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## Insecticides and Fungicides Department

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## INSECTICIDES AND FUNGICIDES DEPARTMENT

By C. POTTER

A number of staff changes occurred during the year, mainly in the form of additions. Dr. Pradhan after taking his London Ph.D. for the work he carried out in the department, returned to India to take charge of insecticide work in that country, and later on in the year Mr. T. D. Mukherjea came over from Dr. Pradhan's laboratory to work with us. Mr. K. A. Lord was awarded his London Ph.D. during the year. Mr. M. E. Elliott has joined the research staff as synthetic organic chemist, and Mr. P. Needham as an Experimental Officer on the Biological side.

The lack of accommodation, and in particular, the lack of facilities for providing controlled environments in which to carry out experiments, continues to be a considerable handicap to the work. A scheme has been put forward for improved accommodation and facilities. There are still some shortages of equipment but considerable progress has been made in remedying deficiencies in this respect.

The work of the department may be conveniently described under five headings. (1) General; (2) Chemical:— (Analytical, Synthetic, Biochemical); (3) Physico-Chemical; (4) Biological; (5) Field work.

### GENERAL

(a) *Ad hoc* work on organic phosphorus insects. (b) Biological evaluation of samples of Benzene Hexachloride containing different proportions of the isomers. (c) Estimations of the insecticidal activity of residues from the preparation of piperidine by the process of hydrogenation of pyridine.

### *Organic phosphorus insecticides*

*Hexaethyl-tetraphosphate* (H.E.T.P.) At the request of the Agricultural Research Council, the insecticidal activity of samples of H.E.T.P. prepared in three different ways have been compared on adult Flour Beetle (*Tribolium castaneum* Hbst.) using a direct spray technique. No significant differences in biological activity were found between any of the three samples.

The toxicity of H.E.T.P. when formulated in three different media has also been estimated, again using adult flour beetle as the test subject. There were considerable differences in the slopes of the probit lines obtained with the three media, so that it was not possible to make direct comparisons of the toxicity. However, when the differences in the weight of poison deposited, using the three media, were taken into account there did not appear to be any considerable differences in toxicity. The three media were:—(a) Odourless distillate (a highly refined light petroleum oil). (b) 10% v/v odourless distillate emulsified in 0.1% w/v sulphonated loral in water. (c) 10% v/v acetone in 0.1% w/v sulphonated loral in water. The stock solutions used were 0.24% w/v H.E.T.P. in odourless distillate, 2.4% w/v H.E.T.P. in odourless distillate and

2.4% w/v H.E.T.P. in acetone. All the solvents used for preparing the stock solutions were dried over calcium chloride. After keeping the stock solutions of H.E.T.P. for one month they were compared biologically with a further set of freshly made solutions. The month old solutions of 0.24% v/v H.E.T.P. in odourless distillate was non-toxic. The probit line obtained with the old 2.4% w/v H.E.T.P. in acetone solution differed slightly in position, but not in slope, from the freshly made solutions, indicating a slight loss in toxicity. The probit line obtained with the old solution of 2.4% w/v H.E.T.P. in odourless distillate sprayed as an emulsion altered its slope so that it approximated to that obtained with the acetone solution in water, rather than to the slope of the corresponding emulsion for the freshly prepared 2.4% w/v H.E.T.P. in oil solution. The main conclusion that may be drawn from these experiments seems to be that, although some decomposition had occurred in the stock solutions, it had been insufficient to reduce the toxicity of the spray solutions, where strong stock solutions were used, although it had caused a change in the slope of the probit lines.

The insecticidal activity of H.E.T.P. has been compared with that of nicotine as a contact insecticide, by means of a direct spraying technique. Both insecticides were formulated in aqueous medium containing 10% v/v acetone, and 0.1% w/v sulphonated lorol. The insects used as test subjects were adult apterous, viviparous parthenogenetic females of pea aphid (*Acyrtosiphon pisum*, Harris) adult mustard beetle (*Phaedon cochleariae* F.) and the larvæ of the diamond back moth (*Plutella maculipennis* Curt.). H.E.T.P. was about 10 times more toxic than nicotine to all these insects under the conditions of test.

