ROTHAMSTED RESEARCH

# Rothamsted Report for 1951

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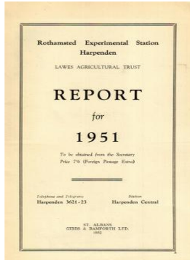
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LAWES AGRICULTURAL TRUST

REPORT

for

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## Insecticides and Fungicides Department

### C. Potter

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# INSECTICIDES AND FUNGICIDES DEPARTMENT

By C. Potter

The continued absence of Mr. M. J. Way on secondment to Zanzibar has decreased the amount of biological work carried out in the current year. Dr. A. H. McIntosh returned from the United States of America and Dr. K. A. Lord from the Biochemistry Department, Cambridge, at the beginning of the year, and resumed their programmes of work. Both these members of the department felt they had greatly benefited by their experience.

Mr. Neely Turner, Entomologist, of the Connecticut Agricultural Experiment Station, U.S.A., arrived at the beginning of the year for a stay of six months in exchange for Dr. A. H. McIntosh. Mr. Turner's visit proved very profitable for the department, the members of which were able to benefit from his wide experience. Mr. Turner expressed himself well satisfied with the experience gained from his stay here. Mr. R. W. Kerr, of the Commonwealth Council of Scientific and Industrial Research Organization, Canberra, returned to Australia in October.

In March the annual conference of research workers on insecticides for plant protection was held at Rothamsted, and the work of the department was exhibited and discussed.

The British Council organized a course on Insecticides and Herbicides for visiting research workers, and in June a week's course on Insecticides was given at Rothamsted by members of the department. Seventeen delegates coming from Finland, Sweden, Norway, France, Holland, Belgium, Egypt, Italy and Australia attended the course.

Mr. Burt and Dr. Potter attended the Ninth International Congress of Entomology held in Amsterdam in August.

Dr. Hammerlund, in charge of the Insecticides testing section of Government Plant Pathological Station, Lyngby, Denmark, visited the department and stayed for about six weeks, studying techniques for the assessment of insecticidal action.

The work of the department during the year is set out below :—

## Chemical

### *Physical chemistry*

(1) The effect of particle size of suspensions of contact insecticides on their toxicity.

Since his return from the U.S.A. Dr. A. H. McIntosh has continued his work on this subject. In 1950, tests of suspensions by injection into adult *Oncopeltus fasciatus* Dall. had suggested that the relative insecticidal behaviour of different-sized particles of solid poisons depends on their fat solubilities. If the ratio of median lethal dose to solubility in oil is high, small particles seem to be more toxic than large ones. The difference is only in speeds of action, the ultimate toxicities of large and small particles being the same. Differences in speed of action are more easily measured if the

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insects are kept cool after treatment. If the ratio of median lethal concentration to solubility in oil is low, the difference in speeds of action between large and small particles may not be noticeable, even with cool insects. All this applies to injection tests.

These ideas are now being applied to the behaviour of suspensions tested for contact action. It is assumed that in contact action, the first step in penetration of the cuticle is solution in the wax layer of the cuticle, whereas in injection tests (*O. fasciatus*) the first step is solution in the fats of the blood stream. The manner in which different-sized particles act, therefore, depends on their physical properties, especially solubility in fats, which may itself depend on particle-size.

With each of about 10 D.D.T. analogues, two suspensions have been made—one of colloidal poison, and one of crystalline particles of about 20-50 $\mu$. These have been tested against *Oryzaephilus surinamensis* L. by the dipping method, the insects being kept cool (11°C) after treatment. If there is a large difference in speeds of action, the difference in toxicity between colloidal and crystalline poison appears to be large if the insects are inspected soon (one day after treatment). The analogues chosen cover a range of fat solubilities: for each analogue the solubility in olive oil at 11°C has been measured.

From the results obtained so far it can be seen that the relation of fat solubility to *difference* in speeds of contact action is not as simple as in injection tests. Other physical properties (speed of flocculation of colloidal poison and speed of solution of poison in wax) are involved. The problem is being investigated further.

(2) Effect of surface active agents on the action of contact insecticides.

Work on this subject has been temporarily suspended while some factors in the techniques of investigation are being studied.

