fiducial limits 0.7690
0.5030

Abs. " " " 4.33 " " " " 5.88
3.I9.

TABLE 6. 2×11.

Ratio of piperonyl cyclohexenone to the pyrethrins :- I6 : I.
Test solutions diluted four times relative to the standards.
Twin Cross - over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standard.	Test.	Sums.	Differences.
	I.	63.4	35.I	98.5	-28.3
I.	2.	58.I	36.9	95.0	-2I.2
	3.	60.7	54.3	II5.0	-06.4
	Sums.			298.5	-55.9
Reduced sums of squares.				2,25I.50	25I.55
	I.	35.I	74.7	II9.8	39.6
2.	2.	35.I	90.0	I25.I	54.9
	3.	36.9	74.7	III.6	37.8
	Sums.			356.5	I32.3
Reduced sums of squares.				I04.4I	I76.58
	I.	63.4	90.0	I53.4	26.6
3.	2.	45.0	77.I	I22.I	32.I
	3.	50.8	7I.6	I22.4	20.8
	Sums.			397.9	79.5
Reduced sums of squares.				660.I9	65.86
	I.	90.0	52,5	I42.5	-37.5
4.	2.	7I.6	62.7	I34.3	-07.9
	3.	90.0	56.9	I26.9	-53.I
	Sums.			403.7	-98.5
Reduced sums of squares.				I08.33	I,057.47.
Sums of reduced sums of squares.				3,I24.43	I,549.46

S^2/w 520.72
s^2/w 258.23

Criterion of parallelism - I7.3
Standard error of criterion 22.82.
Departure from parallelism is not significant at 5% level of P.

T I9.2
U I22.0

U^2 I4,884.00
t^2s^2/w 99I.99
U'^2 I3,892.0I

M' 0.0474
M 0.6495

C 0.07I4
CM' 0.05078
CM'^2 0.0024

Log. relative potency is 0.6495 with 95 % fiducial limits 0.7307
0.5683.

Abs. " " " 4.46 " " " " " 5.38
3.70.

TABLE 6. LXIII.

Ratio of D.H.S. activator to the pyrethrins :- I : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Group.	Replicate.	Responses to Standard.	Test.	Sums.	Differences.
	I.	43.9	29.3	73.2	-I4.6
I.	2.	4I.0	I8.4	59.4	-I2.6
S	3.	59.3	30.7	90.0	-28.6
	Sums.	2282.6		222.6	-55.8
Reduced sums of squares.				469.68	I52.II
	I.	28.7	57.5	66.2	08.8
2.	2.	25.8	40.4	66.2	I4.4
	3.	I8.4	45.0	63.4	26.6
	Sums.			I95.8	49.8
Reduced sums of squares.				8.28	I65.68
	I.	27.3	58.I	85.4	30.8
3.	2.	2I.I	56.8	77.9	335.7
	3.	29.3	56.2	85.5	26.9
	Sums.			248.8	93.4
Reduced sums of squares.				I46.30	42.00
	I.	36.6	28.7	67.3	-09.9
4.	2.	33.2	3I.3	64.5	-0I.9
	3.	45.9	36.I	82.0	-05.8
	Sums.			213.8	-17.6
Reduced sums of squares.				I09.00	3I.42
Sums of reduced sums of squares.				695.06	39I.2I

S^2/w II5.6I
s^2/w 65.I9

Criterion of parallelism -8.7
Standard error of criterion I0.73.
Departure from parallelism is not significant at 5% level of P.

T 23.2 M' 0.0967
U 72.2 M 0.0967.

U^2 5,700.25 C I.0459
t^2g^2/w 850.42 CM' 0.0887
U'^2 5,429.83. CM'^{2} 0.0065

Log. relative potency is 0.0967 with 95% fiducial limits 0.I650
0.0304
Abs. " " " I.25 " " " " I.46
I.07.

TABLE 6. XXIII.

Ratio of D.H.S. activator to the pyrethrins : 5.0 : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Group.	Replicate.	Responses to Standard.	Test.	Sums.	Differences.
I.	I.	46.I	I8.4	64.5	-27.7
	2.	43.3	I8.4	6I.7	-24.9
S	3.	4I.0	25.I	66.I	-I5.9
	Sums.			I92.3	-68.5
Reduced sums of squares.				9.92	78.5I
2.	I.	4I.0	38.6	79.6	-02.4
	2.	I8.4	38.I	56.5	I9.7
	3.	I5.3	33.8	49.I	I8.5
	Sums.			I85.2	35.8
Reduced sums of squares.				5I8.39	3I0.08
3.	I.	I8.4	50.8	69.2	32.4
	2.	30.0	38.I	68.I	28.I
	3.	29.3	56.2	85.5	26.9
	Sums.			242.8	87.4
Reduced sums of squares.				2I7.98	I9.64
4.	I.	39.2	2I.I	60.3	-I8.I
	2.	38.I	27.3	65.4	-I0.8
	3.	39.8	I8.4	58.2	-2I.4
	Sums.			I83.9	-50.3
Reduced sums of squares.				27.42	57.I7
Sums of reduced sums of squares.				767.70	465.20

S^2/w I27.98
s^2/w 77.8I

Criterion of parallelism -27.2
Standard error of criterion II.30
t 2.407, significant at the 5% level of P.

