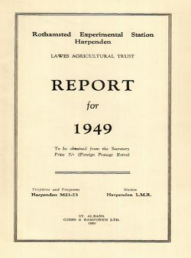
ROTHAMSTED RESEARCH

Report for 1949

Full Table of Content



Insecticides and Fungicides Department

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INSECTICIDES AND FUNGICIDES DEPARTMENT

By C. Potter

The only change in the scientific staff of the department consisted of the appointment of Mr. P. Burt as research entomologist. During the course of the year Mr. A. H. McIntosh was awarded the Ph.D. degree of London University.

The lack of accommodation and in particular lack of facilities for providing controlled environments was felt even more severely than in previous years, but deficiencies in equipment, have, to a very large extent, been remedied.

The central theme of work of the department continues to be the laboratory study of the factors that affect the susceptibility of insects to insecticides. In addition some special studies have been undertaken and some field experiments carried out.

Chemical

Analytical chemistry

Rotenone. During the year a series of analyses were made on a sample sent in by the Board of Greenkeeping Research.

Pyrethrum. (a) World wide collaborative analysis of pyrethrum flowers. Assistance was given by the department in the preparation of a report on this subject, based on the work carried out in the previous years. (b) Colour reaction. It was observed that a colour reaction of carboxylic acid derivatives was also applicable to the pyrethrins.

Hexaethyl tetraphosphate. Estimations of the tetraethyl pyrophosphate content of samples of hexaethyl tetraphosphate were carried out using the method of Jacobson and Hall as modified by Albright and Wilson to avoid interference due to added materials.

Physical chemistry

The effect of particle size of suspensions of contact insecticides on their toxicity as contact poisons. This work has been continued mainly in order to try to explain why D.D.T. in the form of large crystals is easily taken up through the insect cuticle and crystalline rotenone, under the same conditions is not.

It is supposed either that D.D.T. is considerably more soluble than rotenone in the outermost (wax) layer of the insect epicuticle or that the critical particle size, under which solubility becomes greater than normal, is different for D.D.T. in wax than for rotenone in wax. On this basis it would be expected that at a low temperature after treatment, small particles of D.D.T. would be more toxic, relative to large, than at a higher temperature after treatment.

Experiments have been carried out which show that this is so. D.D.T. needles (350μ) are about five times as toxic to adult *Tribolium castaneum* Hbst. as D.D.T. colloid when the insects are kept at 25°C. after treatment, but at 11°C. the colloid is about twice as toxic as the needles. With adults *Oryzaephilus surinamensis* L. as test subjects the results are more marked. Part of this work has been published. Further experiments along these lines are contemplated.

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Surface active agents. The work started in 1946 on the effect of surface active agents on the behaviour of chemicals applied as contact insecticides in aqueous medium is being continued. No consistent large differences in the toxicity of the insecticide due to different surface active agents has as yet been found, but differences up to three or four times may occur. Differences in the surface active agent used may affect considerably the physical state of the deposit of insecticide. This work is being continued.

Biochemical

The survey of the effects of insecticides on the oxygen uptake of insects has been continued and a paper describing the experimental technique devised and some of the results obtained has been accepted for publication.

Preliminary work on insect esterases has been carried out, and extracts of the mealworm *Tenebrio molitor* both adults and larvæ, and of the flour beetle, *Tribolium castaneum* Hbst. adults have been shown to hydrolyse rapidly ethyl butyrate and o-nitrophenyl acetate. Extracts of adult *T. castaneum* did not appear to show any activity in the hydrolysis of acetyl choline.

Some work has been done on the capacity of organo-phosphorus compounds used as insecticides to inhibit the activity of these insect esterases. The hydrolysis of o-nitrophenyl acetate is inhibited by E. 605 (O.O. diethyl-o-p-nitrophenyl thionophosphate) and H.E.T.P. (Hexaethyl tetraphosphate). At pH 6·5 and 25°C. the concentration required to give 50 per cent. inhibition is approximately 10^{-7} M for E. 605 and 10^{-8} M for H.E.T.P. when estimated on the basis of the presumed active constituent—tetraethyl pyrophosphate. An attempt is being made to integrate this work on the mechanism of action of poisons with that on bioassay methods as outlined in the section on bioassay. It is proposed to continue the work both on the effect of poisons on the oxygen uptake of insects and on insect enzyme systems.

Synthetic organic chemistry

Work is proceeding on the synthesis and reactions of keto-alcohols related to the pyrethrins.