*Biochemical*

Previous work has shown that esterases other than choline-esterase were inhibited in high dilution by the organo-phosphorus insecticides and might be concerned in the mechanism of toxic action of these compounds. During his stay in the Biochemistry Department at Cambridge, Dr. Lord made some progress in isolating and studying the properties of one of these esterases, but this work was held up when he returned to Rothamsted owing to lack of equipment.

In the interim while the necessary equipment is being obtained, further studies of the activity of extracts from different species of insects were investigated in order to study the distribution of esterases hydrolysing both acetyl choline and other substrates. The relative amounts of these types of activity varies considerably with the species.

Using organo-phosphorus insecticides as inhibitors, it has been found that there were large differences in the amounts of material required to inactivate a given amount of esterase from different species of insect using phenyl acetate as substrate. This would

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provide a biochemical basis for specificity. It was further found that pure Parathion [0 : 0-diethyl 0-(p-nitrophenyl) thiophosphate] is not an inhibitor of the " non-specific " esterase from *Tribolium castaneum* Hbst. adults, although it is an effective insecticide against this species. Pure parathion has also been reported as having no inhibitory activity against mammalian choline-esterase.

A start has been made by Dr. Lord and Dr. Potter in comparing the relative toxicity of insects of three analogues of parathion with their capacity to inhibit insect esterase. The analogues were chosen because it had been reported that their mammalian toxicity did not run parallel to their insecticidal activity. The mammalian toxicity based on tests on rats of the three compounds has been reported to be as follows: 0 : 0-diethyl S-(p-nitrophenyl) thiophosphate > 0 : S-diethyl 0-(p-nitrophenyl) thiophosphate > 0 : 0-diethyl 0-(p-nitrophenyl) thiophosphate (parathion). The insecticidal activity based on the contact effect on adult *Tribolium castaneum* Hbst. ranks the materials as follows: 0 : 0-diethyl 0-(p-nitrophenyl) thiophosphate (parathion) > 0 : S-diethyl 0-(p-nitrophenyl) thiophosphate > 0 : 0-diethyl S-(p-nitrophenyl) thiophosphate. The anti-insect esterase activity of the three compounds to an extract from *Tribolium castaneum* Hbst. acting on phenyl acetate as substrate ranked the compound as follows: 0 : S-diethyl 0-(p-nitrophenyl) thiophosphate > 0 : 0-diethyl S-(p-nitrophenyl) thiophosphate > 0 : 0-diethyl 0-(p-nitrophenyl) thiophosphate > (parathion) which in pure form does not inhibit in saturated solution. From this data it would appear that the order of contact insecticidal action of this series of analogous compounds to *Tribolium castaneum* Hbst. is exactly the reverse of their order of toxicity to mammals (rats).

The insecticidal activity could be correlated with capacity to inhibit insect esterase with two of the three compounds, the reason for the inability of pure parathion to inhibit esterase in vitro while it is highly toxic in vivo remains to be explained.

A considerable amount of work of an exploratory nature including experiments on insect dehydrogenases has been carried out by Dr. Lord, but has not so far advanced beyond the preliminary stage.

*Relationship between chemical constitution and insecticidal activity*

The work on the biological activity of molecules allied to the pyrethrins has been continued by Mr. Elliott, Mr. Needham, and Dr. Potter.

An investigation of the effects on insecticidal activity of altering the structure of the acid parts of pyrethrin-like esters has been carried out. Most of the compounds used were kindly donated by Dr. S. H. Harper of King's College, University of London.

The contact effects were studied by means of a measured drop technique ; three different insect species were used to detect variations in susceptibility and any possible variations in relative toxicity of any two compounds with species.

A summary of the results is set out in Table 1.

TABLE 1. *The effect of variations in the structure of pyrethrin-like esters on their relative contact toxicity*

| Alcohol | Acid | Relative toxicity *Phaedon cochleariae* | *Tenebrio molitor* | *Dysdercus fasciatus* |
|---|---|---|---|---|
| (±)-allylrethronyl | (+)-*trans*-chrysanthemate | 100 | 100 | 100 |
| ,, | (±)-*trans*- ,, | 44 | 48 | 53 |
| ,, | (±)-*cis*- ,, | 32 | 32 | 25 |
| ,, | (—)-*trans*- ,, | 4 | | |
| (±)-2′-methallylrethronyl | (+)-*trans*- ,, | 69 | | |
| ,, | (±)-*trans*- ,, | 28 | | |
| ,, | (±)-*cis*- ,, | 12 | | |
| (±)-allylrethronyl | (±)-*trans*-dihydro-chrysanthemate | 20 | | |
| ,, | 5-methyl-*n*-hex-2-enoate | <0·1 | | |

(For nomenclature used, see Harper, " Chemistry and Industry," 1949, 636.)

