ROTHAMSTED RESEARCH

Report for 1959

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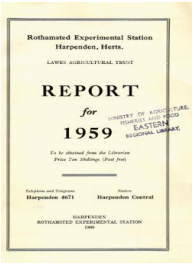

Insecticides and Fungicides Department

Anon

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The purification and properties of esterases of eggs of *Pieris brassicae* L. were further studied. The purification procedure for removing natural inhibitor from new-laid eggs was improved to give an almost inhibitor-free brei, which can be freeze-dried without loss of activity. Preliminary tests indicated that little or no esterase was adsorbed on a polyurethane sponge, so this material was used for electrophoresis of the brei of eggs from 0 to 12 hours old. The patterns obtained can best be explained by the presence of at least three esterases responsible for the hydrolysis of the four substrates used, which were the phenyl esters of acetic, propionic, butyric and caproic acids. A proportion of the acetic and propionic acid esters are hydrolysed by esterase resistant to inhibition by $10^{-4}M$ paraoxon. This concentration of paraoxon inhibited hydrolysis of the butyric and caproic acid esters. Preliminary tests indicate that $10^{-4}M$ eserine inhibits hydrolysis of phenyl acetate but not the hydrolysis of the other three substrates. The more sensitive method of assay used in these tests showed that the breis from 0–12-hour-old eggs slowly hydrolysed phenyl laurate and phenyl stearate.

Paper electrophoresis of purified breis of 5-day-old eggs (incubation period $5\frac{1}{2}$–6 days) gave results best explained by the presence of at least five distinct esterases hydrolysing the four phenyl esters studied. As with the 0–12-hour-old eggs, the hydrolysis of the butyric and caproic acid esters was strongly inhibited by $10^{-4}M$ paraoxon, whereas hydrolysis of the acetic and butyric acid esters was much less inhibited. (Solly.)

Histochemical studies on the action of organo-phosphorus insecticides

Normal flies. Work continued on the effect on the cholinesterases of the central nervous system when houseflies are poisoned by diazinon (O, O-diethyl O-(2-*iso*propyl-6-methyl-4-pyrimidyl thiophosphate). The Koelle technique was used to detect the cholinesterase in whole mounts and frozen sections of the ganglia of treated and untreated insects.

Inhibition of cholinesterases after poisoning with diazinon is most obvious in the thoracic ganglionic mass (i.e., fused thoracic and abdominal ganglia). With an LD/50 dose, there was little inhibition in the thoracic ganglia shortly after poisoning, but inhibition gradually increased with time and was progressive from the outer peripheral region of the ganglia inwards. At a higher concentration, e.g., LD/95, peripheral inhibition appeared within 2–4 hours of poisoning and was complete throughout the ganglia at 24 hours. The condition of the fly at a given time after poisoning, e.g., slightly, or badly affected or moribund, was reflected in the amount of visible inhibition in the thoracic ganglion mass. For instance, a slightly or badly affected fly generally lacks cholinesterase in the sheath and nerves arising from the ganglia, and to a greater or lesser extent in the cortical region immediately beneath the sheath. The interior part of the ganglia or neuropile may still contain considerable amounts of enzyme. A sharp and darkly staining line of demarcation often occurs between the inner enzymic zone and the zone of inhibition at the periphery. A moribund or dead fly may lack cholinesterase completely, or the enzyme may be confined to the central part of the ganglionic mass only.

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INSECTICIDES AND FUNGICIDES DEPARTMENT

Dr. Frederick Tattersfield, founder of the department and the friend of many of its present members, died on 1 May 1959. His death was a great personal loss.

J. Ward left to join Mitchell Cotts and Co. Ltd. Mr. Jose Maria Rey, of Instituto de Edafologia y Fisiologia Vegetal of Spain, and Mr. Talip Öden, of Institute of Plant Protection Chemicals and Equipment, Ankara, Turkey, arrived to work in the department.

At the request of Dr. Rasmussen, K. A. Lord was seconded for biochemical work at the Institute of Plant Systematics and Genetics, Royal Agricultural College of Sweden, on strains of insect resistant to insecticides.

The Analytical Methods Committee of the Society of Analytical Chemistry has retained P. H. Needham to advise and report on the use of bioassay methods for the detection and estimation of pesticide residues in foodstuffs. At the request of the committee he visited laboratories working on this subject in the United Kingdom and several other European countries.

At the invitation of the Ministry of Agriculture, Fisheries and Food C. Potter attended the International Conference on the use of Aviation in Agriculture at Bedford and read a paper on the effect of aerial spraying on wild life.

At the request of Dr. T. F. West of the African Pyrethrum Technical Information Centre Ltd., M. Elliott accompanied him to the works of Messrs. George Scott and Sons Ltd., Leven, Fife, Scotland, and those of Messrs. Leybold Hochvakuum Anlagen G.m.b.H., Germany, to advise at work's scale trials on the molecular distillation of pyrethrum extract.

