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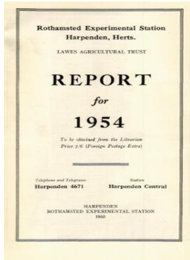
ROTHAMSTED RESEARCH

Report for 1954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REPORT
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1954

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

During the current year F. Last was transferred from the Plant Pathology Department to work on fungicides, R. van Baer from Australia joined the department as a temporary worker, and M. Jeremić and R. Vujadinović from Yugoslavia arrived for a stay of 6 months. Other people who have stayed for short periods are R. J. A. W. Lever, from Malaya, and M. H. Hassanein, from Egypt. C. Potter attended the International Union for the Protection of Nature in Copenhagen at the request of the Society for the Promotionof Nature Reserves. He presented a paper on " The action of insecticides on insect populations in England ".

PHYSICAL CHEMISTRY

A. H. McIntosh and Margaret Macfarlane have continued their work on particle size and toxicity of suspensions of contact insecticides.

In recent years they have carried out tests to show the effect of physical properties on the difference in toxicity between two forms of a single poison—colloid and crystals. This has involved, with each of nine DDT analogues, measurements of: (*a*) retention of insecticide by adult *Oryzaephilus surinamensis* L. from each type of suspension applied; (*b*) rate of crystallization of deposits from colloidal suspension; (*c*) solubility of insecticide in oil; (*d*) rate of solution of crystals in oil. This work has been extended and finished, and is now being published.

If the toxicities of two forms of the same insecticide are compared on one insect species at different after-treatment temperatures, the relative toxicity of the two forms often depends on temperature. We think that it is possible to foretell whether the relative toxicity will increase or decrease when the after-treatment temperature is, for example, lowered. We are now carrying out experiments to test this point, using as many insect species and as many types of insecticide as possible. This work is not complete.

BIOCHEMISTRY

Investigations on the mode of action of organophosphorus insecticides, begun in earlier years by K. A. Lord and C. Potter, have been continued and extended during the past year.

Work reported in previous years on the comparison of a group of phosphorus insecticides and their potency as inhibitors of enzymes hydrolysing acetyl choline and phenyl acetate has now been published. One of the conclusions drawn from the inhibition data was that both types of enzyme activity derived from *Tenebrio molitor* L., *Blattella germanica* L., *Tribolium castaneum* Hbst. and *Dysdercus fasciatus* Sign. differed from species to species. By using paper electrophoresis this has now been confirmed for the soluble enzymes

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which hydrolyse phenyl acetate. Paper electrophoresis also indicated that the enzyme preparations sometimes contained more than one enzyme which hydrolysed phenyl acetate, under the conditions used for the electrophoresis experiments.

Three substrates were investigated for use in locating the enzymes on paper: (*a*) fluorescein acetate, a colourless, non-fluorescent material which on hydrolysis gives rise to fluorescein which is strongly fluorescent; (*b*) indoxyl acetate, which when hydrolysed liberates indoxyl, which in the presence of air gives rise to indigo blue, a deep-blue dye; (*c*) phenyl acetate. The phenol liberated on hydrolysis was detected by coupling, in alkaline conditions, with diazotized *p*-nitro-aniline. The chromogenic substrates were sprayed on to the wet papers in solution in benzene. Other solvents, either more or less volatile or water soluble, did not give such consistently good results. Fluorescein acetate and indoxyl acetate are simpler to use than phenyl acetate, since, in each case, the product of hydrolysis becomes visible without additional treatment. However, since the substrate specificity of the enzymes is not known, it was considered worth while to compare the results obtained using these two substrates with those using phenyl acetate.

Insect enzymes which hydrolyse acetyl choline have proved more difficult to study by electrophoretic methods than the more soluble enzymes which hydrolyse acetyl choline. Some progress has, however, been made; the enzyme from *B. germanica* has been largely brought into solution by the use of sodium taurocholate, and some purification of the enzyme has been effected by dialysis and ammonium sulphate fractionation.

Preliminary tests have been carried out on 3-(diethoxyphosphinyloxy)-*N*-methyl-quinolinium methyl sulphate (Ro 3-0422), which was kindly supplied by Roche Products. This substance is very highly toxic to mammals. An LD50 of 20 μg./kg. by intravenous injection has been reported for mice. It is a very potent inhibitor of mammalian cholinesterases. We have shown that Ro 3-0422 is also a potent inhibitor of the insect esterases hydrolysing both acetyl choline and phenyl acetate. The substance is, however, not a very potent insecticide, and failed to give a significant kill when injected at the rate of 40 mg./kg., into *B. germanica* or *T. molitor*. *B. germanica* were, however, killed at twice this dosage rate. The LD50's of TEPP for *B. germanica* and *T. molitor* by topical application are 4 mg./kg. and 6 mg./kg. respectively. No definite conclusions can be drawn from these findings. Lack of toxicity to insects may be due to the way in which the substance distributes within the insect or to rapid detoxification, but the results are interesting, since they show clearly that anti-esterase activity is not necessarily a guide to insecticidal activity.

The effect of TEPP on the development of *D. oleracea* eggs has been further examined. It was found that aqueous solutions of TEPP applied to newly laid eggs killed but allowed embryonic development to occur. When eggs were placed on films of TEPP or HETP (containing less than 20 per cent TEPP) embryonic development did not occur, providing the eggs were left on the film for a minimum time (20 hours) before the TEPP was removed by washing with water containing a wetter. The shorter the exposure time, the less was the effect of the TEPP.