From these results it appears that the (+) -*trans* and (+) -*cis* forms of chrysanthemum monocarboxylic acid form toxic esters, whilst the (—) -*trans* and the (—) -*cis* forms produce relatively non-toxic compounds.

The comparison of (±) -allylrethronyl (±)—trans-dihydro-chrysanthemate with (±) -allylrethronyl 5-methyl-*n*-hex-2-enoate was carried out to find out if the cyclopropane ring were an essential feature of the acid for high pyrethrin-like activity. The acyclic acid for this comparison was prepared by Mr. Elliott in this department. From the fact that the ester from the acyclic acid and (±) -allethrolone was non-toxic to adult *Phaedon cochleariae* when applied as a measured drop at 5 per cent w/v. in acetone as shown in the table, it appears that the cyclopropane ring structure in the acid is necessary for high toxicity.

It is noteworthy that in the tests given above, the relative toxicity of the compounds tested is very nearly the same with all three species of insects, and with a given species of insect, changes in acidic structure have the same relative effect when the alcoholic component has the allyl or the methallyl side chain.

*Organic chemistry*

Mr. Elliott has continued his chemical work in connection with the study of the relationship between chemical structure and insecticidal activity.

(1) The main subject of work has continued to be the synthesis of ketones related to pyrethrolone, cinerolone and allethrolone (2-*n*-penta-2′ : 4′-dienyl-, 2-*n*-*cis*- but-2′-enyl- and 2-allyl-3 methyl-*cyclo*pent-2-en-4-ol-l-ones respectively). A detailed description of this work will be published shortly and an adequate summary cannot be made here.

(2) In the course of the experiments outlined under (1) above, it has been shown that alkylidene succinic anhydrides absorb in the ultraviolet region at 2350A. (ε max. 10-12,000), whereas the parent alkylidene succinic acids show the expected absorption at ca. 2200A. An examination of the probable causes of this shift in the position of maximum absorption has been made.

(3) Hedenburg and Wachs (J. Amer. Chem. Soc., 1948, **70**, 2216) have shown that 3-alkyl-5(-3 : 4-methylenedioxyphenyl-*cyclo*hex-2-en-1-ones have insecticidal activity themselves to houseflies and also

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synergize the action of the pyrethrins. They concluded that the system $-C=CH-C=O$ was essential for insecticidal activity in these compounds. In particular they described 3-hexyl-5-(3 : 4 methylenedioxyphenyl)-*cyclo*hex-2-en-1-one (" piperonyl cyclonene "). It was thought that 2-allyl-3-methyl-5-(3 : 4 methylenedioxyphenyl)-*cyclo*hex-2-en-1-one would be an interesting compound since it would contain the system $CH_3-C=C\,(CH_2CH=CH_2)-C=O$ which is present in the synthetic insecticide allethrin and it has been suggested (Elliott, Pyrethrum Post, 1951, **2**, No. 3, 18) that the insecticidal activity of the keto esters in the natural pyrethrins and allethrin may to some extent be correlated with the activation of the $\alpha$-methylene grouping in the keto-alcoholic side chains of these compounds. This compound ($\lambda$ max. 2,400A., $\varepsilon$ max. 11,000 ; 2 : 4-dinitrophenylhydrazone, orange needles m. p. 162° (uncorr.) has therefore been made by variations of the published method. Reports on the insecticidal and synergistic properties of this compound (which is related to piperonyl cyclonene) for the pyrethrins and for allethrin will be made.

