ROTHAMSTED RESEARCH

# Rothamsted Report for 1967

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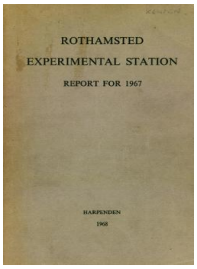


## Insecticides and Fungicides Department

### C. Potter

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# INSECTICIDES AND FUNGICIDES DEPARTMENT

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The installation of a Hitachi Perkin–Elmer RMU 6E double-focusing mass spectrometer was completed in February. It is designed to be coupled to a gas–liquid chromatograph and so provide information on the chemical nature of components separated from mixtures by the chromatograph. Structures can be determined with very small amounts of material. The spectrometer can be operated at high resolution, enabling the mass of a molecule or fragment ion to be measured within 5 ppm, usually sufficient to allow assignment of a unique elemental composition. So far it has mainly been used to study insect secretions and pheromones and synthetic compounds.

More work was done on the factors influencing the amount of poison reaching the site where it acts and on the mechanism of poisoning by organophosphorus compounds. Solids from insect bodies sorbed considerable amounts of various insecticides. Such sorption distributes the poison unevenly in the insect, and may greatly affect the concentration at its site of action.

Evidence was obtained that organophosphorus insecticides kill by acting directly on the central nervous system and that their toxicity depends on the concentration–time curve of the poison in the surrounding haemolymph.

Considerable progress was made both in the genetic analysis of factors that confer resistance to various insecticides on the SKA strain of houseflies and in producing strains containing only single factors. The evidence for factors that decrease penetration by insecticides, and for mechanisms able to detoxify both organophosphorus and organochlorine compounds, provides a reason for resistance often applying to more than one type of compound and for the fact that a population that has become resistant to one insecticide is liable rapidly to become resistant to another.

More evidence was obtained here and elsewhere of the great toxicity to insects of the synthetic pyrethroids produced in the department and fostered by the National Research Development Corporation. The initial interest shown by commercial firms in making these materials continued.

The selectivity of some insecticides was increased by enclosing them in capsules, which diminishes or abolishes contact toxicity while retaining their activity as stomach poisons. Thus the encapsulated poison is toxic to a pest with biting mouth parts, but is unlikely to harm its predators and parasites. Control of persistence by microencapsulation has yet to be studied.

The acreage of oil-seed rape grown in this country increases, and the control of its pests has considerable risk to bees. Malathion, the currently recommended material, has the virtue of being only slightly toxic to



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mammals, but it may not persist long enough always to be effective. Endosulfan did little harm to bees and seemed to control the pollen beetle well. It is used on the continent of Europe, but is very toxic to mammals and fish.

At least three satisfactory substitutes for the organochlorine insecticides were found that control wheat-bulb fly, but none for the control of wireworms.

## Insecticides

### Factors affecting poisoning and the causes of resistance

***Sorption of insecticides by housefly solids from aqueous solution.*** To obtain more information on the factors that may lessen the concentration of poison at the critical site of action, the studies on sorption of diazinon by insect body solids were extended to include organochlorine and other organophosphorus insecticides. Whole flies were macerated in 0·05*M*-tris buffer pH 7·0 containing insecticide and, after equilibration, the distribution of the insecticide between fly solids and equilibrating solution was measured. The distribution coefficient given in Table 1 is the ratio of concentrations of poison in the solid and in the liquid on the basis of

$$\frac{\mu g \text{ poison/single fly abdomen}}{\mu g \text{ poison/1 ml equilibrating solution}}.$$

Detailed work showed that the distribution coefficient with diazinon was independent of the quantities involved. The similarity of the coefficients obtained under different conditions shown in the table indicates that this is also true for most of the poisons tested, but aldrin, paraoxon and chlorthion gave anomalous results.

Table 1 shows that the affinity of different insecticides for fly solids differs greatly. For instance, when 1 μg of dieldrin in 1 ml of aqueous

**TABLE 1**

*Distribution coefficient of insecticides sorbed on fly solids from aqueous solution*

| Insecticide | μg insecticide per fly/μg insecticide per 1 ml aqueous solution | | | | |
|---|---|---|---|---|---|
| | 1* | 2* | | 3* | 4* |
| Diazinon | (0·43) | | Diazoxon | (0·05 | 0·04) |
| Malathion | 0·4 | 0·3 | Malaoxon | 0·03 | 0·03 |
| Parathion | 1·2 | 1·8 | Paraoxon | 0·05 | 0·02 |
| Chlorfenvinphos | 0·7 | 0·7 | | | |
| Chlorthion | 1·2 | 2·3 | | | |
| Dicapthon | 1·6 | 1·5 | γ-BHC | 1·1 | (1·1) |
| Ethion | 5·0 | 4·8 | Aldrin | 4 | 30 |
| Trithion | 5·0 | 6·5 | Dieldrin | 9 | 10 |

* Column 1: Data obtained when 1 ml of aqueous solution containing 1 μg of insecticide was equilibrated with solids from 20 flies.
Column 2: Data obtained when 1 ml of aqueous solution containing 1 μg of insecticide was equilibrated with solids from 5 flies.
Column 3: Data obtained when 1 ml of aqueous solution containing 2 μg of insecticide was equilibrated with solids from 20 flies.
Column 4: Data obtained when 1 ml of aqueous solution containing 2 μg of insecticide was equilibrated with solids from 5 flies.



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solution is equilibrated with an eviscerated fly abdomen, about 90% of the poison is sorbed on the solids, whereas with malaoxon only 3% is sorbed.

The three phosphate insecticides, diazoxon, malaoxon and paraoxon, are all much less strongly sorbed than the corresponding thiophosphate insecticides diazinon, malathion and parathion which are sorbed to about the same extent as chlorfenvinphos, chlorthion, dicapthon and $\gamma$-BHC. Ethion, trithion, aldrin and dieldrin are even more strongly sorbed.

Preliminary work on the nature of the sorbing materials indicate that lipids play a considerable part. Extracting macerated houseflies with chloroform diminishes sorption of diazinon on the remaining solids. Extraction with 2 : 1 mixture of chloroform : methanol diminishes it still more, suggesting that some sorption is by phospholipids. Drops of solvent extracts on filter-paper strongly sorbed $^{14}$C-labelled diazinon from aqueous solution. Autoradiography showed that the labelled diazinon was not easily removed by water from the areas treated with solvent extract. (Lord)

**The factors of resistance to insecticides in the SKA strain of houseflies.** The factors conferring resistance to DDT, dieldrin, diazinon, "ethyl malathion", parathion and chlorthion in the strain of SKA which was selected by exposure to diazinon were located and isolated.

The contribution of each of the five autosomal linkage groups towards resistance is:

***II linkage group.*** The factor R2 on this linkage group delays knock-down and gives a $\times 2$ resistance at death with DDT, dieldrin, diazinon, "ethyl malathion", parathion and zectran, but not chlorthion. R2 probably acts by delaying penetration. About 2·5 times more dieldrin and 5 times more DDT is washed off the outside of flies with R2 and the SKA parent 4 hours after treatment with 1·0 μg dieldrin/fly and 0·5 μg DDT/fly than from flies lacking this factor. R2 is intermediate both for knock-down and kill. It is almost certainly an allele of *kdr-o* which is present in the *organotin-stw* strain, because both factors behave similarly. The strain *organotin-stw* was sent to us recently by Dr. W. Plapp Jr., who had found insecticides penetrated it less readily than other strains.

***III linkage group.*** The factor R3 on this linkage group protects the flies against DDT and diazinon, but not against the other organophosphates tested. This factor is likely to degrade WARF-anti-resistant (N,N-di-*n*-butyl-*p*-chlorobenzenesulphonamide) in addition to DDT. R3 is intermediate, and gives a resistance of *ca.* $\times$ 15 against diazinon and *ca.* $\times$ 10 against DDT when homozygous. It is inhibited by sesamex. This factor, which is very difficult to isolate from SKA flies, may be linked with a lethal gene. We have bred strains in which R3 and the marker for the III linkage group (*ocra*) segregate in conjugation.

***IV linkage group.*** The major factor of resistance to dieldrin DR4 is on this linkage group. DR4 is intermediate and confers, when homozygous, immunity to topically applied dieldrin in acetone during the first 24 hours but increasing numbers of deaths during the next 72 hours



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decrease resistance to *ca.* × 700. This factor is now present in only about 10% of the SKA flies.

***V linkage group.*** Two factors are present on this linkage group: gene ***a***, the gene for low ali-esterase activity which confers resistance to diazinon (×15), "ethyl malathion" (×10), parathion (×15) and chlorthion (×15), and DDT-ase (DDT-dehydrochlorinase) which gives great resistance to DDT. DDT-ase is present in only 20% of the SKA flies. Several attempts to obtain this factor in a homozygous condition failed.