The new departmental glasshouse was completed and is in full use. Progress was made with the three constant environment rooms, but it seems unlikely they will be completed for some months.

Insecticides

Action of organo-phosphorus insecticides

Insect eggs. A possible reason for the embryo of *Pieris brassicae* L. completing its development after an early application of a lethal dose of TEPP would be that the poison is stored in the shell or extra-embryonic membranes and does not reach the embryo until after resorption, when the embryo attempts to eat its way out. Although considered improbable, because TEPP is unstable and so unlikely to remain active in the shell for several days, the idea was tested. The choria and membranes were dissected off poisoned and unpoisoned eggs in the last stages of development, both before and after resorption of the outer membranes. The percentage survival of dissected and undissected eggs was similar, which indicates that the deaths at the end of development were not caused by poison stored in the chorion and membranes. (Molloy.)

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There was rarely any inhibition of cholinesterase in sections of the brain after poisoning, even in dead flies, unless very heavy doses were used, when there was sometimes general inhibition throughout the brain.

The 1-μl. drop of poison was usually applied to the ventral part of the thorax, near the thoracic ganglia, and this could have made the local concentration of poison large enough to swamp the enzyme. However, experiments in which the poison was applied to the dorsal thorax and ventral abdomen showed that the amount of enzyme in the thoracic ganglionic mass was related to the state of the individual fly after poisoning and was irrespective of the site of application of the poison. The poison was not applied to the head, because 50% of acetone-treated controls died when the drop was placed close to the head or mouth parts.

If inhibition of cholinesterase of the nervous system causes death these results suggest that the inhibition occurs predominantly in the thoracic ganglia and not in the cerebral ganglia, which might have been assumed to be the more critical sites. (Molloy.)

Comparison between normal and resistant flies. With the techniques mentioned above, the effect of diazinon on the cholinesterases of the central nervous system of normal and a diazinon-resistant strain of houseflies was compared. A discriminating dose of 0·04% diazinon, which killed 90% of the susceptible strain and less than 5% of the resistant strain, was first used. After 18 hours most of the susceptible flies were dead or dying, and histochemical examination showed that cholinesterase was inhibited in the thoracic ganglia but not in the brain. Most of the resistant flies then seemed unaffected or only slightly affected, and these showed no inhibition or very slight inhibition in the ganglionic sheath only.

After a 100% lethal dose, i.e., 0·4% diazinon on the susceptible flies and 1·0% on the resistant flies, the cholinesterase was almost completely inhibited in both strains in the thoracic ganglia, and very slightly inhibited in the brain. In this experiment the flies were probably grossly overdosed, so the significance of this correlation between death and the inhibition of the cholinesterase of the thoracic ganglia is being further studied. (Molloy.)

The inheritance of resistance to organo-phosphorus compounds

In continued selection and bioassays on diazinon-resistant strains of houseflies, *Musca domestica* L., both the selected and unselected series of the Sacca and Keiding 203a strains maintained their resistance levels—the Sacca selected strain at 10× and the unselected at 4× the normal level, the Keiding 203a at 20× and the unselected at 2·5× the normal level. A cross between the two selected strains (203a × Sacca) resulted in a strain of higher and still increasing resistance; when last measured it was approximately 40× the normal.

A strain of housefly very susceptible to diazinon was obtained in January 1959 from Holland by the courtesy of Dr. Van Asperen of Utrecht University. It is being kept for biological and biochemical comparisons with the resistant strains, and for the genetical analysis.

Breeding experiments on the inheritance of resistance, done in

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collaboration with Dr. H. Kalmus of the Galton Laboratory, University College, London, showed that mass crosses between the resistant 203a strain and the susceptible Dutch strain gave offspring with resistance intermediate between that of the parent strains. This could be explained by resistance to diazinon being controlled either by one intermediate gene or by many genes. To decide between these alternatives, further population tests and single pair matings are being made. (Kruggel and Piall.)

Toxicity and persistence of insecticidal deposits

The factors influencing the toxicity of residual films were further studied and the toxicity was assessed by the thermal-preference technique with houseflies as test subjects and a 5 minutes' exposure time. Experiments on how age affects the toxicity of residual films of DDT showed that deposits from DDT–xylene emulsion on wax-coated glass plates increased their toxicity after 2 days, whereas DDT–xylene–liquid paraffin solutions applied in the same way did not. Suspensions of 60-μ needles of DDT deposited on glass plates increased in toxicity after 16 days. Coumarone resin or aluminium monostearate added to DDT–xylene emulsions in amounts equal to $\frac{1}{10}$ the DDT content, gave deposits on glass which were two to four times as toxic as those from plain emulsion, but the two formulations had similar toxicities when applied to a cabbage leaf. Adding large amounts of Arochlor resin lowered the toxicity of deposits from the emulsion on leaves. (Ward and Gillham.)