*O,O-Diethyl O.p-nitrophenyl thiophosphate (E605)*. The toxicity of this material was compared with that of H.E.T.P. under the same conditions of test as those outlined above for the comparison of H.E.T.P. and Nicotine. The test subjects used were adult flour beetles (*Tribolium castaneum* Hbst.) and the larvæ of diamond back moth (*Plutella maculipennis* Curt.). The E605 proved to be of the order of 10 times as toxic as the H.E.T.P. to these insects.

#### *Benzene hexachloride*

At the request of the Agricultural Research Council two samples of the gamma isomer of benzene hexachloride obtained from different sources were compared biologically for their insecticidal activity, using a direct spraying technique and the adult grain weevil (*Calandra granaria* L.) as test subjects. No significant difference was found between the biological activity of the two samples.

A sample of so-called enriched material, which consisted of the gamma and delta isomers and perhaps other material, was tested at the same time as the two samples mentioned above and was about  $\frac{1}{3}$  as active as the pure gamma isomer. Since the delta isomer comprised approximately  $\frac{2}{3}$  of the so-called enriched material this indicated that the delta isomer had in this instance no insecticidal activity and no synergistic effect.

At the request of the Agricultural Research Council and the Chemical Research Laboratory, Teddington, some tests were carried out to determine the insecticidal activity of a series of fractions of a residue formed in the process of preparation of piperidine by the

vapour phase hydrogenation of pyridine. The crude residue had already been reported to have insecticidal activity.

Ten fractions were sent to us to test. The overall boiling range of these fractions was from below 80° C. to 180° C. at 7 mm. pressure. They were tested in aqueous medium containing 10% v/v acetone and 0.1% Lissapol N (an oil soluble emulsifier consisting of condensed polyethylenes), at 0.1%, 0.5% and 1.0% v/v. The test insects used were adult apterous, viviparous, parthogenetic females of bean aphid (*Aphis Fabae* Scop.), adult saw toothed grain beetle (*Oryzaephilus surinamensis*) eggs and 5th instar larvæ of tomato moth (*Diataraxia oleracea*) and 5th instar larvæ of cabbage moth (*Mamestra brassicae*). Very little toxicity was shown by any of the fractions. The higher boiling fractions at the 1% dilution gave a high percentage kill of aphids, but at this dilution and at the two lower dilutions no appreciable mortality occurred with the other test subjects.

#### CHEMICAL

##### *Analytical*

*Pyrethrum.* During the past year a series of estimations of the pyrethrin contents of samples of pyrethrum flowers have been carried out in connection with a world-wide collaborative scheme. Two samples were examined using three methods, viz., the Wilcoxon, the Seil and the Ripert techniques. Since the results of all the collaborators have not yet been received and examined, it is not possible as yet to reach any conclusions. The purpose of the work is to find a method for routine analyses where producible results may be obtained, irrespective of the operator and location. Additional sets of tests to compare the analytical results obtained with samples stored in the refrigerator with those that had travelled round the world were also carried out. This was done to determine if deterioration or any other effects occurred during travel which might affect the results obtained by workers in different parts of the world.

*Rotenone.* At the request of the National Agricultural Advisory Service (Wye), a series of rotenone analyses were carried out on a group of Derris and Lonchocarpus dusts.

##### *Synthetic*

This work has only been in progress for a few months. The present aim in the first instance, is to study the relationship between insecticidal activity and chemical structure with especial reference to the pyrethrins. For this purpose synthetic routes to keto-alcohols related to pyrethrolone and cinerolone are being explored and it is intended to esterify any such compounds obtained with trans-chrysanthemum mono-carboxylic acid (isolated from a concentrate of the natural pyrethrins) and examine the biological effect of these esters.

