ROTHAMSTED RESEARCH

Report for 1969 - Part 1

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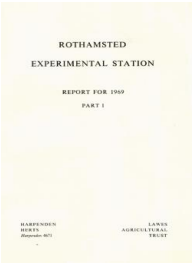

Insecticides and Fungicides Department

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INSECTICIDES AND FUNGICIDES DEPARTMENT

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The genetical work on resistance to the pyrethroids indicates that some of the mechanisms that confer resistance to the natural pyrethrins did not do so to the new synthetic material tested (NRDC 104), so resistance to it is much less than to the natural pyrethrins after equivalent selection. By checking their effectiveness against strains resistant to the pyrethrins, this information could be used to select new insecticides less likely than older ones to lose effectiveness because insects develop resistance. These synthetics are perhaps more effective against susceptible strains because they are less easily degraded. Similarly, biochemical work defined degradation mechanisms responsible for insects becoming resistant to organophosphorus compounds, and by selecting compounds not susceptible to degradation by these mechanisms organophosphorus compounds less likely to lose effectiveness because insects develop resistance could be produced.

Experiments under controlled conditions showed how the amount and distribution of rain can affect the insecticidal efficiency of foliar applications of granular formulations of systemic insecticides applied to leaves of field beans. Granules have several advantages over sprays, especially in safeguarding bees, but in dry weather sprays might be more effective against aphids.

Earlier work showed that commercial application of dry seed dressings for pest control was unsatisfactory. Current work has confirmed that this is also true of liquid seed dressings and that it applies both to insecticides and fungicides. With dry dressings the distribution per seed is good, but the amount retained per seed is smaller, often much smaller, than required. With liquid dressings retention may be adequate but the distribution is poor and may result in inadequate protection and phytotoxicity.

Many fungicides, including some with systemic action, were tested on potted plants for control of potato common scab, but only captafol and tecnazene were as effective as quintozene, which is used commercially in some countries, but it is suspected to be carcinogenic.

Insecticides

The causes of resistance

***Metabolism of organophosphorus insecticides by strains of housefly* (Musca domestica *L.*).** Subcellular preparations of the SKA strain metabolise diazinon, diazoxon and parathion by at least five mechanisms, of which two are associated with resistance factors.

Using pure line strains for each resistance mechanism showed that one mechanism (A), desethylation of all three insecticides by a glutathione-requiring enzyme contained in the supernatant fraction of subcellular preparations, accompanies the gene *a* resistance factor (chromosome II).

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The second mechanism (B), contained in the microsomal fraction of subcellular preparations, metabolises diazoxon to two unidentified compounds. It accompanies a resistance factor on chromosome V. Also both the supernatant and microsomal fractions of preparations, from either resistant or susceptible (*Rothamsted Report for 1968*, Part 2, 171) flies, cleave all three insecticides to either diethyl phosphorothionate or diethyl phosphate (mechanisms C and D), and the microsomal fractions convert the thionates to their more toxic oxygen analogues (mechanism E).

Although resistant and susceptible strains differ little in the rates they cleave diazoxon when diazoxon is the substrate, with diazinon (or parathion) as substrate almost no diethyl phosphate is formed *in vitro* either by susceptible strains or the strain with the chromosome V factor.

Subcellular preparations of houseflies do not convert diethyl phosphorothionate to diethyl phosphate, and so diethyl phosphate results from cleavage of the oxygen analogue. From this, the sum of the amounts of oxon and diethyl phosphate present gives the total oxon formation. Flies with either the resistance factor on chromosomes II or V, or on both as in the SKA strain, produce more of the oxygen analogues than do susceptible flies. However, the actual concentration of oxon in these resistant strains is much less, because it is rapidly detoxified by the resistance mechanisms.

Thus resistance to diazinon and parathion in strains with gene *a* depends on a faster and more efficient detoxification of diazoxon and paraoxon to diethyl phosphate and the desethyl derivatives. In strains with the chromosome V factor, resistance to diazinon is from the detoxification of diazoxon to the two unidentified metabolites (this factor does not confer resistance to parathion). (Lewis)

Because gene *a* gives specific resistance to ethyl chlorthion, it was thought desirable to use this insecticide to study the gene *a* mechanism. [^{14}C-ethoxy]ethyl chlorthion was synthesised by a method analogous to that for parathion (Hilton & O'Brien, *J. econ. Ent.* (1965), **58**, 221). Both resistant and susceptible strains of housefly cleave ethyl chlorthion to diethyl phosphorothionate, *in vivo* and *in vitro*. However, subcellular preparations from strains with gene *a* produce very little of the desethyl derivative and more of an unidentified metabolite, in the presence of glutathione. (Lord and Lewis)

Interaction between the factor delaying penetration of insecticides in houseflies and the desethylation resistance mechanism. The factor delaying the penetration of insecticides in housefly strain 348 was successfully bred into a strain with the desethylation mechanism of resistance (strain 393), using the method of substituting the unmarked chromosomes with the resistance factors by chromosomes with visible mutant markers lacking factors of resistance. This enabled the interaction of the delayed penetration and desethylation to resistance to be measured against the same 14 organophosphate insecticides as used for previous resistance studies (*Rothamsted Report for 1968*, Part 2, 172).

Delayed penetration increased the resistance of the flies with the desethylation mechanisms and their combined activity produced a resistance

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identical to, and thus fully accounting for, the resistance of the SKA strain to parathion methyl, malathion, chloroxon and ethyl chlorthion, and for most of the resistance against chlorthion and ethyl malathion. Delayed penetration increased resistance to the corresponding phosphates except chloroxon little, partly because of the faster penetration of the phosphates, and cannot account for more than half the resistance against these compounds in the SKA strain. The sesamex-inhibited factor (on chromosome V), present in the SKA strain, probably interacts with the desethylation mechanism to increase resistance to the phosphates, although by itself this mechanism gives only moderate to slight resistance to diazinon, diazoxon and malaoxon ethyl and none to other organophosphorus compound tested.

The modifying activity of mechanisms that alone have no apparent effect on resistance, complicates the study of resistance and may explain some of the puzzling results reported in the literature. (Sawicki)

Penetration of insecticides into houseflies with and without the factor delaying penetration. The penetration of insecticides into houseflies, with and without the factor that delays penetration (*Rothamsted Report for 1967*, 165), was studied by measuring the amounts of the insecticides on the surface of the flies at intervals after treatment. The factor slowed the entry of all the insecticides tested, but its effect depended on the dose, and had little or no effect when the dose applied was big (20 μg/fly). Penetration in all strains, susceptible and resistant, increased but not proportionally to the size of the dose. The rate insecticide was lost from the surface slowed with time and increasing age of the insects. It was faster in males than females, and was always faster in flies without the penetration factor. The differences in penetration between the flies with and without the factor was less with n-dodecane as solvent than with acetone.

With all strains, rate of loss from the surface was slowest with dieldrin. Diazinon, parathion and chlorthion ethyl were lost at about the same rate; diazoxon penetrated fastest. (Sawicki and Lord)

Compounds influencing insect behaviour

Glandular secretions of the cotton stainer,* Dysdercus intermedius *Dist. Results from work on the scent-gland system of adult cotton stainers were published. (9.10). (Calam and Scott)

***Sex- and species-specific compounds from male bumblebees* (Bombus *spp*).** Identification of the major components of extracts from heads of male bumblebees of five species was reported last year (p. 174). Authentic samples of *cis*-hexadec-9-en-1-ol and ethyl myristoleate (*cis*-tetradec-9-enoate) were obtained and their analytical behaviour agreed closely with that of the compounds identified in *B. lapidarius* and *B. lucorum* respectively. Unfortunately, insufficient natural material remained for direct comparison and the stereochemistry of the double bonds in the natural compounds remains in doubt. (Calam)

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Salivary gland secretion of the larvae of* Dasyneura *spp. Larvae of *Dasyneura* spp. stimulate the formation of galls in plants, which has been attributed to substance(s) secreted from their salivary glands. Extracts of dissected glands from both *D. urticae* and *D. affinis* larvae were examined by mass spectrometry. Both samples contained 3-indolylacetic acid (1AA) as a major component (identified by comparison with an authentic sample), together with small amounts of interfering material. The average content of 1AA, estimated colorimetrically was 1·24 μg per *D. urticae* larva and 4·39 μg per *D. affinis* larva. 1AA is a plant-growth hormone and its identification provides a possible explanation for the ability of the larvae of the genus *Dasyneura* to promote galls. (Calam and D. Leatherdale, Royal Entomological Society)