Pyrethrins and related compounds

Molecular distillation of the pyrethrins. The possibility of separating the insecticidal esters in pyrethrum extract from inert and coloured materials was studied, using a laboratory 2-inch wiped film molecular still. The conditions for obtaining a pale-coloured distillate containing 60–70% of pyrethrins, together with some waxes and other inert materials, were determined. The undistilled residue contains less than 10% pyrethrins; no thermally isomerised pyrethrins I and II (see *Rep. Rothamst. exp. Sta.* for 1957, p. 137; 1958, p. 121) can be detected. This work was done in collaboration with Mr. J. S. Olejniczak (Messrs. Edwards High Vacuum Ltd.) and Mr. J. J. Garner (Messrs. George Scott and Sons (London) Ltd.). (Elliott.)

Isolation of cinerolone from pyrethrum extract. When pyrethrolone is separated from cinerolone by recrystallisation of its hydrate from ether the more soluble cinerolone hydrate is concentrated in the mother liquors and is difficult to separate and purify. It was found that (+)-cinerolone acetate, free from (+)-pyrethrolone acetate (as shown by ultraviolet and infrared spectra, by refractive index and by analysis) can be obtained by fractionally distilling the mixture through a column packed with stainless-steel gauze rings; this selectively polymerises and holds back the pyrethrolone acetate. Thus (+)-cinerolone, like (+)-pyrethrolone, can now be isolated fairly easily from pyrethrum extract to use in reconstituting pure cinerins I and II. The cinerolone acetate so obtained appears homogeneous, and the analytical results exclude the possibility that keto-alcohols, difficult to detect otherwise and related to cinerolone

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but with singly unsaturated five carbon side chains, occur combined in the extract. (Elliott.)

Characterisation of Pyrethrins I and II as 2 : 4-Dinitrophenylhydrazones. Pyrethrin I with 2 : 4-dinitrophenylhydrazine in methanolic sulphuric acid gives the expected phenylhydrazone of the intact ester. However, pyrethrin II, under the same conditions, gives a mixture of pyrethrin II–2 : 4-dinitrophenylhydrazone and appreciable quantities of pyrethrolone methyl ether 2 : 4-dinitrophenylhydrazone. The required derivative of pyrethrin II is difficult to separate from this mixture, but it can be obtained by another method in which an ether-*iso*-octane solution of pyrethrin II is treated with aqueous 2 : 4-dinitrophenylhydrazine sulphate for some weeks. The greater tendency of pyrethrin II to be cleaved must be caused by the methoxycarbonyl group in pyrethrin II transmitting its effect along the double bond and *cyclo*propane ring of the acid, so making the link to pyrethrolone weaker than in pyrethrin I. (Elliott.)

Biological activity of pyrethrum constituents. The biological activity of reconstituted and naturally derived constituents of commercial pyrethrum extracts were studied on reconstituted constituents prepared by Elliott, and on chromatographed extracts provided by Dr. Thain of the Tropical Products Institute. Five- to six-day-old female houseflies were treated topically with a 1-µl. drop of solution in acetone, kept at 20° C. after treatment and examined 24 hours later for mortality. The relative toxicity of the four constituents at the LD50 level was: Commercial pyrethrum extract 1·0, pyrethrin II 1·5, pyrethrin I 1·0, cinerin II 0·5–0·6, cinerin I 0·4–0·5. The probit lines for the extract and the two pyrethrins had steeper slopes than those of the two cinerins. The reconstituted materials and those obtained by chromatographic separation behaved similarly.

The concentration of the individual constituents of the standard commercial pyrethrum extract were determined chromatographically by Dr. Thain. As the LD50's of the standard and its constituents were also known, the theoretical LD25, 50 and 75 of the extract could be determined in terms of pyrethrin I equivalents. The theoretical and experimental results agreed well, which suggests that the constituents of the extract are neither antagonistic nor synergistic but act in a simple additive manner. This suggestion was supported by making mixtures of pyrethrin I and cinerin I, and pyrethrin II and cinerin II and estimating their toxicity.

Preliminary results with methyl cellosolve as solvent indicate that in this medium pyrethrins I and II are equitoxic and are about 1·5 times more active than the standard. This and other results (*Rep. Rothamst. exp. Sta.* for 1957, p. 140) make it probable that the relative toxicities are not constant for a species of insect, and vary with the technique and solvent used. (Sawicki, Thain and Elliott.)

The biological examination of a commercial pyrethrum extract, chromatographed by Dr. Thain of the Tropical Products Institute, showed that the activity was restricted to the four known active constituents. The sweet-smelling substances coming in front of cinerin I and pyrethrin I, and the pyrethroid coming after pyrethrin II, were biologically inactive. Solutions containing the four pure

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constituents in the same concentrations as in three commercial extracts had the same toxicities as the extracts, so commercial pyrethrum extracts are unlikely to contain insecticidal substances other than the four known ones. (Sawicki and Thain.)