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Examination of the esterases of both untreated and treated eggs showed that in no case was the hydrolysis of phenyl acetate completely inhibited although extracts obtained by grinding (without addition of water) mixtures of treated and untreated eggs in all cases suggested that some residues of TEPP occurred in the eggs. The residues were sufficient to give a partial inhibition of the esterases from the untreated eggs. The amount of inhibition apparent seemed to decrease with the age of the eggs. Enzymes hydrolysing acetyl choline appeared in batches of eggs in which embryonic development was not apparent except in a small proportion of eggs. It is possible that the activity may have occurred only in those eggs showing some development.

There was no apparent inhibition of the enzymes hydrolysing acetyl choline in normal eggs ground (without addition of water) together with eggs in which embryonic development had been inhibited by TEPP. In the course of these studies the high level of hydrolysis of phenyl acetate by extracts of *D. oleracea* eggs at all stages of development and the increase of hydrolysis of acetyl choline as the egg develops (previously reported) has been fully confirmed.

From this work it appears that TEPP only penetrates the chorion of *D. oleracea* fairly slowly, and other species which might have more permeable shells are being examined.

The development of enzymes hydrolysing acetyl choline and phenyl acetate in insect eggs has now been examined in two further species. As in the case of *D. oleracea*, the hydrolysis of acetyl choline becomes evident in the eggs of *Pieris brassicae* only a considerable time after the eggs are laid, and then increases until the eggs hatch. The hydrolysis of phenyl acetate is evident immediately the eggs are laid, and remains at a high level throughout the development of the egg. An attempt has been made to correlate embryonic development with the hydrolysis of acetyl choline by extracts of eggs of *P. brassicae*. An apparently anomalous result has been obtained with *Dysdercus fasciatus*. So far it has not been possible to demonstrate the hydrolysis of acetyl choline by extracts of eggs of *D. fasciatus* at any stage of development, although extracts of the adults and of all stages of the nymphs hydrolyse acetyl choline. Phenyl acetate is rapidly hydrolysed by extracts of eggs of these species at all stages of development.

Work on the action of organophosphorus insecticides inside a plant mentioned in last year's Report has been prepared for publication. The evidence obtained suggests that some organophosphorus compounds can have an effect on plant enzymes *in vivo*. Further work is necessary to elucidate this problem fully.

Organic Chemistry

The relationship between insecticidal activity and chemical constitution in compounds related to the pyrethrins

The compounds being studied by M. Elliott are related to the naturally occurring pyrethrin I

$$(\text{I},\ R = cis\text{-}CH_2{\cdot}CH{=}CH{\cdot}CH{=}CH_2)$$

and cinerin I (I, $R = cis\text{-}CH_2{\cdot}CH{=}CH{\cdot}CH_3$). Work has been

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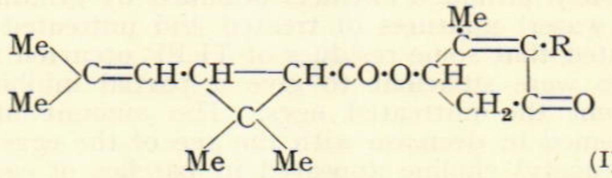
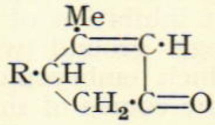
carried out recently to try to throw light on the part played by the side chain R in (I) on the toxicity of the compounds. In



(I)

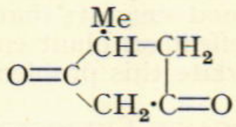
particular, the ester (I, R = H) has been prepared from (II, R = H) and the usefulness of the latter compound as an intermediate in the



(II)

preparation of insecticidally active esters has been examined. The work carried out with (II, R = H) is summarized below.

3-Methyl-*cyclo*pent-2-enone (II, R = H), now available by the cyclization of acetonyl acetone (Acheson & Robinson, *J. chem. Soc.*, 1952, p. 1127), had been obtained, prior to this publication, by the cyclization of ethyl acetonyl acetoacetate by Elliott in this laboratory. Reaction with *N*-bromosuccinimide gave 4-bromo-3-methyl-*cyclo*pent-2-enone (II, R = Br), which with silver acetate produced the acetoxy compound (II, R = O·Ac). Since Conia (*Bull. Soc. chim. France*, 1954, 690) showed that *iso*phorone was alkylated by allyl chloride in the presence of sodium *t*-amylate to give 2-allyl-3 : 6 : 6-trimethyl-*cyclo*hex-2-enone, the possibility of obtaining an analogous sodium salt from (II, R = O·Ac) was investigated. However, this reaction did not occur, since with sodium, sodium hydride and alcoholic sodium ethoxide cleavage of the ester grouping occurred, both in (II, R = O·Ac) and with (II, R = (±)-*cis-trans*-chrysanthemyl—see below). From the latter compound, only (±)-*cis-trans*-chrysanthemic acid could be isolated. In 5 per cent aqueous sodium hydroxide (II, R = O·Ac) showed $\lambda_{max.}$ 2230 Å., $\epsilon_{max.}$ 10,950; after heating to 100° for 30 minutes, $\lambda_{max.}$ was at 2590 Å., $\epsilon_{max.}$ 15,500. This spectral evidence indicated that, after saponification, the αβ-unsaturated γ-ketol system in (II, R = OH) had been isomerized to one or both of the enolic forms of the 1 : 3-diketo-*cyclo*pentane (III). (It is probable that an



(III)

analogous isomerization of naturally derived pyrethrolone and cinerolone takes place when the so-called " *iso*-pyretholone enols " are formed, of which the structure has not been determined.) Such re-arrangements would preclude the alkylation in the 2-position of esters such as (II, R = O·Ac) under alkaline conditions.