The nature of components in wheat extracts that influence the behaviour of Wheat Bulb fly larvae. Methanol extracts of wheat stems, partially purified by chromatography on Sephadex LH20, were assayed by a test for measuring arrestant activity. Concentrating purified extracts yielded an insoluble fraction that contained most of the biological activity. This fraction mostly dissolved in a large volume of methanol. When aliquots of the solution were evaporated and the residues heated, either in the ion source of a mass spectrometer to 260°C at a pressure of 1×10^{-6} mm, or in air to 150°C, they retained activity. Heating in air to 260°C destroyed activity. The involatility and susceptibility to oxidation, together with the changes in solubility associated with concentration, discount the possibility that the active material is inorganic. These properties also severely limit the choice of organic substances, and possibly suggest a phenolic compound of some type. (Scott and Calam)

***Behaviour and control of wireworms* (Agriotes *spp.*).** Results of laboratory tests to see whether a bait could be developed for use with a poison to kill wireworms, or whether substances could be found that would prevent wireworms from feeding on plants, are summarised in 9.18. (Griffiths)

Resistance to the pyrethroids and DDT. To see whether exposure to two structurally related selecting agents would affect the pattern of resistance they developed a strain of houseflies, 213ab, which had been selected intermittently by exposure to an 8 : 1 mixture of piperonyl butoxide and pyrethrum extract, was divided into two sub-strains. Each sub-strain has been selected regularly for more than 50 generations, one (strain *NPR*) with pyrethrum extract alone, the other (strain *104*) with 5-benzyl-3-furylmethyl (±)-*cis-trans*-chrysanthemate (5B3FC). The resulting populations were compared with each other and with the original parent stock.

Strain *NPR* was much more resistant (< 100) to pyrethrum extract than 213ab (approx. 10) and has a slightly increased cross resistance to 5B3FC (from 35- to 80-fold). Strain *104* was less resistant (one-thirtieth) than 213ab to 5B3FC, and also less cross resistant (one-third) to natural pyrethrins.

Both strains remained very resistant to DDT with approximately 70% of the population immune. DDT is not synergised by Sesamex or FDMC

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(bis-(p-chlorophenyl)-trifluoromethyl carbinol), or a combination of both, indicating that the major resistance factor is not DDT-dehydrochlorination or the Sesamex-susceptible factor as it is in the SKA strain. (Farnham)

Site of action of pyrethrin I

Effect of pyrethrin I on conduction of action potentials in insect giant fibres. Previous work (*Rothamsted Report for 1968*, Part 1, 176–177) showed that as little pyrethrin I in saline as 10^{-10} M increased spontaneous nervous activity perceptibly in sixth abdominal ganglia of the cockroach *Periplaneta americana* L., and that a concentration of 10^{-7} M caused serious symptoms of poisoning. Narahashi, however (*J. cell. comp. Physiol.* (1962), **59**, 61–65, 67–76) found that as much as 3×10^{-6} M of the synthetic pyrethroid allethrin was needed to cause first repetitive after-discharge and then conduction-block in the giant fibres of the cockroach abdominal nerve cord.

The effect of pyrethrin I on conduction in giant fibres was tested by irrigating a length of the abdominal cord with solutions of pyrethrin I in saline at a rate of 10 μl per minute while stimulating the cord electrically at intervals and measuring with an oscilloscope the amplitude of the action potentials produced. Pyrethrin I at concentrations of 2×10^{-6} and 2×10^{-7} M steadily decreased the amplitude of the action potentials to 20–30% of their original value 2 hours after treatment began, and soon afterwards the fibres ceased to propagate action potentials even when the stimulus strength was doubled. Irrigation for 2 hours with pyrethrin I at 2×10^{-8} M decreased the amplitude of the action potentials to 50–70% their initial values, though conduction was not blocked even after 3 hours. The effects of irrigation with more dilute pyrethrin I could not be distinguished from those of saline alone, which had decreased the amplitude of action potentials to 85% initial value after 3 hours.

These results resemble those Narahashi obtained with allethrin and suggest that pyrethrin I and allethrin are of similar potency at this site of action. However, the concentration of pyrethrin I in the haemolymph of cockroaches given a lethal dose of this insecticide was shown to be less than 10^{-7} M (*Rothamsted Report for 1968*, Part 1, 178), too small a concentration to affect giant fibre conduction, though much lower concentrations affect spontaneous nervous activity within ganglia.

Condition of ventral nerve cords in cockroaches previously poisoned with lethal doses of pyrethrin I. Cockroaches were treated topically with LD95s (0·45 μg) of pyrethrin I, and at intervals up to 24 hours later the ventral nerve cord was exposed and its condition assessed by electrophysiological methods. Fifteen minutes after dosing, when the insects began to show symptoms of poisoning, spontaneous activity in the sixth abdominal ganglion had doubled; it remained at this level for at least 4 hours after dosing. From 15 minutes to 2 hours after dosing, when the insects showed violent symptoms of poisoning, bursts of intense activity

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occurred in the ganglia, but bursts became fewer as the insects became prostrate.

Until 12 hours after dosing, when the insects were becoming moribund, the cercal nerve–giant fibre pathway through the sixth abdominal ganglion seemed only partially affected. The amplitude of action potentials in giant fibres decreased little until at least 4 hours after dosing, and conduction was not blocked until 6 hours after dosing. Hence, impaired conduction in the cercal nerve–giant fibre pathways, and in the giant fibres themselves, seems to be a very late symptom in the poisoning process and could be associated with a general deterioration in the insects' condition rather than with the primary action of pyrethrin I. However, the amount of spontaneous activity within sixth abdominal ganglia changes when the insects show severe symptoms of poisoning and when the maximum concentration of pyrethrin I within the insects is first reached. Therefore, although the nature of the toxic action of pyrethrin I at the lethal site of action may resemble its action on giant fibres, the fatal lesion is probably situated within the ganglia rather than in the giant fibres or peripheral nerve axons.

The spread of topically applied pyrethrin I to the central nervous systems of cockroaches. Burt and Lord (*Ent. exp. appl.* (1968), **11**, 55–67) showed that 1 hour after LD90s of diazoxon were topically applied in 1 μl drops of acetone to adult cockroaches, 18% of the insecticide had appeared in the tissues, proportioned between fluids and solids according to its partition coefficient, and that the concentration in the haemolymph could account for the severity of the symptoms of poisoning in the central nervous system. We concluded that the insecticide made its way into the nervous system from the haemolymph. Gerolt (*J. Insect Physiol.* (1969), **15**, 563–580) has since produced evidence that the tracheal system conveys insecticides from the cuticle to the central nervous system, the haemolymph playing little or no part. How insecticides spread to the central nervous system and enter it must be taken into account when their mode of action is studied, because the mode of entry may well influence the mode of action. Some tests were therefore done to try to determine the way pyrethrin I spreads from the cuticle to the central nervous system.

(1) Pyrethrin I content of haemolymph. Attempts to determine chemically pyrethrin I in the haemolymph of cockroaches poisoned with this insecticide failed because the method used was not sensitive enough, so biological tests were made.

Haemolymph was collected in capillaries of known volume ('Microcaps') from cockroaches previously poisoned with LD95s (0·45 μg) of pyrethrin I. The haemolymph was transferred to a micrometer syringe specially modified to displace its contents completely. This discharged the haemolymph continuously at a rate of 0·85 μl per minute on to exposed abdominal nerve cords in untreated cockroaches, while the cords were monitored electrophysiologically for abnormal symptoms. Haemolymph from untreated cockroaches had no effect on the nerve preparations, but irrigation for 10 minutes with haemolymph from cockroaches treated with LD95s of pyrethrin I 1–2 hours before sampling, increased spon-

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taneous nervous activity in sixth abdominal ganglia tenfold. Activity then decreased, a sequence of events similar to that produced by irrigating ganglia with saline solutions of pyrethrin I in the range 10^{-8}–10^{-7} M. Haemolymph from treated cockroaches decreased the amplitude of action potentials in the giant fibre of the cord and increased the strength of the stimulus required to excite them to approximately the same extent as solutions of pyrethrin I in the range 10^{-8}–10^{-7} M.

Neuroexcitatory materials released into the haemolymph as a consequence of pyrethrin intoxication could have increased the spontaneous activity in the ganglia, but are much less likely to have affected conduction in the giant fibres.