***VI linkage gronp.*** No measurable factors of resistance were detected on this linkage group. (Sawicki and Farnham)

**The poisoning process in cockroaches treated topically with diazoxon.** Previous electrophysiological and histochemical studies (*Rothamsted Report* for 1966, p. 164) compared the condition of nerve cords from the cockroach *Periplaneta americana* L. treated directly with diazoxon with that of cords from cockroaches poisoned with diazoxon *in vivo*. The results supported the view that organophosphorus insecticides kill insects by affecting synaptic conduction, and allowed an indirect estimation of the concentration of diazoxon in the haemolymph of cockroaches treated topically with an LD90 of this insecticide. Using recently improved methods for assaying organophosphorus insecticides chemically, this

**TABLE 2**

*The penetration, distribution and loss of LD90s of diazoxon applied topically to* P. americana

An LD90 (2·6 μg per cockroach of mean weight 0·8 g, 16% dry matter) would give a concentration of 13·4 μM if evenly distributed through the body fluids. Approximately 25% body weight is haemolymph

| Time—hours after treatment | ½ | 1 | 1½ | 2 |
|---|---|---|---|---|
| | percentages | | | |
| Proportion of diazoxon penetrating cuticle | 24 | 39 | 58 | 73 |
| Proportion of diazoxon penetrating which is found inside insect | 37 | 40 | 22 | 9 |
| Concentration of diazoxon found inside insect: | micro-molar | | | |
| (*a*) If all dissolved in total body fluids | 1·3 | 2·4 | 1·8 | 1·0 |
| (*b*) In total body fluids allowing for sorption | 0·8 | 1·4 | 1·1 | 0·6 |
| Concentration of diazoxon found in haemolymph: | | | | |
| (*a*) Maximum | 2·0 | 3·6 | 3·4 | 3·0 |
| (*b*) Minimum | 0·3 | 0·6 | 0·9 | 0·8 |
| (*c*) "Median" | 0·8 | 1·4 | 1·8 | 1·4 |

Nerve function in metathoracic ganglia from insects treated 1–2 hours previously with LD90s of diazoxon is affected to the same extent as in ganglia irrigated continuously for 1–2 hours with 0·6–1·0 μM diazoxon solution.

estimate was tested by direct measurement; also the individual contributions of such processes as penetration of the cuticle, sorption and detoxication in determining what proportion of externally applied diazoxon ultimately reaches the central nervous system were examined. Table 2 summarises the results.

Microchemical techniques were used to assess the rate diazoxon penetrated into the insect, by measuring its loss from the surface of the cuticle



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after topical application. These techniques were also used to measure what proportion of the amount that entered was decomposed, absorbed by the tissues or circulated in the haemolymph. Penetration of diazoxon applied to the metathoracic sternum of adult male cockroaches is directly proportional to the elapsed time, and about three-quarters of an LD90 (2·6 μg) penetrated after 2 hours. The amount within the insect increases to about one-fifth the applied dose 1 hour after application and declines to about one-twelfth after 2 hours. About two-fifths of the amount within is sorbed on solids and, allowing for this, the maximum concentration attained in the total body fluids was calculated to be 1·4 μM, which is equivalent to about one-eighth the applied dose. The time–concentration curve of diazoxon in the haemolymph of individual cockroaches treated with an LD90 followed a course similar to that for total diazoxon; the concentration reached a maximum 1½ hours after treatment, when it ranged from 0·9 to 3·4 μM, with a "median" value of 1·8 μM. The close relationship between concentration in haemolymph and in total body fluids suggests that they are in approximate equilibrium.

By irrigating cockroach nerve cords *in vitro* with a range of concentrations of diazoxon in saline, while observing symptoms of poisoning in the metathoracic ganglia by an electro-physiological technique, a curve was constructed relating concentration of diazoxon to time taken to damage nerve function irreversibly. By using the same electrophysiological technique to observe the condition of ganglia in cockroaches treated topically at known times previously with LD90s of diazoxon, the concentration of diazoxon in the haemolymph when the ganglia were irreversibly damaged could be estimated from the *in vitro* time–concentration curve. Irreversible damage first occurred 1–2 hours after treatment with an LD90, and the concentration required to produce the same stage of poisoning in exposed cords *in vitro* in the same time (0·6–1·0 μM) is similar to the "median" concentration (1·8 μM) found by the chemical assay in the haemolymph of similarly treated cockroaches. The close agreement between the chemical and biological estimates suggests that diazoxon invades the nerve cord from the haemolymph, and that it acts directly, rather than as a metabolite or by the release of a neuroactive material. (Lord and Burt)

**Action of pyrethrum and synergised pyrethrum on the nervous system of *Periplaneta americana* L.** Further work confirmed that the time taken by pyrethrum to block nerve conduction in nerve cords from the cockroach *P. americana* is shortened by adding sesamex or piperonyl butoxide to the pyrethrum. Nerve cords were exposed by dissecting the isolated abdomens of male cockroaches. One of the cercal nerves was stimulated at intervals of 1 second by a pair of electrodes inserted into the cercus, and the propagated response, recorded from the abdominal cord by a second electrode pair, was observed on a cathode-ray oscilloscope. The sixth abdominal ganglion was irrigated continuously with saline containing the compounds to be tested and the time taken to block all propagated nerve potentials noted.

The time taken by pyrethrum to block conduction when applied alone at a concentration of $3{\cdot}2 \times 10^{-6} M$ (active ingredients) was shortened by



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from 10%, when sesamex was added to the pyrethrum at a molecular ratio of 0·03 : 1, to 75% at 300 : 1. The blocking times with sesamex–pyrethrum mixtures expressed as percentages of the blocking time with pyrethrum alone are approximately proportional to the logarithms of the molar ratios of sesamex to pyrethrum in the mixtures.

The toxicity to whole cockroaches from the same population of pyrethrum and sesamex–pyrethrum mixtures was tested by applying measured drops of the mixtures in acetone to adult males. Large ratios of sesamex to pyrethrum (30 : 1 and 300 : 1) decreased the LD50 of pyrethrum alone by the same proportion as blocking time was shortened by the same ratios applied directly to nerve cords. Smaller ratios decreased the LD50 less than they shortened the blocking time.

Piperonyl butoxide, tested with pyrethrum in the same way but over a smaller range of molecular ratios, gave somewhat similar results; it synergised pyrethrum less than sesamex did, either against whole insects or when applied to nerve cords. (Burt and Goodchild)

**Effects of anoxia on nerve conduction in *Periplaneta americana* L.** To try to decide whether anoxia, a condition that may be created in insects by insecticides, kills insects primarily by damaging their nervous systems, the effects of anoxia on the nervous system of the cockroach *P. americana* were studied in experiments of two kinds. In some, beheaded adult males were dissected to expose their nerve cords from the sixth abdominal ganglion to the metathoracic ganglion. These preparations were then exposed to atmospheres of $N_2$ and $CO_2$ free from $O_2$ and treated with water vapour. Before and after treatment, nerve conduction in several regions of the nervous system was tested by conventional electrophysiological procedures. In other experiments intact cockroaches were kept in atmospheres of $CO_2$ or $N_2$ before their nerve cords were exposed and tested in a similar way. $CO_2$ and $N_2$ had similar effects, so results with the two cannot be distinguished.

The amplitude of action potentials of exposed nerve axons deprived of oxygen for short periods slowly decreased until conduction was completely blocked 5–10 minutes after anoxia began. Recovery of normal function was rapid when air was readmitted and complete in 2–6 minutes. In the sixth abdominal ganglion, conduction was blocked sooner (1–3 minutes) and recovered more slowly (4–10 minutes); just before block there was a transient burst of violent spontaneous activity, similar to the burst preceding block during poisoning by organophosphorus insecticides. All units tested seemed to recover completely from these short periods of anoxia.

Axons of the cercal nerve seemed unaffected by being anoxic for 10 hours, and conducted normally when $O_2$ was restored, whereas conduction through the sixth abdominal ganglion always failed, and it usually failed in the giant fibre pathway through the metathoracic ganglion. However, in some preparations, anoxic for 7 hours, all pathways tested were normal.

When the condition of nerves in cockroaches deprived of $O_2$ for 10 hours was examined, conduction in the cercal nerves and through the metathoracic ganglia of nearly all insects was normal, but conduction



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through the sixth abdominal ganglia was impaired to some extent in half of the preparations, though normal in the other half. Stimulating the crural nerve always failed to cause the leg muscles to contract. All regions of the nervous system tested in cockroaches deprived of $O_2$ for 24 hours either functioned abnormally or failed to function. The period of anoxia necessary to kill cockroaches was determined. All recovered from 5 hours of anoxia, but died after 10 hours anoxia.