It was necessary to prove that 3-methyl-*cyclo*pent-2-enone was brominated in the 4-position in reaction with *N*-bromosuccinimide, so that the structure of the compound obtained by reaction of the

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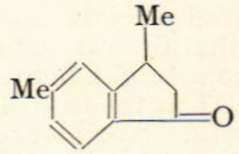
bromide with silver (±)-*cis-trans*-chrysanthemate would be known to be (I, R = H). Other possible sites for attack were the 5-position and the methyl group. The former was unlikely from previous experience of the reactivity of the *cyclo*pent-2-enone ring with *N*-bromosuccinimide. If bromine had replaced a hydrogen atom of the 3-methyl group, hydrogen bromide could not be easily evolved under mild conditions. It was found that the brominated product evolved hydrogen bromide at room temperature on treating with trimethylamine in carbon tetrachloride. The product was expected to be 3-methyl*cyclo*penta-2 : 4-dienone. The latter compound had been described by Pavolini, Gambarin and Verza (*Ann. Chim., Roma* (1952), **42**, 149) as an easily polymerizable yellow liquid b. p. 218–220°, n_D 1·501.

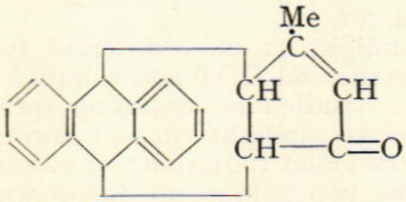
Assuming that the initial stages of the synthesis proceeded as described by Pavolini *et al.*, it seemed probable that the product was 3 : 5-dimethylindanone (IV) (or the isomeric 3 : 6-dimethyl com-



(IV)

pound) rather than 3-methyl*cyclo*penta-2 : 4-dienone. The product obtained by Pavolini *et al.* was characterized only by its refractive index, density, boiling point and carbon–hydrogen analysis. An oxime m.p. 143–145° was prepared, but no analytical figures were quoted for it.

The synthesis was therefore repeated and the Italian findings confirmed up to the last stage. However, when (V) was heated



(V)

above its m. p., carbon monoxide was detected in the evolved gases, and the product and its 2 : 4-dinitrophenylhydrazone had analyses and ultra-violet absorption spectra in agreement with those of a derivative of 3 : 5-(or 3 : 6-)dimethylindanone. It was concluded therefore that the product prepared here under the conditions used by the Italian workers was not 3-methyl*cyclo*penta-2 : 4-dienone but a dimethylindanone.

The dimethylindanone obtained by the anthracene route and its 2 : 4-dinitrophenylhydrazone were not identical with authentic 3 : 6-dimethylindanone, which was obtained by pyrolysis of the product from dehydrobromination of (II, R = Br). The infra-red spectra indicated only minor differences between the two ketones, however, and further work to determine the nature of the difference is in progress.

It was concluded that, first, the ease of elimination of hydrogen

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bromide from (II, R = Br) gave the required proof of its structure and thence of (I, R = H), and, secondly, the claims of Pavolini *et al.* to have prepared 3-methyl-*cyclo*penta-2 : 4-dienone could not be substantiated.

The compound (I, R = H) had negligible insecticidal activity, in contrast to (I, R = allyl), which is highly toxic. It was prepared and tested with a series of other esters derived from (I) that had varying side chains and on which the insecticidal results will be summarized in next year's Report.

Toxicity and Persistence of Insecticidal Deposits

This work has been continued by J. Ward and Eileen M. Gillham. The previous work has been reported in two papers which have been submitted to the *Bull. ent. Res.* for publication.

The rate of evaporation of various insecticidal deposits was studied by exposing sprayed petri dishes in a constant-temperature room (29° C.) in which the air was kept moving by a fan. The deposits were shielded from bright light. Under these conditions, a crystalline deposit of DDT at the rate of 4 μg./sq. cm. evaporated almost completely in 2 weeks. Arochlor resin, which has been shown to decrease the rate of evaporation of BHC, does not prolong the life of DDT residues under the conditions of this test.

The persistence of the bromine analogue of DDT, 1 : 1-*bis*-(*p*-bromophenyl)-2 : 2 : 2-trichloroethane, was compared with that of DDT in the constant-temperature room and was found to be much greater, a deposit of 1 μg./sq. cm. decreasing by only 25 per cent in 19 days, while a similar deposit of DDT decreased by 96 per cent. Previous reports of the toxicity of the bromine compound are conflicting, so it is being tested in comparison with DDT against several species of insect.

The effect of sunlight on three different types of DDT deposit (pure *pp'*-DDT, commercial DDT and a liquid deposit of DDT and Arochlor resin) was studied by exposing sprayed petri dishes to direct sunlight and to sunlight filtered through window-glass to block out short ultra-violet radiation; the temperature and degree of ventilation of the two batches of dishes were the same. After 3 days' exposure, totalling about 24 hours of sunshine, the *pp'*-DDT had diminished by about a quarter of the amount originally present, while the deposits of crude DDT and of DDT with Arochlor had each lost about half. Losses from the dishes exposed to direct sun and to sun screened through glass were about the same. Weather conditions made it impossible to study the effect of longer exposure periods.