(2) ***Toxicity of pyrethrin I applied in different ways.*** LD95s of pyrethrin I were applied to cockroaches in three ways: (a) to the metathoracic sterna dissolved in 1 μl of acetone; (b) to the metathoracic sterna in 1 μl of a solvent-free emulsion in saline; (c) introduced into the tracheal system *via* a metathoracic spiracle in 0·1 μl of a solvent-free emulsion. (Treatments (b) and (c) contained 0·1% of the emulsifier Ethylan-TU.) The treated insects were stored at 20°C for 48 hours and their condition assessed from time to time. All treatments took effect equally quickly except that (b) was a little slower than the others for the first half hour after treatment. Forty-eight hours after treatment all insects treated topically were prostrate or badly affected, and 80% of those receiving pyrethrin I *via* the tracheal system. There was little difference between the effectiveness of topical and tracheal treatments, and the presence of acetone did not increase the speed of action or the ultimate toxicity of pyrethrin I applied topically.

(3) ***Effect on spontaneous nervous activity in the sixth abdominal ganglion of introducing pyrethrin I into the associated tracheal system.*** The isolated abdomen of a cockroach was dissected to expose the sixth abdominal ganglion and associated tracheal system. A fine-tipped glass capillary mounted on a micrometer syringe was used to introduce 0·15 μl quantities of a solvent-free emulsion of pyrethrin I into one of the longitudinal ventral tracheae close to the ganglion, and precautions were taken to prevent the emulsion running out of the cut tracheae on to the preparation. Spontaneous nervous activity in the ganglion was then monitored electrophysiologically. Significant differences were not detected between the amounts of spontaneous nervous activity associated with the following treatments: (a) external irrigation of the ganglia with saline alone; (b) external irrigation with saline after introducing 0·15 μl of a saline solution of emulsifier into the tracheal system; (c) external irrigation after introduction into the tracheal system of 0·15 μl of an emulsion in saline of 0·15 μg of pyrethrin I (about one-third the LD95). When large doses (1·5 and 7·5 μg) of pyrethrin I were introduced into the tracheae, spontaneous activity became much less, though without the initial burst of greatly increased activity usual when ganglia are irrigated externally with high concentrations of pyrethrin I in saline.

Of the series of experiments described, experiment (1) suggests that the haemolymph from cockroaches poisoned with pyrethrin I does contain

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a substance toxic to nerve tissue; this may be pyrethrin I. Experiments (2) and (3) provide no evidence in favour of tracheal transport of pyrethrin I, because placing a similar dose of insecticide actually inside the tracheal system increases neither its speed of action nor its ultimate toxicity compared with the same dose applied externally; also, placing inside part of the tracheal system a dose of pyrethrin I three times as large as the whole pyrethrin I content of a cockroach poisoned one hour previously with an LD95 of this insecticide, fails to produce abnormal symptoms in the associated nerve ganglion. (Burt and Goodchild)

Distribution of pyrethrin I in relation to its toxic action

Sorption of pyrethrin I on cockroach tissues. Tests were made on the distribution of pyrethrin I between aqueous buffer solution (0·05*M* Tris pH 7·0) and solids prepared by macerating whole adult cockroaches (*P. americana*) or dissected nerve cords. Pyrethrin I in aqueous buffer solution was mixed with cockroach solids and after 5 minutes allowed for equilibration, the solid and liquid phases were separated by centrifugation and decantation. In some tests, the solids were then equilibrated with fresh buffer and the separation repeated. The pyrethrin I was extracted from the separated phases with hexane and assayed by gas chromatography using N_2 carrier gas, an electron capture detector and a 2 ft column of 2% SE30 on chromosorb W at 190°C. Pyrethrin I was about equally strongly, though reversibly, sorbed on solids from nerve cords or whole insects. The distribution coefficient between solids and buffer was approximately 2000 in favour of nerve cords (wet weight) and 30 000 in favour of total solids (dry weight).

Pyrethrin I in tissues from poisoned insects. Cockroaches were poisoned with LD95s of pyrethrin I (0·5 μg) and at intervals up to 24 hours nerve cords were removed and assayed for insecticide. The dissected nerve cords were extracted with hexane, the extract purified by chromatography on silica-gel-loaded paper (Whatman SG81), using ethyl acetate : hexane = 1 : 3 as solvent, and examined by gas chromatography. Although the method could detect as little as 1·5 ng pyrethrin I in a single nerve cord (5×10^{-7} M), pyrethrins were not found at any stage of poisoning. This indicates that nervous function is affected with less pyrethrin in the nerve cord than 5×10^{-7} M. This, together with results described in the previous section, suggests that amounts of pyrethrin I in the fluid fraction of the haemolymph $< 2{\cdot}5 \times 10^{-10}$ are toxic to cockroaches. The electrophysiological results indicate that amounts of this order could affect spontaneous activity in the ganglia, but not axonic conduction. The results as a whole therefore support the suggestion that pyrethrin I kills insects by interfering with the function of the nerve ganglia. (Lord)

Insecticidal activity of synthetic pyrethroids. Some new structure–activity relationships bearing on the essential requirements for the structure of the *acid* side of the molecule were investigated. Using the most effective alcohol, 5-benzyl-3-furylmethyl alcohol, esters of a series of cyclopropane acids

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were synthesised and tested for insecticidal activity (Tables 1 and 2). Comparison of the activities of the 5-benzyl-3-furylmethyl esters of cyclopropane and (±)-2,2-dimethylcyclopropane carboxylic acids shows the importance of methyl groups on C-2. The dimethyl analogue also had a far stronger knockdown effect than similar synthetic pyrethroids. In view of these important properties, the acid was resolved, and the corresponding esters of the (+) and (−) acids examined separately. The near equivalence in activity of these two esters (see Tables 1 and 2) contrasts with the results from the (+) and (−) *trans* chrysanthemates (*Rothamsted Report for 1968*, Part 1, 183) and is important evidence for the type of reaction that occurs when the insect is being poisoned.

Other acids containing the 2,2-dimethyl groups, namely the 3,3-dichloro- and 1-methyl-substituted 2,2-dimethyl cyclopropane carboxylic acids gave esters, which although less potent than many of the pyrethroids previously synthesised, were active enough to show that they still contained the structural elements necessary for toxicity to insects. The 3-nitro substituted analogue, though, was much less toxic; it is often observed that a very polar group in this class of compounds diminishes toxicity.

The importance of another centre in the molecule was established by examining an analogue of 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate, with an extra methyl group on the α-carbon atom of the alcohol (Table 1). This small modification to the structure destroys the activity almost completely, suggesting that the new substituent is interfering fundamentally with an essential step in the poisoning process.

TABLE 1

Contact toxicity to adult Phaedon cochleariae *Fab. and adult* Musca domestica *L. of synthetic pyrethroids*

	Relative toxicities to	
	Phaedon cochleariae (F.)	*Musca domestica* L.
5-Benzyl-3-furylmethyl ester of:		
(±)-*cis-trans*-chrysanthemic acid	100	100
cyclopropane carboxylic acid	<0·1	<0·5
(±)-2,2-dimethylcyclopropane carboxylic acid	20	5
(+)-2,2-dimethylcyclopropane carboxylic acid	14	*ca.* 3
(−)-2,2-dimethylcyclopropane carboxylic acid	35	*ca.* 5
1,2,2-trimethylcyclopropane carboxylic acid	28	*ca.* 10
3,3-dichloro-2,2-dimethylcyclopropane carboxylic acid	20	10
3-nitro-2,2-dimethylcyclopropane carboxylic acid	0·2	0·5
(+)-*trans*-chrysanthemic acid	310	300
(+)-*Trans*-chrysanthemic ester of:		
1-(5-benzyl-3-furyl)-ethyl alcohol	0·2	1

Organic chemistry of synthetic pyrethroids. 2,2-dimethyl-, 1,2,2-tri-methyl- and 3-nitro-2,2-dimethyl cyclopropane carboxylic acids were prepared as described in the literature (Nelson, *et al.*, *J. Amer. Chem. Soc.* (1957), **79**, 3467; Cannon *et al.*, *J. Amer. Chem. Soc.* (1959), **81**, 1660; Smith & Engelhardt, *J. Amer. Chem. Soc.* (1949), **71**, 2676 respectively).

