ROTHAMSTED RESEARCH

Report for 1958

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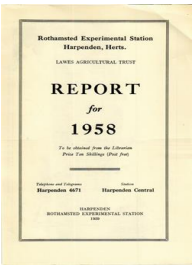

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

Daphne V. Holbrook and Elaine Fairey left and were replaced by Evelyn M. Brown and Gertrude Kruggel. F. T. Last returned from secondment to the Sudan.

Dr. Carlo Corral of the Instituto de Química "Alonso Barba" (Consejo Superior de Investigaciones Cientificas), Madrid, Dr. G. M. Das from India, Mr. McCallum Deighten of Mitchell Cotts and Co. Ltd., London and Mr. E. Mastrandeou from Greece worked in the department for various periods.

K. A. Lord attended the 4th International Congress of Biochemistry in Vienna, and visited laboratories in Austria, Holland, Germany and Switzerland where insecticides are studied.

At the request of Dr. A. D. Hanna, Chief of the Agricultural Research Division, Cotton Research Station, Wad Medani, C. Potter visited the Sudan to discuss the control of insect pests and diseases of cotton there. With Dr. A. B. P. Page, Reader in Entomology at the Imperial College of Science, he went to Uganda, Kenya, Tanganyika, Zanzibar and Southern Rhodesia primarily to study the work of the Colonial Pesticides Research Unit and to consider how the Unit might give more help to these territories. He also visited research laboratories of the Pyrethrum Board of Kenya in Nakuru to study the production and technical development of pyrethrum as an insecticide.

At the request of the Agricultural Research Council's Technical Committee on Insecticide, Fungicide and Herbicide Research, a conference of the research workers on plant protection under the chairmanship of Professor J. W. Munro was organized by the department and held at Rothamsted.

The new departmental glasshouse, though unfinished, is now being used. All four of the new constant-temperature constant-humidity rooms operated during the year and are used by several departments. There have been breakdowns and other difficulties, but it is hoped that these will be overcome. Work has been started on the three new constant-environment rooms.

INSECTICIDES

Effect of temperature on the toxicity of insecticides

The work started by Dr. Das on the effect of temperature on the toxicity of DDT to mosquito larvae was continued. Larvae of *Aëdes aegypti* L. were exposed to DDT at 15° C. and 25° C. for short periods and then transferred to DDT-free rearing medium at 20° C. for 48 hours. DDT had a significantly greater effect on larvae exposed at 25° C. than at 15° C., which together with the results

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of Das, indicates that mosquito larvae pick up more DDT at the higher temperature. (Needham.)

Isolation and properties of insect esterases

As part of the study of the biochemical mode of action of the organo-phosphorus insecticides, work on the isolation and properties of insect esterases was continued. The results previously reported on the properties of cholinesterase from the German cockroach *Blatella germanica* L. were confirmed and extended. Substrate concentration–enzyme activity curves have been obtained for the acetyl, propionyl and butyryl esters of choline, phenol, glycerol and ethanol. The same enzyme seems to hydrolyse all these substrates. The results show that the properties of the enzyme from the German cockroach resemble those of true cholinesterase of mammalian nervous tissues, but the cockroach cholinesterase has a higher affinity for choline esters. (Lord.)

Work on the hydrolysis of non-choline esters by breis of *Pieris brassicae* L. eggs indicated the presence of inhibitors in breis from newly laid eggs (0–12 hours old), and a method for their removal has been devised.

The purified extracts hydrolyse the acetyl, propionyl, butyryl and caproyl esters of phenol and glycerol. Inhibition studies with paraoxon indicate the presence of at least two enzymes; one is readily susceptible to inhibition, another is not inhibited by a concentration of paraoxon as high as $10^{-4}M$. As about 50 per cent of the susceptible enzyme is inhibited by a paraoxon concentration of $10^{-7}M$, it could be concerned in the poisoning process, and is therefore being examined in more detail. Preliminary experiments indicate that the inhibition kinetics may be of special interest.

Inhibitors were detected in breis from older eggs (5 days old at 20° C.), and their removal called for a method different from that effective with the young eggs. Purified breis from the older eggs also hydrolyse the acetyl, propionyl, butyryl and caproyl esters of phenol and glycerol. Inhibition studies also indicate the presence of at least two enzymes, one of which is still inhibited by paraoxon at $10^{-8}M$ whereas the other is not inhibited at $10^{-4}M$. Crude breis of the 5-day-old eggs hydrolyse esters of long-chain fatty acids, but it is not yet known whether purified breis do this. Esters of long-chain fatty acids are not hydrolysed either by crude or purified breis of new-laid eggs. (Solly.)

Toxicological and histochemical studies on the action of organo-phosphorus compounds on insect eggs

The permeability of the chorion, the specialization of the embryo, and the number and kind of enzymes present all alter with changes in age of the egg. Changes in susceptibility with age may therefore help towards an understanding of the mode of action of a poison. The effect of age on the susceptibility of eggs of the cabbage white butterfly *Pieris brassicae* L. to organo-phosphorus compounds was further studied. The methyl and *iso*propyl analogues of paraoxon, like paraoxon and dipterex, showed only small changes in toxicity with changes in the age of the eggs. Methyl paraoxon has about $\frac{1}{10}$ the toxicity of paraoxon and appears to increase in toxicity slightly

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with increasing age of the egg. *iso*Propyl paraoxon has about $\frac{1}{100}$ the toxicity of paraoxon and appears to decrease slightly in toxicity with increase in age of the egg.

