ROTHAMSTED RESEARCH

Report for 1952

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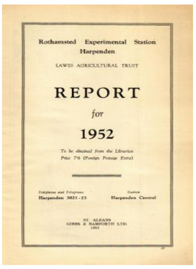
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REPORT for 1952

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. Potter

M. J. Way returned to Rothamsted in July, 1952 after being seconded to the Clove Research Scheme, Zanzibar from 1950. Since his return he has been preparing his work there for publication until November 30th, 1952 when he resumed his work in the Department. His work in Zanzibar on the problem of *Theraptus* on coconuts has been widely commended, and is being followed up by the Colonial Office and the local Departments of Agriculture. Four papers have now been submitted for publication. These describe some effects of controlling the tree nesting ant *Oecophylla longinoda*. This was thought harmful because it attends and greatly benefits a Coccid which once was suspected as a possible vector of a disease of the Clove Tree. During this work it was found that *O. longinoda* is beneficial to coconut palms which it protects from attack by the Coreid bug *Theraptus* sp.; this bug was found to cause severe nutfall and Gummosis, hitherto attributed to poor soil. When attempts were made to encourage *O. longinoda* in coconut plantations, it was found that its relative scarcity was mainly due to three other ant species which although destructive to it do not prey upon *Theraptus* sp. Preliminary experiments were carried out on chemical control of one harmful ant *Anoplolepis custodiens*, and also on direct control of *Theraptus* sp. A Dieldrin emulsion was more effective than either D.D.T. or γ BHC emulsions as a spray treatment against the ant. The coconut yield in one area was increased by more than x10 when the crowns of cocnut palms were dusted fortnightly by hand with 0·4 per cent γ BHC to destroy *Theraptus* sp.

Miss H. Salkeld of Ontario Agricultural College completed her work for a Ph.D. of London University, and was awarded the degree. A paper on the effect of the age and stage of development of eggs on their resistance to insecticides which is based on her thesis has been accepted for publication.

M. Elliott has also been awarded the degree of Ph.D of London University for part of his work on the synthesis of the pyrethrins.

Miss C. Hutt married and left the department, she has been replaced by Miss D. Holbrook.

M. Elliott, K. A. Lord, A. H. McIntosh and C. Potter attended the IIIe Congrès International de Phytopharmacie in Paris. C. Potter presided over the Commission for the standardization of Biological Assay Techniques. At the invitation of the organizers, he also gave a Congress Lecture on the " Mechanism of action of insecticides ", and was awarded a Congressional Medal.

At the invitation of the Kenya and Tanganyika Pyrethrum Boards, C. Potter, in company with J. Furlong, Principal of the

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Colonial Products Advisory Bureau, visited Kenya and Tanganyika on behalf of the Colonial Office, in order to advise the Pyrethrum Boards on the problems of the Pyrethrum industry. A visit was also made to the Belgian Congo at the invitation of the Pyrethrum Growers there. At the invitation of the Colonial Office C. Potter visited Zanzibar to discuss the problem of *Theraptus* on coconuts and to be informed on the work on Sudden Death and die-back of Cloves, he also visited the Colonial Insecticides Research Unit in Arusha, Tanganyika.

Chemical

Physical Chemistry

The effect of particle size of suspensions of contact insecticides on their toxicity. A. H. McIntosh has continued to work on this subject. Previous work, on injection of insecticidal suspensions into adult milkweed bugs (*Oncopeltus fasciatus*), had suggested that the difference in speeds of action between colloidal and crystalline forms of the one poison depended on the ratio of dose to solubility of poison in oil.

Tests, begun last year and now completed, show that this does not hold in contact action. Biological tests were made on adult saw-tooth grain beetles (*Oryzaephilus surinamensis*) with D.D.T. and eight of its analogues, none of which showed fumigant action. Further, there is no relationship at all between absolute toxicity of a D.D.T.-like compound and its solubility in oil.

The first step in penetration of insect cuticle by a poison is considered to be solution in the wax layer of the epicuticle. This wax layer is thin and will easily become saturated with poison from colloidal particles, no matter how high their lipoid solubility is. It is assumed that the rate at which saturation is reached is the same for all colloidal analogues. It has not been possible to measure the speeds at which crystals of analogues dissolve in wax from *O. surinamensis*. However, a method has been worked out for measuring the speeds at which deposits from suspensions of crystals dissolve in olive oil. There are big differences in the rates at which the crystalline forms of different analogues dissolve; crystals of different poisons but of the same size do not necessarily dissolve at the same speed. If the crystals dissolve slowly, there is a large difference in speed of insecticidal action between crystalline and colloidal poison. (This is measured by comparing the kills from crystalline and colloidal poison on insects kept cool after treatment and inspected after one day). There is, in fact, a correlation coefficient of 0·92 between difference in speed of insecticidal action and rate of solution of crystals.