Racemic 2,2-dimethyl cyclopropane carboxylic acid was reacted with either (+) or (−) α-methylbenzylamine to give mixtures of amine salts from which the salts of the (−) and (+) acids respectively could be isolated

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TABLE 2

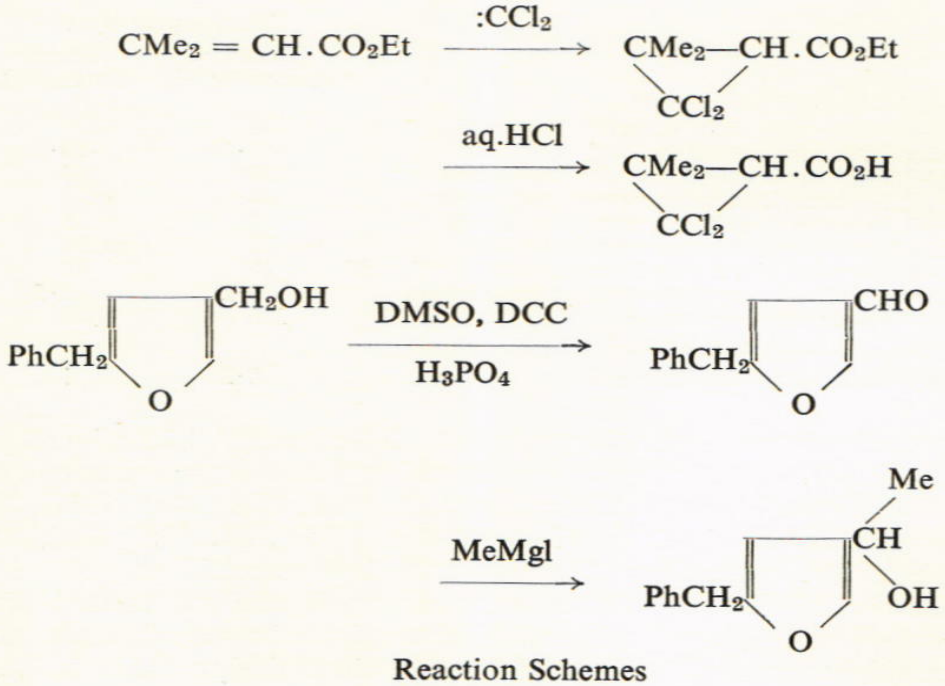
Contact activity to mosquitoes of synthetic pyrethroids

	LD50s in ng per 1–2 day old females	
	Anopheles stephensi	*Aedes aegypti*
5-Benzyl-3-furylmethyl esters of:		
(+)-*trans*-chrysanthemic acid	0·95	1·5
(+)-2,2-dimethylcyclopropane carboxylic acid	2·3	3·7
(−)-2,2-dimethylcyclopropane carboxylic acid	5·1	8·0
cyclopropane carboxylic acid	>315	>315

(The results in this table were obtained by Dr F. Barlow, Tropical Pesticides Research Unit.)

by repeated recrystallisation from benzene. The two amine salts from, say, the (+) amine and the (±) acid had very similar n.m.r. spectra, but a useful difference was noticed for the peaks from one of the C-2 methyl groups. In concentrated solutions in deuterochloroform, these two peaks are about 1 c/s apart, a difference enough to monitor the progress of the resolution at each stage. Decomposition of the purified amine salts, and esterification gave 5-benzyl-3-furylmethyl (+) and (−)-2,2-dimethyl carboxylates, each free from the other enantiomer.

Adding dichlorocarbene (generated from sodium trichloracetate in diglyme and tetrachloroethylene) to β,β-dimethyl acrylic ester gave the ethyl ester of 3,3-dichloro-2,2-dimethyl cyclopropane carboxylic acid. Hydrolysis of this ester in alkali attacked the chloro groups also, but hydrochloric acid in hot aqueous dioxan gave the required acid satisfactorily.



Reaction Schemes

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The reagent developed by Pfitzner and Moffatt (*J. Amer. Chem. Soc.* (1963), **85**, 3027) for the controlled oxidation of primary alcohols to aldehydes proved useful for the synthesis of 5-benzyl furan-3-aldehyde. Thus, 5-benzyl-3-furylmethyl alcohol with dicyclohexylcarbodi-imide and orthophosphoric acid in dimethyl sulphoxide gave a 40% yield of the aldehyde. Reaction with the Grignard reagent from methyl iodide was normal, but attempts to distil the alcohol so formed caused it to be dehydrated. Instead, it was converted directly to the ester, which could be distilled. (Elliott, Janes and Payne)

Synthetic pyrethroids for the control of pollen beetles (*Meligethes* spp.). An experiment was done to investigate the performance, on a field crop, of one of the synthetic pyrethroids now being evaluated for commercial production. NRDC 114 (5-Benzyl-3-furylmethyl (±)-*trans*-chrysanthemate) was sprayed on to oil seed rape to measure its effectiveness against pollen beetles (*Meligethes* spp.). Unfortunately the kill of adult beetles could not be determined because the population in the crop was too small. However, by cutting flower heads and washing out the beetle larvae, it was possible to establish that the kill was comparable to that with malathion. (Needham and Stevenson)

Effects of environment, formulation and substrate on the persistence of insecticides

Volatilisation. Dieldrin crystals (crystal lengths 10–100 μ) suspended in water were sprayed to give deposits of 3–5 μg/cm² on plain glass and sintered glass of porosity grades 2, 3 and 4 (representing mean pore diameters of about 45 μ, 25 μ and 8 μ respectively). When put in 'still air' environments at approximately 5% R.H., 50% R.H. and 95% R.H. at 20°C, slightly but significantly more dieldrin volatilised at 95% R.H. than at the other humidities from all the sintered glass surfaces, but not from plain glass. Whereas double exponential equations usually apply to the rates of loss by volatilisation of dieldrin from glass surfaces (*Rothamsted Report for 1968*, Part 1, 184), losses at 95% R.H. from porous glass surfaces were better fitted by a simple sigmoid (or logistic) curve, with the general equation:

$$y = \frac{Y}{1 + e^{k(t-u)}}$$

where y is the amount of insecticide remaining on the surface at time t, Y is the maximum value of y, u the value of t when $y = \frac{1}{2}Y$, and k is the slope parameter.

Stickers for use with microcapsules. The choice of stickers to increase the times microcapsules are retained (principally against rainwashing) on different surfaces was narrowed to three (all supplied by BASF (U.K.) Ltd. as 50% (w/v) aqueous emulsions), namely 'Lutonal J.65D' (polyvinyl isobutyl ether), 'Acronal 4D' and 'Acronal 7D' (butyl polyacrylates). Their effectiveness was compared by counting the microcapsules washed off young leaves of cotton or kale or glass surfaces, with and without stickers, after different periods in the 'rainwashing' machine (described

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TABLE 3

Percentages of microcapsules aged for different periods at 20°C remaining on surfaces after different rainwashing periods

(Results are the means of 5 replicates)

Ageing period	1 day				1 week				4 weeks				8 weeks			
Rainwashing period (secs)	0	30	150	300	0	30	150	300	0	30	150	300	0	30	150	300
Surface: Glass																
Sticker: Acronal 4D	100	97	89	67	100	97	95	89	100	97	95	91	100	99	96	89
Acronal 7D	100	97	75	49	100	96	77	54	100	96	85	68	100	97	81	55
Lutonal	100	92	56	28	100	95	61	25	100	98	73	36	100	93	77	44
Surface: Kale leaf																
Sticker: Acronal 4D	100	94	83	65	100	96	92	77	100	97	92	78	100	96	93	85
Acronal 7D	100	92	65	36	100	90	62	39	100	97	84	65	100	99	91	75
Lutonal	100	84	47	15	100	81	43	10	100	71	45	20	100	85	39	9
Surface: Cotton leaf																
Sticker: Acronal 4D	100	96	87	65	100	95	85	64	100	77	54	41	100	87	73	55
Acronal 7D	100	91	57	26	100	92	65	35	100	94	77	62	100	96	83	70
Lutonal	100	67	17	3	100	38	5	1	100	47	18	3	100	88	31	4

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in *Chemy Ind.* (1969), **13**, 414–415). The sticker emulsions were diluted with water to give 2½% (w/v) sticker emulsions. Suspensions of a large microcapsule sample (500–750 μ diameter, consisting of a DDT formulation encapsulated in a hardened gelatin-gum arabic wall) were made in these dilute emulsions, and 0·5 ml containing 100–200 microcapsules and 0·01 g sticker spread over 10 cm² circular areas of the surfaces with a small spatula. The prepared surfaces were then aged for periods ranging from 1 day to 2 months at 20°C before testing for rainfastness. The 'rainwashing' machine was modified to collect the microcapsules that were washed off. The collecting cylinders were replaced by a 'Sartorius' filter apparatus, using 'Sartorius' black cellulose nitrate filter-membranes or Whatman No. 29 black filter papers, with the water-pump running at full suction to cope with the efflux from the 'rainwashing' machine. The microcapsules retained by the filters after washing for 30, 150 and 300 seconds were counted under magnification. Table 3 shows the percentages of microcapsules that remained on the 'rainwashed' surfaces in one experiment.