Small measured drops of paraoxon in acetone were applied to the base of the egg and to the micropylar end of eggs of *P. brassicae* throughout their development. No difference in toxicity with site of application was found until the eighth day at 16° C., which is 24 hours before the eggs were due to hatch; then the poison applied to the base was virtually non-toxic, whereas that applied to the micropylar end had approximately the same toxicity as to the previous stages.

Preliminary work on the effect of age on the susceptibility of eggs of house cricket *Gryllus domesticus* L. showed that TEPP was virtually non-toxic except when applied in the earliest stages of development. The toxicity of paraoxon decreases during the first few days of development and then increases slightly with increasing age. At the concentrations tested, full development occurs even when amounts lethal to larvae are applied. (Holbrook.)

The possibility that the poison remains in the chorion after application, and only reaches the embryo just before or during hatching was investigated by dissecting out embryos before hatching. Late-stage embryos from untreated eggs will complete their embryonic development in a Ringer solution, but in preliminary experiments with eggs poisoned with TEPP the embryos dissected out did not complete their development.

Experiments on the enzyme histochemistry of normal embryos of *P. brassicae* and those poisoned with TEPP were studied, together with the penetration by acetyl thiocholine through the embryonic cuticle, the general body tissues and the nerve sheath of older embryos. Treatment of the whole embryo, after removing the egg chorion, with a lipoid solvent, a wetting agent and a proteinase such as trypsin, assist penetration of acetyl thiocholine. An incubation of 2–8 hours at room temperature (20° C.) favours the reaction of the cholinesterase with the substrate. (Molloy.)

The action of organo-phosphorus insecticides on normal and resistant strains of houseflies (Musca domestica *L.*)

Of two strains of houseflies resistant to diazinon (0 : 0-diethyl 0-(2-*iso*propyl-6-methyl-4-pyrimidyl) phosphorothioate), one (Sacca), obtained from Dr. Sacca of Italy, maintained its resistance from generation to generation whether or not adult selection by exposure to the poison was practised in each generation. The other strain (203a), obtained from Dr. Keiding of Denmark, maintained its resistance to diazinon only when there was continual adult selection. Work on the mechanism of inheritance of resistance is being done in collaboration with Dr. H. Kalmus of the Galton Laboratory. Attempts to raise the level of resistance of the 203a strain by selecting at high mortality levels failed. Breeding from the survivors of selection at 75 per cent mortality presents difficulties, and an attempt is being made to improve the technique. (Fairey.)

Histochemical studies by the Koelle technique on frozen sections of isolated brains of *M. domestica* suggest that acetylcholinesterase

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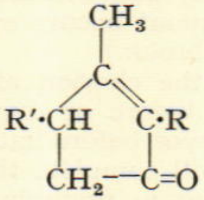
is generally distributed throughout the tissue. There also seem to be localized centres of high cholinesterase activity, but more work is required before anything definite can be said. When brains of *M. domestica* poisoned with lethal doses (0·014 per cent w/v) of diazinon were examined by this technique staining was unaffected up to 24 hours after poisoning, indicating that the cholinesterase of the brain was not being inhibited.

The rate of penetration of organo-phosphorus insecticides is being studied with a labelled poison where penetration and localization can be measured by autoradiographs. (Molloy.)

Pyrethrins and related compounds

Relation between the chemical structure and the insecticidal activity of compounds related to the pyrethrins. A series of compounds related to the pyrethrins was prepared using furan compounds as precursors.



(I) R = $CH_2COOC_2H_5$
(II) R = $CH_2COOC_3H_7$
(III) R = $CH_2COOCH_2CH{-}CH_2$
(IV) R = $CH_2COOC_6H_5$
(V) R = CH_2COCH_3

The parent ketones (I–V, R′ = H) were brominated with *N*-bromosuccinimide and the resulting bromoketones were reacted with the silver salt of (±)-*trans*- or (±)-*cis-trans*-chrysanthemic acid. The structures of the intermediate bromoketones and hence of the esters (I–V, R′ = (±)-*trans*- or (±)-*cis-trans*-chysanthemoyl) were proved by showing that more than 80 per cent of the bromine introduced could be removed as hydrogen bromide by very mild treatment with trimethylamine. These are the first esters related to the pyrethrins to be prepared with ester or keto functional groups in the β position of the alcoholic side chain.

Preliminary bioassay results, using mustard beetles and a measured-drop technique, indicate that these groups do not produce such a high insecticidal activity in the molecule as that produced by one double bond (e.g., cinerins I and II and allethrin) or by conjugated double bonds (e.g., pyrethrins I and II). (Corral and Elliott.)

Isolation, examination and properties of the insecticidal constituents of pyrethrum extract. Work on the isolation and purification of the alcoholic constituents of the pyrethrins has continued. After preparing pure (+)-pyrethrolone by techniques which have enabled adequate supplies of pure pyrethrin I and pyrethrin II to be reconstituted, attention was given to making (+)-cinerolone so that cinerin I and cinerin II may be reconstituted in a similar manner. (+)-cinerolone hydrate has now been obtained as a low-melting crystalline solid.