(4) Photometric methods employing the ultraviolet absorption of the natural pyrethrins have been advocated by Beckley (Pyrethrum Post, 1949, **1**, No. 3, 5 and 1950, **2**, No. 1, 23) and by Shukis, Cristi and Wachs (Soap and Sanitary Chemicals, 1951, 27, No. 11, 124). It was of interest, therefore, to see whether these substances showed deviations from the Beer-Lambert Law due to fluorescence in the region of absorption (c.f. Brande and Timmons (J. Chem. Soc., 1950, 1019)) and especially to determine whether the effects were appreciable in the range of extinction (optical density) values recommended by these workers. It has been shown that the " $\alpha$-dl-*trans* " isomer of allethrin (a specimen of which was available by the generosity of Dr. M. S. Schechter ; see Schechter, La Forge, Zimmerli and Thomas, J. Amer. Chem. Soc., 1951, **73**, 3541) does show such deviations in ethanol and in *n*-hexane, but that the relationship optical density *vs.* concentration is linear at and below the maximum optical density readings suggested by Shukis *et al.*

These results and a discussion of the probable effects of any variation in the pyrethrin to cinerin content in various samples of pyrethrum flowers estimated by photometric and the more conventional chemical methods will be published.

## Insecticides Derived from Plants

### *Pyrethrum*

The insecticidally active principles of Pyrethrum Flowers have been identified in the past by a study of their products of hydrolysis.

They can be re-sinthesized individually from these products but a purely physical method of separation would be valuable, both as a means of improving methods of analysis and as a source of pure compounds for toxicity tests.

In the course of collaborative work with the Colonial Products Advisory Bureau, Dr. K. A. Lord and Mr. J. Ward, using a chromatographic column of alumina, achieved a complete separation of Pyrethrin and Cinerin I from Pyrethrin and Cinerin II.

A method of carrying out this separation by paper chromatography has been evolved and this provides a useful qualitative test

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of the presence of the " Pyrethrins " I and II. Some evidence of a partial separation of the cinerins on the column has also been obtained.

The presence of esters with the monocarboxylic acid and of those with the dicarboxylic acid in the washings was shown by their U.V. absorption and also by chemical methods. This evidence has been supported by bioassay for insecticidal activity.

*Heliopsis* spp.

Following a report from the United States on the insecticidal effectiveness of an amide called " Scabrin " isolated from the roots of *Heliopsis* spp., and in response to a request from the Colonial Products Advisory Bureau, some work has been started on these plants. Seeds of *Heliopsis scabra* and *H. parvifolia* have been obtained through the good offices of the Colonial Products Advisory Bureau and plants of both these species have been grown successfully in the open air and in the glasshouse. Seeds have been collected for germination tests. Dr. Martin Jacobson of the United States Department of Agriculture Bureau of Entomology and Plant Quarantine kindly sent us a sample of Scabrin and Mr. P. H. Needham carried out some preliminary biological tests using Adults of *Tenebrio molitor* L. (Meal worm), *Phaedon cochleariae* Fab. (Mustard beetle) and *Dysdercus fasciatus* (Cotton stainer). The contact effect of the scabrin in these three species were compared with that of (±)-allylrethronyl (±)-*trans*-chrysanthemate. The relative toxicity ranged from approximately 1/30 with male *Dysdercus fasciatus* to approximately 2/3 with *Tenebrio molitor*. It would appear from this that Scabrin is not likely to be as insecticidally active as the pyrethrins and the pyrethrin-like esters over a range of insect species, but is sufficiently active to justify further investigation, particularly since it would appear that other N-*iso*butylamides have considerable insecticidal activity.

It is proposed therefore to continue to work on this plant insecticide.

## Biological

*Bioassay techniques*

*Measured drop technique*

In connection with the work on the bioassay of synthetic pyrethrin like esters, a measured drop technique of testing contact insecticides has been developed by Mr. P. H. Needham.

In the first instance this technique was used because comparisons of insecticidal activity can be made with very much smaller samples of poison than with the " Potter Tower " spraying method, and this is an important factor because the samples of some of the synthetic compounds being examined are extremely small.

The apparatus consists of a standard 1 ml. micrometer syringe of the " Agla " pattern with a cannula affixed in the place of the needle. The poisons are applied as solutions in pure solvents. Acetone, ethyl alcohol and olive oil had so far been used with success.

Adults of *Phaedon cochleariae*, *Tenebrio molitor* and *Dysdercus fasciatus* have been used as test subjects and very consistent and

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satisfactory results have been obtained. The data have consistently provided non-heterogeneous probit lines which could be repeated. So far it has not appeared to be necessary or desirable to provide any of the arrangements that have been described for removing the drop from the needle.