The following general picture can be drawn from these results:

Comparison of ageing effects on the stickers

(a) On glass. Whatever the rainwashing period, microcapsules were better retained after ageing with the stickers for 4–8 weeks.
(b) On kale. Similar to glass, although 'Lutonal' did not improve when aged.
(c) On cotton. Similar to glass, except that 'Acronal 4D' deteriorated slightly with age. 'Lutonal', after initial deterioration, then improved.

Comparison of sticker type

(a) On glass. For all periods of ageing 'Acronal 4D' was better than 'Acronal 7D', which was better than 'Lutonal'.
(b) On kale. There was little difference between the 'Acronal' types, which were better than 'Lutonal'.
(c) On cotton. Similar results as for kale.

'Acronal 4D', in total the best, reached its maximum efficiency sooner than 'Acronal 7D', especially on the leaf surfaces. Further tests with 'rainwashing' for different periods at weekly intervals up to 3 months confirmed these conclusions.

The testing of microcapsules. The rates at which insecticides leak from different types of microcapsules were measured by bioassay and chemical analyses. It depends not only on the internal phase (i.e. the 'carrier' in which the insecticide is dissolved or sorbed) and the wall material, but also on the conditions in which the wall materials were deposited during the encapsulation, because, among other things, these govern the porosity of the walls. Some microcapsules with walls of gelatin-gum arabic plus 'Bakelite' were penetrated readily by sperm wax (in which DDT was dissolved), but leakage was much slower from capsules with double walls of the same material. Humid atmospheres (*ca.* 80% R.H.) increased the 'leaking' rates through hardened gelatin walls. With solid internal phases,

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such as DDT sorbed on china clay, the DDT would not be expected to leak through the walls, but it did from one batch of microcapsules kept in water. This was because the wall material contained carragheenum, which was probably degraded by bacteria. Several types of microcapsules soon leak their contents in very humid atmospheres or in water. (Phillips and Gillham)

Poisoning of honeybees in the field. Forty-one samples of honeybees (*Apis mellifera*), alleged to be poisoned, were received from the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food, two more than in 1968. Of the 28 that contained insecticide, 24 reacted positively to our test for organophosphate insecticide poisoning, three gave inconclusive results, and one contained organochlorine compounds. The test for organophosphate insecticide measures residual cholinesterase and not the insecticide residues themselves. Information supplied with these samples suggested that seven poisonings were caused by spraying field beans from the ground and six by spraying them from the air. Two were from spraying rape, one spraying from the air. Two samples were from hives in which the wax had become contaminated by previous storage with dichlorvos-resin strips. One sample taken from a swarm collected from a field gate contained both dieldrin and BHC.

The incidents where poisoning was confirmed involved an estimated total of 220 colonies, 20 more than in 1968 and 305 fewer than in 1967. (Needham and Stevenson)

Confirmation by mass spectrometry of the presence of organochlorine residues in honeybees. Gas–liquid chromatography using the electron capture detector is very sensitive to organochlorine compounds, and is therefore extensively used to detect small amounts of these insecticides in biological material, such as the honeybees mentioned above. However, the presence of a peak at the correct retention time in the gas chromatogram may not always prove the presence of the insecticide. Monitoring the effluent from the GLC column with a mass spectrometer provided a very selective detection method for dieldrin and BHC in extracts of poisoned bees already examined by GLC and bioassay. The method is sensitive to 0·2 ng BHC and 0·2 ng dieldrin per 1 μl injection. Continuous measurement with the mass spectrometer of the amount of a characteristic molecular fragment (m/e, 263 for dieldrin and 217 for BHC) produced a trace that could be used for qualitative and quantitative analysis in the same way as a conventional gas–liquid chromatogram. The likelihood of wrong identification from coincident retention times is much less, because the spurious compound would also have to give an equally substantial fragment in its mass spectrum at the value chosen.

Of the bees examined, the presence of BHC or dieldrin which had been indicated by gas chromatography was confirmed, but the test showed that another sample with a small peak at the retention time for BHC did not contain this insecticide. (Janes and Stevenson)

Bee poisoning on oilseed rape. Tests in 1967 and 1968 showed the risk to bees of spraying oilseed rape to control pollen beetles (*Meligethes* spp.)

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was greater with malathion or azinphos-methyl than with endosulfan. The relative effectiveness of azinphos-methyl and endosulfan to control pollen beetles was tested and no significant difference found. Estimates of the larval population suggested that the endosulfan remained active longer than azinphos-methyl. Thus, endosulfan may be a suitable insecticide to use against pollen beetles where honeybees are at risk.

The vacuum insect sampler (*Rothamsted Report for 1968*, Part 1, 189) was again used to estimate populations of adult and larval pollen beetles on the rape. The comparison of effectiveness of insecticide treatments was better by this method than one in which only adult beetles could be counted on flower heads in the field. (Needham and Stevenson)

Apparatus and techniques

Suction samplers. Two modified suction samplers, powered by internal combustion engines, were completed and used to sample insect populations from arable crops and grassland. Their efficiency has yet to be determined, but as their air flow and blocked load vacuum exceeds those of the electrically operated samplers, it is expected to be satisfactory. Further consideration is being given to the collecting bag and hose design to speed the transfer of insects from the sampler and to lessen the damage to trapped insects.

Method for confining insects. A new method of confining insects to specific areas was developed. The apparatus consists of a series of orbitally oscillating rings or chambers. The frequency and amplitude of the oscillations required to contain a range of insects on the stationary areas, surrounded by the rings or chambers, were determined and are shown in Table 4. Insects contained over several days by this technique showed no adverse physiological effects. It should be noted that the *Plusia gamma* larvae can only be contained when the larvae are shorter than the height of the barrier walls.

TABLE 4

Frequency in cycles per minute required to retain insects in a barrier oscillating at different amplitudes

Insect species	Amplitude (mm) 3	4	5
Myzus persicae (Sulz.) Peach potato aphid	350	200	150
Acyrthosiphon pisum (Harris) Pea aphid	250	150	100
Drosophila melanogaster (Meig.) Vestigial winged mutation of vinegar fly	300	200	150
Phaedon cochleariae (F.) mustard beetle	200	100	100
Lasius niger (L) Black lawn ant	450	350	200*
Plussia gamma (L.) Silver Y moth 5th-instar larvae	300	250	150

*After being confined at the higher frequency for approximately 5 minutes.

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Limited observations using a linear oscillating device suggested that the two node positions created could be fairly quickly found by some insects, enabling them to escape at these points unless the frequency and amplitude were considerably increased.

Liquid metering device. A dispenser for metering known volumes of liquids to plants, etc., was designed. This is usually coupled to the main water-supply system, although a portable pressurised tank can be used when it is required to dispense nutrients or other solutions. Using this method 50 ml doses of a liquid can be applied accurately to plants at a rate of more than 20 doses per minute. The measured dose is applied to the plant at a low pressure so that neither the plant nor the soil is unduly disturbed and splash is minimal, a distinct advantage when watering in C.T. cabinets and other confined spaces.

Gas–liquid control valve. A further embodiment of the gas–liquid full flow control valve was included when the National Research Development Corporation completed the patent. Arrangements for the manufacture of the valve commercially are being negotiated.

Colony counter. The colony counter developed in 1968 is now in commercial production and is proving valuable in industry and hospitals, in addition to laboratories.