Another factor which may influence difference in speed of insecticidal action is the stability of the film left on an insect's body by treatment with a colloidal suspension; these films show a greater or lesser tendency to crystallize as time passes. If the crystallization is very rapid, the advantages of applying poison as a colloid may be nullified. However, it was found that, although the colloidal analogues showed great differences in their ability to crystallize in films on glass, there was no relationship between speed of crystal-

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lization and difference in speed of action of colloidal and crystalline analogues.

All the biological tests in this series have been carried out on adult *O. surinamensis* by dipping. The tests are now being repeated on adult mustard beetles (*Phaedon cochleariae*) by dipping, and on adult mealworms (*Tenebrio molitor*) by placing measured drops under the wings.

Effect of surface active agents on the toxicity of contact poisons. This work has remained in abeyance during the current year.

Biochemical

The work, started in previous years by K. A. Lord and C. Potter on the study of insect esterases and choline-esterases has been continued and extended during the past year. Non-specific esterases which hydrolyse phenylacetate have been shown to be present in all the insects tested to date. However, evidence based on the insect extracts tested to date indicates that choline esterase activity is less widely distributed.

During the current year much of the work has been designed to provide further information, first on the relationship between mammalian toxicity and insecticidal activity, in the hope that relatively non-toxic compounds of high insecticidal activity might be developed on rational lines; secondly on the correlation between anti-esterase activity and insecticidal activity; thirdly on the relative importance of any particular esterase or groups of esterases in the poisoning process.

A comparison of the relative toxicities of paraoxon, TEPP, parathion and two of its analogues with their capacities to inhibit insect esterases and choline-esterases has been made in four species of insects, viz. *Blattella germanica* adult, *Tenebrio molitor* larvae, *Dysdercus fasciatus* and *Tribolium castaneum* adults. The toxicities of the three isomers of parathion to those four insects species were, in order of increasing toxicity:—

O:O-diethyl O-(p-nitrophenyl) thiophosphate (Parathion) O:S-diethyl O (p-nitrophenyl) thiophosphate O:O diethyl S-(p-nitrophenyl) thiophosphate. This is in the reverse of the order of their toxicities to rats as reported by Aldridge, N. W. and Barnes, J. M. (1952), Nature **169**, 345.

Paraoxon was more toxic than parathion both to the four insect species and to rats. TEPP was of the same order of toxicity as the parathion isomers to all four insects species, but its exact position relative to the other compounds varied with the species.

Taking the substances as a whole, if they are arranged in order of increasing inhibitory power to either general esterase or choline-esterase, this order is not a guide to the order of the compounds in increasing toxicity to the insect from which the enzymes were derived. However, it is perhaps significant that the parathion isomer O:S-diethyl O-(p-nitrophenyl) thiophosphate is a better inhibitor of the general esterase for each of the four insects species tested and is also more toxic to them than O:O-diethyl S-(p-nitrophenyl) thiophosphate, but the reverse is true of inhibitory power to choline-esterases, both insect and mammalian, and of toxicity to rats.

In the case of adults of both *B. germanica* and *Tenebrio molitor* the amount of each inhibitor required for inhibition of the general esterase is less than that required to inhibit the choline-esterase for the corresponding insect. This suggests that it is the inhibitor of the general esterase which usually causes death in the insect, a view which receives support from studies on the *in vivo* inhibition of the two enzymes in *T. molitor* which have been treated with paraoxon.

Pure Parathion, as reported previously, is not an effective esterase inhibitor, but when applied to insects *in vivo* it is rapidly converted into a powerful esterase inhibitor. The evidence for this is that extracts from insects poisoned by external application of pure parathion show marked anti-esterase activity.

Further work of a preliminary nature has been carried out on insect enzymes other than esterases.

Relationship between chemical constitution and insecticidal activity

Materials of plant origin which have insecticidal activity are frequently less toxic to mammals than many of the synthetic compounds which have been widely used for pest control in recent years. Chiefly for this reason compounds related to naturally occurring materials have continued to be those mainly studied in this department in the above connection.

N-iso*butylcarboxyamides*. A number of *N-iso*butyl amides of straight chain acids with varying degrees of unsaturation have been isolated from plant sources by various workers. They have in common a sialogogue activity and several have been claimed to show considerable toxicity to various insect species, especially the housefly, *Musca domestica* L. Leading references are cited by Crombie, J. Chem.Soc., 1952, 2997.