Analysis of γ-BHC in soil

The technique of estimation of γ-BHC in soil was further studied. Cold extraction with solvents did not give satisfactory results when insecticides had been in contact with the soil for any length of time, and hot acetone extracted much interfering matter together with the γ-BHC. The method finally used involved refluxing the soil with 50% aqueous acetone. Nitration of the residue obtained from the soil extract (1958 Report) was the most satisfactory method for removing interfering substances. Some of the nitro compounds formed in this reaction interfered with the final colour test, and the nitrated residue had to be reduced with titanous chloride, because the action of zinc and acid in the next stage, i.e., reduction of γ-BHC, was not sufficient. The Schechter and Hornstein method, involving reduction of the BHC to benzene by zinc powder in glacial acetic acid was therefore modified, and the reduction done in aqueous hydrochloric acid, with zinc amalgam, using the Hancock and Laws apparatus. The benzene was swept into nitrating acid by the hydrogen also evolved. The final colour development was based on that used by Schechter and Hornstein. By this method, recoveries of 90% were obtained. The method as modified was used in the range of 0–10 μg., based on *m*-dinitro-benzene produced in the final nitration, and the blanks were brought to between 0·5 and 1 μg., that is 4–7 μg. on 100 g. soil. (Ward and Jeffs.)

Fifty soil samples from the National Vegetable Research Station, Wellesbourne, were analysed for γ-BHC by this method, which completed the experiment on taint started in 1955 under the auspices of the Agricultural Improvement Council. The samples from the 1955 plots taken in autumn 1957 still contained some γ-BHC, but had lost about 80% of the amount applied. The 1956 Wellesbourne plots show a loss of 75–80% by autumn 1957, and the 1956 Fen soil plots, highly organic soil, showed a loss of 40–60% by autumn 1957. (Jeffs and Gillham.)

Uptake of aldrin and dieldrin in wheat seedlings

As part of a study on how aldrin and dieldrin applied as seed-dressings, kill wheat-bulb-fly larvae, the uptake of these materials from dressed seed was measured. Using materials labelled with radioactive chlorine, 3 μl. of an approximately 50% solution of each insecticide in dioxan was applied directly to the seed, mostly on the embryo. The insecticides tended to crystallize out on the seed, so a second experiment was done, using a 50% solution in dioxan and "Tween 80", when no crystals formed. The treated seeds were germinated in silver sand at 10° C., and after periods of 30–40 days the young plants were dissected into coleoptile, shoot (growing point and leaf base, excluding coleoptile) and leaves. The coleoptile was rinsed with acetone to remove surface-adhering insecticide. The separate parts were then extracted, and the residual radioactivity in each part measured with a scintillation counter. The coleoptile

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contained 5–23 p.p.m. of dieldrin, the shoot, 4–10 p.p.m. and the leaves, 1–3 p.p.m.

An experiment on the solubilities of aldrin and dieldrin in water suggested that the aldrin preparation used contained a water-soluble radioactive impurity; the results obtained for aldrin are therefore not reported. (Jeffs, Ward and Way.)

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.
	Rhopalosiphum padi (L.)
Lepidoptera	*Pieris brassicae* L.
	Diataraxia oleracea L.
Coleoptera	*Phaedon cochleariae* F.
Diptera	*Leptohylemyia coarctata* Fall.

Stored product, domestic and medical

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

Bioassay techniques

A technique for the knockdown assessment of topically treated normally active houseflies. A vacuum technique enabled houseflies to be immobilised, sexed, dosed and the knockdown effect assessed within 15 minutes or less after dosing. Techniques in which the insects are anaesthetised or chilled to immobilise them are thought not to assess knockdown reliably. The flies were sucked in batches from a modified fly cage on to a terylene gauze attached to a suction platform on the hose of a vacuum cleaner. The insects were not affected by suction, and when the number dosed was between 8 and 10, the dosing time difference was small. Drops of 0·125 µl. or smaller had to be used to prevent them from being sucked off the fly by air flow. The fate of the dose under the airflow conditions of test was studied under ultraviolet light using acetone, cellosolve

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and odourless kerosene containing fluorescent dyes. Kerosene spread very rapidly over the body of the insects; the other solvents evaporated rapidly. This technique has been devised to test the knockdown activity of the constituents of pyrethrum. (Sawicki.)

Technique of microbioassay with larvae of Aëdes aegypti

The bioassay technique used to detect insecticides in samples of poisoned bees made use of 2nd instar larvae of *Aëdes aegypti*, which were exposed to the purified extract in 2 ml. of water (*Rep. Rothamst. exp. Sta.* for 1957, p. 143) for 2 hours, when the effect on the response of the larvae to light was measured. In the absence of bee extract, a 50% response of a replicate of 15 larvae was obtained to the following quantities of toxicant in 2 ml. of water:

BHC	0·0056 μg.
Dieldrin	0·009 μg.
DDT	0·0072 μg.