Radial outflow turbine. The radial outflow turbine is now being fitted to recording volumetric spore traps for use in the field and in buildings. A separate bench-mounted model for general laboratory use is also being manufactured. (Arnold)

Improved silver staining of insect central nervous system. Further use of the Bodian protargol staining technique in histological studies of the central nervous system of the cockroach *Periplaneta americana* (*Rothamsted Report for 1968*, Part 1, 190) showed additional factors affecting the result. Fixation of ventral nerve cord ganglia was improved by ageing the alcoholic Bouin fixative for at least 40 days at 60°C. Tissues shrank less and nerve fibres stained more vigorously against a paler background than with freshly made fixative. During impregnation of paraffin sections of ganglia in the silver-protein protargol, increasing the metallic copper in the solution and decreasing the pH gives a paler staining, which is more selective for nerve fibres. Prolonging impregnation from 24 to 48 hours weakens the stain and decreases selectivity. The intensity of the stain depends chiefly on the amount of unreduced (developable) silver combined with the tissue components during impregnation; selectivity is determined mainly by the number and distribution of the minute particles ('nuclei') of reduced silver also formed during impregnation. These act during later development of the stain as catalytic centres for the reduction of the unreduced silver, which is thought to be deposited around them. Differentiation of the stain during development depends on the ratio of developing agent, hydroquinone, to sodium sulphite. Increased sulphite gives more differentiation, increased hydroquinone gives less. Optimum developer composition depends on the conditions of impregnation, and thick sections

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need more differentiation than thinner ones. The factors controlling the stain interact, so that similar results can be given by different combinations of conditions, but by suitable adjustment of the conditions the result can be varied within a wide range. For general neuroanatomical work that requires most nerve fibres to be stained, 10 μm sections are impregnated with little copper (0·5–1 g per 65 ml of 2% protargol solution) and differentiated vigorously in developer containing 10% sodium sulphite (hydrate) in 0·25–0·5% hydroquinone; to trace individual nerve fibres, 20 μm sections can be used with much copper (4–5 g per 65 ml) and more moderate differentiation (5–10% sulphite in 1% hydroquinone). The former conditions give a dark red stain, the latter a blue one. (Gregory)

Systemic insecticides

Uptake of organophosphorus insecticides from solution by wheat. Calculations of the supply of organophosphorus insecticides to roots by mass flow and diffusion (*Rothamsted Report for 1967*, 179) indicated that quantities taken up by wheat when insecticides are uniformly incorporated in soil are limited by processes in the plant and not by movement through the soil to the roots. To test this and to learn more about absorption and translocation of these compounds by plants, uptake of P-32 labelled dimethoate and disulfoton by wheat from solutions was studied under different conditions. Most results so far are with dimethoate.

Wheat was grown in nutrient solutions in controlled environment rooms with the following conditions: temperature: day 22°C, night 17°C, relative humidity: day 70%, night 89%, day length: 16 hours. At the two-leaf stage plants were transferred to solutions containing the radiolabelled insecticides at concentrations ranging from 0·1 to 30 ppm and radiotracer activity up to 10 μC/L. These solutions were renewed every 6 days. Plants were harvested at intervals from 1 hour to 28 days, separated into root and shoot and extracted by maceration with chloroform and water. Radioactivity in the extracts was assayed using a liquid-well G.M. tube and Panax autoscaler. Preliminary results from the early stages of the experiment indicate that uptake was approximately proportional to concentration. With a concentration similar to that in the soil solution in the previous pot experiments (as calculated from adsorption isotherms), the uptake by wheat from the nutrient solution was comparable to that from soil. (Graham-Bryce and Etheridge)

Factors influencing the effectiveness of granules of systemic insecticides applied to beans. Further studies on the performance of granular disulfoton and phorate applied to field beans in controlled environment rooms (*Rothamsted Report for 1968*, Part 1, 191) indicated that several additional environmental factors influence effectiveness. The importance of simulated rain was reported last year. More detailed experiments, using the same general methods, showed that the precise effect of rain depends on its frequency and intensity. Rain was applied to the plants at different intervals in amounts that gave the same weekly average of 18 cm to all. Toxicity decreased when the interval between applications was lengthened from one to three or more days, but did not increase significantly when

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they were more frequent than once a day. However, when the amount in each individual application was increased to give larger overall rates at the same range of frequences, toxicity increased considerably. When rain was delayed so that there was an interval of up to 14 days between dosing with insecticide and the start of daily rains, there was little toxic effect until the first rain was applied. However, although toxicity persisted somewhat longer when rain was delayed, the total effectiveness was less than when rain followed closely after dosing.

With daily rain, temperature changes had large effects. When temperature was 24° during the day and 19° during the night, initial toxicity was greater than with the standard conditions (20° day, 15° night), but toxicity decreased faster so that the insecticides were less effective for most of the experiment. At 15° during the day and 10° during the night, the insecticides were least effective and toxicities were less than the other regimes throughout the experiment.

The commercially available 7·5% a.i. formulation of disulfoton on pumice was usually more effective than the corresponding 10% a.i. formulation of phorate on fullers earth. The extent to which this reflected differences in formulation was of interest and, with the kind co-operation of the manufacturers and Mr. P. W. Lloyd of Pan Britannica Industries Ltd., batches were prepared of each insecticide formulated on the two carriers at both 7·5 and 10% a.i. With standard conditions and daily rainfall, the formulations on pumice were more effective than the corresponding ones on fullers earth, and usually the 7·5% loading was more toxic to aphids caged on the plants than 10%. However, disulfoton remained somewhat more toxic than phorate even when the formulations were identical, so that other factors are involved.

In all experiments except those where rain was delayed, the general pattern of the bioassay results was similar. Toxicity decreased steadily during the 2 months of the tests. Differences between treatments appeared as differences between initial toxicities and the slopes of the toxicity–time curves. However, the curves often overlapped and differences were not clear cut. During the early stages of some experiments greater mortality on untreated control plants in the controlled environment rooms compared with similar plants isolated from the insecticides, indicated a fumigant effect, but this was not consistent. The questions raised by these results are being examined further by investigating the behaviour of granules and uptake by plants in more detail, and whether the results obtained in the controlled environment rooms apply to field conditions will be tested by field experiments. (Graham-Bryce, Stevenson and Etheridge)

Analytical work

Polarography of organophosphorus insecticides. Previous studies (*Rothamsted Report for 1968*, Part 1, 192) indicated that the polarographic response for disulfoton and phorate was due to substituted thiols formed by hydrolysis in the support electrolyte. Measurements with demeton using the C.R.O. polarograph confirmed this and gave information about the electrode process.

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As with disulfoton and phorate, peak heights for demeton-S in 2% tetraethyl ammonium hydroxide increased rapidly during the first few hours after preparing solutions and then decreased slowly over a period of days. The solutions were extracted with hexane at intervals corresponding to the polarograph readings and analysis of the extracts by GLC showed that the decrease in concentration of the original compounds due to hydrolysis coincided with the initial increase in polarograph readings. Demeton-S gives the same peak potential as disulfoton (—0·74V *vs* S.C.E.). This supports the suggestion that the thiol is the polarographically active compound because disulfoton and demeton-S give the same hydrolysis product (HS CH_2CH_2S Et). Comparison of the behaviour of demeton-S, disulfoton and a sample of this thiol synthesised by an independent route further confirmed that the thiol is responsible for the polarographic activity. Hence, previously reported differences in polarographic sensitivity for freshly made up solutions of demeton-S, disulfoton, phorate and related compounds may be attributed to differences in rates of hydrolysis. Peaks given by demeton-O were indistinct and unsatisfactory for analysis. This provides additional evidence that hydrolysis to SH-containing compounds is necessary for polarographic activity.

Although all solutions that gave satisfactory polarograms with the C.R.O. polarograph also gave clear peaks with the Cambridge Univector A.C. instrument, significant waves were not found when solutions of disulfoton were investigated over a wide range of pH, using conventional D.C. polarography, by Mr. R. Jee of Imperial College. This suggests that the electrode reaction does not involve simple reduction. However, peak currents with the C.R.O. instrument were similar to those of conventional reductions at comparable concentrations and therefore do not suggest catalytic processes. Electrocapillary curves for HS CH_2CH_2S Et were therefore measured to give more information about the electrode process. The thiol caused a significant flattening of the electrocapillary curve for N/100 KOH characteristic of adsorption until the peak potential was reached. It is therefore concluded that the electrode process involves adsorption/desorption at the mercury drop. (Graham-Bryce)

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

Control of aphids on field beans in relation to toxicity to bees and the growth and yield of the crop. Collaborative work on these problems is described in the work of the Entomology Department. (Stevenson)

Comparison of the biology of resistant and susceptible aphids. Apterous *Myzus persicae* resistant to dimethoate reproduce for the same length of time as susceptible aphids, but the resistant ones reproduce significantly faster during the first ten days of adult life (especially during the first five days). Thereafter they reproduce more slowly, so that eventually the susceptible adults produce approximately the same number of larvae. Resistant aphids die significantly earlier.