The optical rotation of pure undistilled pyrethrin I $[\alpha]_D^{20}$ –13·4° changes greatly on distillation, the extent depending on the heating conditions. The rotation of cinerin I is unchanged on heating, so the change with pyrethrin I is most likely to be associated with the isomerization of the double bonds in the side chain. It follows that

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any distilled samples of pyrethrin I or pyrethrin II may contain some isomeric esters. This can explain some of the inconsistent optical rotations recorded in the literature and could affect estimates of toxicity. The work on isomerization was helped by the gift by Dr. M. Thain of the Tropical Products Institute of a generous sample of pure pyrethrin I obtained by chromatography of the natural material. This permitted a definitive measurement of the optical rotation to be made. (Elliott.)

Examination of the crude pyrethrum extract for additional insecticidal compounds. This work (on the biological activity of fractions of a crude pyrethrum extract from the Belgian Congo, rich in cinerins) is being done in collaboration with Dr. Thain of the Tropical Products Institute, who is concerned with the chemical investigations.

The capacity of nitromethane to extract all the substances with insecticidal activity from the crude extract was studied as part of an investigation into the possibilities of producing pyrethrum extracts which do not stain. After seven successive extractions with nitromethane all the biological activity is transferred to the nitromethane portion and none remains in the crude extract. The recombined extract made up from the nitromethane extracted material and the residue is equal in toxicity to the crude extract and the nitromethane portion. Therefore, judged on the mortality of houseflies after 24 hours, it is unlikely that the residue contains any substance which could activate the insecticidal material present in the nitromethane fraction. (Sawicki and Elliott.)

Synergists for pyrethrum. At the request of the British Petroleum Company the effect on the toxicity of pyrethrum of adding diphenyl methane was compared with that of piperonyl butoxide using the flour beetle *Tribolium castaneum* Hbst. A measured drop technique was used, together with an after-treatment temperature of 20° C., a 7-day inspection time and ratios of pyrethrins to possible synergist of 1 : 1, 1 : 2·5, 1 : 4 and 1 : 9. No evidence of synergism was found with the diphenyl methane; indeed, the toxicity of the pyrethrins was consistently lower in the presence of the diphenyl methane, although the difference was not significant.

Piperonyl butoxide applied in the same way showed some syngeristic effects, for the mixture was 1·36 times as toxic as the extract at the 1 : 1 ratio and 2·4 times as toxic at the 1 : 9 ratio. (McCallum Deighton.)

Four substances have been claimed to activate pyrethrum extract: piperonyl butoxide (3 : 4-methylenedioxy-6-propylbenzyl *n*-butyl diethyleneglycol ether), " Sesoxane " (2-(2-ethoxyethoxy) ethyl-3 : 4-methylene-dioxyphenyl acetal of acetaldehyde), " S.421 " (octochlorodipropyl ether) and " B.P.50 " (diphenyl methane). The synergism of these substances was tested by a measured-drop technique, at fixed ratios with pyrethrum, on 5–6-day-old female houseflies with an after-treatment temperature of 20° C. and an inspection time of 24 hours. Acetone was used as the solvent. The ratios of synergist to pyrethrins were 1 : 1, 1 : 2, 1 : 4, 1 : 8 and 1 : 20. " Sesoxane ", piperonyl butoxide and " S.431 " all increased the activity of the extract. " Sesoxane " had most activity, closely followed by piperonyl butoxide; " S.421 " was considerably less

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active. Diphenyl methane depressed activity, as also found with *Tribolium castaneum*.

To determine whether these materials act simply as synergists or whether they are insecticides, their toxicity when applied alone was tested. Another syngerist, " Bucarpolate " (the ester of piperonylic acid with mono-*n*-butyl ether of diethylene glycol) was included: it was non-toxic 24 hours after dosing and $\frac{1}{2500}$ the toxicity of pyrethrin after 48 hours. " Sesoxane " had $\frac{1}{50}$ and piperonyl butoxide $\frac{1}{100}$ the toxicity of pyrethrum to houseflies, 24 hours after dosing. The flies were not knocked down by these compounds. " S.421 " was fairly toxic, about $\frac{1}{10}$ the toxicity of pyrethrum; it also had a definite knockdown action. (Sawicki.)

Physiological studies on the mode of action of pyrethrins

As pyrethrum is often stated to act as a nerve poison, the action of pyrethrins on the insect nervous system is being studied by measuring their effects on the membrane potentials, both normal and anoxic, of the giant fibre axons of the American cockroach *Periplaneta americana* L. Pyrethrin I (reconstituted from the naturally-derived alcohol and acid) depresses the normal membrane potential slightly, to an extent that is roughly proportional to its concentration; the depression is negligible at $10^{-6}M$ and about 12 per cent at $10^{-3}M$. At the lower concentrations the membrane potential recovers slightly after its initial depression. As the anoxic membrane potentials were less steady, it was more difficult to measure the effects of pyrethrin I on them; however, they appeared to be depressed about the same extent as the normal potentials. Pyrethrin I delayed the return of the anoxic membrane potentials to normal levels when nerves were restored to oxygen. (+)-allylrethronyl(+)-*trans*-chrysanthemate and α-(±)-*trans*-allethrin had much less effect than pyrethrin I on the membrane potentials, though there were small depressions at $10^{-3}M$. There appeared to be little difference between these two compounds, but there is not yet enough information to draw reliable conclusions. (Burt and Weevers.)