The aim of the work under this heading is the study of the relationship between molecular constitution and configuration and insecticidal activity. The work is at present confined to the pyrethrins. Contact has been established with Dr. F. B. LaForge in the Bureau of Entomology and Plant Quarantine, U.S.A., and collaboration established with Dr. S. H. Harper in England. An account of a toxicity study of two compounds closely related to the pyrethrins obtained by the courtesy of Dr. LaForge is given in the bioassay section.

Bioassay

Organo-phosphorus compounds

Insecticidal activity of E. 605, T.E.P.P. and H.E.T.P. Following up work done in the previous year additional laboratory tests of insecticidal activity of samples of E. 605 (O.O. diethyl O.p. nitrophenyl thionophosphate), H.E.T.P. (Hexaethyl tetraphosphate) and T.E.P.P. (Tetraethyl pyrophosphate) were carried out.

The materials were tested for their effect both as contact and

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stomach poisons and some preliminary experiments made on their ovicidal effect.

Contact effects

Larvæ of *Plutella maculipennis Curt.* (Diamond back moth), E. 605 (M.L.D. 0·0055% w/v) was approximately six times as toxic as T.E.P.P. (40% Tetraethyl pyrophosphate) (M.L.D. 0·035% w/v). When measured at LD50 level, D.D.T. (M.L.D. 0·00386% w/v) was slightly more toxic than the E. 605.

Adults of *Phaedon cochleariae* (Mustard Beetle). E. 605 (M.L.D. 0·00615% w/v) was twenty-five times as toxic as T.E.P.P. (40% Tetraethyl pyrophosphate) (M.L.D. 0·0153% w/v) measured at LD50 level.

The T.E.P.P. was approximately sixteen times as toxic as nicotine (M.L.D. 0·234% w/v).

Adults of *Tribolium castaneum* Hbst. (Flour Beetle). E. 605 (M.L.D. 0·000775% w/v) was twenty seven times as toxic as T.E.P.P. (40% Tetraethyl pyrophosphate) (M.L.D. 0·0211% w/v) forty four times as toxic as H.E.T.P. (15% Tetraethyl pyrophosphate) (M.L.D. 0·0342% w/v).

Stomach poison effects

Strips of food leaf bearing known doses of insecticides were fed to the last instar larvæ of *Diataraxia oleracea* (Tomato moth).

The figures for the M.L.D. were taken from the provisional probit lines and were estimated in terms of mg. per larva, the larvæ being approximately 0·4 gm. each.

E. 605 (commercial sample) 0·0035–0·0045 mg., T.E.P.P. (75% Tetraethyl pyrophosphate) 0·070 mg., lead arsenate (99% pure) 0·075–0·090 mg., D.D.T. (pure p.p. 'isomer) 0·012 mg. Thus E. 605 was the most toxic, being approximately three times as toxic as D.D.T. and about twenty times as toxic as T.E.P.P. and lead arsenate.

Ovicidal effects

Preliminary tests were carried out with E. 605 and T.E.P.P. on the eggs of *Diataraxia eleracea* (Tomato moth), *Plutella maculipennis* (Diamond back moth) and *Oligonychus ulmi* (Fruit tree red spider).

E. 605 showed high ovicidal effect to the lepidopterous eggs at concentrations from 0·1% w/v to 0·01% w/v but the T.E.P.P. showed little or no toxicity at the same concentrations. Variable results were obtained with E. 605 on the red spider eggs, in one test it appeared to be less toxic than D.N.O.C. at 0·1% w/v. T.E.P.P. showed no action until the concentrations were raised to 0·8% w/v when there was some evidence of toxicity.

Relationship between the tretaethyl pyrophosphate content of H.E.T.P. and its insecticidal and anti-esterase activity

It has been stated that the insecticidal activity of H.E.T.P. is entirely due to tetraethyl pyrophosphate present in it. A series of samples of H.E.T.P. were received from Messrs. Albright and Wilson, with a request that their insecticidal activity should be investigated. It was decided to carry out this work and at the same time to determine what correlation, if any, there was between the insecticidal activity of the samples, their content of tetraethyl pyrophosphate and their capacity to inhibit the esterase activity. The esterase was

https://doi.org/10.23637/ERADOC-1-71

obtained from larvæ of the mealworm (*Tenebrio molitor*).

It is proposed to do some more work on this subject and to publish a full account later.

From the figures already obtained it would appear that while the tetraethyl pyrophosphate content gives some indication of both insecticidal activity and of anti-esterase activity, other active components are present in the mixtures.

The possibility of using anti-esterase activity as a method for the estimation of insecticidal potency of samples of organo-phosphorus compounds of similar constitution is under consideration.