The contact toxicity to *T. castaneum* of liquid deposits of DDT and Arochlor resin has been measured. It has been found that under certain circumstances the liquid deposits can be several times more effective than the crystalline, when equal weights of DDT per sq. cm. are compared. When the DDT is applied as a measured drop, the addition of Arochlor resin causes a slight reduction in the toxicity, so the enhanced contact effect is presumably due to increased pick-up by the insects, and not to improved penetration of the cuticle or to a synergistic action of the Arochlor resin.

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Bio-assay

Insecticidal activity of N-iso*butylcarboxyamides*

M. Elliott and P. H. Needham have carried out tests of some further compounds of this type, continuing the work mentioned in the Report of Rothamsted Experimental Station for 1952 to assist Dr. L. Crombie (Organic Chemistry Department, Imperial College of Science and Technology). The bio-assay tests were performed on *Tenebrio molitor* L. by a measured-drop technique previously described.

The following results were obtained :

Compound	Structure	% kill at 3% w/v in acetone
Anacyclin [1]	$CH_3(CH_2)_2C{\equiv}C{\cdot}C{\equiv}C(CH_2)_2CH\overset{t}{=}CH{\cdot}CH\overset{t}{=}CH{\cdot}CO{\cdot}NH{\cdot}CH_2CH(CH_3)_2$	6
Tetrahydro-anacyclin [1]	$CH_3(CH_2)_2CH\overset{c}{=}CH{\cdot}CH\overset{c}{=}CH(CH_2)_2CH\overset{t}{=}CH{\cdot}CH\overset{t}{=}CH{\cdot}CO{\cdot}NH{\cdot}CH_2CH(CH_3)_2$	100
Pellitorine [1, 2, 3]	Distilled sample isolated from *Anacyclus pyrethrum* D.C. (Pellitory).	100
N-*iso*butyl dodeca-*trans*-2 : *trans*-4-dienamide	$CH_3(CH_3)_6{\cdot}CH\overset{t}{=}CH{\cdot}CH\overset{t}{=}CH{\cdot}CO{\cdot}NH{\cdot}CH_2CH(CH_3)_2$	6

[1] Crombie, *Nature, Lond.*, 1954, **174**, 832.
[2] *idem, J. chem. Soc.*, 1952, 4338.
[3] *idem, J. chem. Soc.* (in the press).

A group of amides derived from deca-2 : 4-dienoic acid was available from Dr. Crombie, and an insect susceptible enough to enable their insecticidal activity to be compared was sought. This would enable information about the effect of the amine portion of the molecules on activity to be obtained. However, *N*-*iso*butyl-dodeca-2 : 4-dienoic acid gave such very low kills when applied topically to *Megoura viciae* and *Phaedon cochleariae* that further tests were abandoned.

Insecticidal activity of organo-phosphorus compounds

The series of experiments started last year has been continued by P. H. Needham. In addition, he has carried out some experiments in which the systemic action of " Systox " and " iso-Systox " has been tested in several ways against the aphid *Megoura viciae*. An attempt has been made to assess separately the contact and fumigant action, as well as the toxicity to feeding aphids, of leaves from broad-bean plants which have taken up through their roots 20 ml. of solution of the insecticides mentioned. A concentration of either " Systox " or " iso-Systox " required to produce just 100 per cent mortality of feeding aphids was found to exhibit no fumigant action through the leaves. The surface of these leaves did not kill by contact action aphids with severed mouth parts.

Bio-assay techniques

Methods of bio-assay of residual films were examined by Eileen M. Gillham and J. Ward in connection with the work on the persistance of insecticidal deposits. An attempt was made to use the Mustard Beetle, *Phaedon cochleariae* Fab., as an alternative test insect to *Tribolium castaneum* Hbst. It was not found possible to obtain reproducible results with the Mustard Beetle, so work with

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it was discontinued. A method of test using *Drosophila melanogaster* Meig. has been evolved, but so far it has not been used with DDT deposits.

A. H. McIntosh has observed that when adult *Tenebrio molitor* L. are sealed up in a small volume of air without any insecticide, they become paralysed in about 24 hours. The exact time depends on the degree of crowding; it decreases as the crowding increases. If the paralysed insects are now allowed a plentiful supply of air, they recover in an hour or so. If they are sealed up again they do not become paralysed again, however long they are kept. It is assumed that the paralysis is due to poisoning by carbon dioxide, and that the insects become "conditioned" to it (cf. Report of Rothamsted Experimental Station for 1953, p. 109, and below).

This type of paralysis may upset the results of laboratory tests of insecticides as fumigants, and may also interfere with other tests if insufficient aeration is allowed.

Pyrethrum

Synergism

The work on the study of synergism of the pyrethrins has been continued by Daphne Holbrook, K. A. Lord and C. Potter. *T. molitor* has so far proved to be the most successful test insect, and the results obtained with three synergists (piperonyl butoxide, Sulfoxide and *Melaleuca bracteata* oil) have been confirmed and subjected to probit analysis. This analysis shows that piperonyl butoxide and Sulfoxide have similar synergistic properties. The probit lines for these two compounds were roughly parallel at the various concentrations. Whereas with *Melaleuca bracteata* oil the probit lines converged at the LD75–LD100 levels.