Toxicity and persistence of insecticidal deposits

A method for assaying toxicity of residual films of insecticide using adult *Musca domestica* L. was developed and is described in the next section. It permits short exposure times, whereas long exposures are necessary with adult *Tribolium castaneum* Hbst. Plates sprayed with a DDT emulsion consisting of 0·1 per cent w/v DDT, 0·3 per cent v/v xylene and 0·1 per cent w/v lissapol N in water gave rather variable results, probably because of irregular crystallization. The plates increased in toxicity for at least 2 days after spraying. Plates sprayed with a solution of DDT in 78 per cent v/v xylene, 20 per cent v/v ethyl cellosolve (ethylene glycol monoethyl ether) and 2 per cent v/v medicinal paraffin gave more reproducible results. The concentrations tested ranged between 0·05 and 0·5 per cent w/v DDT, and with this formulation the film remained liquid on storage. This liquid paraffin formulation is more toxic on either plain or wax-coated plates than on cabbage leaves. The emulsion deposits, by contrast, are more toxic on

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leaves than on plates. A suspension of 60μ needle crystals of DDT containing 0·1 per cent w/v sulphonated lorol, 10 per cent v/v ethyl alcohol and 90 per cent water gives a more toxic deposit than the emulsion on both plates and leaves. These relative toxicities, determined with houseflies and exposure times of 2–8 minutes, may differ with other species and longer exposure times. (Ward and Gillham.)

Chemical analytical techniques

Work on the analysis of BHC in soil was resumed. The method previously used (*Rep. Rothamst. exp. Sta for 1956*) was unsatisfactory with soils rich in organic matter and gave erratic results with some other soils. It was found that steam distillation of BHC from the soil gave either incomplete extraction or, when superheated steam was used, there was some decomposition. Solvent extraction gave an extract with a lot of interfering matter which could not easily be removed. The Schechter–Hornstein method for BHC estimation was also investigated in conjunction with solvent extraction of the soil, but without satisfactory results. Nitration of the residue from soil extract followed by reduction with titanous chloride appears promising, but has not yet been fully investigated. (Ward and Jeffs.)

Bioassay techniques and standardization of test insects

Bioassay of contact toxicity of insecticide deposits. In the bioassay method using adult houseflies (*Musca domestica* L.), the flies are chilled, then sorted into batches of ten and allowed to recover. Each batch of ten is then exposed to the treated surface in an exposure chamber. The base of the chamber is perspex with a glass plate for the floor. The glass plate can be slid out and replaced by the treated surface. The side of the chamber is a metal ring cooled by circulating ice water to make it repel the flies. The top of the chamber is of perspex, with a hole for the introduction of the test insects. The exposure chamber is placed on a plate kept at 30–35° C. and the glass plate on the floor allowed to warm to this temperature. The test insects are introduced and allowed to settle down on the attractive warm surface. The test surface, now at the temperature of the warm plate, is slid in to replace the glass plate as the floor of the chamber, and the flies settle on it. Ice may be placed on the roof of the chamber to make it repellent. After the given exposure time the flies are anaesthetized and removed to clean tubes. They are allowed to feed on sugar water during the 24-hour holding period at 20° C., after which mortality is assessed.

This method of bioassay gives results with shorter exposure times and is more sensitive than the one formerly used with *Tribolium castaneum* Hbst. as test insect. (Ward and Gillham.)

Standardization of Musca domestica *L. for bioassay of the contact toxicity of pyrethrin-type molecules.* Improved methods of rearing and standardization of houseflies for bioassay tests on the pyrethrins have been developed. Female houseflies oviposit more readily on cotton-wool packs soaked with a solution containing 30 per cent yeast and 60 per cent milk than on milk-soaked packs or bran. Eggs can be simply separated from the ovipositing medium and a

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technique has been developed for inoculating the medium with a constant number. Substituting polythene wash bowls 12 inches diameter for jam jars allows the amount of medium required to rear 5,000 flies to be halved, for the larvae do not burrow below a depth of 1 inch in the medium. This is probably because the semi-liquid state of the food causes the tunnels to collapse, depriving the burrowing larvae of air. The collected pupae are cleaned and weighed and larval mortality and pupal weight recorded. The figures have been fairly constant since the rearing technique has been standardized.

Only flies that emerge within a given 24 hours are used. Females are sorted on the fourth day after emergence and dosed on the fifth. The insects are kept in the rearing room at 27° C. until dosing; after treatment they are kept at 20° C. Although the post-treatment life of these flies is shorter than with younger flies, the end point, i.e., the point beyond which no appreciable recovery or additional kill takes place, is reached within 24 hours. This was confirmed when flies lived for 48 hours.

The rather high mortality of the young larvae (about 30 per cent) was investigated, but no explanation was found. Eggs that float in water are not necessarily infertile. Most of the eggs laid within 6 hours are hydrophobic and float for some time before sinking. About 10 per cent of the eggs which sink do not hatch within 24 hours at 28° C.