*Injection techniques*

Mr. P. H. Needham has continued to work on this subject. It was thought that earlier failures may have been due to the formulation of the poisons and the relatively large volumes injected.

Previously the insecticides, in this case " synthetic pyrethrins", were formulated in a sulphonated lorol/water medium containing 10 per cent acetone. Now by using hot olive oil and an oil soluble emulsifier, aqueous injection media giving satisfactory results have been obtained.

Only *Tenebrio molitor* adults have been used for this work so far, but we hope to increase the range of test subjects in the near future.

*Spraying techniques*

Dr. Potter and Mr. A. J. Arnold have made an examination of the physical factors affecting the performance of the improved model of the laboratory contact spraying apparatus (the Potter Tower) in use in the department, and this work has been sent in for publication. With distilled water a difference in the environmental temperature of 20°F between 60°F and 80°F produced approximately 10 per cent difference in the deposit of spray droplets and a difference of 20 per cent in the relative humidity of the environment between 60 per cent and 80 per cent produced approximately 5 per cent difference in the deposit. A short investigation of the possible influence of electrostatic effects on the amount of spray deposit indicated that variations in the charge on the droplets did not influence the amount deposited. The deposits remained the same when potentials up to 1·5 kilovolts were applied to the spray target.

During the course of these investigations it was found that the nature of the surface active agent used in the spray greatly affected the charge on the spray droplets. With a solution of anionic wetting agent no charge could be measured while in the presence of two non-ionic materials the charge was greatly increased over that on distilled water. In a given set of conditions a droplet of distilled water of average weight 0·00013 mg. at a potential of 25 volts carries a change of 0·00026 e.s.u. and a droplet of 5 per cent v/v. lissapol N. (non-ionic surface active agent) of average weight 0·000084 mg. at a potential of 49 volts carries a change of 0·00044 e.s.u. By the process of elimination it appears likely that the major factor producing variations in the deposit is variations in the turbulence induced in the tower.

*Technique of estimation of contact poisons*

Dr. Potter and Miss Christine Hutt have continued their examination of the relative importance of direct contact effects and residual film effects in laboratory insecticidal assay and on the factors influencing these effects. This work is nearing completion.

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*Synergism between nicotine and the pyrethrins*

Mr. Neely Turner had previously found evidence of the occurrence of synergism between nicotine and the pyrethrins when these two insecticides were injected into adult *Oncopeltus fasciatus* Dal.

During his visit he studied the action of these two poisons applied jointly as contact poisons to adult *Tribolium castaneum* Hbst. by means of a dipping technique.

When the two poisons were applied simultaneously no evidence of synergism was found, but when the nicotine was applied first and the pyrethrins subsequently after an interval of three-quarters of an hour there were indications of synergism. A possible reason to explain these facts is the unequal rates of penetration of the two poisons.

This work has been accepted for publication.

*The effect of stage of development on insect resistance*

Miss Helen Salkeld from Canada has continued her work on the changes in resistance of insect eggs to insecticides that occur during development. The majority of the work has been done on eggs of the tomato moth *Diataraxia oleracae,* and in an attempt to determine the basis of changes in susceptibility during development Miss Salkeld has made a detailed study of the structure and changes that occur during development of the layers composing the egg shell in this species of egg. In order to facilitate this study Dr. V. B. Wigglesworth kindly agreed to her spending a month working under the supervision of Mr. Beament in the Entomology Department at Cambridge. In addition to the eggs of *Diataraxia oleracea* the eggs of *Ephestia kuhniella* and *Dysdercus fasciatus* have been included in the study. The effects of D.D.T., the pyrethrins, allethrin, T.E.P.P., Parathion and the triethanolamine salt of 3·5 dinitro-ortho-cresol have been investigated.

Differences in resistance during development have been found with all three species of eggs, but the nature of the curve for susceptibility differs for each species. It has further been found that there are considerable differences between the different insecticides in the ratio between the dose required to give approximately 100 per cent kill and that required to inhibit the development of the embryo.

*Factors affecting the toxicity and permanence of insecticidal deposits on plants*

Mr. P. Burt and Mr. J. Ward have continued their studies on the behaviour of D.D.T. deposits on films of plant waxes and have extended their work to include the behaviour of D.D.T. deposits on living plants.

