ROTHAMSTED RESEARCH

Report for 1960

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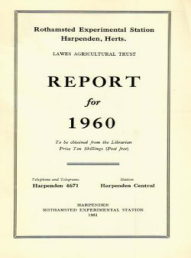

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

M. J. Way, a valued member of the department for seventeen years, left to become Reader in Applied Entomology at the Imperial College of Science and Technology. F. T. Phillips was appointed to replace J. Ward for work on problems of interest to the Colonial Pesticides Research Committee. The Society for Analytical Chemistry has awarded a fellowship to Dr. J. H. Stevenson to work in the department on techniques of microbioassay of residues of insecticides, particularly in plant material. C. Potter, accompanied by B. M. Church (Statistics Department), went to Kenya to investigate methods of sampling pyrethrum flowers. P. E. Burt, C. Potter, M. J. Way and R. Bardner attended the XIth International Congress of Entomology in Vienna, after which M. J. Way visited European laboratories working on insecticides.

By invitation, M. Elliott lectured in Paris on the " Molecular Distillation of the Pyrethrins " to the Comité Français pour l'Etude des Applications des Pyréthrines.

INSECTICIDES

The action of organophosphorus insecticides

Inhibition of cholinesterase from Blatella germanica (*L.*). A preparation of cholinesterase from *B. germanica* was strongly inhibited by " Para-oxon " (*OO*, diethyl-*O*-*p*-nitrophenyl phosphate), physostigmine and " Sevin " (1-naphthyl-*N*-methyl carbamate). The degree of inhibition does not depend on the concentration of the substrate. Both carbamates (physostigmine and " Sevin ") inhibited in the presence of substrate, but higher concentrations were needed than when carbamate and enzyme were briefly incubated before the substrate was added. The inhibition does not appear to be progressive, either in the presence or absence of substrate. The results cannot readily be explained, but the presence in the cholinesterase preparation of an enzyme decomposing carbamates would provide an explanation.

Tests made with " Para-oxon " and physostigmine confirm that phenyl acetate, phenyl propionate and triacetin are all hydrolysed by cholinesterase and not by another esterase. (Lord.)

The mode of action of organophosphorus insecticides on houseflies (Musca domestica *L*). Previous histochemical work showed that treatment with diazinon (*OO*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate) inhibited cholinesterase locally in the thoracic ganglion of *M. domestica* while the brain was less affected. Improved techniques have now shown that, after minimum lethal doses, there is an inhibition in the brain which is usually confined

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to the suboesophageal and other motor centres. Inhibition is usually greater in affected than in unaffected flies.

The selective early inhibition of the cholinesterase of the suboesophageal ganglion and the thoracic ganglion, which together control locomotor activity, may account for the poison symptoms of hyperactivity followed by paralysis and twitching of the limbs.

Examining flies at different times after applying the poison showed that there was often considerable inhibition in samples taken immediately after application, whereas symptoms of poisoning did not occur until at least 10–15 minutes afterwards. This examination also provided evidence that the enzyme was being reactivated *in vivo*. Examination of moribund and dead insects showed that in some individuals there was considerable cholinesterase activity in the ganglia, in others there was very little, indicating that there is not a strict correlation between toxicity and degree of inhibition of cholinesterase at these sites. (Molloy.)

Some experiments were done to find whether the apparent reversibility of inhibition of cholinesterase by diazinon *in vivo* also occurred *in vitro*. The oxygen analogue ("oxydiazinon") of diazinon (diethyl 2-isopropyl-4-methyl-6-pyrimidyl phosphonate) was used because this is the likely inhibitor produced from diazinon *in vivo*, but no indication of reversibility was found. With 5×10^{-7} *M*-"oxydiazinon" inhibition increased progressively with time, even in the presence of substrate, and when cholinesterase from fly heads was incubated for 30 minutes with 10^{-7} *M*-"oxydiazinon" it was completely inhibited. (Lord and Molloy.)

Resistance to organophosphorus compounds in houseflies. Histochemical examination of resistant and susceptible strains of *M. domestica* using pure diazinon confirmed that cholinesterase of the thoracic ganglion was considerably inhibited in the susceptible flies but not in the resistant flies 40 minutes after applying a discriminative dose, i.e., a dose that killed the susceptible but not the resistant. With a higher dose both strains showed inhibition. With a dose giving approximately 100% kill, the enzyme was reactivated *in vivo* in both strains 24 hours after application. In the susceptible flies a larger dose inhibited irreversibly. (Molloy.)

Van Asperen and Oppenoorth (*Ent. exp. & appl.* **2** (1959), 48) reported that their strains of resistant flies showed a lower aliesterase activity than their susceptible flies. The rate ethyl butyrate was hydrolysed by extracts of our resistant strains was between $\frac{2}{3}$ and $\frac{3}{4}$ that of our susceptible strains, whereas the ratios obtained by the Dutch workers were between $\frac{1}{4}$ and $\frac{1}{5}$. (Lord.)