It is intended to repeat this work with another test insect—the housefly, *Musca domestica*, when these insects have been reared in sufficient numbers.

The constituents of pyrethrum flowers

An automatic fraction collector for the chromatographic work on the separation of the constituents of extract of pyrethrum flowers was built by A. J. Arnold and brought into use by J. Ward in July. The degree of separation obtained under various conditions was studied, using purified and decolorized extracts. Attempts to use finely-ground alumina as a packing for the chromatographic column instead of the standard 100–200-mesh material were not successful, owing to the difficulty of obtaining uniform packing of the columns. The very low rates of percolation made possible by the use of the automatic fraction collector resulted in an improved separation. Work has begun on the examination of the constituents, both insecticidal and inert, of crude extracts of pyrethrum flowers.

Insect Resistance to Insecticides

F. Tattersfield and Jill R. Kerridge have continued their work on this subject. *Drosophila melanogaster* has been used, so far, as test subject, and it has been shown that by a process of selection resistant strains can be obtained, but some attention has necessarily been given to factors other than selection.

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The problem of conditioning

The strain of *Drosophila* which was used for selection work developed a susceptibility to carbon dioxide, from which highly resistant strains could be readily obtained by selection. In addition, however, this strain showed the property of becoming more resistant if treated with sublethal doses of carbon dioxide given in two ways: (*a*) by administering pure carbon dioxide for different periods of time, short enough so that, on the average, little or no lethal effect was produced, and on recovery subjecting them to more prolonged periods, comparisons with fresh untreated insects being made; (*b*) by administering non-lethal dilutions of carbon dioxide in air, but increasing concentrations stepwise, and later submitting to pure carbon dioxide for various periods of time, and the lethal effect being compared with fresh untreated insects.

(*a*) It was found that for a short period after the administration of pure carbon dioxide for a few seconds, very susceptible flies became highly resistant for a short period of time. With less susceptible flies the resistance persisted for a longer period. Thus, in one experiment 24 hours after conditioning all the survivors of the treatment for 7·5, 15, 30 seconds were immune to a treatment of 600 seconds, and counting all those dying during conditioning, 75 per cent survived against a survival of 44 per cent with fresh insects. This result was significant.

(*b*) Conditioning with increasing concentrations of carbon dioxide in air for different exposure times yielded similar results. The data were analysed by ourselves, and by M. J. Healy by a different technique, and similar conclusions drawn:

(i) The average proportion surviving in the "conditioned" insects is significantly greater than that with "fresh" insects.
(ii) There is a slight tendency for the proportion surviving to increase with increasing time of exposure during "conditioning".
(iii) There is a definite tendency for the proportion of survivors to increase with increasing time of exposure to 100 per cent carbon dioxide. (This is not easy to understand, unless it is explained by the possibility that, when passing the carbon dioxide over insects, it takes time to replace the air, during which the insect is being "conditioned" and its survival prolonged.)

Conditioning experiments with DDT and BHC

Some recent experiments suggest that a resistant strain of *Musca domestica*, having lost some of its resistance, might have it restored by conditioning. The testing of such a hypothesis would require appropriate insects. It was considered advisable to carry out a series of trials to ascertain whether a normal culture of insects (*Drosophila melanogaster*) could be conditioned, either positively (i.e., towards greater resistance) or negatively (towards greater sensitivity), and whether one or other of these effects was handed on to their progeny.

Reliance in the main had to be put on the females, as the males are more sensitive, and when segregated may die out during the tests.

In two series in which the conditioning spraying ranged from 0·0025 to 0·01 per cent in three and four stages, there was a slight

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decline in resistance after conditioning as judged by the value of *m* in the log median lethal concentration, but there was a decline in slope of the regression line, indicating a more pronounced effect on the less resistant insects, so that, judged at a kill of about 90 per cent, the effect is almost nil.

There is, however, very considerable difficulty in allowing for deaths during conditioning, when conditioning is started at a low and succeeded by higher concentrations. On reversing the process, and starting conditioning at the highest concentration 0·01 per cent DDT followed by 0·005 per cent and 0·0025 per cent, the log median lethal concentrations run in declining order, untreated insects > insects treated once > insects treated three times, showing a decline again in resistance with conditioning, *but* there is an *increase* in the slope of the regression line with conditioning, indicating that the more resistant portion of the population is being relatively more affected than the less resistant portion.

Tests with the medium only showed that there was no significant difference between the value of the median lethal doses between fresh insects and those sprayed once and three times, but that one spraying increased the slope of the regression line, and three slightly lowered it.

A number of experiments were carried out to ascertain the effect upon the progeny of the conditioned insects. With the females, after five conditioning sprays with concentrations of DDT that showed little if any selective power, the resistance declined significantly, but where the concentrations had been high enough to show selection (over 90 per cent kill) resistance increased significantly. With the males subjected to five conditioning sprays, the final tests upon the male progeny showed a slight but not significant rise in resistance; but where the treatments had given rise to a kill, likely to be selective (about 90 per cent), the resistance of the male progeny had increased significantly.

Our experimental results did not show, when judged at the median lethal dose, that the resistance of adult *Drosophila melanogaster* could be increased either in parents or progeny by conditioning with sub-lethal doses of DDT.

Experiments carried out with benzene hexachloride were less exhaustive, but the results obtained indicated that this conclusion was true of this insecticide also.