Many units of the cockroach nervous system seem resistant to damage from anoxia, for even in cockroaches fatally injured by anoxia many units produced propagated action potentials. The primary fatal lesion caused by anoxia may therefore lie outside the nervous system. The lack of response in the leg muscles of cockroaches fatally exposed to anoxia when their crural nerves were stimulated suggests that the neuromuscular junctions or the muscles themselves may be more susceptible than the nervous system to anoxia. However, we tested only a few of the units in the nervous system, so susceptible units within it may have been irreversibly damaged at an earlier stage. The fact that units within the sixth abdominal ganglion failed to conduct while axons outside it still did so, does suggest that units differ in their susceptibility. The similar actions of $CO_2$ and $N_2$ suggest that $CO_2$ affects the nervous system by causing anoxia and not by any direct effect of the $CO_2$ molecule itself, such as a decrease in pH. (Burt)

**Structure of the central nervous system of the cockroach *Periplaneta americana*.** The distribution of cholinesterase in the central nervous system of *P. americana* is being studied histochemically, as a basis for investigating local cholinesterase inhibition in the central nervous system by organophosphates (*Rothamsted Report* for 1966, p. 168). To deduce how inhibition affects function requires more detailed knowledge about the structure of the central nervous system than exists, so a histological study of the central nervous system became necessary. The thoracic ganglia were examined first, using serial paraffin sections stained by the Bodian Protargol, Blest–Holmes silver nitrate or Wigglesworth osmium-ethyl gallate methods.

The three thoracic ganglia are similar in general structure, but differ in detail. In the mesothoracic ganglion studied most intensively so far, the central neuropile contains seven pairs of major longitudinal nerve tracts, ten transverse commissural tracts, linking the two halves of the ganglion, and various oblique and dorsoventral tracts. The paths of the bundles of motor and sensory fibres forming the roots of the seven pairs of segmental nerves and single median nerve were traced into the neuropile. The motor and association neuron-cell bodies lie outside the neuropile in the surrounding glial cell layer, and are arranged in seven unpaired median and 20 paired ventral and lateral groups. The courses of their axon bundles passing into the neuropile were traced, and some of the motor fibres followed to the nerves in which they leave the ganglion. (Gregory)

**Glandular secretions of the cotton stainer *Dysdercus intermedius* Dist.** Earlier work showed that the cotton stainer produces substances of which some cause a population to aggregate and others to disperse.



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These substances might be used to control this pest, so they were further studied. The larvae possess three dorsal abdominal scent glands. Secretion from the two anterior glands was less complex than from the posterior gland when analysed by gas chromatography. The main component was identified as *n*-tetradecane, from its mass spectrum.

The posterior gland secretion was analysed by combined gas chromatography–mass spectrometry. Eight components which usually comprise 99·9% or more of the total were identified: dodecane, tridecane, pentadecane, hexanal, hex-2-en-1-al, 4-ketohex-2-en-1-al, oct-2-en-1-al, 4-keto-oct-2-en-1-al. The composition of the secretion differs from one individual to another. Oct-2-en-1-al is often the main component (more than 40%), and together with 4-ketohexenal, 4-keto-octenal and tridecane comprises 95% of the total. All these compounds have previously been identified in arthropod defensive secretions, but the composition of the *Dysdercus* material is unique. Aggregations of *Dysdercus* are dispersed by the whole secretion and the two aldehydes tested (hexanal and hexenal), but not by the hydrocarbons. The whole secretion is more effective. This mechanism probably protects most individuals in a group from attack by a predator. (Calam)

## Persistence and toxicity of chlorohydrocarbon insecticides

**Effect of environment on the persistence of dieldrin crystals.** Further work on the rates of dieldrin crystals (labelled with radioactive $Cl^{36}$ as a tracer) in deposits of different densities volatilise from glass and cotton-leaf surfaces in still air at 20° C confirmed previous conclusions on the form of the volatilisation curve. The rate is steep and almost linear with large deposits (*ca.* 10 μg/cm²), but is approximately halved with deposits about 3 μg/cm². At around 1 μg/cm² the curve becomes very flat; this flattening occurs with larger deposits on leaf surfaces than on glass, and finally the curve becomes asymptotic to the time axis.

**Effect of formulation on persistence of deposits of DDT on foliage.** How formulation affects the removal of deposits by rain washing was further tested. Using a washing period of 5 minutes instead of 30 seconds con-

TABLE 3

*Amounts (μg/cm²) of DDT removed by "rainwashing" (measured from zero time)*

| | Cotton leaf | | Glass | |
|---|---|---|---|---|
| Time (seconds) | Std W.P. | 5% "LOVO"-W.P. | Std W.P. | 5% "LOVO"-W.P. |
| 0 | 0 | 0 | 0 | 0 |
| 2 | — | — | 6·2 | 0·2 |
| 4 | 18·5 | 3·6 | 7·6 | 0·4 |
| 6 | — | — | 8·4 | 0·5 |
| 8 | 20·8 | 5·1 | — | — |
| 16 | 21·5 | 6·6 | — | — |
| 30 | 21·9 | 8·2 | 9·0 | 1·5 |
| Original amount of DDT in surface deposit (μg/cm²) | 22·8 | 18·3 | 9·3 | 12·0 |
| % washed off in 30 seconds | 96% | 45% | 97% | 13% |



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firmed last year's results that adding "LOVO 192" (a mixture of amine stearates) to wettable powder formulations increased "rainfastness". This happened with cotton leaves both on and off the plant, and with fresh and 6-week-old deposits of DDT. The formulations tested were 0·4% DDT WHO standard wettable powder and wettable powders containing 5% and 10% "LOVO 192".

Table 3 shows that the greater part of the DDT in the deposit from the standard wettable powder was washed off during the first 5 seconds, whereas release was much slower and more gradual from the formulations containing "LOVO 192".

**Formulation using microencapsulation.** "LOVO 192" not only affects the persistence of deposits when enough is added but also lessens the contact action of insecticide without affecting stomach-poison action, thus conferring some selectivity. Stomach-poison action was tested with cabbage white caterpillars (*Pieris brassicae*). Diminished contact activity was thought to be caused by a matrix of stearic acid forming around the DDT particles. Microencapsulation, a recently developed technique, seemed likely to be a more effective way of preventing contact activity, and might also remove repellency and perhaps prolong persistence. Microcapsules (ranging in diameter from 500 to 750 μ) of a kerosene solution containing 2·5% DDT and 0·5% BHC enveloped in hardened (cross-linked) gelatin were supplied by the National Cash Register Company, who originated the method for encapsulating dyes. In a standard bioassay neither they nor the residue from water in which capsules were boiled affected houseflies, but the contents of ruptured capsules were lethal to houseflies.

A stomach-poison test, using a leaf-sandwich method and cabbage white caterpillars (*Pieris brassicae*) of 4th instar and above, showed effects when the caterpillars were able to ingest the capsules, but the capsules were mostly too large.

A smaller size range, 370–600 μ diameter, was tested in a similar manner for stomach-poison action on cabbage white caterpillars and the larvae (4th instar and above) of mustard beetles (*Phaedon cochleariae*). Stomach-poison action was shown with both species.

Much smaller capsules are required if this technique is to be applicable to insects such as 1st instar codling moth, and experiments which include the effects of ageing and humidity are now in progress with 10–40 μ diameter capsules. (Phillips and Gillham)

## Pyrethrins and related compounds

**Relationship between structure and toxicity.** 5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate (I) in laboratory tests was more toxic than most other known insecticides to at least three insect species (*Rothamsted Report* for 1966). It, and related compounds, were studied further.

5-Phenyl-3-furylmethyl chrysanthemate (II), in which the benzyl side chain of (I) was replaced by a phenyl group, was less than $\frac{1}{500}$ as toxic as (I) to adult houseflies and $\frac{1}{125}$th as toxic to mustard beetles. This shows that, in this series of compounds, the methylene group in the side chain is an important feature for toxicity. By contrast, in the cyclopentenolone esters



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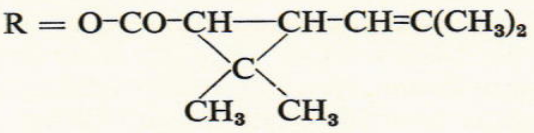
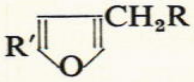
to which the natural pyrethrins belong, the phenyl compound (IV) was more toxic than the benzyl compound (III), and both were considerably toxic to houseflies.

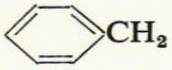
Compounds were examined in which the benzene ring of the benzyl side chain of (I) was modified. A methyl group in the *p*-position (V) diminished toxicity to mustard beetles to $\frac{1}{400}$ and to houseflies to $\frac{1}{4}$. With a *p*-methoxy group (VI), compounds were $\frac{1}{350}$ and $\frac{1}{5}$ as toxic to these two insects, and about half as toxic when the *p*-substituent was chlorine (compound (VII)).

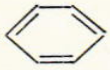
The new compounds mentioned were synthesised from the appropriate δ-substituted laevulic ester by the route described (*Rothamsted Report* for 1966).