To examine the mechanism of inheritance of resistance and to obtain homogeneous material for biochemical studies work was continued on the breeding of housefly strains highly resistant to organophosphorus compounds. The stock strains of *M. domestica* (Sacca selected and unselected, Keiding 203a selected ≡203aA and unselected, the susceptible Dutch strain NH and a strain resulting from the cross Sacca × 203a) all maintained their resistance levels during the year. In an attempt to decide whether diazinon resistance in the Keiding strain is polygenic or due to a single intermediate gene, the distribution of resistance in the population of Keiding 203aA and the susceptible NH strain and crosses and back crosses of these

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two strains was determined by a repeated dose technique. The NH strain was fairly homogeneous, but individuals from the 203aA strain differed widely in their resistance, the more susceptible individuals being as susceptible as those from the NH strain. The results from the crossing experiments showed neither the continuous variation characteristic of polygenic inheritance nor clear-cut segregation characteristic of a monogenic factor.

Selection was then started to obtain more homogeneous material from the resistant 203aA strain, both for use in biochemical work and to repeat the experiment on the mechanism of inheritance. Flies were taken from the mass culture of 203aA and given a dose producing about 80% kill. Thirty-five single-pair matings were set up from the survivors. Samples of the offspring of each pair that produced enough offspring were tested for resistance to diazinon. The sibs of four of these groups of offspring which showed most homogeneity for resistance were made up into single pairs, seven or eight from each group. The offspring of these were tested, and those showing too much heterogeneity were discarded; sibs of the remainder were kept for breeding. This was repeated each generation. Now, after eleven generations, there are seven strains of flies derived from the Keiding 203aA, all showing high resistance and a much greater degree of homogeneity. It is proposed to do similar selective breeding of the susceptible NH strain to obtain a homogeneous more susceptible strain and then repeat the experiments on the mechanism of inheritance of diazinon resistance using these " purified " strains. In the meantime the selected resistant strains can be used for biochemical work. (Kruggel and Piall.)

Esterases in eggs of Pieris brassicae *L.* The object of the work is to detect as many of the esterases as possible in the eggs of *P. brassicae*, to isolate them and study their properties, particularly their inhibition by organophosphorus compounds.

Esterase activity was measured using as substrates the phenyl and triglyceryl esters of acetic, *n*-propionic, *n*-butyric and *n*-caproic acids and acetyl choline chloride, acetyl β-methyl choline chloride, propionyl choline perchlorate, butyryl choline perchlorate and benzoyl choline chloride.

Crude breis of 0-10-hour-old eggs hydrolysed the phenyl and triglyceryl esters but not the choline esters. Crude breis of 5-day-old eggs hydrolysed all the substrates.

Crude breis were placed on polyurethane sponge and subjected to electrophoresis at pH 7·4, $\mu = 0{\cdot}05$. The sponge was divided into 30 sections. Using both 0-10-hour- and 5-day-old eggs, the procedure concentrated the protein and nucleic acid, as well as the esterase activity, into three peaks, but all the enzymes moved as a group and there was no separation of enzymes. Differences in movement producing the three peaks could be caused by absorption on different " inert " materials or by the presence of isozymes. (Solly.)

Ovicidal action of organophosphorus compounds. When organophosphorus compounds are applied in doses just sufficient to kill, the embryo develops fully before death occurs; with heavier doses embryonic development is incomplete. The toxic mechanism that

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kills but allows full embryonic development may differ from that where death occurs earlier. The temperature coefficient of toxicity of TEPP to eggs of *Pieris brassicae* was studied, first at lethal doses permitting full development, and secondly, at dosages that prevented development. Difference in temperature (15° and 25°) had little effect on the concentrations required to prevent hatching with full development, but at higher temperatures more TEPP is required to prevent development than at lower temperatures. The toxicity of " Para-oxon " depends less on temperature. The difference in the effect of temperature on the toxicity of TEPP at the different dose levels supports the hypotheses that more than one toxic mechanism may be involved. (Lord and Molloy.)

Further evidence was obtained on the significance of inhibition of esterase systems as a cause of death of the egg of *Pieris brassicae* by TEPP and " Para-oxon " poisoning. New-laid eggs, treated with a dose of TEPP just sufficient to prevent hatch, were kept at 15° and examined for esterase activity after 7–8 days, i.e., when they were due to hatch. Hydrolysis of acetyl choline or phenyl acetate was only slightly inhibited. Tests on homogenates of mixtures of treated and untreated eggs indicated that no free poison remained at the time of examination.