##### *Biochemical*

Work on the effect of insecticides on the respiration of insects has been continued. An electro-magnetic device has been elaborated for distributing dusts inside a modified Barcroft apparatus without disturbing the thermal equilibrium of the apparatus. It is now

possible to observe the effects, if any, of poisons, on the oxygen uptake of insects during the first few minutes after treatment.

A survey of the effects of a variety of insecticides of widely different chemical types is at present being carried out and a number of substances of known physiological importance are being included.

Data has already been obtained on the action of several chemicals on the oxygen uptake of adult *Tribolium castaneum* Hbst. but as yet the results have not been examined in detail. It is not thought wise to report any data from this section of the work until it has been carried further, since great care has to be used in the interpretation of the results.

#### PHYSICO-CHEMICAL

##### *The effect of particle size on toxicity*

Work on the effect of crystal size on the toxicity as direct contact insecticides of suspensions of pure 2,2 bis (parachlorophenyl) 1,1,1, trichloroethane (D.D.T.) and rotenone has been continued.

It has been shown that the results previously obtained with D.D.T. on adult *Tribolium castaneum* Hbst. (which is not normally susceptible to rotenone) also applies to adult *Oryzæphilus surinamensis*. These results show that toxicity increases as the crystal size increases within the limits tested. It has now been found that when rotenone is used on adult *O. surinamensis* exactly the opposite effect occurs, that is, the toxicity increases as the crystal size decreases.

With both poisons it appears that a larger amount of poison is retained on the surface of the body with the larger crystal sizes, and this may be a partial explanation of the results obtained with D.D.T. but obviously cannot explain the results obtained with rotenone. Some reason for the effects obtained and the cause of the differences in behaviour between rotenone and D.D.T. are now being sought.

Assuming that rate of penetration of the cuticle is a limiting factor and that this, in its turn, is governed by the lipid solubility of the rotenone, the results obtained with this poison may be explained by the enhanced solubility of small crystals.

Working on the same hypothesis with D.D.T. it would appear that overall lipid solubility of the crystals was not the limiting factor, and that the increase in the amount retained was therefore more important. This heavier dosage might be increasing the amount of poison penetrating the cuticle over the whole area of the body, or it might be having its effect at some specially sensitive point. It was thought that the legs, particularly the tarsi, might be sensitive areas and since in addition the large needle shaped crystals of D.D.T. are retained somewhat preferentially in this area, some experiments were made with insects and their legs removed, but these gave negative results.

It is now being inferred that the cuticle as a whole is more easily penetrated by D.D.T. than rotenone, and an approach to the problem is being made by taking into account the structure and properties of the epicuticle and by studying the effect of various treatments of the epicuticle on the toxicity of the two poisons.

##### *Surface-active agents*

A study of the effect of surface active agents on the toxicity of contact insecticides, applied directly to the body of the insect, was started by Dr. Potter in the U.S.A. in the latter part of 1946

and carried on in that country to the end of the year. This work has been continued at Rothamsted, and a large quantity of data has been obtained. The object of this study was to determine the magnitude of the difference, if any, of the toxicity of contact poisons produce by different surface active agents, and to find, if possible, a general principle on which the differences could be based. The data obtained are not yet complete, and have not been fully analysed and examined.

#### BIOLOGICAL

##### *Natural variation in resistance*

During the course of the year, work was resumed on the variation that occurs naturally over a long period in insect populations. *The effect of differences in host plant on the resistance of a given insect species*

The pea aphid *Acyrtosiphum pisum* was reared on two different host plants, beans and clover, side by side in a glass house. The strain from the bean was used to colonize the clover.

Samples of populations from both host plants taken on the same day were tested for their resistance to rotenone.