Purified bee extract depresses the toxicity of the poison and makes the test less sensitive. With dieldrin, for example, the factor of depression is approximately 4. (Needham.)

Assessment of cholinesterase inhibition in poisoned bees

Methods of detecting organo-phosphorus insecticides were studied in connexion with the work on bee poisoning. Dewaxed extracts of bees suspected of being killed by " Rogor " (*Rep. Rothamst. exp. Sta.* for 1957, p. 143) showed no insecticidal action on larvae of *Aëdes aegypti* and did not inhibit esterase. Extracts of bees killed by topical application of " Rogor " also gave negative results in these tests. Other methods of extracting the poison were tried without success.

Following a suggestion of Dr. Edson of Fison's Pest Control, a different method of detecting organo-phosphorus poisoning was developed. This depended on the amount of esterase inhibition occurring in the insect and not on extracting the poison and measuring its anti-esterase activity *in vitro*. Bees killed by carbon dioxide and ether and stored at 20° C. and 55% R.H. in the dark, retained the cholinesterase (ChE) and phenyl acetate esterase activities in the head (measured by the Warburg technique) for at least 19 days after death, whereas bees killed by topical application to the ventral surface of the thorax of an LD50 of " Rogor " and stored under the same conditions never contained more than 20% of the ChE activity of the controls. The ChE activity in the heads of all the samples of bees received from the National Agricultural Advisory Service (N.A.A.S.) was measured and the samples classified according to their state of preservation. Samples in good condition and with less than 33% of the normal ChE activity were assumed to have been poisoned by an organo-phosphorus or other ChE-inhibiting insecticide.

The occurrence of bee poisoning in the field

Of twenty-nine samples of bees received from Mr. Milne the Chief Bee Advisory Officer of the N.A.A.S., ten contained esterase-

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inhibiting material. Judging from the results of the tests and the information received with the samples, it is likely that four contained parathion and six "Rogor". Tests indicated that six further samples contained esterase-inhibiting material, but the decayed state of the bees made the results inconclusive.

Of seven samples containing chlorinated hydrocarbon poisons, three gave positive tests for dieldrin and three for lindane. One contained both DDT and dieldrin. The level of the DDT residue in this sample was only 0·02 μg./bee. Because the LD50 for DDT on bees by topical application is about 9·2 μg./bee and for dieldrin is only 0·15 μg., dieldrin probably caused their death.

One sample contained too few bees for a complete range of tests to be done. Esterase-inhibiting substances were excluded by cholinesterase measurement. A bioassay with mosquito larvae showed the presence of insecticidal substances, probably a chlorinated hydrocarbon, but no test for arsenic was done.

In five samples no insecticide was detected. (Needham and Lolly.)

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

Effect of plant density of spring-sown beans on infestation and control. The experiment described on p. 127 of the 1958 Annual Report was repeated and gave the following results:

TABLE 1

Plants per yd. row ...	4·7	9·3	17·2	24·0	36·8	48·4	25·1 *
Yield (cwt./acre) unsprayed	3·0	1·6	2·0	3·4	3·0	5·9	9·2
Sprayed	12·4	17·7	19·8	21·6	23·5	22·5	23·9
Peak aphid Nos. per stem on untreated	4,900	7,800	8,900	4,700	3,100	1,590	680

Row spacing = 22 inches except * = 11 inches.

Big aphid populations developed on all treatments in favourable weather after a large primary migration from the overwintering host. This contrasts with 1958, when initial populations were small and weather unfavourable for aphid development. The following factors are among those influencing the build-up and control of aphids on different densities of crop: amount and time of primary migration, plant height and condition of crop canopy at the time of migration, weather for about 1 month after migration.

Effect of aphicides on beneficial insects. Replicated plots of field beans infested with *Aphis fabae* were sprayed at field strength with different aphicides in 60 gallons water per acre on 6 July and dead aphids and aphid predators collected on sheets placed between the rows of the crop. Stem samples were collected 2 days after spraying and examined in the laboratory for surviving aphids and predators. Surviving predators were also collected by shaking stems over sheets 3 days after spraying. Table 2 shows numbers of dead predators collected on 9-foot lengths of sheet per treatment 1 day after spraying.