When the experiments were repeated with " Para-oxon " enzyme activity was decreased more, but the tests on homogenates of mixtures of treated and untreated eggs indicated that with this more stable chemical there were contaminating residues which caused inhibition when the eggs were ground up. The technique of using excess substrate was tried to overcome the difficulty of contamination, but it failed. The results with TEPP provide further evidence that, if death is caused by inhibition of either cholinesterase or aliesterase, the inhibition is local and not general. (Lord, Solly and Potter.)

Electrophysiological techniques

Several electrophysiological techniques were investigated to see whether they can be used to obtain information on the mode of action of pyrethrins. It is desired to experiment with separate nerve units of an insect whose response to pyrethrins has already been widely studied. Potentials have been recorded from the stimulated labellar hairs of *Musca domestica*, a preparation which has the desired properties. (Burt.)

Pick-up and penetration of dieldrin from residual films

The persistent chlorinated hydrocarbon insecticides are often used to control insect pests by residual films of poison which kill insects coming into contact with them. Little is known of the mechanics of poisoning in this way, so the present work is designed to study the pick-up, distribution and penetration of the poison, and the relationship between the amount picked up and penetrating and the toxicity to the insect.

Adult *Tenebrio molitor* L. were placed on films of dieldrin labelled with radioactive ^{36}Cl, made by evaporating the solvent from 4 ml. of aqueous suspension of dieldrin in 100-ml. wide-necked conical flasks. After a given exposure time, the insects were re-

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moved to clean containers and were subsequently analysed, by scintillation counting, for radioactive dieldrin outside and inside the body. The amount of poison left by the insects on the container was also estimated.

Two experiments were done on the relationship between the amount picked up and penetrating and the toxicity to the insect. In the first the insects were exposed for 2 hours to a dose of 500 γ per 33·2 sq. cm. and were kept during exposure and afterwards at 25°. After 30 hours all the insects were either moribund or dead. The mean pick-up was 5·5 γ per individual, the moribund insects averaging rather less (4·4 γ) than the dead insects (6·9 γ). There was a mean of 0·5 γ outside and 3·9 γ inside the moribund insects and 1·1 γ outside and 5·8 γ inside the dead insects.

In the second experiment, a shorter exposure time of 30 minutes was used, the conditions being otherwise the same. Because of the shorter exposure time, less poison was picked up and the insects took longer to die; a mortality of 22% was recorded after 2 days and 84% after 4 days. The mean pick-up per individual of the insects dead after 2 days was 2·7 γ (0·2 γ outside and 2·5 γ inside). A random sample of the insects alive on the 2nd day showed a mean individual pick-up of 2·6 γ (0·2 γ outside and 2·4 γ inside). An examination of the individuals dead after 4 days showed a mean individual pick-up of 1·7 γ with 0·1 γ on the outside and 1·6 γ on the inside. The insects still alive after 4 days had picked up a mean of 1·1 γ per individual (0·1 γ outside and 1·0 γ inside). The results give some information on the actual dose of dieldrin picked up from the film that will kill in a given time, and indicate that the rate at which the insects die is correlated with the amount they pick up from the film.

A third experiment gave some information on the initial rate of penetration into the insect and the amount of poison left by the insects on the container. Nine batches of 15 insects were exposed for 30 minutes to a deposit of 100 γ per 33·2 sq. cm. at 25°, removed to clean containers and kept at 25° until assessing.

TABLE 1

Penetration of radioactive dieldrin into adult Tenebrio molitor *L.*

Time after placing on film (min.)	No. of insects	Dieldrin lost to container (γ/individual)	Dieldrin on surface of insect (γ/individual)	Dieldrin penetrated inside insect (γ/individual)	Total dieldrin on and in insects (γ/individual)
30	45	0	0·30	0·06	0·36
90	45	0·03	0·20	0·11	0·31
150	45	0·04	0·12	0·24	0·36

Table 1 shows that $\frac{1}{6}$ of the dose picked up had penetrated at the time the insects were removed from the film, $\frac{1}{3}$ had penetrated after $1\frac{1}{2}$ hours and $\frac{2}{3}$ after $2\frac{1}{2}$ hours. Approximately $\frac{1}{10}$ of the total dose was lost to the container.

The $^{36}Cl^-$ dieldrin was not active enough to enable individual insects to be examined. More active ^{14}C labelled dieldrin has now been obtained through the courtesy of Shell Chemicals, so that this

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work may be extended and individual insects examined for the pick-up, distribution and penetration of the poison. (Rey.)

Pyrethrins and related compounds

Characterisation of cinerins I and II as 2 : 4-dinitrophenylhydrazones. In addition to the pyrethrins I and II, the cinerins have now been characterised as 2 : 4-dinitrophenylhydrazones. Identical derivatives were formed from cinerins obtained by displacement chromatography of pyrethrum extract and by reconstitution from the alcoholic and acid components. This is further evidence that structure does not change during the reconstitution procedure. (Elliott and Thain.)