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Larvae of resistant aphids develop faster than larvae of susceptible ones and are significantly heavier when they first become adult.

The resistant aphids do not excrete faster during the first four days of adult life but as they produce more larvae during the first ten days of adult life and have developed faster, they probably fed faster during development and early adult life or have obtained more nutritious food.

Resistant aphids were at least as effective as susceptible ones in transmitting sugar-beet mosaic and pea mosaic viruses. Indeed, the results seem to suggest that plants showed symptoms sooner when resistant aphids were the vectors. (Banks)

Insect rearing. The following species were reared:

PLANT FEEDERS

Homoptera	*Aphis fabae* (Scop.)
	Myzus persicae (Sulz.)
	and a dichlorvos/dimethoate resistant strain
	Megoura viciae Buckt.
Hemiptera	*Dysdercus intermedius* Distant
Coleoptera	*Phaedon cochleariae* (F.)
	Rhynchophorus palmarum (L.)

OTHERS

Orthoptera	*Blaberus discoidalis* (L.)
	Periplaneta americana (L.)
Lepidoptera	*Pieris brassicae* (L.)
Coleoptera	*Tribolium castaneum* (Herbst.)
	Tribolium confusum J. du V.
	Trogoderma granarium Everts
Diptera	*Drosophila melanogaster* (Meig.)
	Strains. Normal
	Vestigial wings
	Ebony bodied, brown eyed
	White eyed
	Musca domestica L.
	Strains. *ac; ar; bwb; ocra* SRS—fully susceptible to DDT, dieldrin and organophosphorus insecticides
	SKA—diazinon selected, very resistant to many organophosphorus insecticides
	A number of strains derived from SKA, each with one or more factors of resistance to organophosphorus insecticides, DDT or dieldrin
	NPR—pyrethrum extract selected, very resistant to pyrethroid insecticides
	104–5B3FC selected, very resistant to 5B3FC
	ac; ar; bwb; ocra—called 608Q, fully susceptible to pyrethroid insecticides, to pyrethrum knockdown and to carbamates
Hymenoptera	*Acromyrmex octospinosus* (Reich)

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Distribution and retention of liquid seed dressings. A small survey in 1967 of seeds dressed with liquid preparations of insecticides suggested that neither the average loading nor the distribution of insecticide on seeds was as required (see *Rothamsted Report for 1967*, p. 181). In a larger survey now completed, 14 merchants sent either three or six samples taken at various stages of the dressing process, and some taken on wet and others on dry days. The insecticides were γ-BHC, aldrin or chlorfenvinphos, applied by two types of machinery. Each sample was assayed for the average loading, and single seeds from each sample were assayed to find the range of distribution. The differences between samples from the beginning, middle and end of runs showed no regular pattern but samples from the same merchants varied less than between different merchants. Weather had no detectable effect on the seed loading.

Two merchants obtained average loadings near the desired amount: 116 and 110% of the theoretical loading of γ-BHC. Of the remainder treated with γ-BHC—one had about 75%, three 50–60% and three 40–45% of the desired loading. With aldrin, only one merchant obtained an average amount that exceeded 70% of the desired loading and the averages from the other five merchants ranged from 20 to 60%. Only one merchant sent samples treated with chlorfenvinphos, and the average loading was 60% of the desired.

Single seed analysis. Up to 100 single seeds were analysed from each merchant's series of samples. Differences of distribution patterns between merchants using the same insecticide and process (Methods 1 or 2) were very small. The range attained with the four most common combinations of process and insecticides, as obtained by all the merchants using them, were as follows:

Method 1: γ-BHC. The average loading, calculated from the bulk assays was 81% of the target. Approximately three-fifths of the individual seeds had less than half the target dose, which was 12·5 μg per seed, about one-fortieth had doses of several hundred μg per seed and about one-sixth were close to the target dose.

Method 1: Aldrin. The average loading, calculated from the bulk assays was 40% of the target. Approximately four-fifths of the seed had less than half the target dose of 37 μg/seed. One-tenth had doses of several hundred μg/seed and the remaining one-tenth were about right.

Method 2: γ-BHC. The average loading, calculated from the bulk assays was 51% of the target. Just under half the individual seeds had less than half the target dose, which was 10 μg/seed, a few had doses of up to 240 μg/seed and about half were close to the target dose.

Method 2: Aldrin. The target dose was 30 μg/seed, and approximately two-thirds of the individual seeds had less than half this, there was a

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fairly large spread around the target and a few seeds had doses of over 100 μg/seed. The average loading taken from bulk analyses was 60% of the target.

Clearly there are large average differences in the efficiency with which aldrin, γ-BHC liquid seed dressings are applied. Although two merchants using the same insecticide and method of application obtained average dressings near to the desired dose, analyses on single seeds show that the distribution is poor; a small proportion of seeds received large doses, and a proportion, one-tenth to about three-fifths received a dose near the target, and the rest had less, usually much less, than the target. Neither method is therefore uniformly satisfactory at present. (Jeffs with Mr R. Tuppen, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory)

Assay of mercury on single seeds and observations on distribution of insecticide and fungicide on seeds treated with liquid formulations. Because the amounts of insecticide on commercially dressed seed were not those intended, some measurements were made with fungicides. A method of measuring mercury on individual cereal (wheat) seeds treated with fungicides was devised in collaboration with G. Brown and R. Harden (Pedology Department). Seeds were pressed flat on to a support (filter paper) after initial softening with water or dilute ammonia solution, and the mercury content measured by X-ray spectrophotometry. After the mercury was assayed, the insecticide on the same seeds was extracted and measured chemically (by the method of Jeffs, Lord & Tuppen, *J. Sci. Fd Agric.* (1968), **19**, 195).

The method was used to measure the amount of insecticide and mercury (fungicide) on individual seeds in samples taken at three stages—the beginning, middle and end of runs of two types of seed dressing machinery that apply the fungicide and insecticide at the same time, but from different sources. The fungicide was either methyl mercury dicyandiamide (Method 1) or phenyl mercury acetate (Method 2).

The amount of mercury on most individual seeds ranged between one-third and three times the average on a mass of seeds, but between 5 and 10% of the seeds carried at least four times the average amount. The distribution of mercury on individual seeds was similar irrespective of the stage of dressing procedure in each of two tests with two types of machinery (four tests in all). Within each test, the average amount of mercury on seeds varied but it was not related to the stage of the operation.

Insecticide and fungicide were distributed between seeds similarly (see Jeffs, Lord & Tupper, *J. Sci. Fd Agric.* (1968), **19**, 195, already reported for insecticide), although the range of doses on individual seeds was usually greater with insecticides than with fungicides, and the relative amounts of each on individual seeds were not closely related. Thus an individual seed with a little fungicide is almost as likely to carry a lot as a little insecticide. In the four tests, the average amount of mercury on seeds was between one-fifth and one-tenth that expected and the amount of insecticide was less than half in two tests and more than two-thirds the expected amount in the other two tests. (Lord)

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Biological efficiency of seed dressings against Wheat-Bulb fly *Leptohylemyia coarctata* (Fall.)