Work has been started to test the effect of different food media upon susceptibility to DDT and BHC, and interesting preliminary results have been obtained of the bearing this factor may have upon the reproduction, both in numbers and size of the progeny, as well as upon resistance to insecticides.

It is now proposed to repeat the work with greater care, since we have found that the adults carry yeasts on their bodies, and this food has considerable bearing upon the results obtained. With the help of F. Last, considerable headway has been made on a suitable technique for obtaining yeast-sterile adult *Drosophila*.

Insect Rearing

Thirty-one species of insect have been reared during the year, fifteen plant feeders and fourteen stored-product insects, together with *Drosophila melanogaster* Meig. and *Musca domestica* L.

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Of these, five species have been discontinued and six are new to last year's list. The new species are the bird-cherry aphid *Rhopalosiphum padi* L., the cabbage root fly *Hylemyia brassicae* Bouché, the large cabbage white butterfly *Pieris brassicae* L., the American cockroach *Periplaneta americana* L., the house cricket *Gryllus domesticus* L. and the housefly *Musca domestica* L.

Joyce Kenten has written up her work on the factors influencing the production of the various forms of the aphid *Acyrthosiphon pisum* Harris, and it has been accepted for publication.

Most of the experimental work on rearing techniques, including diapause, has been done on the wheat bulb fly *Leptohylemyia coarctata* Fall. and is described in the next section, together with the other work on this species.

Joyce Kenten has done a considerable amount of work on the embryological development of *Pieris brassicae* in connection with studies of the action of organo-phosphorus compounds on insect eggs.

Control and Laboratory Rearing of *Leptohylemyia coarctata* Fall., the Wheat Bulb Fly

Work on the wheat bulb fly (*Leptohylemyia coarctata* Fall.) included laboratory, box and field experiments on insecticidal control, and studies on laboratory rearing of the insect. M. J. Way, R. Bardner and Joyce Kenten have collaborated on this work.

Studies on the action of insecticides

(a) *Laboratory experiments.* 2nd and 3rd instar larvae were collected in the field and a dipping technique was used to treat them with different insecticide emulsions. The larvae were kept at 20° C. after treatment and mortalities recorded after three days. The order of toxicity was dieldrin = endrin < parathion < aldrin < γ-BHC < DDT < chlordane.

(b) *Seed-box experiments.* Wheat was grown outdoors in seed-boxes containing known numbers of wheat bulb fly eggs and various formulations of insecticides. Dusts "drilled" with the wheat seed included 2 per cent γ-BHC at 3 g. dust per foot of drill, and 2 per cent dieldrin and 2 per cent demeton ("Systox") at 4 g./foot. All gave good control of attacking larvae, especially γ-BHC and demeton. Seed dressings (containing 70 per cent active ingredient) of γ-BHC at 0·4 g. dressing per 100 g. wheat, and dieldrin at 1 g. dressing per 100 g. wheat were less effective. An interesting effect was observed with the dieldrin treatments, in which the larvae attacked and damaged the first tillers but died before damaging further ones.

This season, more insecticides, including endrin, isodrin, aldrin, DDT and parathion, are being tested as seed dressings and as dusts drilled with the seeds. A method which uses a methyl cellulose sticker for coating the seed with large quantities of insecticide has been developed. Other experiments in progress are designed to throw light on the mode of action of some of the insecticides tested last year, and on their persistence in the soil.

(c) *Field experiments.* In March small plots of wheat attacked by wheat bulb fly larvae were sprayed by hand with parathion and

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demeton; 0·05 per cent parathion gave almost 100 per cent control. This result suggests that a suitable spray application in March might be a practical alternative to the ploughing up and drilling with another crop which is usually advised. This work is being followed up.

Laboratory rearing

Eggs laid during the previous summer (1953) were stored outside or at 8° C., until January and February, when they were placed in pots of wheat. These were kept under various conditions, (*a*) outside; (*b*) in a cool greenhouse; (*c*) at 20° C., and (*d*) at 8° C. 7 per cent of the eggs stored at 8° C. and 38 per cent of those stored outside, produced adult flies. It is considered that the eggs stored at 8° C. failed to produce adult flies because diapause of the egg had not been broken. Some larvae were reared individually in cut stems of wheat in specimen tubes at a constant temperature of 20° C. Under these conditions the length of the various stages was : *larval stage*—males 17–20 days, females 17–26 days; *pupal stage*—males 22–23 days, females 22–27 days. These adults emerged during March, 3 months earlier than those reared outside or in the cool glasshouse, although the eggs had hatched at the same time.

Satisfactory techniques for rearing larvae on a large scale have not yet been developed. Suitable oviposition cages have been designed. The adults were fed on a mixture of honey and condensed milk, and on mammalian blood. They oviposited freely through perforated zinc at the bottom of the cage. This method made egg-collection simple and easy, and about 15,000 eggs were obtained for insecticide testing and laboratory rearing.

The 6-month diapause during the egg stage is a disadvantage for laboratory rearing, and special attempts have been made to shorten the diapause period. Eggs were kept at constant and varying temperatures from 0° to 30° C., but none hatched until over 4 months after they were laid. The histology of the developing embryo was studied in an attempt to determine the effects of the different temperature treatments. A continuous temperature record was kept from August 1953 to March 1954 in soil containing wheat bulb fly eggs, and the time of egg hatch was related to changes in soil temperature. This year, temperatures similar to those outdoors, but more extreme, are being used in the laboratory in an attempt to reduce the period of the diapause.