The addition of vitamins A, D and C was not significantly beneficial, and at high doses was detrimental to the larvae. The addition of cholesterol to the medium did not affect weight or the mortality of the larvae, although it has been claimed to increase the weight.

Preliminary results suggest that although the variation in the population density of the larvae affects the pupal and imaginal weights, the influence of variation in weight, within the limits tested, on the results of bioassay with pyrethrins is not statistically significant. By the standardization of the breeding and handling techniques, fluctuations in the LD50 values were so decreased as to obtain reproducible results from week to week. (Sawicki.)

Detection and identification of toxic substances in poisoned bees

Eight samples of bees submitted during the year were examined for insecticide. Three were found to contain an esterase-inhibiting material; from the details submitted by the beekeepers it seems likely that the bees had been poisoned by parathion. One sample, thought to have been exposed to lead arsenate, contained 1·6 μg. of arsenic per bee, which is enough to account for the deaths. One further sample contained metabolized DDT. No residues were found in the remaining three samples; of these, two came from fields known to be near fields of beans sprayed with " Metasystox " just before the bees died.

A bioassay method is used in the preliminary examination for the presence of insecticide, and mosquito larvae (*Aedes aegypti*) had previously been used as the test insect. As the toxicity of extraneous material in the extracts caused difficulties, an alternative method was developed using adult fruitflies (*Drosophila melanogaster*

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Meig). Tubes were coated with a deposit of the extract and the test insects exposed to the deposit for 2 hours and then transferred to clean tubes containing food for a further period of 24 hours at 28° C., when the mortality was assessed. An even coating on the tube was obtained by dissolving the extract in low-boiling petroleum ether and rotating the tube in a horizontal position in a special device while the solvent was evaporated off. With this technique, extraneous materials were not a problem, but the method proved limited because the test insects were particularly insensitive to DDT and not very sensitive to " Metasystox ". Improved methods of separating the insecticide from the extraneous materials have now been developed, and with these purified extracts bioassay tests using mosquito larvae have been giving good results.

Samples of bees submitted during the past two summers thought by beekeepers to have been poisoned with " Metasystox " have not yet been found to contain material toxic to mosquito larvae. Mosquito larvae are not very sensitive to " Metasystox " (threshold about 0·8 p.p.m.), but " Metasystox " is not very toxic to bees, the oral LD50 being about 1 μg. per bee, so that poisoned bees should contain a detectable residue if there were no decomposition. A test was made to determine whether the " Metasystox " was liable to decompose in the dead bees between the time of poisoning and extraction. The bees were treated with 3 μg. of " Metasystox " by topical application and kept at 20° C. after treatment. Determinations of loss of " Metasystox " with time were made by mosquito larvae bioassays and esterase-inhibition tests. The bioassay tests indicated that some insecticidal material was left in the bees after 8 days, but this was not detectable by the esterase-inhibition tests. However, as the samples sent by beekeepers had often been exposed to sun and rain before the sample was taken and were rarely received in under 5 days, weathering may explain the failure to detect the poison.

Dinitro-*o*-cresol, which has a low toxicity to the larvae of *Aëdes aegypti* and to adult *Drosophila melanogaster* Meig., is highly toxic to bees. A colorimetric method for detecting DNC in the extracts has been developed, depending on the partition coefficient of the DNC in different solvents and the development of the yellow colour in a buffer solution at a pH of 7·5. This test is relatively non-specific. As smaller amounts of DNC than expected have been found in poisoned bees, a test with the colorimetric method was made with this poison to determine the loss of insecticide with time. The conditions of the test were the same as those for " Metasystox ". The amount of DNC diminished with time of keeping, but was still detectable after 8 days.

A more specific sensitive spot-test for DNC was developed. It consists of putting a spot of the bee extract on filter-paper, treating with titanous chloride and exposing to bromine vapour, when the DNC gives a purple colour. This test failed even with bees extracted 3 hours after applying DNC, probably because the colour was masked by coloured extractives from the bees. (Ward and Needham.)

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Insect rearing

The following insects were reared in the department during the year:

Plant feeding

Hemiptera	*Megoura viciae* Buck.
	Aphis fabae Scop.
	Acyrthosiphon pisum Harris.
	Myzus circumflexus Buck.
Lepidoptera	*Pieris brassicae* L.
	Diataraxia oleracea L.
	Plutella maculipennis Curtis.
Coleoptera	*Phaedon cochleariae* F.
Diptera	*Leptohylemyia coarctata* Fall.
	Hylemyia antiqua Meig.
	Chortophila brassicae Bouché.

Stored product, domestic and medical

Orthoptera	*Blatella germanica* L.
	Blatta orientalis L.
	Periplaneta americana L.
	Gryllus domesticus L.
Coleoptera	*Oryzaephilus surinamensis* L.
	Oryzaephilus mercator Fouv.
	Tribolium castaneum Hbst.
	Tribolium confusum Duval.
	Tenebrio molitor L.
	Trogoderma granarium Everts.
Diptera	*Aëdes aegypti* L.
	Drosophila melanogaster Meig.
	Musca domestica L.

The technique of rearing *Pieris brassicae* L. was modified to avoid handling the adults.