The objects of the work described below have been two-fold:—(1) to assist Dr. L. Crombie (Imperial College of Science and Technology, Organic Chemistry Department) in chemical investigations he is carrying out on the structure and synthesis of the insecticidal materials, pellitorine and herculin, and (2) to try to throw light on structure-toxicity relationships in compounds of this type. This study continues work outlined in the Annual Reports for 1949 and 1951. The results obtained so far give fair promise of revealing interesting structure-toxicity relationships in a field hitherto almost unexplored and an indication of a considerable degree of species selectivity in the potency of some such materials has been obtained.

The compounds tested and their insecticidal activity are shown in Table I. The bioassay was performed by P.H. Needham using a technique described elsewhere.

Jacobson (J. Amer. Chem. Soc., 1949, **71**, 366) proposed the gross structure *N-iso*butylnona-1:5-diene-l-carboxyamide for the insecticidal sialogogue, pellitorine, isolated from the roots of *Anacyclus pyrethrum* D.C. (for references, see Jacobson, *loc cit.*, and

Crombie, J. Chem. Soc., 1952, 4338). Compounds 1–4 in the table are the four possible geometrical isomers of this structure, specimens of which were supplied by Dr. L. Crombie. None showed appreciable toxicity as tested here and Crombie, *loc cit.*, on the basis of chemical evidence and these results has concluded that the above structure does not represent the insecticidal material from this source. Chemical and light absorption evidence (Crombie, Chemistry and Industry, 1952, 1034) indicated that the toxic compound in pellitory root extract might be *N-iso*butylnona-*trans*-l-*trans*-3-diene-l-carboxyamide (Compound 7). Whilst a synthetic specimen of this *iso*butylamide showed appreciable toxicity to *Musca domestica* L. (Crombie, Chemistry and Industry, *loc cit.*) it had negligible toxicity to *Tenebrio molitor* adults even at a concentration of above 10 per cent w/v. in acetone, whilst pellitorine, m.p. 72°, gave a 45 per cent kill at 3·13 per cent w/v. and a crude material, m.p. 49°, a 96 per cent kill at 3·15 per cent w/v. However, preliminary results indicate that the crude material, m.p. 49°, is only 4·4 times as toxic as the synthetic *N-iso*butylnona-*trans*-l-*trans*-3-diene-l-carboxyamide to *Blatella germanica*. Therefore it appears that the relative toxicity of the latter synthetic material and the crude natural extract varies with the species examined and this phenomenon is being investigated further.

Again Jacobson (J. Amer. Chem. Soc., 1948, **70**, 4234) proposed the gross structure *N-iso*butylundeca-1:7-diene-l-carboxyamide for herculin, the insecticidal sialogogue from the bark of *xanthoxylum clava-herculis* L. (for further references, see Crombie, J. Chem. Soc., 1952, 2997). Specimens of compounds 11–14 in the table have kindly been given by Dr. L. Crombie and are the four possible geometrical isomers of Jacobson's structure for herculin. None showed appreciable toxicity as tested here and this provides some support for Crombie's conclusion that herculin is not a stereoisomer of *N-iso*butylundeca-1:7-diene-l-carboxyamide.

A few other *N-iso*butylamides were also examined, but as shown in the table, none showed appreciable toxicity.

Pyrethrin-like compounds. The examination of the relationship between insecticidal activity and structure of synthetic compounds to the natural pyrethrins related has been continued.

The compounds in this series are esters of 2-alkyl and -alkenyl-3-methyl*cyclo*pent-2-en-4-ol-l-ones with various optical and geometrical isomers of 2:2-dimethyl-3-*iso*butenyl-*cyclo*propane-l-carboxylic acid (chrysanthemic acid). In the Report for 1951 some results on the toxicity of various forms of this acid and compounds related to it were presented. Work can now be reported which relates to variations in the alcoholic part of the molecule.

1. The effect of reducing the >C=O group to >CHOH in the keto-alcoholic part of the molecule has been examined. M. Elliott has found that allethrin (the ester of (±)-2-allyl-3-methyl-*cyclo*pent-2-en-4-ol-l-one with (±)-*cis-trans*-chrysanthemic acid) can be reduced by means of sodium borohydride to (±)-2-allyl-3-methyl*cyclo*pent-2-en-1:4-diol-4-(±)-*cis-trans* chrysanthemate. Only the ketone group in the *cyclo*pentenone ring is affected in this reaction. The reduced compound is only 1/10th as toxic to *Phaedon cochleariae* adults as the parent keto-ester.