γ-BHC and organophosphates. Work continued on how known amounts of γ-BHC affect growth of Cappelle winter wheat and to what extent they protect young plants from attack by larvae of Wheat Bulb fly. Seeds were dressed with powder containing γ-BHC and fungicide combined, instead of as before with liquid organomercury fungicide and powder γ-BHC. Seed dressings containing mercury and the organophosphorus insecticides chlorfenvinphos or ethion were also included on one trial site. The main conclusions from the complete series of trials are: (1) Powder dressing containing ethion and mercury adhered better to the seeds than the other dressings which, when dressed in the laboratory, had only about 80% of the intended amount of insecticide. (2) Seeds treated with all the powders lost some insecticide during handling and drilling. Those treated with γ-BHC powders and sown with a 'Bast Seed Drill' had only 50–60% of the intended amount of insecticide as they entered the ground. (3) Small amounts of insecticide, <10 μg/seed of γ-BHC or <15 μg/seed of the organophosphates, did not protect plants from insect attack. Increasing amounts successively decreased the percentage shoots damaged and the percentage plants with live larvae, but greater amounts of the organophosphorus insecticides, especially ethion, were needed to give the same protection as by γ-BHC. (4) In the peaty loams and sandy clay loams, no seed treatments damaged seedlings, and the best plant stands arose from seeds treated with the largest amounts of insecticide tested, approximately 50 μg/seed of γ-BHC, 70 μg/seed chlorfenvinphos and 120 μg/seed ethion. But in two sandy loams, germination of seeds was badly affected by treatment with liquid fungicide and powder γ-BHC so that, despite the protection the dressings gave against insect attack, most dosages tested were harmful. (Griffiths and Scott)

Seed dressings of experimental compounds. Organochlorines, phosphates and carbamates are the main classes of insecticide that have been tested against Wheat Bulb fly, and less stable insecticides have rarely been tried. However insecticides that are very toxic to Diptera are worth testing, especially when formulated to give slow release. Seed dressings were made of two synthetic pyrethroids, compound A (5-benzyl-3-furylmethyl (±)-*cis-trans*-chrysanthemate) (=NRDC 104) and compound B (5-benzyl-3-furylmethyl 2,2,3,3-tetramethyl cyclopropane carboxylate), using 5% w/w antioxidant (2,5-di-*tert* butylquinol) and either talc as an absorbent or polyvinyl acetate, as a slow-release absorbent. The talc dressings were stuck to seeds with methyl cellulose. Seeds treated with both pyrethroids were sown out-of-doors in boxes of soil to which Wheat Bulb fly eggs were added later, and seeds treated with compound A were sown in a field containing 3 million eggs/acre (7·5 million eggs/ha) in the East Midlands Region. Counts of damaged plants in spring showed that neither formulation of the pyrethroids protected plants from insect attack in the boxes but that the talc formulation of compound A at 1% a.i. to weight of seeds significantly decreased the number of shoots damaged by Wheat Bulb fly

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larvae in the field. The discrepancy between box and field results needs investigation and further tests will be made with NRDC 104 and several analogues. (Griffiths and Scott)

Fungicides

Tests of fungicides to control blight (caused by *Phytophthora infestans*) on potato haulms, common scab (caused by *Streptomyces scabies*) on potato tubers, and cereal take-all (caused by *Ophiobolus graminis*) continued. Many of the materials were kindly given by the makers; 'M2452' is the Dow Chemical Company's code number for 0,0-diethyl phthalimidophosphonothioate, and 'N-Serve' their name for 2-chloro-6-(trichloromethyl)-pyridine.

Laboratory tests

Potato-haulm blight. Organo-tin compounds were tested, as aqueous saponin suspensions, for possible control of potato blight. In most tests ('1-day' tests), detached potato leaflets were sprayed, 'rainwashed' and inoculated with zoospore suspensions the next day, and then incubated for a further week for the lesions to develop. In some tests ('2-day' tests), inoculation was done the day after 'rainwashing', i.e. 2 days after spraying; these tests imitated field conditions slightly better than the '1-day' tests. In all tests, fentin acetate was used as standard for comparison.

Although diphenyltin dichloride is quite a good fungicide (*Rothamsted Report for 1968*, Part 1, 197), the following other diaryltin compounds were inactive: diphenyltin di(thiophenoxide), dibenzyltin dichloride, diacetate and other dicarboxylates.

Table 5 shows results with some triphenyltin compounds. Triphenyltin thiophenoxide and *p*-chlorothiophenoxide were as effective as fentin acetate, but not significantly more so. The relative effectiveness of the sulphide, but not of the thiophenoxide, was significantly increased ($P < 0{\cdot}01$) by changing from '1-day' to '2-day' tests, i.e. by making the conditions slightly closer to those in a field trial. It is not possible to go much further in this direction in laboratory tests on detached leaflets; however, field trials of the sulphide are being continued.

TABLE 5

Relative effectiveness of organo-tin compounds for control of P. infestans

Compound	Relative effectiveness (fentin acetate = 100) in '1-day' tests	'2-day' tests
Bis(triphenyltin) sulphide	8	30
Triphenyltin thiophenoxide	115	112
Triphenyltin *p*-chlorothiophenoxide	74	—

Potato common scab. Potato plants can be grown in pots in the glasshouse or growth room throughout most of the year under the conditions needed for severe infection of tubers from soil by common scab; possible

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scab-control chemicals can thus be assessed, in about 8–10 weeks, by adding suitable amounts to the soil. Damage to plants, and effects on yield, can also be estimated. Results agree closely with those from field trials.

Quintozene, which is now suspected of being carcinogenic, was used as a standard for comparison. In all glasshouse tests it controlled scab very effectively ($P \simeq 0{\cdot}001$), and we assume that no chemical has any chance of success in practice unless it is at least as effective as quintozene.

The following chemicals either failed to control scab ($P = 0{\cdot}05$), or damaged the plants, or both, at 50 ppm or at the rates shown: benquinox, bis(triphenyltin) sulphide, captan, carboxin (at 20 ppm), dibutyltin diacetate, dichlofluanid, dichlone, dimethirimol, dodine, Dow 'M2452', drazoxolon, fentin acetate, folpet, glyodin (2-heptadecyl-2-imidazoline acetate), 'N-Serve' (at 12 ppm), oxycarboxin (at 20 ppm), pentachloroaniline, quinazamid, quinomethionate, sultropen, 'Thiabendazole' (2-(4-thiazolyl)benzimidazole), thiram, triamiphos and zineb. The following chemicals gave significant control ($P = 0{\cdot}05$), but could hardly compare with quintozene: benomyl, chloroneb, dicloran, maneb, 1-phenylthiosemicarbazide and tetrachloroisophthalonitrile. Table 6 shows results, from three separate tests, with quintozene, captafol and tecnazene, all of which controlled scab at $P \leqslant 0{\cdot}001$.

TABLE 6

Scab indices (BMS scale) on glasshouse-grown Majestic tubers after soil-treatments

	Test No.		
Treatment, 50 ppm	1	2	3
Nil	22	18	32
Quintozene	4	1	5
Captafol	2	1	—
Tecnazene	—	—	4
LSD ($P = 0{\cdot}001$)	13	11	15

Field trials

Potato-haulm blight. The 1968 trial at Rothamsted was repeated, with minor changes. However, there was no blight attack in the unusually dry summer, and no useful information was gained.

Potato common scab. A trial at Woburn with the variety Maris Piper tested the control of common scab by soil-treatments before planting. 'N-Serve', at 2% of the N in the fertiliser (ammonium sulphate), was broadcast at 3 lb/acre, and the other chemicals as dusts at 70 lb a.i./acre, on 18 April; all plots were rotavated within 1 hour of application, and potatoes were planted the same day. Scab indices (BMS scale) were calculated at harvest from samples of 50 tubers per plot (Table 7).

The dry summer favoured a more severe attack of scab than in 1968. Quintozene, used at 70 lb/acre, was less effective than in 1968, when it was used at 150 lb/acre; it clearly decreased scab, but did not increase yield, as it did in 1968. 'N-Serve' decreased both yield and percentage of

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TABLE 7

Effect of soil-treatments on yield and incidence of potato common scab (BMS scale)

Treatment	Total tubers, tons/acre	% Ware	Scab index
Quintozene	19·3	97·3	19
Folpet	19·9	96·8	34
Thiram	19·8	96·9	39
Zineb	19·4	97·3	34
'N-Serve'	16·6	95·2	36
Nil	18·7	97·0	31
LSD ($P = 0·05$)	1·8	1·2	7
LSD ($P = 0·01$)	—	1·7	9

ware, without affecting scab. The increase in scab from treatment with thiram, which was also found in glasshouse tests, is unexplained. (McIntosh)

Cereal take-all. In a field trial, the systemic fungicides benomyl, Dow 'M2452' and oxycarboxin failed to affect take-all, but there was some control of eyespot by benomyl. Details are given in the report of the Plant Pathology Department. (McIntosh, with Prew, Plant Pathology Department)

Staff and visiting workers

D. H. Calam left and was replaced by A. R. Greenway. A. Mudd was appointed to work on behalf of the Ministry of Overseas Development. C. Potter attended the F.A.O. Symposium in Rome on Insect Resistance to Insecticides where he read a paper. At the request of the Indian Government, he spent a month in India to advise on the ways and means of increasing pyrethrum production in that country. During 1969, M. Elliott worked with the Toxicology Group of Professor J. E. Casida, in the Division of Entomology, University of California, Berkeley, U.S.A. Mr. Srinivasan from Madras University visited the department to learn techniques of assessment of fungicidal action.

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