Modifications have been made in the Schechter-Haller method for determining D.D.T. in order to adapt it to the analysis of deposits on leaves. Reliable results may now be obtained on samples containing 15 micrograms of D.D.T.

Previous work had shown that deposits of D.D.T. crystals on glass plates, either plain, or coated with sisal wax, when stored at 45°C showed a loss of approximately 50 per cent in a fortnight, both by biological and chemical estimation. This was presumed to be due to volatilization. Further experiments were done to obtain some evidence on this point and to find out if similar effects would be obtained with a more typical plant wax. A 10 gm. sample of wax from cabbage plants was prepared, and $0{\cdot}5\mu$ thick films of this wax were sprayed on glass plates.

Waxed plates sprayed with crystalline D.D.T. suspensions of different concentrations together with unsprayed plates were kept at 45°C in an atmosphere saturated with D.D.T. vapour. No loss of insecticidal activity was found in the high concentrations of D.D.T. after three weeks and at the lower concentrations both the activity and D.D.T. content had increased. The waxed control plates had also become insecticidal. It was shown in a later experiment under the same conditions that the untreated waxed plates could pick up from the atmosphere 0·8 micrograms of D.D.T. per $cm^2$ in 11 days, which was enough to produce a 1·6 per cent solution in the wax.

Taken as a whole these experiments indicate that at 45°C in the open considerable losses of D.D.T. from a surface may occur due to volatilization in a few days. They further show that D.D.T. can go into solution in plant wax via the vapour phase.

Further experiments showed that the median lethal dose to adult *Tribolium castaneum* of wax films containing D.D.T. absorbed from the vapour phase did not differ greatly from films coated with an equivalent amount of D.D.T. in the form of $50\mu$ needles or from films prepared by spraying a solution of wax and D.D.T. in benzene, although it was clear that the slopes of the probit regression lines of mortality on concentration were different with the different treatments.

The experiments so far have indicated, although the evidence is by no means conclusive, that the D.D.T. absorbed from the vapour phase is evenly distributed in the wax layer. Further experiments are in progress on this subject and on the effect of the physical state of the applied poison on its toxicity.

Preliminary work on leaf surfaces has shown that differences in contact insecticidal activity occur with different species of plants, when the leaves are sprayed to the same deposit density, and there is a much more marked difference between all leaf surfaces so far tested and glass plates, the latter invariably being considerably less toxic. Work is in progress to determine the reasons for these differences.

For the work on leaf surfaces it was necessary to provide a technique for the application of known repeatable doses evenly over the leaf surface. The " Potter Tower " which had been used to coat the plates was not suitable for coating leaves on the living plants. A technique has been worked out giving a coefficient of variation of the deposit of about 5 per cent over a sampling area of 67 sq. cm.

*Mechanism of selection of strains of insect resistant to insecticides*

Dr. Tattersfield has continued his work on this subject assisted during the year by Mr. R. W. Kerr from Australia and Miss Jill Kerridge.

Continuous selection by treatment at three-weekly intervals over the period of a year, of adults of a wild stock of *Drosophila melanogaster* with D.D.T. was carried out in 1950. The effect of selection was tested at two levels of survival, approximately 90 per cent kill and approximately 60 per cent kill. Considerable fluctuations occurred throughout the period of selection, but it was finally estimated that at the end of the year the stock selected from the high level kill was two to three times as resistant as the untreated stock. Little if any change in resistance had occurred in the stock selected from the lower level kill. A comparison of the respiration rates of resistant adults with those of normal adults indicated that the resistant individuals had a higher rate. Further work is being done on this point.

Various possible sources of the fluctuations in susceptibility that occurred during the course of this work were investigated including (1) carbon dioxide susceptibility and (2) temperature effects. A series of experiments showed that at least a considerable proportion of the variation was due to the presence in the stock of a strain susceptible to $CO_2$, the $CO_2$ susceptibility having a considerable negative temperature coefficient. Ph. L'Héritier discovered the phenomenon of $CO_2$ susceptibility in a strain of *Drosophila* between 1938 and 1940 and some of his stock was sent to Rothamsted in 1941. It seems probable that this strain became incorporated in the wild stock used for these experiments.

The $CO_2$ susceptibility has been ascribed to a plasmagene or a virus that can be transmitted to the progeny more potently through the female than the male.