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TABLE 2

Insecticide	Oz. active ingredient/ acre	Coccinellidae Adults	Coccinellidae Larvae	Syrphid larvae	Antho-coridae
" Phosdrin "	3	7	19	147	3
" Rogor "	6	10	18	171	1
Methyl-demeton (new formulation)	3	3	35	273	1
" P.P. 175 "	3	2	9	297	0
Fluoracetamide... ...	3	3	2	72	1
Untreated (sprayed with wetter)	—	3	1	25	0

Live predators collected 3 days later by " beating " the plants over the same lengths of sheet were:

TABLE 3

	Coccinellidae Adults	Coccinellidae Larvae	Syrphid larvae	Antho-coridae
" Phosdrin "	0	0	1	0
" Rogor "	0	0	0	0
Methyl-demeton ...	9	0	0	0
" P.P. 175 "	1	8	10	3
Fluoroacetamide ...	7	6	14	4
Control	11	19	41	0

The numbers of live predators found on about 100 stems collected from each treatment 2 days after spraying were as follows:

TABLE 4

	Coccinellidae Adults	Coccinellidae Larvae	Syrphidae larvae	Antho-coridae
" Phosdrin "	0	0	1	1
" Rogor "	0	0	0	0
Methyl-demeton ...	1	0	1	0
" P.P. 175 "	5	2	9	1
Fluoroacetamide ...	2	2	47	0
Control	4	5	131	1

No live aphids were found on stem samples from " Phosdrin ", " Rogor " and demeton-methyl-treated plots; 35 and 44 live aphids were counted on fluoroacetamide and " P.P. 175 " samples, and very large numbers on the untreated. All the insecticides, except possibly fluoroacetamide, killed most aphid predators. The results with fluoroacetamide need confirmation because it may act slowly against predators, as it does against aphids.

When honeybees were enclosed in sleeves on stems immediately after spraying and examined 1 day afterwards, fluoroacetamide and " P.P. 175 " seemed less poisonous than the other insecticides, but this needs confirmation.

Natural control in relation to chemical control. Population studies of the active stages of *Aphis fabae* on its overwintering host *Euonymus europaeus* done in collaboration with C. J. Banks of the Entomology Department are now complete, except for further collections of natural enemies. Experiments on overwintering eggs

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showed that from 15 to 46% failed to hatch on different *E. europaeus* bushes. Most of these were non-viable, though a few were killed by Anthocorids and incidentally by birds while they were pecking the buds of the host plant, but there was no evidence that birds preyed directly on the eggs in 1958–59.

Big *A. fabae* populations developed in June on the summer hosts *Vicia faba* and *Chenopodium album*, and numbers remained high during July and August. Some predators, notably Coccinellidae, were inexplicably scarce, though Syrphidae became abundant.

Glasshouse experiments confirmed the importance of intra-specific competition in quickly checking the rate *Aphis fabae* multiply early in the development of the population, thereby allowing other factors, such as natural enemies, to overtake the host and check further increase. The results also explain why aphid populations can rise so quickly to their original numbers after inefficient use of insecticides, which may merely decrease numbers to levels where optimum rate of increase is possible. (Way.)

Soil insecticides. The results of an experiment done in 1958 to find whether insecticides applied in the soil affected the spread of aphid-transmitted viruses from infected to healthy potato plants (*Rep. Rothamst. exp. Sta.* for 1958, pp. 128–129), are given on p. 98.

In addition to the chemical assays for insecticide residues in the tubers harvested from this experiment, harvested tubers were planted in pots and kept in a greenhouse at 17°–20° C. until they had produced shoots 1–1½ inches high. Each shoot was then infested with 10 adult apterous *Myzus persicae* and a week later the aphids on the shoots were counted (Table 5). Test 1 began on 14 November 1958 and Test 2 on 3 March 1959.

TABLE 5

M. persicae *found on shoots from daughter tubers 1 week after infestation with 10 adult apterae*

Treatment	Test 1 (13 tubers)		Test 2 (15 tubers)	
	Average No. of aphids per shoot	% untreated	Average No. of aphids per shoot	% untreated
" Thimet ", individual doses (1)	56	92	58	95
" Thimet " with fertilizer (2)	40	66	51	84
" Rogor ", individual doses (3)	31	51	32	52
Control (4)	61	100	61	100

In both tests insecticide treatments always depressed the aphid populations below the corresponding untreated levels, but the depression was statistically significant only in test 2 with tubers given treatment 3.

The time taken for the shoots to grow to a height of 1 inch was measured and, in test 2, their height when the aphids were counted. (Table 6.)

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TABLE 6

	Test 1 (13 tubers)	Test 2 (15 tubers)	
	Average time for growth of shoots to 1 inch (days)	Average time for growth of shoots to 1 inch (days)	Average height at end of test (inches)
"Thimet", individual doses (1)	66	16·9	4·4
"Thimet" with fertilizer (2)	64	20·3	4·6
"Rogor", individual doses (3)	64	19·7	2·0
Control (4)	69	12·3	4·7

In test 1 shoots took three to 5·5 times as long as similarly treated shoots in test 2 to grow to a height of 1 inch. In test 1 shoots from tubers given insecticidal treatments took about the same time to reach a height of 1 inch as shoots from untreated tubers, but in test 2 they took from 37 to 60% longer. Treatment 3 decreased the height of the shoots at the end of the test to about one-half that of the untreated ones. Although chemical tests detected little insecticide residue in the tubers, growth was nevertheless abnormal. When such tubers were planted in the field in 1959 the plants from them yielded as well as those from untreated tubers, so effects on young shoots seem not to be reflected in the later growth of the plants.