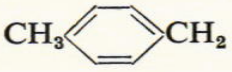
The nature and substituents of the ring to which the benzyl side chain and the $CH_2OH$ group were attached were also important. 5-Benzylfurfuryl chrysanthemate was 10–20 times less toxic than 5-benzyl-3-furylmethyl chrysanthemate and a 2-methyl group adjacent to the $CH_2O$ group in the alcoholic portion also lessened toxicity (*Rothamsted Report* for 1966). The oxazole (VIII) was less than $\frac{1}{100}$ as toxic as the related furan compound (I). The alcohol for this ester was synthesised by reduction (lithium aluminium hydride) of the known corresponding oxazole ester.

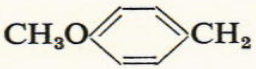


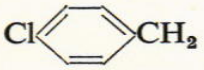


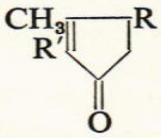
I R′ = 

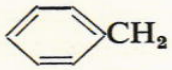
II R′ = 

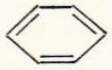
V R′ = 

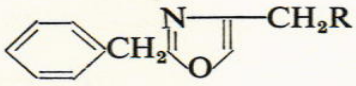
VI R′ = 

VII R′ = 



III R′ = 

IV R′ = 

VIII 

To investigate the reason for the great toxicity of 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate, the action on houseflies with and without the synergist sesamex was examined. One mode of action of synergists is considered to involve inhibition of the mechanism by which insects detoxify insecticides. A dose of 2 μg/fly of sesamex, applied 3 hours before the insecticide, produced the greatest synergistic effect, but did not otherwise affect the fly. This quantity of sesamex decreased the LD50 of pyrethrum from 0·35 to 0·0017 μg/insect (see Table 4), a synergistic factor of 200. Under the same conditions, the synergistic factor for 5-benzyl-3-furyl-



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methyl (+)-*trans*-chrysanthemate was only 7 (LD50 from 0·0079 to 0·0011). This indicates that both pyrethrins and benzylfurylmethyl chrysanthemate owe their toxicity to similar structures but that the pyrethrins are more readily detoxified. (Elliott, Janes, Pearson, Farnham and Needham)

**TABLE 4**

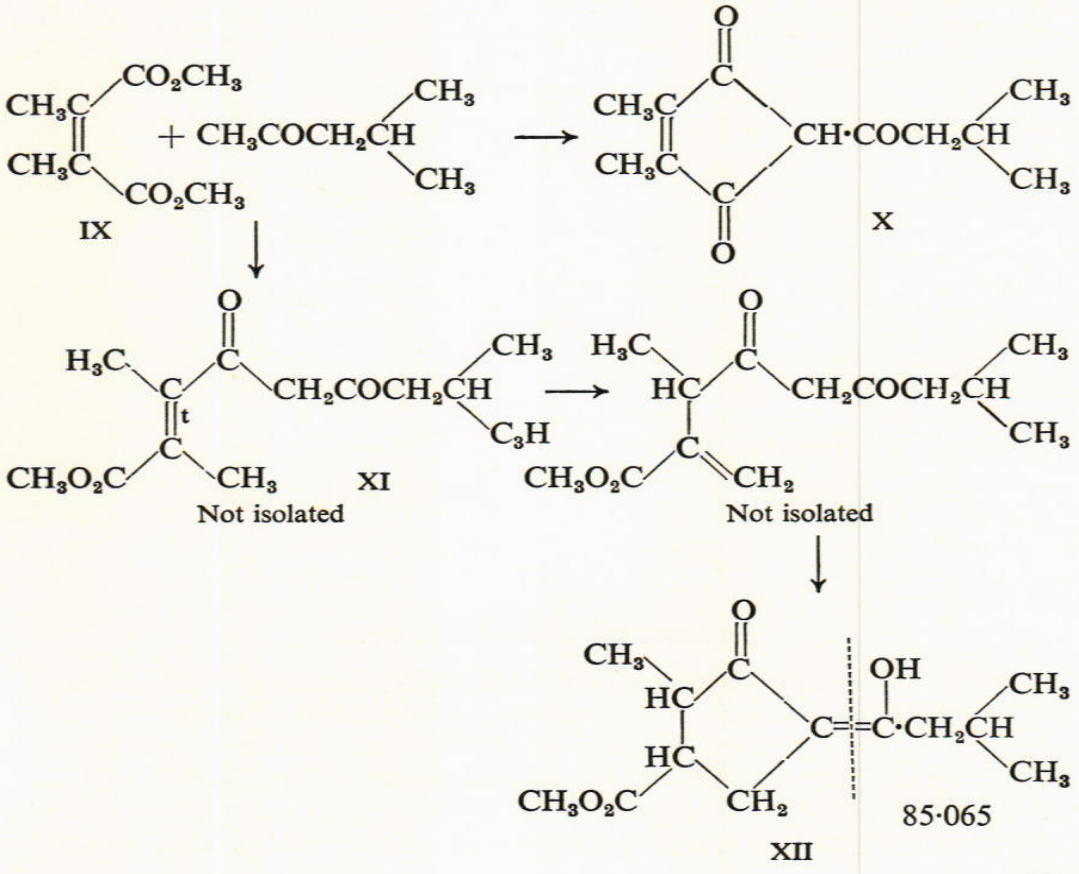
*Synergism, houseflies: LD50 values (μg/♀ fly)—topical application, acetone*

| | Compound alone | After Pretreatment with 2 μg sesamex | Synergistic factor |
|---|---|---|---|
| Pyrethrins | 0·35 | 0·0017 | 200 |
| Allethrin | 0·50 | 0·0082 | 60 |
| 5-Benzyl-3-furylmethyl (+)-*trans* chrysanthemate | 0·0079 | 0·0011 | 7 |
| 5-Benzyl-3-furylmethyl (±)-*cis-trans* chrysanthemate | 0·016 | 0·0013 | 12 |
| 5-Benzyl-3-furylmethyl (+)-*trans* pyrethrate | 0·028 | 0·0026 | 10 |

## Organic Chemistry

The use of physical methods to determine structures of organic compounds is illustrated by work with two compounds, both side products isolated during work on the chemistry of various insecticides.

1. Calythrone (X), a natural product of which a specimen was required



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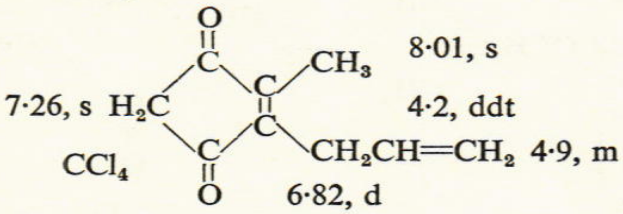
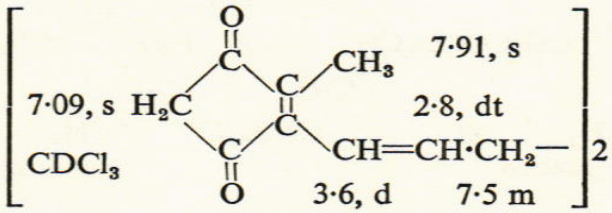
for biological examination, was synthesised by condensing dimethyl dimethyl maleate (IX) with isobutyl methyl ketone in the presence of sodium hydride (*Rothamsted Report* for 1961). The major product was a 1,3-diketone (copper chelate) at first considered to be (XI) because it did not cyclise to calythrone (X) with more sodium hydride. NMR evidence proved structure (XI) incorrect (absence of olefinic $CH_3$) and indicated a cyclic product (presence of $CH_3CH$ and $CH_2$ with no protons on adjacent Cs) in the enolic form shown (no H on C=C). Mass spectral evidence proved structure (XII) correct, and distinguished it from 15 other possible structures that can be formulated for condensation products of dimethyl dimethyl maleate and isobutyl methyl ketone, with subsequent internal Michael addition (parent ion, 240; $C_{13}H_{20}O_4 = 240$; fragment of mass 85·067 by accurate mass measurement under high-resolution conditions: calculated for $C_5H_9O$, 85·065).

2. Cyclopentenolones such as allethrolone are readily oxidised to cyclopentenediones, e.g. allethredione (XIII) by activated manganese dioxide (*Rothamsted Report* for 1960). A side product obtained in *ca.* 1% yield was shown to be (XIV) by the following evidence: (*a*) molecular weight (mass spectrum) = 298, i.e. (2 × allethredione-2H); (*b*) NMR evidence (on formulae) showed the cyclopentenedione ring unchanged, but detected a profound alteration in the side-chain consistent with the structure shown; (*c*) these allocations were confirmed by the collapse of the multiplet at 7·5$\tau$ to a singlet on irradiation at 2·8$\tau$, analogous to the behaviour of the multiplet due to the non-olefinic protons in biallyl $(CH_2{=}CH{\cdot}CH_2)_2$. (Elliott, Janes and Jeffs)




XIII




XIV

## Toxicity of pesticides to bees

**Poisoning of honeybees in the field.** Fifty-three samples of honeybees (*Apis mellifera*), alleged to be poisoned, were received from the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food, 22 more than last year. Of the 37 containing insecticide, 33 had organophosphates. Information supplied with these samples showed that 20 of them were affected by spraying bean crops, nine of which were sprayed



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from aircraft. Information on two of the other samples indicated that they were caused by spraying peas, on one occasion from the air. Two further samples were also attributed to aerial spraying of unknown crops. Another contained dieldrin in addition to an organosphosphate.