Field Experiments

Control of bean aphid (Aphis fabae, *Scop.*)

M. J. Way was responsible for this work. He was assisted by R. van Baer.

(a) *Comparison of different insecticides.* This experiment was done in conjunction with the Ministry of Agriculture Plant Pathology Laboratory, Harpenden, and was made possible by Mr. F. Harvey of Bishops Stortford, who provided a field of spring-sown beans and co-operated in the spraying operations.

The insecticides were applied once only (22 June 1954) by tractor-drawn machinery. Spray nozzles on the spray boom were set over the rows of beans, with three nozzles per row, one spraying

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vertically down and two obliquely down from each side of the row. The volume of spray applied was based on 100 gal./acre for a row spacing of 22 inches.

The sprays applied were all commercially formulated emulsions, except nicotine. The treatments were replicated four times, and yields of seed on some were obtained by a sampling technique which proved satisfactory. Full details are given in the following table:

TABLE 1

Insecticide used	Conc. of active ingredient, % w/v *	Approx. no. of aphids per stem at peak (26.7.54)	Seed yield, cwt./acre
Parathion	0·02	248	22·6
Malathion	0·1	238	—
Demeton ("Systox")	0·025	50	—
"Metasystox" ..	0·05	16	24·2
Nicotine	0·05	221	25·4
DDT	0·2	668	22·9
γ-BHC	0·05	191	—
Endrin	0·05	435	—
Untreated		1589	16·7

* Except nicotine which is % v/v.

At the doses applied, demeton and "Metasystox" (the systemic insecticides) were most effective, while DDT and endrin were probably the least effective. All, however, gave useful control.

The success of the experiment suggests that some of the insecticides may still remain effective when applied through only one nozzle per row and at relatively low volume. It is hoped to test this in 1955.

(b) *Timing of spray application.* In work on *A. fabae* control, mentioned in previous Annual Reports, the insecticide was applied once only when it was considered that migration of aphids into the bean crop had just ceased. This year the movement of adult aphids from *Euonymus* to beans was studied quantitatively in relation to the time of spraying, and this will be continued in future years to check that early spraying is practicable.

(c) *General effects of insecticides.* In a field trial at Rothamsted, quantitative data were obtained on the effects of a DDT spray suspension, and a demeton spray, on insects other than the bean aphid. Most time was spent developing and comparing different insect sampling methods, including beating-tray techniques, sticky traps and removal of stems for subsequent examination in the laboratory. The DDT suspension had a relatively small effect on the *Aphis fabae* population, but was highly toxic to the pea aphid, *Acyrthosiphum pisum*, the pea and bean weevil *Sitona lineatus* (which was abundant on the experimental area) and to pollen beetles (*Meligethes* sp.). It was also toxic to Coccinellid, Anthocorid and Staphylinid predators of aphids, but did not affect oviposition by Syrphid adults or reduce the populations of their larvae.

The effect of demeton on aphid predators was complicated by its virtual extermination of aphids, resulting in loss of attraction of predators to the demeton-treated plots. Demeton had less effect than DDT on phytophagous beetles. It is hoped to continue this work in 1955.

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(d) *Effect of insecticides on yield.* In the field experiment at Rothamsted the yields of seed from spring and autumn beans sprayed once on 21 June 1954 were as follows :

TABLE 2

Insecticide used	Conc. active ingredient, % w/v	Spring beans Approx. peak population, aphids/stem	Spring beans Seed yield, cwt./acre	Autumn beans Approx. peak population, aphids/stem	Autumn beans Seed yield, cwt./acre
Untreated ..	—	2772	16·8	3218	18·8
Demeton ..	0·1	48	35·5	70	28·8

It is interesting to note that in addition to showing a very large increase in yield with both spring- and autumn-sown beans the figures also indicate that spring beans may give a larger crop than autumn beans if they are kept free from aphid attack.

(e) *Nature of* A. fabae *damage.* Work was done on build-up of aphids in relation to the stage of growth of the crop. This suggested that the " setting " of the flowers is a critical stage, and if even a relatively small aphid population is at, or near, its maximum when this is occurring, serious crop loss may result. If most flowers have set while the aphid numbers are still low the subsequent increase to a large population may do relatively little harm. This may have an important bearing on the economics of aphid control.

Control of the vectors of potato virus

P. E. Burt of this department with L. Broadbent, of the Plant Pathology Department, continued their field experiments on the control of potato virus diseases.

Another experiment of a similar nature to that done in 1953 was carried out (see Report of Rothamsted Experimental Station for 1953, p. 115), but a change in the design was made which reduced the number of treatments to four. The treatments used were DDT, endrin, parathion and demeton (" Systox "), all formulated as emulsions. The apparatus and methods of spraying employed were substantially the same as in 1953, and the crop was sprayed seven times during the period 16 June to 31 August. Cold, rainy weather persisted throughout the season and aphids were few. Unlike 1953, when *Myzus persicae* Sulzer predominated, *Aphis symphiti* Schrank was the most numerous species.

Aphid control was effective with all the treatments. For example, on 13 July, the total number of aphids found on 150 leaves from the control plots was 703, of which 559 were *A. symphiti* and 95 *M. persicae*; the greatest total from any of the treated series was ten. Figures for the amount of virus spread will not be available until next year.

Further information about the result of the 1953 virus control experiments is given in the report of the Plant Pathology Department.