N Isobutylamides

At the request of the Agricultural Research Council three amides of structures related to that proposed for Herculin (an insecticidal material isolated from prickly ash bark) which had been synthesized by Drs. Raphael and Sondheimer (Nature, 1949, **164,** 707) were tested for their activity as contact insecticides.

Materials

A. $C_3H_7CH\overset{cis}{=}CH(CH_2)_4CH\overset{cis}{=}CO.NH.CH_2.CH<\begin{matrix}CH_3\\CH_3\end{matrix}$

B. $C_3H_7CH\overset{cis}{=}CH(CH_2)_4\ C\equiv CO.NH.CH_2.CH<\begin{matrix}CH_3\\CH_3\end{matrix}$

C. $C_3H_7C\ =\ C(CH_2)_4\ C=CO.NH.CH_2CH\ <\begin{matrix}CH_3\\CH_3\end{matrix}$

The materials were tested on apterous, viviparous, parthenogenetic females of *Macrosiphum solanifolii* (potato aphid), fully grown larvæ of *Plutella maculipennis* (diamond back moth), and adult *Phaedon cochleariæ* (mustard beetle). None of the materials showed any appreciable toxicity at concentrations ranging from 0·5%–0·005% w/v when applied in aqeous medium containing 10% w/v acteone and 0·1% w/v sulphonated lorol.

Synthetic compounds allied to the pyrethrins

The announcement by Dr. LaForge and his colleagues of the synthesis of highly active esters of similar constitution to the pyrethrins was of special interest to us, since work was in progress in the department on the relationship between the chemical structure and biological activity of the pyrethrins and similar molecules.

By the kindness of Dr. LaForge and of the Chemical Biological Co-ordination Committee of the National Research Council of America, we obtained samples of the esters of (±)–3–methyl–2–allyl–cyclopent–2–ene–4–ol–1–one with the natural (+)–trans– and the synthetic (±)–cis–trans–chrysanthemum monocarboxylic acids.

The toxicity of these two esters as contact insecticides was compared with that of an extract of pyrethrum flowers to four insect species. It was found that both the absolute and relative toxicity of the materials varied with the species of insect used. The compound with the natural acid and synthetic alcohol varied from about four times the toxicity of the natural pyrethrins to the larvæ of the diamond back moth (*Plutella maculipennis*), to about one eight as

https://doi.org/10.23637/ERADOC-1-71

toxic to the adult apterous viviparous females of the potato aphid (*Macrosiphum solanifolii* Ashm.). The fully synthetic material varied from approximately twice as toxic as the natural pyrethrins—test subject, *Plutella maculipennis* Curt., to approximately one sixteenth as toxic—test subject *Macrosiphum solanifolii*.

This variation in relative toxicity with the species of insect used as test subject is interesting since the compounds are chemically closely related. The variation in relative toxicity with test subject is further illustrated since the ester with the natural acid is approximately as twice as toxic as the ester with the synthetic acid to three of the test subjects, but was about 4/5 as toxic to the fourth. These specific variations render the task of relating molecular structure and configuration to toxicity difficult.

Biological

Bioassay techniques

A micropipette has been constructed for the injection of small quantities of insecticide into the body cavity of insects. The design of the pipette is based on the Gilmont ultra-microburette and the Heal and Menusan injection pipette. A mechanical manipulator for holding and positioning the insect for injection has also been built, to work in conjunction with the pipette. No other apparatus for precision injection has so far been described, and, in addition to its use for the study of the action of insecticides, it is proposed to study the various factors involved in injection techniques which influence the results of the experiments.

During the course of the year some advances have been made in the decision of the atomizing nozzle and other parts of the Potter spray tower. Some work has also been done on the relative importance of the direct contact effect on the body of the insect and the residual film effect using this technique normally employed with this instrument. In general the results are an estimate of the sum of the two effects. It appears that, in some instances, at least, an exposure to the film alone gives a higher toxicity than direct contact on the body of the insect alone.

The effect of stage development on resistance

Using the tomato moth (*Diataraxia oleracea*), the mealworm (*Tenebrio molitor*), and the cockroach. (*Periplaneta americana*) with D.D.T. and pyrethrum as insecticides, a study has been made of how resistance varies with the stage of development. Large differences in resistance, between different instars of a given species have been found, and even within a given instar these differences may be considerable.

Persistence of insecticides on foliage

Work on this subject has been in progress in the department for some time and has now been intensified at the request of the Colonial Insecticides Committee, which is providing additional finance.