Several commercial firms showed interest in the work to control potato virus disease by insecticides applied in the soil and made trials with our co-operation. The spraying trials at Efford Horticultural Research Station continued.

Systemic insecticidal seed dressings

Following up the preliminary results reported in 1958, formulation was found to have a considerable effect on the phytotoxicity and on the strength and persistence of the insecticidal effect of seed dressings. The insecticidal activity of foliage of plants grown from seeds of wheat and white mustard dressed with γ-BHC or "Thimet", was tested with the aphid *Rhopalosiphon padi* for wheat and the beetle *Phaedon cochleariae* for mustard. Formulations used as standards were γ-BHC plus a solution of methyl cellulose which acted as a sticker and "Thimet" plus siliceous earth and methyl cellulose solution. Polyvinyl acetate or methyl cellulose solution plus activated carbon considerably lessened the phytotoxicity of γ-BHC seed-dressings to wheat. Polyethylene glycol and a solution of polyvinyl alcohol had similar but smaller effects. No aphids were killed on wheat by any of the γ-BHC formulations.

γ-BHC dressings in the formulations were not toxic to mustard, but killed beetles fed on the foliage. Judging from the insecticidal activity of the foliage, activated carbon plus methyl cellulose and polyvinyl acetate emulsions slowed the uptake of γ-BHC. Plants grown from seed treated with these materials attained their maximum toxicity slowly and were insecticidal for less time than those given the standard formulation.

"Thimet" formulations containing carbon plus methyl cellulose solution or polyvinyl acetate were less toxic than standard formula-

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tions to wheat, but when the ratio of polyvinyl acetate or carbon to " Thimet " was suitable their aphicidal effect persisted much longer. Like γ-BHC, " Thimet " was not toxic to seedlings of mustard, but activated carbon or polyvinyl acetate prolonged the period of insecticidal activity against mustard beetles.

These effects did not happen because the amount of insecticide sticking to the seeds differed with the different formulations, or because the insecticide decomposed at different rates. Activated carbon and polyvinyl acetate probably absorb insecticide and release it slowly, so that it can be taken up by the plant over a long period. Activated carbon and polyvinyl acetate also lowered the toxicity of sodium fluoracetate and pyrolan to wheat. " Aroclor 5460 ", a chlorinated diphenyl resin also released insecticide slowly.

By using a suitable formulation, the rate insecticides are released from a seed-dressing can be greatly affected, with the desirable results of decreasing phytotoxicity and prolonging insecticidal action.

Many field crops are sown at a mean spacing of ½–1½ inches, and when wheat and mustard seeds treated with " Thimet " were sown at these distances, untreated seeds grown in the soil absorbed enough insecticide to become toxic to insects that fed on their foliage. This did not happen at 3 inches spacing. Similar effects were noticed with mustard seed treated with γ-BHC. For unknown reasons, plants from untreated seeds absorbed more insecticide when grown near live treated seeds than when grown near dead treated seeds. (Bardner.)

Seed dressings against wheat-bulb fly (Leptohylemyia coarctata)

Five new compounds were tested as seed-dressings in box tests against wheat-bulb-fly larvae. They were " Sevin ", " Trithion ", dibutyl tin S S′ (bisisoctyl thioglycollate), dibutyl tin di(thiobenzoate) and the new chlorinated hydrocarbon " W.L. 1650 " using a siliceous earth formulation with methyl cellulose solution as a sticker. Only " W.L. 1650 " was as good as heptachlor or dieldrin, which were included in the trial as standards. " Sevin " was very phytotoxic. (Bardner.)

Seed-dressings to control mustard pests

It has been suggested that γ-BHC seed-dressings could control attacks of the stem weevil *Ceuthorrhynchus quadridens* on " Trowse " mustard. Possible seed-dressings for controlling this pest were compared in the glasshouse tests during winter, using the mustard beetle *Phaedon cochleariae* as a test insect. Insecticide was applied at 10% of seed weight, with siliceous earth as a filler. Seeds were sown in potting compost at a rate equivalent to that used in the field. Insecticides tested were γ-BHC, heptachlor, parathion, aldrin, dieldrin, endrin, DDT, " Toxaphene ", " Dipterex ", " Rogor ", " Sevin ", " Thimet " and a new chlorinated hydrocarbon known as " W.L. 1650 ". " Thimet " was the best, killing 84% of beetles caged on foliage for 48 hours. " Rogor " killed 54%, but with the possible exception of γ-BHC, none of the other insecticides had any effect. This test was done 6½ weeks after sowing. Plants were about 2½ inches high and had two pairs of rough leaves. A similar

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experiment in summer showed that there was no insecticidal effect with any of the treatments 4 weeks after sowing, when the plants were 6–9 inches high. This lack of persistence of insecticidal effect on rapidly growing plants accounts for the poor results of a small field experiment on stem weevil control. "Thimet" and γ-BHC were tested at three different rates and three sowing dates. Both insecticides protected the seedling for over a month against flea-beetle attack, but did not control stem weevil. A serious attack of *Brevicoryne brassicae* decreased yields of the two later sowings. With the early sowing, the high rate of γ-BHC, applied at 20% of seed weight, gave a significant increase, doubling the yield compared with the control. These experiments show that, although seed dressings may be useful in controlling some mustard pests, they are of little use against stem weevil. (Bardner and Doherty.)