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Toxicity of various N-iso*butylcarboxyamides to* Tenebrio molitor *adults*

No.		*Concentration* (% w/v. in acetone)	*% Kill*
1.	*N-iso*butylnona-*trans*-1-*trans*-5-diene-1-carboxyamide $Pr^{n}CH\overset{t}{=}CH.(CH_2)_2.CH\overset{t}{=}CH.CO.NH.Bu^{i}$	1·92	4
2.	,, ,, ,, *cis*-1-*trans*-5- ,, ,, ,, $Pr^{n}CH\overset{t}{=}CH.(CH_2)_2.CH\overset{c}{=}CH.CO.NH.Bu^{i}$	2·02	0
3.	,, ,, ,, *trans*-1-*cis*-5- ,, ,, ,, $Pr^{n}CH\overset{c}{=}CH.(CH_2)_2.CH\overset{t}{=}CH.CO.NH.Bu^{i}$	1·92	4
4.	,, ,, ,, *cis*-1-*cis*-5- ,, ,, ,, $Pr^{n}CH\overset{c}{=}CH.(CH_2)_2.CH\overset{c}{=}CH.CO.NH.Bu^{i}$	2·64	0
5.	Pellitorine, m.p. 72-4°, isolated by Jacobson's method	3·13	45
6.	Pellitory root extract, m.p. 49°, isolated by chromatography	3·15	96
7.	*N-iso*butylnona-*trans*-1-*trans*-3-diene-1-carboxyamide $Am^{n}CH\overset{t}{=}CH.CH\overset{t}{=}CH.CO.NH.Bu^{i}$	3·37 10·81	0 4
8.	Stillingic acid *N-iso*butylamide $Am^{n}CH{=}CH{\cdot}CH{=}CH{\cdot}CO{\cdot}NH{\cdot}Bu^{i}$ (stereo-isomer of 7)	4·09 10·50	4 4
9.	*N-iso*butylnona-*trans*-1-ene-1-carboxyamide $Pr^{n}(CH_2)_3.CH\overset{t}{=}CH.CO.NH.Bu^{i}$	2·19	0
10.	,, ,, ,, *cis*-1- ,, ,, ,, $Pr^{n}(CH_2)_3{\cdot}CH\overset{c}{=}CH{\cdot}CO{\cdot}NH{\cdot}Bu^{i}$ Nos. 1–10 are compounds related to pellitorine	1·97	0
11.	*N-iso*butylundeca-*trans*-1-*trans*-7-diene-1-carboxyamide $Pr^{n}CH\overset{t}{=}CH.(CH_2)_4.CH\overset{t}{=}CH.CO.NH.Bu^{i}$	2·13	0
12.	,, ,, ,, *trans*-1-*cis*-7- ,, ,, ,, $Pr^{n}CH\overset{c}{=}CH.(CH_2)_4.CH\overset{t}{=}CH.CO.NH.Bu^{i}$	1·09	3
13.	,, ,, ,, *cis*-1-*trans*-7- ,, ,, ,, $Pr^{n}CH\overset{t}{=}CH.(CH_2)_4.CH\overset{c}{=}CH.CO.NH.Bu^{i}$	1·96	0
14.	,, ,, ,, *cis*-1-*cis*-7- ,, ,, ,, $Pr^{n}CH\overset{c}{=}CH.(CH_2)_4.CH\overset{c}{=}CH.CO.NH.Bu^{i}$	2·01	0
15.	" neo-Herculin " m.p. 64·5-66° (cf. Crombie, *loc. cit.*)	2·00	70
16.	*N-iso*butylauramide $Me.(CH_2)_{10}.CO.NH.Bu^{i}$ Compounds 11–16 are related to herculin as formulated by Jacobson	2·33	3

No.		Concentration (% w/v. in acetone)	% Kill
17.	*N-iso*butylpenta-*trans*-1-*trans*-3-diene-1-carboxyamide Me.CH$\overset{t}{=}$CH.CH$\overset{t}{=}$CH.CO.NH.Bu^i	2·31	0
18.	*N-iso*butylpenta-*cis*-1-*trans*-3-diene-1-carboxyamide Me.CH$\overset{t}{=}$CH.CH$\overset{c}{=}$CH.CO.NH.Bu.	2·38	0
19.	*N-iso*butylheptadeca-16-ene-8:10-diyne-1-carboxyamide CH_2=CH.$(CH_2)_4$.C≡C.C≡C.$(CH_2)_7$.CO.NH.Bu^i	1·61	0
20.	Capsisicin Extract (B.P.)	4·61	0