Infra-red spectra of the components of pyrethrum extract. The infra-red spectra of pyrethrins I and II, of cinerins I and II, of the alcohols and acids constituting these esters and of other constituents of pyrethrum extract, were recorded to assist their identification. (Elliott.)

The enols of pyrethrolone. The structures of the enols of pyrethrolone, compounds formed when pyrethrolone is boiled with methanolic sodium methoxide, were determined. The lower boiling enol is a cyclopentenedione in which the double bond nearer the ring in the diene side chain has been saturated. The higher-boiling enol is a cyclopentane-1 : 3-dione. Both types of compounds are of considerable chemical interest. (Elliott.)

Synthesis of cyclopentenediones. A synthesis of cyclopentenediones by oxidation of cyclopentenolones with manganese dioxide in chloroform has been developed. Many cyclopentenolones are available by synthesis, so this route can be used to make a variety of 2 : 3-disubstituted cyclopentenediones. (Elliott and Jeffs.)

Toxicity to houseflies (Musca domestica *L.*) *of the four active constituents of pyrethrum extract.* Earlier work on the insecticidal activity of the four active constituents of pyrethrum extract used alone (*Rep. Rothamst. exp. Sta.* for 1959, pp. 121–122) was followed by a study of their insecticidal activity when synergised, of their knockdown activity and their full toxicity curves, including knockdown recovery and death. The synergistic factors of the four constituents and their order of relative insecticidal activity were determined over the ratios 0·1 : 1, 0·5 : 1, 4 : 1, 8 : 1 and 20 : 1 synergist : active constituent, using piperonyl butoxide as synergist, acetone as solvent, a measured drop technique and 5-6-day-old flies. Both the synergistic factor [antilog of (log LD50 pure constituent — log LD50 synergised constituent)] and the relative insecticidal activities of the synergised constituents [antilog of (log LD50 of synergised pyrethrum extract — log LD50 of synergised active constituent)] vary with the ratio of synergist to active constituent. At the ratio of 8 : 1 the synergistic factors of the extract and its four active constituents were: pyrethrum extract, 12·0; pyrethrin I, 15·0; pyrethrin II, 6·0; cinerin I, 26·0; cinerin II, 14·0. The relative insecticidal activities of the synergised materials at this ratio were: pyrethrum extract, 1·0 (1·0); pyrethrin I, 1·3 (1·0); pyrethrin II, 0·9 (1·4); cinerin I, 1·3 (0·5); cinerin II, 0·6 (0·6). The figures in brackets show the relative toxicities of the four constituents without synergist.

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These results show that the relative insecticidal activity of the constituents alone differs from their relative insecticidal activity in the presence of synergist, because the synergistic factors for the four constituents differ greatly.

Studies on the toxicity curves of pyrethrum and its constituents show that, at a fixed concentration, the progress of the responses to poison can usually be divided into: (1) a very short latent period,

TABLE 2

Knockdown of houseflies (M. domestica *L.*) *by the four active constituents of pyrethrum extract*

Substance	KD50 (% w/v)	KD end point (min. after treatment)	Relative toxicity
Pyrethrum extract ...	0·043	Approx. 13	1·0
Pyrethrin I	0·055	,, 20	0·78
Pyrethrin II	0·021	,, 10	2·05
Cinerin I	0·072	,, 20	0·60
Cinerin II	0·058	,, 10	0·75

when only a few insects are paralysed; (2) the knockdown period, when the number paralysed rapidly reaches a maximum and remains steady during (3) the period of maximum effect, which is followed by (4) recovery of some insects and death of others. Some experiments were done to determine knockdown effects and subsequent recovery or death of flies treated with pyrethrum extract and its active constituents, both alone and synergised. Using the suction technique (*Rep. Rothamst. exp. Sta.* for 1959, pp. 123–124) and applying the materials in *n*-dodecane to 5-6-day-old flies with a treatment and after treatment temperature of 20°, the knockdown

TABLE 3

Concentrations (% w/v) of pyrethrum extract and its four constituents affecting 50% of the insects, 15 and 45 minutes, 2 and 24 hrs. after treatment

	Concentrations (% w/v) affecting 50% of the insects at specified times							
	15 min.		45 min.		2 hr.		24 hr.	
Substance	U *	S †	U	S	U	S	U	S
Pyrethrum extract ...	0·040	0·016	0·065	0·012	0·080	0·013	—	0·028
Pyrethrin I	0·052	0·012	0·065	0·011	0·070	0·0098	—	0·019
Cinerin I ...	0·082	0·022	0·096	0·016	0·120	0·013	—	0·034
Pyrethrum extract ...	0·050	0·013	0·068	0·0094	0·086	0·0090	—	0·027
Pyrethrin II	0·022	0·0098	0·034	0·0094	0·043	0·0090	—	0·027
Cinerin II ...	0·066	0·018	0·098	0·016	0·014	0·015	—	0·040