Preliminary experiments on the persistence of D.D.T. on foliage which were carried out during previous years has been followed up in greater detail in 1949. Using turnip and cabbage foliage and a number of different formulations it was found that the type of foliage (sp. of plant) and the nature of the spray medium have a considerable effect on the toxicity of residual films of D.D.T. Using

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D.D.T. in simple crystalline suspension it was shown that on cabbage leaves the residual films rapidly lose their effectiveness as a result of attenuation of the deposit due to plant growth. Under these conditions loss of D.D.T. due to sunlight and rainfall is of negligible importance. It was found, however, that within two days after treatment and before a significant amount of leaf growth had taken place noticeable changes had taken place in the effectiveness of the deposits. There was a reduction in toxicity to insects which only walk the surface but do not eat the leaf and an increase in toxicity to insects that eat the leaf as well as walking the surface. Chemical analyses showed that over a period of one month there was no loss of D.D.T. films on cabbage provided the plants were not exposed to rainfall or to short wave ultra violet light.

Natural fluctuation in the resistance of insect populations and effect of host plant on resistance

Very little work was done on these subjects owing to difficulties with the aphid, mainly due to attacks by parasites and fungal disease.

Mechanism of selection of resistant strains of insect

Some work preparatory to a study of this subject has been carried out. Selected strains of *Drosophila melanogaster* are being reared of known genetical constitution. Spraying techniques for use with these insects are being elaborated and methods for the permanent preparation of individual specimens have been studied so that any differences that occur before and after treatment may be compared.

Effect of environment on the toxicity of contact insecticides

Work on this subject is being continued.

Insect rearing

The twenty five insect species listed in the 1948 report have all been reared during the present year. In addition stocks of *Locusta migratoria* (African migratory locust) and *Pionea forficalis* (Garden pebble moth) have also been reared. A particular study has been made of the biology of *Diataraxia oleracea* L. (Tomato moth) and *Phaedon cochleariæ* F. (Mustard beetle), which have proved to be among the most satisfactory test subjects.

A great deal of trouble has been experienced during the current year with the aphid stocks due to parasites and fungus disease and probably other factors. It has not so far been possible to work on improvements of the rearing technique but this will be done as opportunity offers.

Studies on insect diapause

Some work has been done on the factors influencing the diapause in mustard beetle and tomato moth since this is a major factor when considering rearing techniques.

The mustard beetle overwinters as a resting adult. This can be activated and induced to oviposit within a few days by placing it in a temperature of 30°C. At 12–24°C. activation does not occur or is delayed unless the insect is subjected to 16 hours light per 24 hours. It was found with tomato moth that climatic conditions during the larval instar alone are responsible for inducing or preventing diapause in the pupa. Sixteen hours light per 24 hours prevents diapause

https://doi.org/10.23637/ERADOC-1-71

except at low temperatures (12°C.). Eight hours light per 24 hours induced diapause except at high temperatures (34°C.).

Field Experiments

Control of wireworms

The experiment started in the autumn of 1947 was continued in order to study the residual effects of the various treatments. In addition, a part of the experimental area was set aside for testing possible tainting effects, resulting from the treatments with benzene hexachloride. Judging on crop yields it was found that the autumn 1947 treatments of benzene hexachloride (12–14% gamma isomer) broadcast at 7·9 lb. crude B.H.C./acre, benzene hexachloride drilled with the seed at 2·9 lb. crude B.H.C./acre and D.D.T. drilled with the seed at the rate of 7·2 lb./acre had the greatest residual effect, with yields of 39·6, 37·3 and 36·4 cwt./acre respectively in 1949. The soil fumigants ethylene dibromide and D.D. had less residual effect, applications of 45·5 lb./acre of ethylene dibromide and 120 lb./acre of D.D. in 1947 giving yields of 34·1 and 31·8 cwt./acre in 1949. Seed treatment had no residual effect. The control yield was 28·4 cwt./acre. Taking into account the previous year's results it would appear therefore, that the treatment where benzene hexachloride was drilled with the seed and broadcast gave good immediate results and good residual effects, ethylene dibromide and to a lesser extent D.D. gave good immediate results and fair residual effects, seed dressing with high gamma isomer B.H.C. gave good immediate effects and no residual effects and D.D.T. gave poor immediate effects but good residual effects. Evidence was obtained that there was a risk of taint occurring in root crops planted in soil previously treated with crude Benzene hexachloride at dosages from 0·98–5·92 lb./acre.

Control of flea beetle

A large scale experiment on the use of benzene hexachloride, D.D.T. and E. 605 to control flea beetles failed owing to lack of attack, but small scale trials indicated that benzene hexachloride sown with the seed might give good protection. It is hoped to follow up this work.

Control of aphids carrying virus on potatoes

In collaboration with the Plant Pathology department a preliminary experiment was carried out on the control of aphids carrying virus in potatoes. The results of this experiment are not yet available.

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