Four experiments were carried out, but only one gave satisfactory results. This experiment gave non-heterogeneous data and an L.D.50 of 0.00015% w/v of rotenone for the insects taken from beans, as compared with 0.00021% w/v for the population on clover. A previous experiment (23.8.43) had given an L.D.50 of 0.00015 for the population on beans as compared with 0.00037 for that on clover. These figures indicate that the insects feeding on clover are more resistant than those feeding on beans, at least where rotenone is the poison. The other experiments were not satisfactory, owing either to considerable heterogeneity of the data or disease in the populations which only showed up after treatment. Owing to difficulty with disease and synchronization of the development of suitable populations it is difficult to obtain satisfactory data on this point, but the work is being continued.

##### *Cuticle structure*

M. J. Way is concluding studies started at Cambridge on the structure and physiology of the larval cuticle of *Diataraxia oleracea*—the tomato moth.

Soon after work was begun it became clear that the fine structure of the cuticle would have to be studied in detail before it would be possible to commence work on the penetration of insecticides.

The formation of the new cuticle has been studied in order to determine the presence and position of various layers which are difficult to define in the mature cuticle. In addition the electron microscope has been used to study fine structure.

Fundamentally the cuticle of this insect is typical of insects in general, and consists of a well defined epicuticle and endocuticle. Particular study has been made of the former, which probably acts as the main barrier to the penetration of materials through the cuticle. The formation of the cement layer, the formation and regeneration of the wax layer, and the "chemistry" of the lipoprotein layers of the epicuticle have been studied.

The pore canals have been suggested as a convenient system along which insecticides may penetrate into the body of the insect.

Their structure and functions in the *Diataraxia* cuticle have been studied in detail. Of interest is the fact that they are functional as conducting canals only in the early stages of development of the cuticle. Afterwards their contents become chitinized and sclerotized and they appear to develop a skeletal function.

The electron microscope has been used to determine the relationship of the pore canals to both the epicuticle and the outer cuticle. Laminae of the latter observed in surface view in the electron microscope showed a clear picture of pore canals and the surrounding chitin structure. No information was obtained on the relationship between chitin and protein in the endocuticle. In the 60  $\mu$  thick inner endocuticle of the mature insect pore canals are absent, but the porous structure of this layer as shown by sections examined in the electron microscope suggests that even in the absence of pore canals it need not act as a barrier to the passage of materials.

#### *Insect rearing*

The search for suitable species of insects that feed on the growing plant and are suitable subjects for the study of insecticides, continues to be an important part of the work of the department.

Studies of the effect of temperature and light of the biology of several species have been made, and it has been found that additional light will break the diapause in some species. So far as we are aware this is the first time this effect has been recorded.

The following species of insects have been worked on during the current year:—

*Insects feeding on growing plants*, Hymenoptera:—*Athalia colibri* F. (Turnip sawfly). Lepidoptera:—*Diataraxia oleracea* L. (Tomato moth); *Plutella maculipennis* Curt. (Diamond back moth); *Mamestra brassicae* L. (Cabbage moth); *Lymantria dispar* L. (Gypsy moth); *Pieris brassicae* L. (large cabbage white butterfly); *Plusia gamma* L. (Silvery moth); *Phragmatobia fuliginosa* L. (Ruby tiger); *Sphinx ligustri* L. (Privet hawk moth); Coleoptera:—*Phaedon cochleariae* F. (Mustard beetle); Rhynchota Aphididæ:—*Macrosiphoniella sanborni* Gill. (Chrysanthemum aphid); *Macrosiphum euphorbiae* Thos. (Potato aphid); *Acyrtosiphon pisum* Harris. (*Macrosiphum pisi*) (Pea aphid); *Aphis fabae* Scop. (Bean aphid); *Myzus circumflexus* Buct. (Mottled arum aphid); *Myzus persicae* Sulz. (Peach-potato aphid).