* U = unsynergised.
† S = pyrethroid with piperonyl butoxide (1 : 10).

activity of each of the four constituents was compared with that of the extract. Knockdown reached a peak (KD end point) after an interval of time, the length of which depends on the chemical and its concentration. The following table shows the KD50 (concentration to give 50% knockdown at the KD end point) and the KD end point for each KD50. Pyrethrin II and cinerin II have a higher initial

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knockdown activity and a faster recovery than pyrethrin I and cinerin I. Adding piperonyl butoxide to pyrethrum extract or its active constituents prolongs the period during which knockdown occurs. At the end of the first 15 minutes the increase in the toxicity of synergised pyrethrum and its constituents is already considerable. Recovery (which begins 2 hours after treatment) is much slowed down and fewer flies recover. In general, the effect of piperonyl butoxide is small initially; it increases with the passage of time. Table 3 shows the extent to which piperonyl butoxide increases the toxicity of pyrethrum or its constituents and blocks recovery. (Sawicki.)

Analytical procedures

The department has taken part in collaborative work on the examination of analytical methods for:

1. Pyrethrum. Collaboration organised by the Joint Panel on the analysis of pyrethrum of the Pharmaceutical Society and Society of Analytical Chemistry. (Elliott and Jeffs.)
2. BHC. Collaboration organised by the Joint BHC/DDT panel of the Scientific Sub-Committee on Poisonous Substances used in Agriculture and Food Storage. (Jeffs.)
3. Demeton-methyl. Collaboration organised by the demeton-methyl residues panel of the Scientific Sub-Committee on Poisonous Substances used in Agriculture and Food Storage. (Lord and Solly.)

Insect rearing

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

Plant feeding

Hemiptera	*Acyrthosiphon pisum* Harris
	Aphis fabae Scop.
	Brevicoryne brassicae L.
	Megoura viciae Buckt.
	Myzus persicae Sulz.
	Rhopalosiphum padi L.
Lepidoptera	*Diataraxia oleracea* L.
	Pieris brassicae L.
Coleoptera	*Phaedon cochleariae* F.

Stored product, domestic and medical

Orthoptera	*Acheta domesticus* L.
	Blaberus discoidalis L.
	Blatella germanica L.
	Blatta orientalis L.
	Periplaneta americana L.
	Ryparobia maderae Fab.
Lepidoptera	*Anagasta kühniella* Zell.

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Coleoptera	*Oryzaephilus mercator* Fauv.	
	Tenebrio molitor L.	
	Tribolium castaneum Herbst	
	Tribolium confusum J. du V.	
	Trogoderma granarium Everts	
Diptera	*Aëdes aegypti* L.	
	Drosophila melanogaster Meig	3 strains
	Musca domestica L.	4 strains

In addition, guppies (*Lebistes reticulatus*) were reared for microbioassay studies.

Bioassay techniques

Because of the sensitivity of larvae of *Aëdes aegypti* L. to contaminating materials other than insecticides, a microbioassay technique with *Drosophila melanogaster* Meig as test insect was used this year for the preliminary examination of bees suspected of being killed by insecticides. A purified extract of the bees was used to produce a film in $1\frac{1}{4} \times 3$-inch glass vials by putting the extract in them and evaporating it while the vials were kept horizontal and continuously rotated. Adult *D. melanogaster* were exposed to the film for 24 hours at 25° and between 60 and 70% R.H. Mortality was then assessed. The flies had access to 10% sucrose solution during exposure.

A " direct assay " method using adult *D. melanogaster* was also developed to determine residues of " Rogor ", the systemic insecticide containing dimethoate (*OO*-dimethyl *S*-(*N*-methylcarbamoylmethyl)phosphorodithioate) in potatoes. This was in connection with work on the use of " Rogor " to check the spread of viruses. The tubers were washed, cut into pieces suitable for mincing and then deep frozen below —10°. The deep-frozen material was minced and homogenised and 25-g. aliquots of the pulp placed in 7-cm. crystallising dishes. Flies were introduced into the test dishes, which were then covered with cellophane and kept at 25° and the mortality assessed at intervals to construct a time–mortality curve. The mortality from test tubers was compared with that obtained in a series where different concentrations of dimethoate were added to the pulp of untreated tubers. This technique detected insecticidal activity in the tubers from the lowered application of " Rogor ", equal to 0·047 p.p.m. dimethoate. This was not the limit of its sensitivity. (Needham.)