T 0I.4
U 80.6

U^2 6,496.36
t^2s^2/w 297.75
U'^2 6,I98.6I

M' 0.0052
M 0.0052

C I.0480
CM' 0.0054
CM'2 0.0000

Log. relative potency is 0.0052 with 95% fiducial limits $\frac{0.07II}{I.9393}$

Abs. " " " I.0I " " " " $\frac{I.I8}{0.87}$.

TABLE 6. LXV.

Ratio of D.H.S. activator to the pyrethrins :- 10.0 : 1.
Test solutions not diluted relative to the standards.
Twin Cross-Over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
	1.	51.4	15.3	66.7	-36.1
1.	2.	61.3	24.4	85.7	-36.9
	3.	54.3	24.4	78.7	-25.9
	Sums.			231.1	-98.9
Reduced sums of squares.				192.37	81.62
	1.	15.3	30.6	54.9	25.3
2½	2.	14.2	41.0	55.2	26.8
	3.	21.1	63.4	84.5	42.3
	Sums.			194.6	94.4
Reduced sums of squares.				556.76	304.50
	1.	18.4	42.1	60.5	23.7
5.	2.	10.0	42.1	52.1	32.1
	3.	13.4	40.1	54.5	27.7
	Sums.			177.1	83.5
Reduced sums of squares.				80.01	38.09
	1.	55.1	19.4	72.5	-35.7
4.	2.	46.7	19.4	66.1	-27.3
	3.	36.6	60.0	96.6	-36.6
	Sums.			177.3	-109.6
Reduced sums of squares.				642.90	16.54
Sums of reduced sums of squares.			1,430.06		340.95

S^2/v 246.62
s^2/v 42.62

Criterion of parallelism 12.2
Standard error of criterion 15.56
Departure from parallelism is not significant at the 5% level of P.

T	-10.8	M'	-0.0844
U	126.0	M	1.9156
U^2	16,384.00	C	1.0101
t^2g^2/v	168.72	CM'	-0.0853
U'^2	16,220.28	CM'^2	0.0072

Log relative potency is 1.9156. with 95% fiducial limits 1.8856
1.9456
Abs. " " " 0.82 " " " " 0.77
0.88.

TABLE 6. LXVI.

Ratio of D.H.S. activator to the pyrethrins :- I5.0 : I.
Test solutions not diluted relative to the standards.
Twin Cross-over design and analysis of relative potency.

Groups.	Replicates.	Responses to Standards.	Responses to Test.	Sums.	Differences.
I.	I.	59.3	I8.4	77.7	-40.9
	2.	4I.0	I5.3	56.3	-25.7
	3.	3I.3	I0.0	4I.3	-2I.3
	Sums.			I75.3	-87.9
Reduced sums of squares.				675.I5	2II.52
2.	I.	26.6	37.5	64.I	I0.9
	2.	24.4	4I.6	66.0	I7.3
	3.	24.4	36.9	6I.3	I2.5
	Sums.			I9I.4	40.6
Reduced sums of squares.				II.I8	22.80
3.	I.	I0.0	33.2	43.2	23.2
	2.	00.0	4I.0	4I.0	4I.0
	3.	I5.3	35.2	48.5	I7.9
	Sums.			I32.7	82.I
Reduced sums of squares.				34.I5	298.32
4.	I.	2I.I	I8.4	39.5	-02.7
	2.	28.7	I5.3	42.0	-I3.7
	3.	33.8	00.0	33.8	-33.8
	Sums.			I15.3	-80.9
Reduced sums of squares.				39.I7	500.95
Sums of reduced sums of squares.				759.65	I,033.59

S^2/w I26.58
s^2/w I72.22

Criterion of parallelism -II.2
Standard error of criterionII.24.
Departure from parallelism is not significant at 5% level of P.

T	-5.I	M0	0.0367
U	86.7	M	I.94I3
U^2	7,5I6.89	C	I.0965
s^2t^2/w	66I.57	CM'	-0.0644
U'^2	6,855.57	CM'^2	0.0058.

Log. relative potency is I.94I3 with 95% fiducial limits 0.0367 I.8459
Abs. " " " 0.87 " " " " I.09 0.70.