*Insects feeding on stored products*. Lepidoptera:—*Ephestia kuehniella* Zell. (Mediterranean flour moth); *Sitotroga cerealella* Ol. (Angoumis grain moth); *Galleria melonella* L. (Large wasp moth); Coleoptera:—*Tribolium castaneum* Hbst. (Flour beetle); *Tribolium confusum* Duv. (confused flour beetle); *Oryzaephilus mercator* L. (Merchant grain beetle); *Calandra granaria* L. (grain weevil); *Tenebrio molitor* L. (meal worm). Orthoptera:—*Periplaneta americana* L. (American cockroach).

#### *Studies of the effect of herbicides and repellents on bees*

The work commenced in 1947, in collaboration with the bee department, on the effect of the newly developed herbicides on bees, was continued during this year, and most of the time was spent on the development of techniques for the study of the toxic and repellent properties of various chemicals to the Honey Bee.

An investigation of the toxicity of films of the acid 3:5 Dinitro-*o*-cresol (DNOC) and its sodium salt, which are both used as herbicides, showed that a film of the acid was a rapidly acting poison under a wide range of conditions, whilst a film of the sodium salt was hardly effective unless the humidity of the atmosphere over the films was such that actual condensation of the moisture took place at the treated surface. When this occurred the bees were as quickly affected as with the film of the acid.

Using films of the acid 3:5 DNOC deposited on a leaf surface (cabbage), it was found that such a film rapidly became more effective as judged by rate of 'knock-down' when the deposit was increased from 0.010 mg./sq. cm. to 0.40 mg./sq. cm., and it was calculated that under field conditions when the DNOC was used as a herbicide, the maximum deposit was unlikely to be 0.066 mg./sq. cm. From this it is assumed that continued foraging on the treated flowers by bees was likely to prove fatal.

A technique was developed for the evaluation of various repellent materials to the Honey Bee. The experiments were conducted in a greenhouse, which contained a small colony and allowed normal flight. This arrangement made it possible for reproducible results to be obtained under semi-controlled conditions, and experiments conducted in the field indicated that differences in repellent properties recorded in the greenhouse, were applicable under normal and foraging conditions.

Of the various chemicals tested, which included the recently developed mosquito repellents, a proprietary wetting agent which consisted of a solution of sodium salts of sulphonated secondary alcohols showed the most promise as a repellent.

#### FIELD EXPERIMENTS

The two experiments on the control of wireworms in Little Hoosfield, started in the autumn of 1947 using wheat as a test crop, have been carried on, and the crop yields for the various treatments have been obtained. In experiment 1, on the effect of different chemicals Ethylene dibromide at 45.4 lb. per acre gave the best results—giving a yield of 32.1 cwt. per acre compared with 8.9 cwt. per acre for the controls. A Gammexane dust (3.5% crude benzene hexachloride containing 12–14% gamma isomer) broadcast at 2 cwt. per acre gave a yield of 30.6 cwt. per acre; D.D. (said to be a mixture of about equal parts of 1, 3 dichloropropene and 1, 2 dichloropropane with small quantities of other chlorinated compounds) 28.3 cwt. per acre; gammexane combine drilled with the seed at the rate of  $\frac{3}{4}$  cwt. per acre gave a yield of 24.8 cwt. per acre. Seed dressed with an experimental mixture prepared by Imperial Chemical Industries containing organic mercurials equivalent to 1% mercury and 20% technical gamma isomer, at the rate of 2 oz. per bushel of seed gave a yield of 24.0 cwt. per acre. A dust containing 5% D.D.T. combine drilled with the seed at 1.2 cwt. per acre gave a yield of 20.7 cwt. per acre.

The second experiment comparing the effects of treatment with benzene hexachloride in the form of gammexane, applied in different ways, confirmed the good results obtained with the seed dressing and indicated that combine drilling at the rate of 1 cwt. per acre, gammexane had slight deleterious effects while dressing below  $\frac{1}{4}$  cwt. per acre might well prove satisfactory.