No information was received about one sample that contained BHC, one that contained dieldrin and aldrin, and a third containing BHC and dieldrin. A fourth sample containing BHC and dieldrin was attributed to malicious poisoning.

The increase in number of organophosphate poisonings probably reflects the large aphid infestation on field beans, which led to sprays being applied while the plants were flowering. Table 3 on p. 197 shows numbers of aphids caught in the Entomology Department's suction traps; catches were larger than usual at Broom's Barn Experimental Station, which is in the part of eastern England where poisoning of bees by bean spraying is most common.

Only one sample of bees containing organophosphate seemed to have been caused by spraying oil-seed rape, despite the increased acreage of this crop. (Needham and Stevenson)

**Toxicity of dimethoate to five strains of honeybee.** To see whether strains of honeybee differ in their susceptibilities to insecticides, the toxicity of dimethoate to workers of five strains of honeybee, kindly supplied by Brother Adam of Buckfast Abbey, and to our Harpenden bees was compared. Brother Adam's bees (Pure Buckfast, Pure Greek, Hybrid Saharan, Hybrid Carniolian and Hybrid Anatolian) were all affected similarly by dimethoate, and none was significantly more resistant or more susceptible to the insecticide than our bees. (Stevenson)

**Poisoning of honeybees on oil-seed rape.** The acreage of oil-seed rape in the United Kingdom increased from 5000 in 1966 to 30 000 in 1967, and as a break crop between cereals may increase further. Although J. B. Free and P. M. Nuttall found that bees are not important as pollinators of the crop, they are greatly attracted to the flowers. The recommended use of malathion at yellow-bud stage to control pollen beetles (*Meligethes* spp.), therefore, is a serious potential danger to bees. Because it is active for less than a day, malathion is also not an ideal insecticide for the purpose. Hence we compared the effects on honeybees of applying three insecticide formulations to spring-sown oil-seed rape in flower: malathion because it is recommended; azinphos-methyl because its use on rape was currently being investigated by the National Agricultural Advisory Service, and it is slightly more persistent than malathion; endosulfan because it is used extensively on rape in northern Europe and is said to do little harm to bees.

The comparison was made on two adjacent farms near Salisbury, where about 150 acres of rape were being grown in three fields. Approximately 10 acres of rape were sprayed with each insecticide. Azinphos-methyl was sprayed in one field, and malathion and endosulfan were applied to opposite ends of a 100-acre field. A further field was left unsprayed as a control. Five colonies of bees were placed adjacent to each area one day before spraying, and bees were working the crop well when the insecticides were



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applied. Dead bees were collected in a trap (Free, Needham, Racey and Stevenson, *J. Sci. Fd Agric.* (1967) *18*, 133) placed in front of each hive for 28 days after the spraying, with results shown in Table 5. The endosulfan wettable powder killed very few bees, whereas malathion and azinphos-methyl killed many.

**TABLE 5**

*Dead bees collected from hives at the experimental insecticide spraying of oil-seed rape (average numbers of dead bees per hive per day)*

| Date | Control | Azinphos-methyl* | Malathion† | Endosulfan‡ |
|---|---|---|---|---|
| 26 June 1967 | 4·6 | 16·4 | 23·8 | 35·8 |
| 27 June 1967 | | Spray application | | |
| 28 June 1967 (10.00 a.m.) | 9·4 | 780·8 | 1557·8 | 67·2 |
| 29 June 1967 | 2·4 | 30·0 | 8·2 | 1·8 |
| 30 June 1967 | 0·8 | 9·4 | 0·6 | 1·2 |
| 1 July to 3 July 1967 | 0·3 | 5·4 | 0·4 | 0·2 |
| 4 July to 5 July 1967 | 0·5 | 9·2 | 0·3 | 0·4 |
| 6 July to 10 July 1967 | 0·2 | 1·5 | 0·6 | 8·6 |
| 11 July to 25 July 1967 | 0·6 | 6·4 | 2·1 | 21·5 |

* 30 fl oz of 22% azinphos-methyl emulsifiable concentrate in 20 gal water per acre.
† 30 fl oz of 60% malathion emulsifiable concentrate in 20 gal water per acre.
‡ 1·3 lb of 35% endosulfan wettable powder in 20 gal water per acre.

Because of a heavy infestation of pollen beetles, all the fields were sprayed with malathion 7 days before the experimental treatments were applied. This reason made it impossible to obtain reliable information on the control of pollen beetles achieved with azinphos-methyl and endosulfan, but counts of larvae, and of the few remaining adults, indicated that endosulfan was effective. (Needham and Stevenson)

**Secretion of systemic insecticides into nectar.** Dimethoate and phorate were applied to fuchsia (Glasshouse c.v. "Duchess of Albany") and nasturtium (*Tropaeolium* dwarf variety), chosen as test plants because of their copious nectar flow. The plants were grown in pots containing vermiculite or soil-less compost, to which the insecticides were applied. Bioassays with nectar gave variable results and were too insensitive a test, so gas–liquid chromatography was used to estimate the amounts in the nectar. Six days after treatment with 50 ml of water containing 25 mg dimethoate the nectar of nasturtium contained 741 $\pm$ 259 ng/ml (parts per thousand million), and the nectar of fuchsia contained 2890 $\pm$ 550 ng/ml (1 ng = $10^{-9}$ g). With phorate used similarly, nectar from fuchsia contained only 100 ng/ml, which was too little to measure accurately. Much more dimethoate than phorate was also recovered in the nectar of field beans sprayed with the two insecticides. The concentration in the nectar of field beans depended on the amount sprayed on the plants and time since spraying; amount in the nectar increased with increase in the dose applied, and was greatest 4 days after treatment. With a dose similar to that used in the field, the maximum concentration in the nectaries was 0·5 ppm. (Lord and Stevenson)



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## Apparatus and techniques

Both the automatic and hand-operated micro-applicators developed in the department are now commercially produced. An adaptor, which allows a larger syringe to be fitted, gives a $\times 10$ increase to all drop sizes, and has proved satisfactory for both instruments.

Two provisional patents were filed by the National Research Development Corporation to protect apparatus developed in the department; one relates to Atomiser Heads and the other to a Variable Sieve. The development of the Atomiser Heads is a direct result of work involving the entrainment of particles into the flow streams of compressible and incompressible fluids. The Patent Specification embodies several forms of atomiser heads, one designed to give a continuous linear band of atomiser droplets. The simplicity of the design suggests the possibility of production by moulding, etching or pressure die-casting, as in making some fluidic devices.

The Variable Sieve was developed to separate from random samples of dead insects fractions according to the smallest cross-sectional dimensions of their bodies. It will probably allow samples to be sorted in a continuous-flow system, instead of by a batch process as at present.

Work on methods of counting trapped insects was postponed until the value of the variable sieve is established, as this may greatly influence the sophistication of the counting equipment.

A prototype primary-sorting apparatus was passed to the Entomology Department for evaluation. Present evidence suggests that its main value is in speeding the extraction of Thrips from the main catch, cleaning dirty catches and presenting fractions that are more readily identified and counted than would otherwise be possible.

A versatile gas–liquid valve was developed to permit the fast full-flow switching of particulate material from one channel to another and the simultaneous or phased switching of a gas or liquid at gauge pressures up to 60 lb in$^{-2}$. (Arnold)

## Systemic insecticides

Work on soil factors affecting uptake of organophosphorus insecticides by plants continued.

**Sorption by soil.** The sorption of dimethoate and menazon was studied using similar equilibration methods to those used with disulfoton and phorate (see *Rothamsted Report* for 1965, p. 168). Progress with dimethoate was slow because of difficulties with analysis, but a satisfactory technique using solvent extraction and gas–liquid chromatography was devised.

Menazon was determined polarographically using a Davis Differential Cathode-ray Polarograph. So far, 0·1*N*-HCl has proved a suitable supporting electrolyte for analysing aqueous solutions and soil extracts.