Wheat-bulb-fly (Leptohylemyia coarctata *Fall.*) *control*

Box experiment. Insecticides tested as seed dressings included " Disyston ", phenyl mercury monofluoro acetate and its ammonium carbonate complex, " Phosdrin ", " Rogor " and " Thiodan ". " Thiodan " was the only material to show any promise, but was not obviously superior to dieldrin and heptachlor, which are being used in the field experiments.

Field experiments. Experiments at Newborough near Peterborough and Southwick, Northants, in collaboration with Dr. H. C. Gough and his colleagues of the National Agricultural Advisory Service, tested the effect of seed dressings applied at two to twelve times the conventional dosage rate. The insecticides used were aldrin, dieldrin and heptachlor applied in an aqueous solution of methyl-cellulose to bind the toxic material to the seed. At Newborough, where there was a heavy wheat-bulb-fly attack, all three insecticides greatly increased yield, from 11·5 cwt./acre for the untreated to 25·2–27·6 cwt./acre for the treated. Heptachlor

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appeared slightly better than aldrin and dieldrin, and the dressings at the rate of 1 per cent insecticide to weight of seed were only a little better than the 0·15 per cent rate. The seed treatments were superior to combine-drill treatments with aldrin and dieldrin at 1·5 lb./acre, which gave yields of 19·7 and 17·0 cwt./acre respectively.

Results at Southwick, where the attack was less severe, were comparable. This trial included a standard seed dressing with 40 per cent γ-BHC at 2 oz./bushel of seed. This proved inferior to the other dressings, perhaps because the early November sowing is less favourable to BHC than the other chlorinated hydrocarbons. None of the seed dressings were phytotoxic. (Bardner.)

Bean aphid (Aphis fabae *Scop.*) *control*

Effect of plant density of spring-sown beans on infestation and control. The effect of plant density of sprayed and unsprayed crops on yield of spring beans was studied. Beans were sown on 18 March 1958 in rows 22 inches apart to give six levels of plant density ranging from 5·3 to 106 plants/yard of row. Each plot was split for spraying with demeton methyl at 6 fluid oz. of active ingredient per acre for aphid control, versus unsprayed. The results are given in Table 1.

TABLE 1

Plants per yard row	5·3	11·3	20·7	41·4	57·0	106·0
Yield (cwt./acre):						
Untreated ...	16·6	24·2	27·9	27·9	26·2	20·5
Treated	24·1	31·2	32·3	29·8	25·1	21·3

The aphid counts have not yet been analysed, but it is evident that initial infestation and subsequent build-up was greatest, both on individual plants and per unit area of plot, where there was a thin plant stand. There were few aphids in plots with 57 and 106 plants per yard of row, and this is reflected in the yield, which did not benefit from the spray. As the plant population fell yield increases from spraying rose. (Way and Doherty.)

Natural control in relation to chemical control. This work was continued in collaboration with C. J. Banks of the Entomology Department. *A. fabae* laid many eggs in the autumn on some of the *Euonymus europaeus* bushes selected for study. Some hatched in February 1958, but did not survive the long cold period in March. Overwintering adults of Coccinellid and Anthocorid predators were abundant in April and May. The Coccinellids were probably plentiful because the big populations of many aphid species in May–July 1957 provided a good food supply. The predators helped to reduce the *A. fabae* populations on the winter host and also destroyed many young colonies developing later on the summer hosts—field beans, *Vicia faba* and fat hen, *Chenopodium album*—on which counts were made periodically.

Revised sampling methods, whereby plants were examined in detail in the laboratory, showed that Syrphid larvae preying on the aphids were abundant in July on summer hosts. These new sampling methods also showed that some of the previous methods were inadequate for the examination of numbers of larval predators.

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Unsuitable weather and natural enemies kept *A. fabae* populations lower from May to July than might have been expected from the many overwintering eggs on some *E. europaeus* bushes. Small populations remained on the summer hosts (field beans, sugar beet and fat hen) from August to October, from which migrants returned to *E. europaeus*, where large numbers developed in some bushes in October and November. (Way.)

Control of potato virus spread

Work in collaboration with the Plant Pathology Department on the control of potato virus spread continued, and the experiment on spray timing done in 1957 was repeated; it is described in the report of the Plant Pathology Department (p. 101 above).

In 1957 " Thimet " (*O, O*-diethyl *S*-(ethylthionomethyl) phosphorothiolothionate) mixed with activated charcoal and placed under potato tubers at planting time prevented aphids from breeding on the plants for at least 4 weeks after the plants emerged. This year this type of treatment was tested to see whether it could produce healthy seed tubers by controlling aphids on the plants until roguing became possible.

Four treatments were tested:

(1) Individual doses of " Thimet " with activated charcoal were placed below each tuber, each dose containing 0·31 g. of active ingredient.

(2) The same " Thimet " formulation applied at the same rate but mixed with granular fertilizer and applied evenly along the row.

(3) " Rogor " (*O, O*-dimethyl S-(*N*-methylcarbamoylmethyl) phosphorothiolothionate), with activated charcoal applied in the same way as treatment (1) at the rate of 0·35 g. of active ingredient per tuber.

(4) A control untreated.

The cost of the treatments (1) and (2) was slightly greater than that of spraying three times with DDT emulsion at 2 lb. of active ingredient per acre. Greenhouse tests showed that the treatments were only slightly toxic to the potatoes. Each treatment was repeated four times, and each plot contained one tuber with leaf roll and one with virus Y. The tubers were planted on 21 April, and all the plants had emerged by 6 June.