The occurrence of bee poisoning in the field. Thirty-three samples of bees suspected of being poisoned in the field were received from Mr. P. S. Milne, the chief bee advisory officer of the National Agricultural Advisory Service. Fifteen were judged to have been poisoned either by organophosphorus or carbamate insecticides, because they gave negative bioassay tests but a positive result by the residual choline esterase technique (*Rep. Rothamst. exp. Sta.* for 1959, p. 124). Two samples thought to contain organophosphorus insecticides gave inconclusive results, but the bees were in an advanced state of decomposition when received.

Six samples contained chlorinated hydrocarbon insecticides,

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two dieldrin, one BHC and one DDT. A further sample gave a positive bioassay, but the chemical could not be identified.

Insecticides could not be detected in ten of the samples. One of these, sent as dead, arrived alive and apparently healthy. (Needham and Solly.)

Occurrence of dieldrin in bees. Extracts from some bees, apparently unaffected by poison, proved toxic when used as controls in bioassay procedures. Tests indicated that dieldrin or aldrin was the contaminating material. Through the co-operation of Advisory Officers, nine samples of apparently normal bees were obtained from different localities in England and Wales, and four of these gave a positive bioassay result. Analysis showed that the toxic agent in all four was aldrin or dieldrin, in amount estimated by bioassay to be about 0·04 μg./bee.

Five of the samples examined by us, two of which had given negative results by bioassay, were analysed by gas/liquid chromatography by Mr. Reynolds of the Woodstock Agricultural Research Centre, Sittingbourne, Kent. This technique showed all five samples to contain dieldrin; three had 0·03 μg./bee and two had 0·02 μg./bee. These results suggest that it is common for bees to contain sublethal doses of dieldrin. (Needham and Solly.)

Control of virus spread

Spray timing experiment. The results of the experiment done in 1959 are presented in the Report of the Plant Pathology Department (p. 117). (Burt.)

Soil insecticides to control virus spread. Following the encouraging results of the small experiment done in 1958 (*Rep. Rothamst. exp. Sta.* for 1959, pp. 98, 127–128), a larger trial on early potatoes was done at Efford Experimental Horticultural Station in 1960, using the Latin Square design previously used at Rothamsted. Each plot contained plants with leaf roll and virus Y and there were five treatments: control, DDT emulsion at 2 lb./acre active ingredient, sprayed at fortnightly intervals, and " Rogor " applied in the soil at planting at 2, 4 and 5 lb./acre active ingredient (dimethoate). The potato aphid population was fairly small; 397 aphids per 100 leaves on 22 June was the highest number recorded. The dimethoate treatments all completely controlled the aphids and were considerably better than the DDT sprays. Effects on virus spread will not be known until 1961.

The toxicity to adult *Drosophila melanogaster* of sample tubers from the lifted crop was measured (see section on bioassay). The samples showed toxicity to these insects equal to 0·047–0·076 p.p.m. of pure dimethoate, which agreed well with chemical analyses done by the Laboratory of the Government Chemist and by Messrs. Fisons Pest Control Ltd. Even though the crop was lifted late and sampling therefore delayed, these are very small residues. (Burt.)

Effects of insecticides on beneficial insects

A field experiment similar to last year's *Rep.* (*Rothamst. exp. Sta.* for 1959, p. 125) was done to determine the effects of different aphicides on insect predators of the bean aphid *Aphis fabae* Scop. Some

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insecticides were applied at less than the recommended rates in an attempt selectively to control the aphid, leaving predators relatively unharmed. Although there were many different predator species on the plots, only Coccinellid and Syrphid larvae were common. Replicated plots were sprayed on 22 June; live and dead predators collected 3 days later from sheets laid between the bean rows before spraying were:

Insecticide	Dose (oz./acre)	Coccinellidae Adults Live	Coccinellidae Adults Dead	Coccinellidae Larvae Live	Coccinellidae Larvae Dead	Syrphid larvae Live	Syrphid larvae Dead
Demeton-methyl ...	2	0	1	4	25	59	22
Demeton	1	0	3	14	39	41	9
Menazon (" PP175 ")	2	1	1	11	17	80	19
Mevinphos (" Phosdrin ")	1	0	7	0	78	59	61
Fluoroacetamide ...	3	1	1	7	3	48	4
Untreated	—	0	0	15	0	59	10

Mevinphos at about $\frac{1}{2}$ of the recommended rate still killed many Coccinellidae and Syrphid larvae. Demeton-methyl and demeton at $\frac{1}{3}$ of the recommended rate still killed some Coccinellid larvae, as did menazon (" PP175 ") at about $\frac{2}{3}$ of the recommended rate. Fluoroacetamide at the recommended rate apparently did least harm.

The best control of aphids was by the demeton-methyl formulation. The mevinphos, menazon and fluoroacetamide preparations also gave what was considered to be good commercial control, but demeton at 1 oz./acre was inadequate.