2. Allethrin is a mixture of the eight possible isomeric forms derived from (±)-*cis-trans*-chrysanthemic acid (4 isomers) and (±)-allethrolone (2 isomers). Crystals of the " α-dl-trans " isomer of allethrin separate from this mixture on standing (Schechter, LaForge, Zimmerli and Thomas, J. Amer. Chem. Soc., 1951, **73**, 3541). This crystalline isomer must consist of " one of the racemic ester pairs, *d-trans* acid with *d*-allethrolone plus 1-*trans* acid with *l*-allethrolone or *d-trans* acid with *l*-allethrolone plus *l-trans* with *d*-allethrolone ".

The ratio: $\dfrac{\text{toxicity of " } \alpha\text{-}dl\text{-}trans \text{ " isomer}}{\text{toxicity of } (\pm)\text{-allylrethronyl}(\pm)\text{-}trans\text{-chrysanthemate}}$

is $\frac{11}{95}$ to *Tenebrio molitor* adults, so that the remaining part, the non-crystalline " β-*dl-trans* isomer ", must be correspondingly more toxic than (±)-allylrethronyl(±)-*trans* chrysanthemate, which is a mixture of the " α-*dl-trans* " and " β-*dl-trans* " isomers.

It can be argued from this evidence that one optical isomer of allethrolone gives more toxic esters than its enantiomer. Furthermore this comparison is of interest because the " α-*dl-trans* " isomer has been suggested as an absolute standard of comparison for pyrethrin-like compounds.

3. Previous results (for summary, see Elliott, Pyrethrum Post, 1951, **2**, (3), 24) have indicated that the toxicity of esters of the type under consideration are independent of the optical form of the alcohol (which has an asymmetric carbon atom at position 4 in the ring). Dr. F. B. LaForge and co-workers have recently resolved cinerolone ((±)-2-*cis-n*-but-2′-enyl-3-methyl*cyclo*pent-2-en-4-ol-1-one) and through his kindness the esters of (+)-*trans*-chrysanthemic acid with the (+)-and (—)-forms of the above alcohol have been available to us. Against *Phaedon cochleariae* and *Tenebrio molitor* adults, the ester from the dextro rotatory form of the alcohol has proved to be 4 to 5 times as toxic as that from the corresponding laevo form of the alcohol. Mixtures of equal proportions of the esters from these two optical isomers of the keto-alcohols show the toxicity to be expected if these two forms act independently without synergism or mutual depression of activity.

4. The effect of altering the side chain of the alcohol in esters of chrysanthemic acid with various 2-alkyl and alkenyl-3-methyl-

*cyclo*pent-2-en-4-ol-1-ones has been examined. Most of the compounds used here were prepared by Dr. S. H. Harper and co-workers, to whom gratitude is expressed for the donation of samples. (c.f. *J. Chem. Soc.*, 1950, 971, 1152, 3552; 1951, 2445; 1952, 869). The results are summarised below.

The evidence indicates that the toxicity of the esters is greatest when there is a double bond in the 2′-position of the side chain of the alcohol and progressively decreases as this ethylenic linkage is separated by more methylene groups from the *cyclo*pentenone ring. The toxicity of esters from two independently prepared samples of cinerolone, which has the *cis*-but-2-enyl side chain, was appreciably greater than those from allethrolone. Saturation of the alcoholic, *cis*-but-2-enyl, side chain produced nearly the same decrease in toxicity as reduction of the ketone grouping in the *cyclo*pentenone ring. It may be significant that both these changes decrease the extent to which the α-methylene group in the side chain is activated. Again, when the double bond is in the 2′ position a methylene group is most highly activated and progressively less so when double bonds are in the 3′ and 4′ positions.

The overall conclusion is that the characteristic biological activity shown by pyrethrin-like esters is associated with the precise stereochemical configuration in which the acidic part of the molecule is held in relationship to the keto-alcoholic part of the molecule, and, in particular, the unsaturated side chain.

Organic chemistry

M. Elliott has continued his work on the synthesis of compounds related to the alcoholic and acid components of the pyrethrins.

Insecticides derived from plants

Pyrethrum. 1. The constituents of pyrethrum flowers.

The work on the chromatographic separation of the constituents of extract of pyrethrum flowers by K. A. Lord and J. Ward has been continued with a view to obtaining a complete separation of all the insecticidally active materials from one another and from the inactive constituents.