Few soils have been examined, but results show that dimethoate and menazon are much less strongly sorbed than disulfoton and phorate. The



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large differences could significantly affect behaviour in the field. So little dimethoate is sorbed that sorption is difficult to measure and soil/solution ratios have had to be larger than for the other insecticides. As with disulfoton and phorate, isotherms so far determined for both menazon and dimethoate are approximately linear. As examples, the slopes of these isotherms (expressed as ppm insecticide in soil/ppm insecticide in solution) were 0·15 for dimethoate and 2·2 for menazon with Woburn soil. (Graham-Bryce)

**Diffusion of organophosphorus insecticides in soils.** A knowledge of diffusion rates is necessary to estimate how far an insecticide moves from where it is added to the soil, and to decide whether transport to roots limits uptake by plants. We therefore measured diffusion of disulfoton and dimethoate, chosen because they have contrasting physical properties and affinities for soil. Apparent diffusion coefficients were measured by a method modified from that used by Phillips and Brown (*J. Soil Sci.* (1966), **17**, 290). Soil is packed into a brass cylinder so that initially half is uniformly mixed with insecticide and the other half contains no insecticide. After a given time the movement by diffusion is found by measuring the amount of insecticide in narrow transverse sections of the soil column. The apparent diffusion coefficient at any concentration is obtained by inserting quantities obtained from the distribution curve into the appropriate solution of the differential equation expressing Fick's law of diffusion. In Woburn soil at 20° C and 11% w/w moisture content, average apparent diffusion coefficients were $2{\cdot}62 \times 10^{-8}$ cm²/s for disulfoton and $3{\cdot}14 \times 10^{-7}$ cm²/s for dimethoate. Disulfoton diffuses less because more of it is sorbed by soil. (Graham-Bryce)

**Uptake of organophosphorus insecticides by plants.** Field trials suggested that the relative effectiveness of different insecticides depended on soil moisture (*Rothamsted Report* for 1966, p. 178), so the effects of soil moisture content on their uptake by plants were studied in experiments where other factors that could influence effectiveness were kept constant. Wheat was grown for 4 weeks in constant-environment rooms in pots containing soil mixed uniformly with different quantities of P-32 labelled dimethoate and disulfoton. Soil moisture was controlled by the osmotic method described in last year's report (pp. 178–179). The pots, made from dialysis tubing supported inside polythene pipe, were placed in tanks of polyethylene glycol solution, which controls the potential at which water enters the soil through the membrane. At frequent intervals the toxicity of the foliage was tested by bioassay with caged aphids (*Rhopalosiphum padi*) and plants were also removed to measure the radioactivity of extracts.

Dimethoate was more effective than disulfoton in killing aphids, although its potency decreased significantly during the 28 days of the experiment, and the potency of disulfoton did not. Changing the moisture content of soil had little effect, but statistical analysis of all the results shows that the effectiveness of disulfoton increased significantly with increasing moisture content, and the effectiveness of dimethoate decreased.



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This is contrary to conclusions drawn from the previous field experiments, which suggested that diffusion of dimethoate and menazon through soil to plant roots depended more on soil water than did diffusion of the more volatile phorate and disulfoton, which can move as vapour through air-filled pores in dry soil.

To try to account for the results in the pots, and to see whether the rate of movement through soil to the roots limited uptake, the maximum possible quantities that could move were calculated and compared with the measured uptake of P-32. Insecticide can reach the root by mass flow in the soil solution moving to the root in response to transpiration by the plant, and by diffusion. Quantities carried by mass flow were calculated from the product of solution concentration (obtained from the adsorption isotherm) and the quantity of water transpired (estimated from the weight of plant produced). Quantities that could move by diffusion were calculated by inserting values for the diffusion coefficient into mathematical solutions of Fick's law that apply for diffusion to a model cylindrical root. The calculations indicated that, at the intermediate moisture content, considerably more insecticide could have moved to the root than was taken up, so that uptake was probably limited by processes in the plant and not by movement to the roots. The results in the pots can then be partly accounted for by the concentration in the soil solution at the roots. More dimethoate would be taken up from dry than wet soil, because dimethoate is very weakly sorbed, and the concentration in the soil solution increases as the soil becomes drier. Disulfoton, by contrast, was less effective than dimethoate because it is more strongly sorbed, and so was less concentrated in the solution at the roots. This concentration is almost independent of moisture content, so that disulfoton is not influenced in the same way as dimethoate. (Graham-Bryce and Etheridge)

**Field experiments.** The effect of moisture on the movement in soils and uptake by plants of organophosphorus insecticides was further studied in irrigated plots of potatoes at Woburn. Disulfoton, phorate, dimethoate and menazon were applied as granules at the equivalent of 3 lb a.i./acre in the furrows below potato tubers (Maris Piper) at the time of planting (27 April 1967).

Four different moisture regimes were established as follows:

1. Covered plots receiving neither rain nor irrigation.
2. Plots receiving rain only.
3. Plots receiving rain and irrigation early.
4. Plots receiving rain and irrigation throughout the season.

The total water supplied to the treatments during the experimental period was: covered plots, nil; rain only, 7·8 in., early irrigation, 10·9 in. and fully irrigated, 17·3 in. Soil-moisture contents, determined frequently, gave the following average values during the experiment: covered plots, 5·3% w/w, rain only, 7·9% w/w, and fully irrigated, 9·5% w/w. The toxicity of the plants was assessed regularly by counting natural aphid populations and by confining aphids (*Myzus persicae*) on leaves in the field.

Both bioassay and natural population counts showed that disulfoton



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and phorate were very effective and persisted well throughout the season at all soil moistures. Dimethoate and menazon were initially as potent as disulfoton and phorate, but soon became less so. The degree of control obtained is well illustrated by the natural aphid populations, which at the time of maximum infestation (11 July) on the rain-only plots were: disulfoton, 0 aphids/100 leaves; phorate, 10 aphids/100 leaves; dimethoate, 60 aphids/100 leaves; menazon, 60 aphids/100 leaves; and without insecticide, 2220 aphids/100 leaves.

Soil moisture had little effect on the behaviour of the insecticides. With disulfoton and phorate there was no significant effect, except in the final test with disulfoton, when plants on irrigated plots were significantly more toxic than on non-irrigated plots. With dimethoate and menazon, moisture had more influence, and they were significantly more effective on the wetter plots in several of the tests. Thus, these results support previous ones in the field and differ in two main ways from those from the pot experiments. First, dimethoate was less effective than disulfoton; secondly, increasing soil moisture increased the effectiveness of dimethoate. Possibly uptake by plants in the field, unlike those in pots, is limited by the amount reaching the roots, and this is greater with disulfoton than dimethoate. However, our measurements show that, although soil water affects diffusion of dimethoate more than of disulfoton, dimethoate diffuses faster, even in dry soil, because it is less strongly sorbed. Dimethoate is probably less effective because it decomposes quicker (as suggested in the pot experiments), and much may have decomposed when the biological tests were done in the field. A smaller concentration of dimethoate than of disulfoton, combined with its greater dependence for diffusion on moisture, could then explain both differences between results in the pots and in the field. However, there are other differences between pot and field experiments, and further experiments are required to interpret the results with certainty. (Graham-Bryce and Etheridge)

**Control of aphids and virus diseases of lucerne.** Collaborative work is described in the report of the Entomology Department. (Etheridge)

**Insect rearing.** The following were reared during the year:

| | |
|---|---|
| | PLANT FEEDERS |
| Hemiptera | *Aphis fabae* Scop. |
| | *Myzus persicae* (Sulz.) |
| | *Dysdercus intermedius* Distant |
| Coleoptera | *Phaedon cochleariae* (F.) |
| | OTHERS |
| Orthoptera | *Blaberus discoidalis* (L.) |
| | *Periplaneta americana* (L.) |
| Lepidoptera | *Pieris brassicae* (L.) |
| Coleoptera | *Tribolium castaneum* (Herbst.) |
| | *Tribolium confusum* J. du V. |
| | *Trogoderma granarium* Everts |



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Diptera

*Drosophila melanogaster* (Meig.)
and a wingless mutant
*Musca domestica* L.
Strains. *bwb; ocra; ar; ac* SRS
SKA (diazinon-selected)
R2; *ocra; ar; ac* (R2 penetration factor)
*bwb;* R3; *ar*; *ac* (R3 sesamex susceptible factor)
*bwb*; *ocra*; *a* DDT-ase: *ac* (low ali-esterase and DDT-dehydrochlorinase)
*bwb*; *ocra*; DR4; *ar*; *ac* (dieldrin resistant)
*organotin*; *stw* (homozygous for R2)
213b (pyrethrum resistant)

**Commercial seed-dressings.** A survey of samples from 38 merchants of seed dressed with BHC powder to control wheat-bulb fly made by some commercial firms and the Plant Pathology Laboratory of the Ministry of Agriculture, Fisheries and Food confirmed what we reported last year, that seeds often carry much less insecticide than they should.

In collaboration with the Plant Pathology Laboratory and the National Institute of Agricultural Engineering, the reasons for this are being sought.

The peformance of two commercial processes of applying liquid seed-dressings of aldrin and γ-BHC was tested. Because liquid dressings seem to penetrate seeds more deeply than dry powder dressings, the procedure for recovering insecticides had to be modified. Extracting by standing seeds for 24 hours in a 1:1 acetone:hexane mixture recovered approximately 95%. In addition to seed dressed by commercial methods, seed dressed in the laboratory with carbophenothion or chlorfenvinphos was also analysed.