The numbers of live predators on samples of 100 stems collected from each treatment 6–8 days after spraying were:

	Coccinellid larvae Old	Coccinellid larvae Young	Syrphid larvae
Demeton-methyl	5	9	34
Demeton	36	43	32
Menazon (" PP175 ")	31	34	43
Mevinphos (" Phosdrin ")	3	6	31
Fluoroacetamide	50	29	50
Untreated	96	67	55

The old Coccinellid larvae were probably present as larvae at the time of spraying; young larvae and Syrphid larvae were probably present as eggs. The numbers partly reflect numbers of aphids, but show that many eggs survived in all treatments, though there were remarkably few Coccinellid larvae in plots sprayed with demeton-methyl or mevinphos. This experiment and last year's suggest that fluoroacetamide and menazon may have a useful selective action for aphid control. Demeton-methyl may also act selectively when applied at less than the normally recommended rate. Tests, in which bees were caged on plants before the spray had dried, suggested that demeton-methyl at 2 oz./acre and mevinphos at 1 oz./acre may still kill some bees working the crop at the time of spraying. (Way.)

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Effect of plant density on infestation and control of A. fabae *on beans*

Two experiments were done in co-operation with G. D. Heathcote of the Plant Pathology Department, one at Rothamsted and one at Woburn similar to those described in *Rep. Rothamst. exp. Sta.* for 1958, p. 127, and for 1959, p. 125. These completed the series; provisional results are:

Rothamsted

Plants per yd. row ...	8·3	15·0	26·6	34·8	54·4	67·6	32·5 *
Yield, cwt./acre:							
Unsprayed ...	8·8	7·7	9·2	11·1	12·7	16·0	20·4
Sprayed	15·1	16·5	20·5	18·6	19·6	19·2	20·7
Peak aphid nos. per plant on untreated	1748	1885	1424	932	279	43	37

Woburn

Plants per yd. row ...	6·1	13·2	21·3	32·1	50·5	69·2	26·9
Yield, cwt./acre:							
Unsprayed ...	2·2	2·4	2·7	5·9	12·0	17·3	22·2
Sprayed	12·1	19·8	22·7	24·5	24·6	26·8	32·2
Peak aphid nos. per plant on untreated	3241	3569	2656	1407	246	87	33

Row spacing = 22 inches except * = 11 inches.

Big effects of crop density in all experiments show that initial aphid infestation is usually greater in the thinly sown plots, in which populations also increase faster than in the thickly sown plots. (Way and Heathcote.)

Natural control in relation to chemical control

This work is in collaboration with C. J. Banks of the Entomology Department. Experiments on death of *Aphis fabae* eggs during winter were continued. Results were similar to those in 1958–59 except at two sites; one at Rothamsted, where on one bush exceptionally many eggs were eaten by birds; the other at Hackthorne, Lincs., where many eggs appeared to be diseased. Elsewhere predation by birds and losses from diseases were insignificant. In more severe winters than those of 1958–59 and 1959–60 predation by birds may be more important.

To test whether low temperatures are directly harmful, eggs were kept for varying periods in the laboratory at temperatures down to −20°. Overwintering eggs were killed when kept at −20° for 7 days, but were unaffected by −10° for 14 days and by a diurnal change from −10° to + 5° for 4 weeks. As temperatures seldom fall to below −10° in this country, cold is unlikely to kill overwintering eggs of *A. fabae*.

The experiment on the effect of intra-specific competition on rate of *Aphis fabae* increase was repeated in cages outdoors and in greater detail than last year. The results confirmed that such competition is likely to be important in relation to both the natural and chemical control of aphids. (Way and Banks.)

Insecticidal seed dressings

Effects of differences in soil type and leaching on the persistence and toxicity of systemic insecticidal seed dressings. Alkaline fen peat,

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acid sedge peat and clay were each mixed with sand in varying proportions to give a number of soils with different characteristics. Wheat seeds, treated with a slurry of phorate and siliceous earth, were planted in pots containing these different soils. Half the replicas were watered every day with excess culture solution to produce leaching, and the remainder of the pots were placed in shallow dishes of culture solution to prevent leaching. Persistence of toxicity was measured by confining aphids (*Rhopalosiphum padi*) on the plants.

With no leaching, toxicity persisted longest with the sand and clay and shortest with peat. With leaching, plants grown in sand, clay or peat lost their toxicity rapidly, and toxicity persisted longest in a peat–sand mixture. Neither sand nor clay seem to hold the insecticide, which rapidly leaches away; it is strongly held on the peat, and is therefore not leached, but only when the peat is diluted with sand and the amount of absorbing material per unit volume reduced, does the insecticide become sufficiently free to be taken up by the plant.