2. The insecticidal activity of strains of Pyrethrum.

At the request of the Kenya Pyrethrum Board a study has been made by E. M. Gillham and P. H. Needham of the insecticidal activity of some different strains of pyrethrum in relation to their pyrethrin content, as determined by chemical analysis. The work is part of a co-operative effort which is being organized by the Colonial Products Advisory Bureau.

The toxicity tests carried out in this department indicate that equal weights of total pyrethrins from different strains have not necessarily equal insecticidal activity. The results obtained depend on the test insect and technique of testing. The reason for the difference may lie in the differences in the ratio of " pyrethrin I " to " pyrethrin II ".

Heliopsis. Miss P. Smith has continued the cultivation of *Heliopsis scabra* and *Heliopsis parvifolia* for a second season. A proportion of both species did not survive the 1951–52 winter and the remaining plants made insufficient root growth for extraction of the toxic principle. The two species are therefore being cultivated for a third season.

The work on insecticidal amides from other plant species are outlined in the section the relationship between chemical constitution and insecticidal activity.

Biological

Bioassay techniques

Measured drop technique. The measured drop technique has been in use throughout the year for the assessment of insecticidal action and has again proved to be very satisfactory.

P. H. Needham and A. J. Arnold have incorporated some modifications of the micrometer syringe designed to improve accuracy and speed of operation. A " clicking " device has been fitted to the micrometer syringe which gives one click for each microlitre delivered. In this way the operator can devote his whole attention to the positioning of the drop on the insect, and not have to watch the micrometer graduations.

Injection technique. The micrometer syringe used in connection with the measured drop technique may also be used for the injection of insects.

Extracts of three strains of pyrethrum have been tested in this way for both relative toxicity and depreciation due to the storage of the flowers. The results obtained showed the technique to be satisfactory and compared favourably with tests carried out on the same materials by our own measured drop technique and by other workers using different methods.

The test insects for these injections were male and female adult *Tenebrio molitor*, and both odourless distillate, and n-hexane were used successfully as media. The volume injected was 0·001 ml.

Technique of estimation of contact poisons. C. Potter and E. M. Gillham have continued the work on the examination of the relative importance of direct contact effects and residual film effects in laboratory insecticidal assay.

The effect of stage of development on insect resistance

Miss H. Salkeld has completed her work on the changes in resistance of insect eggs to insectidies that occur during development. This work has now been accepted for publication.

Toxicity and permanence of insecticidal deposits on plants

P. Burt and J. Ward have continued their work on the behaviour of insecticides on plant surfaces. A detailed report of the work to date has been submitted to the Colonial Insecticides Committee and is being prepared for publication.

Experiments using deposits of crystalline D.D.T. on films of plant wax spread on glass plates have been continued. It was found that when the D.D.T. was dissolved in the wax, it was more readily destroyed by irradiation with short-wave ultra-violet light than D.D.T. crystals. By working with duplicate plates, irradiating one of them with U.V. and then analysing both for D.D.T., an estimate can be made of the proportion of D.D.T. dissolved in the wax. In this way, it was shown that D.D.T. crystals 50μ long and about 1μ in diameter did not dissolve to any appreciable extent in the wax layer on which they are lying, under conditions of free ventilation. This is true for plates stored for up to 14 days at either 18°C or 43°C.

The rate of loss of D.D.T. and of contact activity from crystalline deposits on leaves of plants kept in a greenhouse has been studied under summer and winter conditions, and compared with the rate of loss from glass plates kept alongside the plants. The difference in toxicity between fresh deposits on leaves and on plates, mentioned in the Report last year, seems to be due largely to the greater humidity of the environment of the test insects confined on leaves. For when insects were confined on deposits on glass plates at a higher humidity than usually, the kill was greater than that obtained under standard conditions. To obtain further confirmation, Cellophane was sprayed with a range of deposits of D.D.T. It was found, in a duplicated experiment, that the ratio of LD50's obtained from insects confined on moist and dry cellophane respectively was similar to the ratio on leaf and glass surfaces.