Work is in progress to ascertain whether selection for $CO_2$ resistance will lead to D.D.T. resistance and whether insects selected for D.D.T. resistance using nitrogen for anæsthesia, will be $CO_2$ resistant.

Mr. Kerr has carried out investigations on the techniques of rearing and handling *Drosophila* for the specific purpose of investigating the factors affecting their susceptibility to insecticides, and his work has resulted in considerable improvements and the speeding up of the spraying technique. He further devised a micro-technique for applying very small drops of insecticide quantitatively to individual insects.

Using the micro drop technique he has shown that the susceptibility to D.D.T. of adult *Drosophila melanogaster* varies with age and has determined the age of maximum resistance.

Other factors influencing resistance that have been studied, include the effect of the age of the parent on the susceptibility of the progeny and the effect of the temperature of rearing upon susceptibility.

The work as a whole has been seriously hampered by lack of facilities for providing constant temperature and humidity.

*Insect rearing*

Seventeen species of plant feeding insects and 12 species of stored products insect have been reared during the course of the year. *Phaedon cochleariae* the mustard beetle has proved to be a useful test insect and Miss P. Smith has continued her work on the technique of rearing this insect and on the environmental factors affecting its diapause.

The methods of rearing aphids in the department have been unsatisfactory, since, in addition to difficulties due to the attacks of parasites and predators, the populations fluctuated greatly for reasons that are not yet understood. Mrs. J. Kenten has started to work on improved methods of rearing and to study the underlying causes of fluctuations in the reproduction rate and in the proportions of the different morphological forms in the population.

*Toxicity of plant protective chemicals to bees*

Mr. Glynne-Jones has continued to work on this subject at Seale-Hayne Agricultural College in collaboration with this department.

The following laboratory estimations have been made of the toxicity of three of the newer insecticides.

*Approximate L.D. 50 in 24 hours*

| | Stomach poison | Contact poison | |
|---|---|---|---|
| | mg./bee | conc : w/v. % | deposit/sq.cm.mg. |
| Aldrin ... ... | 0·00027 | 0·0065 | 0·00063 |
| Chlordane ... ... | 0·0012 | 0·06 | 0·0059 |
| Toxaphene ... ... | 0·04 | 0·5 | 0·048 |

The low toxicity of toxaphene is noteworthy.

In view of the importance of dust formulations in plant protection some work has been started on the action of dusts on bees.

A detailed study of the external morphology of the bee as affecting the entrance of dusts has been made.

Serial sections through the abdomen of dusted bees have shown that hairs within the vestibule are efficient filters of particles greater than 5$\mu$ in diameter and it was never possible to find dust particles in the tracheae.

It has been shown that a number of different materials in the form of dusts can increase water loss by the bee when they are brought in contact with the cuticle. This occurs in both living and dead bees. Alumina-aluminium oxide (Almicide) was the most effective, but others such as activated charcoal and calcium carbonate had some effect, perhaps due to their adsorptive properties.

The possible function of the dense covering of hairs in protecting bees from dusts is also being investigated.

Further consideration has been given to the development of a technique for the evaluation of repellent materials and the effects of a series of chemicals allied to phenol have been examined. The results have so far failed to reveal any predictable relationship between chemical constitution and repellancy.

## Field Work

*Control of wireworms*

A preliminary experiment to obtain some data on the possible use of Aldrin and Chlordane for wireworm control was carried out in 1950-51 on the site of the previous experiments of 1947-50, using the control and seed-dressed plots of the earlier experiment. A B.H.C. combine-drilled treatment was included as a standard.

A dust contining 2½ per cent Aldrin was combine-drilled at 190 lb. per acre (4·75 lb. Aldrin per acre). A dust containing 5 per cent Chlordane was combine-drilled at 100 lb. per acre (5 lb. Chlordane per acre), and a dust containing 3·5 per cent technical B.H.C. (technical B.H.C. containing 12-14 per cent gamma isomer) combine-drilled at 75 lb. per acre (2·62 lb. technical B.H.C. per acre). The figures for grain yields in cwt. per acre from the treated plots were as follows :—

Experiment 1 : Untreated 19·8 ± 0·32, Aldrin 26·2 ± 0·45
B.H.C. 25·7 ± 0·45.

Experiment 2 : Untreated 14·7 ± 0·84, Chlordane 18·2 ± 1·18,
B.H.C. 20·1 ± 1·18.