Aphids were counted throughout the season; treatments (2) and (3) kept the plants almost free from aphids throughout the period when they were present in the rest of the crop, and treatment (1) was only slightly less efficient. Plants given treatment (2) became aphicidal a little earlier than those in other plots.

Tests using laboratory-reared aphids confined on the plants by sleeves showed that the plants were still toxic to aphids in mid-August, although by then their toxicity was diminishing. Plant heights were measured three times during the season; treatments (2) and (3) caused very slight stunting. Treatment (3) caused a little browning of the leaf edges. At lifting there were only small decreases in yield in treatments (2) and (3) and none in (1). There were no significant differences between the yields from any of the

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treated plots and the control. The infector plants were rogued on 25 June, about a week after it had become possible to recognize them with certainty as diseased. Just before lifting, tuber samples were taken from plants near the infectors to estimate virus spread. The amount of spread will not be known until next year.

In mid-November the toxic residues in the tubers after lifting were kindly estimated by Dr. E. I. Johnson of the Government Chemical Laboratory. Chloroform-soluble extracts of the tubers were examined for anticholinesterase activity. Tubers from the control plots showed no activity; those from treatment (1) showed a trace, those from (2) showed activity equivalent to 3 p.p.m. " Thimet " and those from (3) activity equal to 5 p.p.m. of " Rogor ". A chemical method showed that tubers from treatment (3) contained not more than 0·1 p.p.m. of " Rogor " or any other chloroform-soluble phosphorus-containing residue. It seems likely that the tubers contained cholinesterase-inhibiting metabolites of greater activity than the insecticides from which they were derived. It is proposed also to make insecticidal bioassay tests for residues.

The effect of the distance between the insecticide and the tuber on aphicidal potency was investigated by placing a dose of 0·31 g. of " Thimet " mixed with activated charcoal at different distances from the tuber. The five treatments were: (1) no " Thimet "; (2) " Thimet " just below tuber; (3) " Thimet " 3 inches to one side of each tuber; (4) " Thimet " 6 inches to one side of each tuber; (5) " Thimet " combined with the fertilizer and applied evenly along the row. The tubers were planted on 21 April. Four sleeve tests were made, the first on 3 June at 95 per cent emergence of the plants and the last on 15 July. Treatment (5) was the most effective and caused almost 100 per cent mortality in all tests except the earliest which shows that the aphicide did not appear in the plants at its most effective concentration until a little after the shoots emerged. Of plants derived from tubers dosed individually with " Thimet ", those from tubers separated by distances of 3 inches and 6 inches took longer to become aphicidal than those in contact with it. The greater the distance, the greater was the delay. Treatment (2) was consistently more effective than treatments (3) and (4), which were about equally effective after the first test. It is concluded that where potatoes are dosed individually the insecticide should be placed as close as possible to the tuber for maximum efficiency. (Burt, Broadbent and Heathcote.)

Pests of mustard

As part of collaboration with Dr. H. C. Gough of the N.A.A.S. and Messrs. Colman, work started on the pests of mustard, particularly of *Brassica juncea* var. Trowse. The damage caused by *Meligethes aeneus* and its related species, *Ceutorrhynchus quadridens* and *C. assimilis*, on four test species of brassica, White mustard (*Brassica alba*, indigenous), Brown mustard (*Brassica juncea* var. Trowse, exotic, Radish (*Raphanus sativus*, indigenous) and Chinese cabbage (*Brassica cernua*, exotic) was studied. In general, the two exotic varieties were more susceptible to attack and damage by all the insect pests than were the two indigenous species. A collection of the parasites and predators of the pests is being made. (Doherty.)

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Seed treatment with insecticides

Seed treatment with insecticides is a convenient and effective method of controlling some insect pests, and work was done on methods of formulating and applying insecticides to seeds and on their mode of action. One of the limitations of seed dressing is that relatively small amounts of active material will adhere to the seed unless special formulations are used, and various methods of applying liquid and solid insecticides were examined, using wheat and white mustard as test seeds. The stickers and fillers tested included aqueous emulsions of polyvinyl acetate and polystyrene, aqueous solutions of polyvinyl alcohol and methyl cellulose, polyethylene glycol, a siliceous earth, activated carbon and various proprietary insecticide fillers. Materials used in the formulation can affect the toxicity of the active ingredient, both to the insect and to the plant. Under some conditions both activated carbon and polyvinyl acetate depress the insecticidal activity of " Thimet ", γ-BHC and dieldrin, and decrease the phytoxic effects of " Thimet " and γ-BHC. This may have considerable practical implications, because carbon is being used increasingly as a carrier for liquid organo-phosphorus insecticides. The possibility that the length of time the seed-dressing remains effective may be prolonged by means of materials in the formulation is being investigated.

Results this year confirmed that seed-dressings are more toxic to plants and insects in sand than in soil. (Bardner.)