Leaves exposed in winter in the greenhouse (air temperature 10–20°C) lost about a third of their D.D.T. and of their contact activity after thirty-seven days, only a small part of this loss being due to leaf growth. In the Summer experiment, the plants were exposed to as much sunlight as they would stand, and air temperatures of 35°C were sometimes reached. Under these conditions, losses of D.D.T. amounted to about half in a fortnight. The losses during a given period appeared to be correlated with the daily maximum air temperature. In both the winter and the summer experiments, but particularly the latter, losses from the glass plates were greater than from the similarly treated leaves. The plates were found to reach a higher temperature than the leaves in sunlight, and this presumably caused the more rapid losses. It is concluded that the most important causes of loss of toxicity of deposits of crystalline D.D.T. on plants in a greenhouse are growth of the plant and volatilization of the D.D.T., and further that when a comparable formulation (e.g. a wettable powder) is used, volatilization is unlikely to be of practical importance as a source of loss of toxicity in a temperate climate, but may well be important in the tropics. Adult *Tribolium castaneum* has been used as the biological indicator of insecticidal activity throughout these investigations. A modified Schecter-Haller method was used for the chemical analysis.

The mechanism of selection of strains of insect resistant to insecticides

Dr. Tattersfield has completed the first stage of his work and two papers have been accepted for publication.

It was shown that the effect of repeated sprayings with D.D.T. upon the adults of a wild colony of *Drosophila melanogaster* found at Rothamsted gave rise to an increase in resistance. This was characterized by marked fluctuations, and the rate of increase depended upon the concentration of the insecticide used, the higher the concentration and percentage initial kill, up to a limit, the quicker resistance developed, but it was apparent that if continued for a sufficient length of time the same end-point was reached finally if the concentrations used gave rise to any marked degree of selection. In the experiments there was no sign of any degree of adaptation, but the proportion of resistant insects in the initial population seemed of some importance. The process was a slow one.

During the course of this work the test insect developed a susceptibility to carbon dioxide and much time was spent examining the effect of this characteristic upon susceptibility to D.D.T. It was found that the insects whether susceptible or selected for their resistance to CO_2, gave the same probit-log. concentration regression line for D.D.T. if nitrogen was sued for anaesthesis prior to spraying. If tested at the temperature 25°C at which the CO_2-susceptibility disappeared, the same regression line resulted whether carbon dioxide or nitrogen were used for anaesthesis. Nitrogen has no toxic effect. Also if tested at a temperature of 13–15°C, at which the CO_2 killed 50 per cent of the test subjects against 2 per cent for nitrogen, and if allowance was made for deaths in the controls, the regression lines whether CO_2 or N is used for anaesthesis were identical. The conclusion was drawn that the effect of CO_2 is merely to limit the population from which selection for resistance to D.D.T. is made.

Work has continued and some data on the effect of D.D.T. on rate of reproduction have been accumulated; the evidence procured suggests a decline in the reproductive rate. An examination of the effect of the age of parents on susceptibility of the offspring was not conclusive.

A good deal of work has been carried out on the susceptibility to CO_2 shown by our strain of *D. melanogaster* and how it has arisen. The subject is most elusive as no physiological reason for its occurrence has yet been discovered. Such gases as nitrogen, ethylene acetylene, nitrous oxide are without effect, beyond a temporary anaesthesis. In contrast to the selection for D.D.T.-resistance, the resistance to CO_2 can be rapidly achieved. More detailed work is being carried out in collaboration with Dr. Kalmus of University College, London. Professor L'Heritier of France has kindly let us have samples of his CO_2-sensitive strain.

The work carried out by Mr. Kerr when at Rothamsted and described in the previous report has been written up and three short papers submitted for publication.

Insect rearing

The stock of plant feeding and stored product species have been maintained during 1952 and *Blatta orientalis* (Oriental cockroach), *Blatella germanica* (German cockroach) and *Oncopeltus fasciatus* (Milkweed bug) added to the list of insect cultures available for experimental work.

O. fasciatus are being reared on crushed Black Nigerian sunflower seed; blanched Nigerian peanuts were found not to be satisfactory for this purpose.

Mrs. J. Kenton has continued to work on aphid rearing and as a consequence the aphid stocks have improved during the course of the year. Mrs. Kenten has carried out detailed experiments to test the effect of different photoperiods and temperatures on the production of the various forms of the aphid *Acrythosiphon pisum* (Harris).

Toxicity of plant-protective chemicals to bees

Mr. G. D. Glynne-Jones and Miss Connell have continued the work on the toxicity of plant-protective chemicals to honey bees at Seale-Hayne in collaboration with the department. In addition to the derived toxicity studies, a special investigation has been made into the possibility of systemic insecticides contaminating nectar and hence appearing in honey. Using Schradan labelled with 32p, this was shown to occur following the application of the insecticide as a foliage spray. Considerable evidence was obtained to support the view that contaminated nectar may result in Schradan appearing in honey in an unchanged form.