Pests and pollination of brown mustard (Brassica juncea *var. "Trowse"*)

Some experiments were done to find whether the Pollen Beetle, *Meligethes aeneus* F., decreases yield of mustard and whether yield could be increased by the pollinating activity of bees when the pollen beetle was not present. In the first, 0, 160, 320 and 480 adult *M. aeneus* were put into cages 5 × 4 × 3 feet, each cage containing 4 mustard plants. The beetles were introduced when the principal racemes of the plant had formed but before flowering had begun. The numbers of flowers, buds, pods set and pods aborted were counted through the season, and a final record was made of the numbers of pods set and aborted and the weight of seed produced when the crop had ripened. The weight of seed in g./plant was:

0 beetles	25·1 g.	± 3·53
160 beetles	18·6 g.	
320 beetles	12·6 g.	
480 beetles	10·9 g.	

Heavy populations of beetles clearly cause much loss of seed, and loss is correlated with the level of infestation. This experiment did not show whether insect pollinators are necessary as agents in increasing the yield; but it showed that a crop could be set in the absence of insects.

In an experiment done in collaboration with the Bee Department, plots of "Trowse" were covered with large cages from which insects were excluded; six cages were used and bees were introduced into half of them and the weight of seed produced at harvest was recorded. Without bees the average yield per plant was 2·14 g. of seed, and with them 2·79 g., an increase not statistically significant. (Doherty.)

Fungicides

Assessment of fungicidal action

The standard method of testing fungicides is to estimate the concentrations necessary to inhibit spore germination. These are, however, not necessarily the minimal concentrations to inhibit infection, because the dose of ultraviolet radiation needed to inhibit infection of broad beans by conidia of *Botrytis* sp. is half that needed

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to prevent germination. The relation between germination and infection was therefore studied. Most conidia of our isolate of *Botrytis* retain the ability to germinate until cultures are 50 days old, but lose their ability to infect, when suspended in water, within 20 days. Infectivity of conidia from cultures 20–50 days old was partially restored by adding sucrose, mannose, maltose and honeydew of *Aphis fabae* to the infection drop, but not by amino-acids and vitamins. Lesions were also formed by water suspensions of these old conidia when inoculated to abraded leaves.

Mechanism of action of fungicides

Work began on problems connected with eradicant and protectant properties of fungicides. One of the fungicides of particular interest, triphenyltin acetate, was also used in field trials against potato blight. It greatly decreased the incidence of disease at apparently non-phytotoxic concentrations (0·025–0·15% active material), but the slower rate of defoliation was not reflected in significantly larger yields of tubers. (Last.)

Effect of time of exposure and temperature on the toxicity of fungicides

Work on the effects of time and temperature of exposure on the toxicity of water-soluble fungicides to spores of some strains of *Botrytis fabae* was finished. Spores were kept in solutions of poisons at 10° and 25° C. for about 10 days, during which germination was repeatedly assessed and ED50 values were obtained by conventional methods.

The following summarises the results obtained with twenty poisons, including inorganic metallic salts, organo-mercury compounds, alcohols, phenols, bases and some others.

The difference between the behaviour of different strains of *B. fabae* was one of degree only, but with most strains the effect of temperature on toxicity was quite small; a high sensitivity to temperature was exceptional.

In tests on one typical strain only, all the poisons gave results of the same general pattern. The ED50 increased with the passage of time at each temperature, finally reaching a steady value at the " end-point ". The ED50 was usually greater at 10° C. than at 25° C. throughout the test, and the increase in ED50 was always greater at 10° C. than 25° C. Thus, in the most common case the apparent " temperature coefficient of toxicity " was positive throughout the test; when this was so, it increased in size throughout the test. Less commonly (e.g., phenol, β-picoline), the apparent temperature coefficient was negative when counts were made early in the test, and positive later on.

Poisons whose end-point temperature coefficients were greater than 2·0 when tested on an insensitive strain were: mercuric cyanide (5·1 after 3 days); methyl mercury nitrile (2·2 after 6 days); tertiary butyl alcohol (3·2 after 10 days); phenol (2·6 after 13 days) and pyridine (2·4 after 10 days).

The size of the end-point temperature coefficient bore no relation to the chemical, physical or other biological properties of the poisons used. (McIntosh.)

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