Control of pea weevil (Sitona lineatus *L.*)

Experiments were done in the Peterborough area by P. H. Needham and M. J. Way in co-operation with the Home Grown Threshed Peas Joint Committee to test the effectiveness of insecti

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cidal seed dressings. Two types of seed dressing were used: (1) a 50 per cent dust shaken with the seed. By this method the seed would hold a maximum of 0·02 per cent of its weight of active ingredient, and this dose was used in the field experiments for γ-BHC, demeton ("Systox") and dieldrin. (2) A method was developed whereby the insecticide was stuck to the seed with a resin "sticker" and then covered with a film of methyl cellulose. The latter stopped the insecticide flaking off the seed, and prevented operators being contaminated by it. Using this method, any dose of insecticide could be applied to the seed, the limit depending on the phytotoxicity of the chemical. Table 3 shows results of phytotoxicity tests using γ-BHC, demeton and dieldrin dressed seeds planted in

TABLE 3

Preliminary phytotoxicity tests

Treatment	% active ingredient of wt. of seed	% Germination
γBHC + coating	0·25	48
	0·063	46
	0·016	72
Demeton + coating	1·0	10
	0·25	54
	0·063	62
Dieldrin + coating	1·0	72
	0·25	86
	0·063	84
Control + coating	—	80
Control untreated	—	84

soil. Using the sticker, the doses of active ingredient for the field trials expressed as per cent of weight of seed were 0·02 per cent for BHC, 0·04 per cent for demeton and 1·0 per cent for dieldrin. Plant counts showed that these dosages were not significantly phytotoxic.

Weevil control in the field experiments was assessed by counts of feeding damage (degree of leaf notching) by adult weevils, by the amount of nodule damage caused by larvae, by counts of larvae and pupae, and by crop yield. Counts of feeding damage (Table 4) showed no significant control of adults; root nodule damage by larvae was almost 100 per cent in all plots. Preliminary larval and pupal counts showed that populations in the treatments were not obviously different from those in the controls, and seed yields

TABLE 4

Mean percentage of margin notched on the youngest and oldest fully opened leaves 6 weeks after sowing

Site	Demeton		BHC		Dieldrin		Control	
	Coated	Dusted	Coated	Dusted	Coated	Dusted	Coated	Dusted
Wansford ..	98	98	98	99	99	98	99	98
Crowland ..	40	43	50	32	38	46	45	59

of the treated plots were not significantly different from those of the untreated ones. We conclude therefore that the seed dressings used are useless for controlling *S. lineatus*.

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In a further experiment at Rothamsted, relatively heavy doses of demeton, aldrin and γ-BHC were applied as dusts on the seed drill, rates of active ingredient per 20-foot row being 2 g. for demeton, and 2 g. and 0·02 per cent g. for aldrin and γ-BHC. Plant counts showed that these treatments did not reduce the plant stand. Adult weevil damage was assessed by counting feeding notches on the youngest fully-formed leaves 6 and 8 weeks after sowing, and the results are shown in Table 5.

TABLE 5

Estimate of damage by weevils. Counts of notches on the youngest fully opened leaves

Insecticide	Rate of active ingredient per 20 row feet	Number of notches per pair of leaflets (average) of three replicates: 6 weeks after sowing	8 weeks after sowing
Demeton	2 g.	3	1
Aldrin	2 g.	14	5
	0·2 g.	18	7
BHC	2 g.	15	8
	0·2 g.	21	9
Control		18	10
Control		19	9

It can be seen that demeton greatly reduced the adult feeding damage while the high aldrin and γ-BHC treatments had a slight effect. Larval attack was slight, and we had not the facilities to deal with large samples needed to show differences between treatments. However, it was apparent that no treatment had protected the roots against larval attack. In view of the high doses of insecticide which were used, it seems that soil insecticides are unlikely to control larvae of *Sitona lineatus*, although a systemic insecticide may be useful in the control of adults.

Control of wireworms

The experiment started in 1951, in collaboration with the Entomology Department, was continued by F. Raw and C. Potter.

The treatments applied in autumn 1951 were: none (0); BHC seed dressing 2 oz. of 20 per cent γ-isomer per bushel (S); 3·5 per cent BHC dust (0·42–0·45 per cent γ-isomer) combine drilled with seed at 56 lb./acre (G); 1·78 per cent aldrin dust combine drilled with seed at 200 lb./acre (A); 5 per cent chlordane dust combine drilled with the seed at 100 lb./acre (C); 5 per cent DDT dust combine drilled at 150 lb./acre (D).

The land has been kept under continuous wheat, and no further insecticide treatments have been made.

The yields of wheat in autumn 1954 were as follows:

TABLE 6

	0	S	G	A	C	D	Mean
		Grain (at 85% dry matter): cwt./acre					
Mean (± 1·03) ..	27·2[1]	29·4	32·9	31·0	29·7	29·2	29·2
Increase (± 1·19) ..		2·2	5·7	3·8	2·5	2·0	—

[1] ± 0·60. Mean dry matter % as harvested: 74·9.

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These figures show that there was a significantly higher yield on plots previously treated with BHC (G), aldrin (A) and chlordane (C) combine drilled, compared with the control. There was no significant difference between the yields of the control and those from plots that had been treated with DDT (D) or had been sown with seed treated with BHC (S). Owing to the low general level of infestation, the experiment has now been discontinued, and the work is being prepared for publication.

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