**TABLE 6**

*Comparisons of commercially and laboratory applied liquid seed dressings*

| Method of treatment | Insecticide | μg of insecticide per seed: Expected | Determined: Mean | Min. | Max. | Number of seeds with amounts: less than mean | more than mean |
|---|---|---|---|---|---|---|---|
| Commercial A | Aldrin | 40 | 36 | 3 | 315 | 46 | 11 |
| Commercial A | γ-BHC | 20 | 10 | 1 | 200 | 43 | 6 |
| Commercial B | Aldrin | 40 | 27 | 2 | 97 | 29 | 20 |
| Commercial B | γ-BHC | 20 | 8 | 2 | 84 | 41 | 9 |
| Laboratory | (25 seeds) carbophenothion | 60 | 83 | 25 | 165 | 13 | 12 |
| Laboratory | (25 seeds) chlorfenvinphos | 74 | 57 | 30 | 115 | 14 | 11 |

Table 6, from analyses of 50 single seeds from each commercial sample and of 25 from each laboratory sample, shows that the insecticide was very unevenly distributed, especially on the commercially dressed samples, of which a small percentage of seeds contained a lot and most had much less than the average amount. (Lord, Jeffs with Mr. R. J. Tuppen, M.A.F.F.)

**Feeding and reproduction of aphids.** Studies on the feeding and reproduction of economically important aphid pests continued (see *Rothamsted Report* for 1964, p. 299). Special attention was given to *Myzus persicae*,



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the peach-potato aphid, a ubiquitous pest that is the subject of world-wide study started this year as part of the International Biological Programme. It exists in clones that differ in various ways. Three of these are being studied. One is "resistant" to "Rogor" and two are "susceptible". They differ morphologically, and V. F. Eastop (British Museum (Natural History)) is collaborating in examining them to try to find better criteria for distinguishing than the morphological differences now known.

The clones also differ in their reproductive physiology. The resistant and susceptible forms have the same length of reproductive life and longevity, but the resistant have a much shorter post-reproductive life. Both are equally fecund, but the resistant ones reproduce faster in their early reproductive period, develop faster in the larval stage and are larger as adults. The resistant form seems generally more metabolically active. The difference in susceptibility of the two strains to dimethoate applied topically differed between tests from approximately 12 to 160 times. These differences may be because it is difficult to compare individuals of the same age and size when the two strains have different reproductive characteristics. Selection for resistance and susceptibility is being attempted to produce uniformly "resistant" and "susceptible" clones. (Needham and Banks)

Aphids are usually regarded as feeding exclusively on plant sap, but evidence was obtained that, in some conditions, they feed as cannibals. Cannibalism among aphids was repeatedly observed in *Megoura viciae* (the vetch aphid), also in *Myzus persicae* and *Acrythosiphon pisum* (the pea aphid). Several records also exist of aphids feeding on other insects (especially insect eggs), not only in the laboratory conditions but in the open. Cannibalism seems to occur when plants deteriorate, so that sap is inadequate and the aphids starve. When feeding on other aphids, predatory aphids may have to suck, but the pressure of body liquids in the prey may assist feeding, just as the pressure of plant sap does. (Banks)

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

**Biological studies.** Further tests on the ability of aqueous extracts of plants to stop wheat-bulb fly larvae from moving confirmed that extracts of stems of Cappelle wheat were more effective than extracts from seeds, roots or leaves, and that extracts from oat plants were ineffective. The biologically active part of the wheat-stem extract was partially soluble in acetone and alcohols; methyl alcohol was the most efficient solvent and gave extracts that arrested larval movements when diluted 100 times. Attempts to isolate and identify the active component or components in the extract, by chromatography, have so far failed.

Egg laying by wheat-bulb fly was studied in cages with their bases divided into different coloured halves. Flies laid more eggs on black than on white surfaces, and when offered an alternative colour to black laid more eggs on brown and green.

In another series of tests one-half of a filter-paper forming the cage base was treated with an aqueous soil extract and the other with distilled water.



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Flies laid twice as many eggs over an area treated with an extract from a peat/sand compost as with water but showed no preference for an extract of soil from Stackyard Field. (Griffiths and Scott)

**Single-row trials of insecticides**

***Experimental insecticides.*** Eight experimental insecticides were compared with heptachlor and ethion standards in co-operative field trials with entomologists of the N.A.A.S. (Eastern Region and East Midlands Region). Insecticides formulated as 20% dusts from the technical materials were: "Aspon" (Tetra-*n*-propyl dithionopyrophosphate), dioxathion ("Delnav"), "Dursban" (*O*-*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), an experimental carbamate (3-*sec*-butylphenyl *N*-phenoxyacetyl-*N*-methyl carbamate), "Aphidan" (*S*-(ethylsulfinylmethyl) *O*,*O*-di*iso*propyl phosphorodithioate), ethoate-methyl ("Fitios") and P1973 (*S*-(*N*-methoxycarbonyl-*N*-methylcarbamoylmethyl) dimethyl phosphorothiolothionate). The other materials, which were supplied as manufacturer's formulations, included a 50% wettable powder of coumaphos, a 30% seed-dressing of ethion and a 40% seed-dressing of heptachlor. The insecticides were used for dressing Cappelle wheat at 0·1% and 0·5% active ingredient to weight of seeds, and the seeds were sown in rows 10 ft long on 15 November at a sandy loam site with 1·2 million eggs/acre, and on 4 January at a peaty loam site with 1·5 million eggs/acre.

Examinations of plants from the peaty loam site in early April showed that "Dursban" was the most promising new material; plants grown from seeds treated with "Dursban" at 0·1% and 0·5% had only 2% and 1% damaged shoots respectively, compared with 16% damaged shoots in the controls. On the sandy loam site "Dursban" damaged the young seedlings, but later the plants recovered to a large extent.

***γ-BHC seed-dressings.*** As a result of the work with commercial seed-dressings described in this report, it became apparent that many earlier results with γ-BHC seed-dressings were probably unreliable, because the amounts of active ingredient presumed to be on the seeds were not necessarily present. Experiments were therefore done to get information about phytotoxicity and insecticidal efficiency with known amounts of chemical checked by GLC analysis. Cappelle wheat, treated with a liquid fungicide, and dressed at 1 oz/bu, 2 oz/bu and 4 oz/bu with 40% γ-BHC ("Abol" seed-dressing) was sown in the same conditions as seed treated with the experimental insecticides—described above. GLC analysis showed that about 80% of each of the three dressings applied remained on the treated seeds. This amount was retained after sowing at the sandy loam site, but fell to 70% at the peaty loam site. The difference probably reflects different sowing techniques. The largest dressing damaged the seedlings, especially in the sandy loam. Seeds treated with 1 oz/bu and 2 oz/bu produced 15% and 8% damaged shoots respectively at both sites; whereas untreated seeds produced 32% damaged shoots at the peaty loam site and 58% at the sandy loam site.

A similar trial was started in the autumn of 1967 with seed-dressings of ¼, ½, 1, 2 and 4 oz/bu. Analysis of the seeds showed a similar retention of



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seed-dressing, with 80% remaining on the seeds after treating, but only 60–70% after sowing because of loss during drilling. (Griffiths and Scott)

**A field trial with three organophosphorus seed-dressings.** The persistence and biological efficiency of three organophosphorus insecticides were measured on small plots on a medium boulder clay site with 2 350 000 wheat-bulb fly eggs/acre. The chemicals tested had already shown promise as substitutes for the organochlorine compounds in controlling wheat-bulb fly. Cappelle wheat seeds were dressed at dosages similar to 2 oz/bu, using commercial formulations of 60% carbophenothion, 40% chlorfenvinphos or 67% ethion, all of which contained fungicide, or with fungicide alone (controls). The seeds were sown on 13 October, 9 November and 5 December in a replicated trial. Plant samples were taken at the time of attack on 9 February to extract and estimate insecticides, and further samples were taken when damage was most evident on 1 March to assess the biological effects of the treatments.

Plant samples for chemical analysis were divided into: (*a*) seeds; (*b*) roots with a small amount of adhering soil; (*c*) "bulbs". Crushed material was extracted with hexane/acetone and analysed by gas chromatography using an Aerograph phosphorus detector. With each of the three insecticides the amounts were similar in samples from all three sowing-dates showing that they persisted in the conditions of the experiment. About 10 times more insecticide was recovered from the seeds than from the roots, although these had more than the "bulbs", which usually yielded less than 1 μg insecticide/g bulb. The combined amounts recovered from seed, roots and bulbs were about 30% of the carbophenothion, 2% of the chlorfenvinphos and 15% of the ethion applied to the seed.