Further laboratory and field trials on the mode of action of dieldrin and γ-BHC when used as a seed-dressing against the wheat-bulb fly *Leptohylemyia coarctata* Fall. showed that kill by dieldrin, probably by contact action only, occurred when treated seed was shallowly sown, i.e., less than 1 inch from the soil surface. These results fall into line with those in which it was shown that most larvae come close to the soil surface before searching for and attacking a host plant, irrespective of the position of the egg from which they hatch. Deep sowing not only reduced kill by contact action of dieldrin outside the plant but also greatly reduced kill by systemic action after the larvae had entered the plant. Thus in one field trial using wheat dressed with 0·058 mg. dieldrin per seed, the percentage kills of larvae which entered the plant was 64 for sowings $\frac{1}{2}$ inch deep and 0 for sowings 3 inches deep.

Preliminary experiments on the action of seed-dressings for the control of the onion fly *Delia antiqua* Meig. showed that dieldrin could prevent damage, probably because it killed the larvae by contact before they attacked the host plant. The choice of the underside of the bulb as the site of entry, even when the seed is sown 1 inch deep, ensures that the larva passes close to the treated seed. The evidence obtained indicated that the larvae were not killed by systemic action after entry in the plant.

Experiments with dieldrin seed-dressing on oat and wheat seed for the control of frit fly *Oscinella frit* L. showed that some larvae, probably those hatching from eggs in the soil, were killed by contact action outside the plant when the seed was shallow sown, but there was little or no effect when the seed was sown 1 or more inches deep. There was no evidence of systemic action, and thus, in prac-

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tice, a dieldrin seed-dressing is not likely to be of value in Frit fly control.

The difference in the effectiveness of dieldrin seed-dressings for the control of these three pests may depend on differences in the environmental conditions when the attack occurs and differences in larval behaviour, as well as differences in absolute resistance of the larvae. Wheat-bulb-fly larvae may be poisoned on the way to the plant, and if not they go down to the growing point soon after entry, where there is likely to be a lethal concentration of poison in the tissues. Onion fly enters via the base of the bulb, and thus is likely to go through a zone of poison before entry. Apparently the conditions of growth are such that a lethal concentration of poison is not built up in the tissues of the onion. The frit-fly larva may enter the plant either above or below ground and does not necessarily pass through a zone of poison before entering the plant. Once in the plant, it may feed and not descend to the growing point for some time, and it is not therefore liable to be killed within the plant, even when the conditions of growth at the time of the year allow the presence of a lethal concentration in the tissues at the base of the plant. (Way.)

Fungicides

Results of spore-germination tests

Evaporation. It is common practice when making spore-germination tests with fungicides to contain drops of spore suspensions in moist chambers on microscope slides. It has been assumed that the volume of the drop, and hence the concentration of the fungicide in the drop, will remain constant, but this is not so. Changes in volume of drops have been followed by measuring the change in concentration of a dilute solution of dye (Naphthalene Scarlet 4 RS). When Böttcher's slides containing 0·3-ml. drops are kept at a constant temperature, the volume usually decreases by about 7 per cent in 48 hours. The size of the loss depends on the conditions. It may be as much as 47 per cent; or water vapour from the water seal may even condense on the drop and increase in volume by about 5 per cent.

Loss of mercury compounds on glass and other surfaces. Glass surfaces remove mercury from neutral aqueous solutions of inorganic mercury compounds. This may appreciably lower the nominal concentration of mercury in the course of spore-germination tests. The extent of these losses has been investigated. The proportion of mercury lost increases with time of contact with the surface, with the area of contact and with the degree of dilution. On microscope slides the losses increase with increase in temperature in the range 10–25° C. The losses may occur during serial dilution of the solutions and on the microscope slides themselves. Thus, when a solution of mercuric chloride containing 60 mg. Hg/litre is diluted in three stages to 1·0 mg. Hg/litre in soda-glass tubes, the loss in strength of the final solution is about 25 per cent. When 0·3-ml. drops of mercuric chloride solution, containing 1·0 mg. Hg/litre, are placed on glass Böttcher's slides under cover-glasses, the losses are 15 per cent in 15 minutes and 60 per cent in 24 hours. The

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combined loss from both stages (tubes and slides) is over 70 per cent in 24 hours. Cavity slides have been made from perspex and polystyrene sheet. The losses on perspex slides are almost the same as on glass; it is likely that the losses on polystyrene will be less.

The uptake of phenyl mercuric acetate by glass from neutral solutions is small. Thus, the loss on Böttcher's slides from 0·3-ml. drops containing 1·0 mg. Hg/litre is only about 7 per cent in 24 hours, compared with 60 per cent for mercuric chloride. The loss on perspex slides is about 35 per cent.

Spores take up mercury rapidly from solution, so that in a spore germination test the mercury will be taken up by the spores and by the surface of the slide as well. The same may be true of other water-soluble fungicides.

The effect of temperature on the toxicity of fungicides

Five strains of *Botrytis fabae* Sardiña were obtained from other laboratories and tests made to determine the temperature coefficient of toxicity of aqueous solutions of mercuric chloride to spores of these strains, and to one strain of *Botrytis cinerea* Pers. ex. Fr. and one of *Sclerotinia laxa* Aderh. & Ruhl. With none of these fungi was the temperature coefficient (10–25° C.) of a significant size, so the results obtained earlier with mercury salts on the Rothamsted strain of *B. fabae* seem to be peculiar to that strain. (McIntosh.)

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