Movement of insecticide from seed to soil. Wheat and mustard seed treated with a slurry of phorate and siliceous earth were sown in John Innes Compost and allowed to germinate and were then transplanted. With wheat aphids and mustard beetles as test insects, transplanted seedlings lost their toxicity much more rapidly than undisturbed plants. This was so even when wheat plants were transplanted with the parent seed attached, suggesting that some of the insecticide moves from the seed to the soil where it is taken up by the plant if undisturbed.

Movement of insecticide in the soil. To obtain information on the behaviour in the soil of insoluble particulate seed dressings, such as the chlorinated hydrocarbons BHC and dieldrin, it was assumed that powdered Saturn Yellow, an insoluble fluorescent dye, would behave like these insecticides. Wheat seeds were treated with a slurry of this dye and planted in John Innes compost, and the distribution of the dye in the soil at intervals after sowing was determined by studying soil sections under ultraviolet light. The effect of different drainage systems on the distribution of the dye was studied.

A method to determine the distribution of organophosphorus insecticides in soil sections was developed in collaboration with Lord and Solly. A gelatin-coated glass slide placed in contact with the soil section absorbs the insecticide on the pattern present in the section. An image of this pattern is produced on filter-paper by moistening the paper with a solution of insect esterases, applying it to the gelatin slide and then treating it with indoxyl acetate. In the areas where insecticide is present the esterases are inhibited and no staining is produced with the indoxyl acetate, whereas the rest of the area is stained. This method was used to study the behaviour of phorate on wheat seeds in John Innes compost at a number of time intervals after sowing. Both sets of experiments showed that some of the insecticide passes into the soil from the seed. The experiments with the fluorescent dye indicated that distribution of insoluble insecticide is assisted by percolation of water through the soil and by germination of the seed. (Bardner.)

Seed dressings for wireworm control. In collaboration with F.

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Raw, experiments on the use of seed dressings for wireworm control were started. These are described below (p. 157). (Raw, Potter and Bardner.)

Fungicides

Partial soil sterilisation

Effects of treating soil with formalin and chloropicrin, and with a range of seed treatments, on the distribution of fungi and bacteria in forest nursery soils, planted with Sitka (*Picea sitchensis*), were studied with M. A. Ram Reddy (see above, p. 127). Preliminary experiments were also done with D. B. Slope to see whether the incidence of take-all of cereals, caused by *Ophiobolus graminis*, is affected by applying chlordane, heptachlor and aldrin (insecticides known to decrease the incidence of some fungal diseases) to the soil. (Last.)

Control of potato blight

Triphenyltin acetate, which gave promising results in 1959, was compared in the field with copper oxychloride and zinc ethylene bisdithiocarbamate used at rates of 0·75, 2·5 and 1·3 lb. of active material/100 gallons respectively. Blight was severe in the unsprayed controls, which yielded 9·9 tons/acre of healthy tubers. Spraying with zinc, copper and tin compounds delayed the destruction of the foliage (50% stage) by 8, 10 and 18 days and increased yields by 0·4, 1·0 and 3·8 tons/acre respectively. The increases with tin occurred despite its phytotoxicity, which showed by the appearance of necrotic spots. (Last.)

Cereal powdery mildews

Using fungicidal sprays, the effects of *Erysiphe graminis* DC on the development of spring-sown barley were analysed fully. Soon after leaf infections appeared, decreases in the dry weights of roots were detected. Infection later decreased leaf area and numbers of tillers. The decreases in the root system were disproportionately large, causing the ratio (w/w) of roots to living leaf lamina to decrease. Infected plants produced fewer and smaller ears—a decrease which may be associated with a lower Net Assimilation Rate. (Last.)

Saprophytic organisms in the phyllosphere

Surfaces of living leaves, like roots, appear to provide a substrate suitable for the development of a saprophytic microflora. Factors controlling the incidence of two members of the phyllosphere flora, *Sporobolomyces* and *Tilletiopsis*, were investigated. *Sporobolomyces* occurs on a wide range of plant hosts, but rarely on ferns and conifers; *Tilletiopsis* is less widely distributed and is rare on trees. Colonies of both fungi are usually more numerous on lower than on upper leaf surfaces, *Sporobolomyces* are more abundant on diseased leaves whether (*a*) infected by rust fungi or (*b*) infected with *Aphelenchoides ritzemabosi* (a nematode) and *Eriophyses macrorrhyncus* (a gall-forming mite), than on healthy leaves. Applying superphosphate to

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winter wheat increased the numbers of *Sporobolomyces* colonies per unit leaf area; potash decreased numbers and nitrogen had no effect. (Last.)

Laboratory techniques of assessing fungicidal activity

Some variants and possible errors in the test-tube dilution and slide-germination methods of assessing fungicidal action were studied and the results prepared for publication. (McIntosh.)

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