Despite the smaller amounts of chlorfenvinphos recovered, the numbers of healthy shoots, damaged shoots, live and dead larvae in March samples were similar with all three insecticides. The differences between plants from insecticide-treated seed and from untreated seed were greatest with the latest sowing, in which untreated seed gave plants with twice as many damaged shoots and only half as many healthy shoots as treated seed. Also, most dead larvae were found in the December sowings. Because of rain in November and December, sowing was uneven, so yields were taken only from plots sown in October. All seed treatments increased yields but not significantly, and experiments with large plots are needed to determine the commercial advantage of such treatments. (Griffiths and Scott)

## Control of wireworms (*Agriotes* spp.)

**Field trials with three organophosphorus insecticides.** In co-operation with the Entomology Department and the N.A.A.S. East Midlands Region, small-plot field trials were done on land ploughed from old grass where wireworms were plentiful. Trials were done in Yorkshire with spring barley, in Lincolnshire with potatoes and in Cambridgeshire with sugar beet. The insecticides were applied to the soil as sprays or granules, usually at 3·0 lb active ingredient/acre, before the crops were drilled; the organophosphorus insecticides were N2790 = "Dyfonate" (*O*-ethyl *S*-phenyl ethyl phos-



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phonodithioate) and phorate, both applied in granular form, and "Dursban" (*O*-*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) applied as a spray. Sprays of an organochlorine standard were also included, γ-BHC at 0·5 lb active ingredient/acre in the sugar-beet trial, and aldrin at 3·0 lb active ingredient/acre in the other two trials.

Wireworm attack was early enough to damage the barley and sugar-beet seedlings before they emerged, and significantly more plants emerged in the treated than in the untreated plots. Losses on the plots treated with organophosphorus insecticides were no greater than on aldrin-treated plots during this early period. In late June, 14 weeks after sowing, the barley on the untreated plots was obviously retarded by wireworm attack, but effects on yield were not meaningful because there was so much lodging.

In the potato trial treatment effects were assessed by examining the tubers for wireworm damage at harvest, 25 weeks after sowing. None of the organophosphorus insecticides rivalled aldrin in preventing damage. Aldrin-treated plots had only 4% damaged tubers, whereas there were 15% with "Dursban", 20% with phorate, 21% with N2790 and 27% without any insecticide.

The wireworm population was also decreased more by aldrin than by the organophosphates. Eight soil cores of 4 in. diameter and 8-in. deep were taken on 30 August 1967 from each plot in the barley experiment; those from untreated plots contained 25 wireworms; compared with 18 with "Dursban", 13 with N2790, 10 with phorate and 7 with aldrin. (Griffiths, Scott with Lofty, Entomology Department)

## Fungicides

Laboratory and field testing of formulations of fungicides to control blight (caused by *Phytophthora infestans*) on potato haulms and tubers, and trials of fungicides for possible control of cereal take-all (caused by *Ophiobolus graminis*) were continued. Most of the materials were kindly given by the makers.

### Laboratory tests

***Bioassay.*** Our bioassay method for testing formulations against blight of potato haulm (see previous *Reports*) was further improved. Leaflets are inoculated with suspensions of 100 000 zoospores/ml, instead of 5000 sporangia/ml. Although these suspensions are disproportionately concentrated, they are more readily made at the exact strength required, and they decrease day-to-day variations in results.

***Formulation.*** Fentin acetate and other organo-tin compounds can be formulated as stable colloidal dispersions by adding small volumes of solutions in alcohol, etc., to aqueous saponin solution. Fentin acetate, formulated in this way, was more than three times as effective in bioassays as the available wettable powder ("Brestan 60": Hoechst Chemicals Ltd.).



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***Apparent systemic action.*** We failed to repeat, either with Woburn soil or with a potting mixture rich in organic matter, the results obtained last year indicating that two organo-tin compounds (fentin acetate and decyltriphenylphosphonium bromochlorotriphenyl stannate) became systemically fungitoxic to *P. infestans* after applying them to the soil round King Edward plants in pots. In similar tests the following compounds were applied, in three equal successive doses, each corresponding to 0·07 g of metallic Sn per plant: fentin chloride and bis(triphenyltin) sulphide, tributyltin acetate and fluoride (all from Pure Chemicals Ltd.). *O-O*, diethyl phthalimidophosphonothioate ("M 2452": Dow Chemical Co. (U.K.) Ltd.), in three successive doses of 1 g a.i./plant, was also tested. None had any detectable systemic action.

However, another test, in which the following organo-tin compounds were applied in the same way, again at 0·07 g metallic Sn per plant on each of three occasions, gave evidence of the material becoming systemic: A, fentin acetate (Hoechst Chemicals Ltd.); B, bis(triphenyltin) sulphide, and C, fentin chloride (both from Pure Chemicals Ltd.); D, decyltriphenylphosphonium bromochlorotriphenyl stannate ("A36": C. H. Boehringer Sohn). One week after the third application leaflets and tubers were harvested; Dr. F. Vernon, Sheffield College of Technology, kindly undertook their analysis. Results for the leaflets, expressed as ppm of tin as triphenyltin at the time of analysis, were: from treatment A, 1 ppm; from treatments B, C and D, 1·5 ppm; and from untreated plants, $<0·05$ ppm. Results of tuber analyses are not yet available.

Thus, the systemic fungitoxic action of these organotin compounds is weak and erratic, even when very large amounts are applied to the soil, but a little organic tin does seem to be taken up into the haulms through the roots. (McIntosh)

***Effect of griseofulvin on insect larvae.*** Griseofulvin, at about 20 ppm, is reported to affect cuticle formation in mosquito larvae, and thereby to produce abnormal or short-lived larvae or adults. However, when we added eggs of rust-red flour beetles (*Tribolium castaneum*) to whole-meal flour impregnated with griseofulvin at 1000 ppm, larvae and adults developed normally. (McIntosh and Banks)

**Field trials**

***Control of take-all.*** Preliminary tests in the glasshouse measured the phytotoxicity of the following compounds, either by mixing them with potting soil in which wheat was at once sown or by spraying them on to 2-week-old wheat plants: 1-phenylthiosemicarbazide; fentin chloride (Pure Chemicals Ltd.); tetrachloroisophthalonitrile ("DAC 2787": Farm Protection Ltd.); chloroneb ("Demosan": Du Pont (U.K.) Ltd.); 1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester ("1991": Du Pont); *O,O*-diethyl phthalimidophosphonothioate ("M 2452": Dow Chemical Co (U.K.) Ltd.); captan; captafol and folpet (both from Murphy Chemical Co. Ltd.); drazoxolon (Plant Protection Ltd.); and quintozene.

In a field trial the first four of the above compounds, plus decyltriphenylphosphonium bromochlorotriphenyl stannate ("A36": C. H. Boeh-



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ringer Sohn), were applied as kaolin dusts to the soil on 17 October 1966; wheat (var. Cappelle) was sown on 27 October; and triamiphos ("Wepsyn": Mi-dox Ltd.) was sprayed on to the young plants on 7 March. The plants were not damaged by the treatments. Sampling the plots in May and July gave no significant evidence that take-all or any other disease was controlled. However, disease incidence differed more than usual between plots, and the results given by the largest amounts of fentin chloride (4 lb a.i./acre on soil) and triamiphos (3·6 lb a.i./acre on plants) seem to make further trials with them worth while. (Slope and Waller, Plant Pathology Department, and McIntosh)

***Control of potato-haulm blight.*** Mr. S. C. Melville, N.A.A.S., Starcross, kindly included one of our wax formulations of fentin acetate in his spraying trial with the variety Arran Consul in Cornwall. However, the blight attack was late, and all treatments controlled it completely, so no comparisons can be made.

***Control of potato-tuber blight.*** In a microplot trial with the variety King Edward at Woburn the following compounds were applied in late June or early July in an attempt to protect the tubers from infection by spores of *P. infestans* washed down from haulms to soil; copper oxychloride, fentin acetate (Hoechst Chemicals Ltd.), tetrachloroisophthalonitrile (Farm Protection Ltd.), captafol (Murphy Chemical Co. Ltd.) and tetraphenyltin (Pure Chemicals Ltd.) as plaster of Paris granules placed on the soil close to the stems or (except tetraphenyltin) sprayed on the lower stems themselves; and the skin-forming "Epok V8020" (British Resin Products Ltd.; see treatment H, 1966 experiment), sprayed on the tops of the ridges. The plants were not damaged by the treatments, but the crop was not attacked by blight, so comparisons cannot be made. (McIntosh)

## Staff and visiting workers

J. B. Lewis was appointed to work on the biochemical basis of resistance to pesticides. J. B. Srivastava came for training in techniques of bio-assay of insecticides. At the request of the Ministry of Overseas Development, K. A. Lord was seconded to Pakistan to help the Department of Plant Protection to develop techniques of analysis of residues of pesticides. A. H. McIntosh, M. Elliott and N. F. Janes attended the 6th International Congress of Plant Protection at Vienna and F. T. Phillips the symposium on the use of isotopes and radiation in entomology held there under the aegis of the International Atomic Energy Authority and F.A.O. R. K. Callow, D. H. Calam and N. F. Janes went to Zürich to take a course of instruction on the use of the mass spectrometer.

Several members of the department contributed to the 4th British Insecticide and Fungicide Conference.



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