Field Experiments

Control of wireworms

In the experiment started in the autumn of 1951 in collaboration with Dr. Raw of the Entomology Department, only minor damage occurred on any of the plots so treatment differences were small. Since 1952 was the first year after ploughing in grass damage may occur in 1953. The results so far confirm the results of the previous experiment. Aldrin 200 lbs. per acre 1·78 per cent dust gave the best crop followed by Chlordane 100 lbs. per acre 5 per cent dust and BHC 56 lbs. per acre 3·5 per cent dust combine-drilled all of which were significantly better than the control. BHC seed dressing and D.D.T. 150 lbs. per acre 5 per cent dust did not improve the crop significantly above the controls.

Control of bean aphis (A. fabae *Scop.*) *on field beans*

M. J. Way, P. Smith and C. Potter continued to work on this subject.

A field experiment was carried out as described in the 1951 report but using Parathion, Systox, Nicotine, Pyrethrum with piperonyl butoxide, and D.D.T. in two formulations. These were applied once—on the 12th June soon after the beans became infested with aphid migrants from the winter host; the mean aphid population at this time being 2·2 per stem.

The yield figures confirmed the result of the previous experiments that a large increase could be obtained by means of a single application of insecticides.

Aphid counts at weekly intervals showed that the best insecticides, Systox (0·05 per cent active ingredient), Parathion (0·02 per cent w/v.), and Nicotine (0·1 per cent alkaloid), by effectively destroying the primary migrants and their first progeny, prevented build-up of aphids in July. Pyrethrum (0·02 per cent w/v. total pyrethrins) + piperonyl butoxide (0·2 per cent w/v.), and D.D.T. (0·1 per cent w/v.) emulsion though not so effective destroyed most of the primary migrants. In the untreated control plots and in those treated with a 0·1 per cent D.D.T. crystalline suspension

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the mean aphid population rose in July to peaks of 1,660 and 2,455 per stem respectively and caused serious damage. Clearly if D.D.T. is required as an aphicide its formulation is important.

The growth of the beans in untreated control plots and in the Parathion treated plots was compared; it was found that the heavy aphid attack had little effect on flower production or flower set. However it prevented many pods from developing to maturity, and reduced the size of seed as well as the yield. Observations were made in the field, and trials carried out in the laboratory on the effect of the different insecticides on Coccinellid predators of the aphids. All except nicotine were toxic as contact insecticides; *Coccinella septempunctata* was less susceptible than *Adelia bipunctata*.

Control of virus vectors

Potatoes. In collaboration with L. Broadbent of the Plant Pathology Department, the department assisted in experiments on the control of potato virus by controlling the insect vectors. One experiment was carried out on the farm of Mr. Soper of Harlow, Essex and another at the farm of Messrs. Tinney and Hitchcock, Clavering, Essex. In the latter experiment the collaboration was extended to Dr. Gough of the National Agricultural Advisory Service and Pest Control Limited. The results of these experiments will not be available until next year.

Brassicas. Two small-scale experiments on the prevention of introduction of Cauliflower mosaic and cabbage black ringspot virus by controlling the aphid vectors were carried out by G. D. Heathcote of the Plant Pathology Department and J. Ward of this department. Systox (0·1 per cent w/v. active ingredient), Pestox iii (0·1 per cent w/v. commercial), Parathion (0·5 per cent w/v. technical), Toxaphene (0·125 per cent w/v. technical), Pyrethrins + piperonyl butoxide (0·01 per cent w/v. total pyrethrins + 0·03 per cent w/v. piperonyl butoxide) and pyrolan (0·1 w/v.) were used, and in the second experiment D.D.T. (0·2 per cent w/v. technical), Pyrolan (0·1 per cent w/v.), Systox (0·1 per cent w/v. active ingredient) and the pyrethrins + piperonyl butoxide (0·03 per cent w/v. total pyrethrins + 0·03 per cent w/v. piperonyl butoxide) were used.

The materials were sprayed in aqueous media at intervals of 10 days starting as soon as possible after the emergence of the seedlings.

In the first experiment the maximum recorded total infection was 3·3 per cent; in the second experiment the maximum recorded was 56·7 per cent. None of the insecticides produced any significant reduction in infection. Pyrolan gave the greatest difference in both experiments with an infection rate of 1·2 per cent in the first experiment and 43·8 per cent in the second experiment.

G. D. Heathcote of the Plant Pathology Department and J. Ward of this department are carrying out an investigation in the greenhouse of the behaviour of aphids towards plants treated with a variety of insecticides to provide data on which to select insecticides for virus control.

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