From these figures it appears that both the Aldrin and Chlordane treatments give an increase in yield not significantly different from the B.H.C. treatment and that all the treated plots gave yields significantly better than the untreated plots. In view of these results a more detailed experiment on a fresh site has been started in collaboration with the Entomology Department. In these experiments it is proposed to study and compare the direct and residual effects of Aldrin, Chlordane, D.D.T., and B.H.C.

*Effect of insecticides on the population of Aphis fabae scop. (bean aphis) on field beans and on the resulting crop*

Experiments reported in 1950 had shown that the population of *Aphis fabae* on field beans could be markedly reduced by a single application of Parathion, H.E.T.P. or Nicotine ; plots treated with D.D.T. subsequently had a higher population than the controls. In the current year a similar experiment was carried out using Parathion (0·02 per cent), Nicotine (0·1 per cent), Isopestox (0·05 per cent), (proprietary material containing Bis (monoisopropylamino) fluorophosphine oxide). Pyrethrum (0·05 per cent total pyrethrins), allethrin (0·05 per cent) and D.D.T. (0·1 per cent). Spring-sown beans were sprayed on 3rd July very soon after infestation was first recorded, at the rate of approximately 150-166 gall. per acre. Records were kept of the changes in the population of both the aphids and their predators and parasites throughout the growing season. All the treatments reduced the aphid population directly after application, and this reduction was maintained to a greater or lesser degree until harvest time. The rapid build-up of population that occurred in the D.D.T. treated plots in 1950 did not occur in 1951. The reason for this difference in effect is not known, but it may be due to a difference in the formulation of the D.D.T. or to differences in the effects on the parasites and predators in the two seasons.

The yield of seed was greatly increased with the more effective treatments. The following figures show the yields in pounds from the differently treated plots ; for each treatment the yield is from four replicate plots each of seven rows 20 in. apart and 10 yds. long : Isopestox 63·5 lbs., Parathion 62·0 lbs., Pyrethrum 59·0 lbs., D.D.T. 56·5 lbs., Nicotine 46·0 lbs., Allethrin 35·75 lbs., Untreated 23·5 lbs.

In view of the considerable increase in yield obtained by the use of insecticides these experiments are being continued.

*Control of virus spread by the use of insecticides*

These experiments have been carried out in collaboration with the Plant Pathology Department.

*Potatoes*

Following the indications of a small-scale experiment in 1949 that spread of leaf roll virus could be reduced with insecticides, a further small-scale trial was carried out in 1950 using Parathion (0·125 per cent v/v. technical), Toxaphene (0·05 per cent v/v. technical), Schradan (0·125 per cent v/v. technical), Dieldrin (0·08 per cent w/v. technical) and D.D.T. (0·2 per cent w/v.). Treatments were given on May 23rd, June 2nd, June 6th, June 20th, June 27th, July 12th, July 31st. The figures for the spread of Virus Y which became available in 1951 were: Control × 31, Parathion × 19, Toxaphene × 19, Schradan × 22, Dieldrin × 26, D.D.T. × 37. There was little spread of leaf roll virus in any plot.

Aphid counts in 1950 had shown a reduction in population in all the treated plots but there appeared to be no necessary correlation with the degree of reduction of population and the degree of reduction of virus spread. D.D.T. appeared to reduce the population on the plants to a low level although it is not known whether it had a toxic or repellent effect; the high degree of spread with this insecticide may have been due to its causing increased movement of the population within the sprayed area.

During 1951 a large scale experiment has been carried out on the farm the results of which will not be available until 1952.

*Brassicas*

A small plot trial on the effect of insecticides on the spread of cauliflower mosaic virus was carried out in 1951 using D.D.T. (0·1 per cent w/v.), Parathion (0·057 per cent) and Isopestox (0·1 per cent) applied to Roscoff Broccoli sown June 20th, 1951. The plants were sprayed at weekly intervals from July 3rd to August 7th. The first spraying was given when the first leaves appeared. No detailed figures for aphids present on the plants were obtained, but very few were present at any time.

The following figures for percentage infections of plants suitable for planting out from the treated plots were obtained: Control 16·9, Parathion 9·8, Isopestox 13·6, D.D.T. 12·3. The reduction in spread caused by the parathion is significant at 10 per cent.

It is proposed to continue this investigation.

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