ROTHAMSTED RESEARCH

Report for 1978 - Part 1

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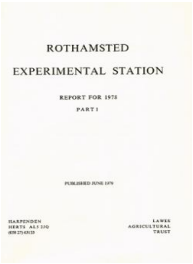

Insecticides and Fungicides Department

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Introduction

In the pursuit of more effective and safer methods of crop protection, which are the essential objectives of the Department's research, chemical approaches to pest and disease control can be regarded as an exercise in selective toxicity. Our investigations into the molecular basis for the toxicological properties of pyrethroid insecticides, which have led to outstandingly potent and safe compounds of great commercial importance represent a notable contribution to this theme. This work received further international recognition during the year with the award of the UNESCO Science Prize to the team of chemists and biologists in the Department associated with the project. Continuing studies on this increasingly important group are reported this year.

While pyrethroids are characterised by very low toxicity to mammals, the commercial insecticides so far developed have had a relatively broad spectrum of insecticidal activity, although different species do vary significantly in their susceptibility. In other studies the Department has for many years sought ways of minimising hazards to beneficial insects, both pollinators and natural enemies of pests. Increasing recognition of the importance of integrated approaches to pest control has underlined the need for such work. Recently we have taken steps to relate these studies more closely to our expertise in insecticide toxicology and structure/activity relationships of pyrethroids with the objective of devising insecticides which act selectively between beneficial and harmful insects. Preliminary results from this initiative are described this year.

Our search for such favourable selectivity is not confined to investigation of the intrinsic toxicological properties of active compounds. Hazards to unintended recipients may also be reduced by ensuring that delivery of the toxicant is confined as far as possible to the pest. As a basis for developing better procedures, an important part of the programme of the Department and the Chemical Liaison Unit is devoted to establishing the principles determining the fate of pesticides following different methods of application in relation to the behaviour of target and non-target organisms. Associated studies are directed towards the improvement of application systems: work described in this report includes examination of controlled droplet application (CDA) and electrostatic systems which are currently attracting much attention because they allow greater control of drop size and deposition than conventional hydraulic spray systems. This should make possible much more precise initial distribution of pesticide. A complementary requirement in many cases is more accurate timing of pesticide application so that pest and disease infestations can be arrested at the optimum stage with the minimum amount of chemical. The use of behaviour-controlling chemicals to monitor pest populations is potentially very valuable in this connection. It is a matter of considerable satisfaction to record that the pea moth pheromone monitoring system, developed jointly with Entomology Department, is now in routine commercial use only a few years after the first investigations were initiated at Rothamsted.

Significant advances have been made in other studies on the manipulation of behaviour by chemicals. The larval mandibular gland secretion of important lepidopterous stored-products pests, which has a range of interesting behavioural effects, has been shown to contain biologically active compounds of a type not previously identified in animals. Good progress has also been made in studies on chemicals influencing behaviour in a diverse range of other organisms including honeybees, aphids and slugs.

In the longer term it is hoped that such behaviour-controlling chemicals may be deployed to replace conventional pesticides entirely in suitable situations, in addition to providing means of using them more effectively. The need for such complementary methods is emphasised by our work on resistance to pesticides, an ever-present concern. Practical studies have again confirmed the widespread distribution of resistance to organophosphates and carbamates in aphids in Britain, while we have now detected

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resistance to pyrethroids and organophosphates in houseflies for the first time in this country, underlining the gravity of the problem and the need for constant vigilance. More fundamental studies have provided basic information of considerable potential importance, including an explanation for the very interesting spontaneous loss of resistance which can occur in certain highly resistant strains of aphids.

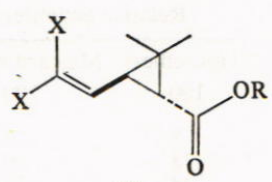
In the case of disease control, selectivity between pathogen and host plant is of particular concern. Detailed studies with compounds shown in previous work to be effective against soil-borne potato scab have now revealed the basis of their selective action. These compounds are particularly interesting because they are active following application to the foliage. It is widely recognised that such downward-moving activity has great advantages for the control of soil-inhabiting pests and diseases but despite intensive investigation it has proved very difficult to find insecticides and fungicides with this property. In recent years we have therefore been developing a comprehensive programme on the factors determining the mobility of exogenous chemicals in plants. The multidisciplinary group working on these problems, which was expanded with the arrival of staff from the Unit of Developmental Botany last year, was further strengthened this year when we welcomed two chemists transferred to the Department following the closure of the Unit of Systemic Fungicides on the retirement of Professor Wain.

Insecticides

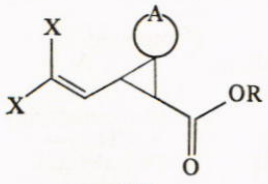
Relationships between molecular structure and insecticidal activity of pyrethroids

Cyclopropylphenylacetates and related compounds. Investigations of isosteric replacements for groups in the side chains of the acidic and alcoholic components of pyrethroids led to compounds with improved properties, such as permethrin, cypermethrin and decamethrin, now commercial insecticides. We now report a similar examination at another site in the molecule, the dimethyl group on the cyclopropane ring of chrysanthemates (eg. I; X=Me) and related compounds and at the equivalent position, the isopropyl group, in 2-aryl-3-methylbutyrates such as fenvalerate (III).

The effect of substituting chlorine for these methyls in pyrethroid esters was examined previously, but only in less active examples (3-aryl-2,2-disubstituted-cyclopropane carboxylates; Novak, Farkas & Sorm, *Coll. Czech Chemical Communications* (1961), **26,** 2090) or those where the possibility of metabolic dehydrochlorination complicated



(I)



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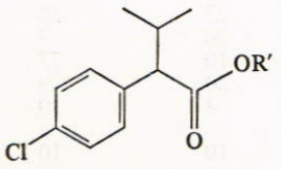

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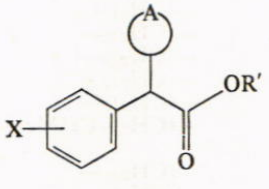

(IV)

R = 5-benzyl-3-furylmethyl
R′ = α-cyano-3-phenoxybenzyl

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interpretation of results (Elliott, *Synthetic pyrethroids, ACS Symposium Series* (1977) No. 42, p. 11).

Incorporating the two methyl groups into an enlarged ring (II; A=$(CH_2)_4$) (Searle and Davis, *ibid.*, p. 37) diminished activity, but no compound in which the dimethyl group was replaced by another of equivalent size had been examined. A spiropentane (II; A=$(CH_2)_2$) in which this condition was fulfilled was sufficiently active (see Table 1) to prompt further investigations of the effect of this modification in other systems, for example in fenvalerate and related esters. The compounds required were synthesised as shown; the results (Table 1) show that cyclopropyl is generally as effective at this site in

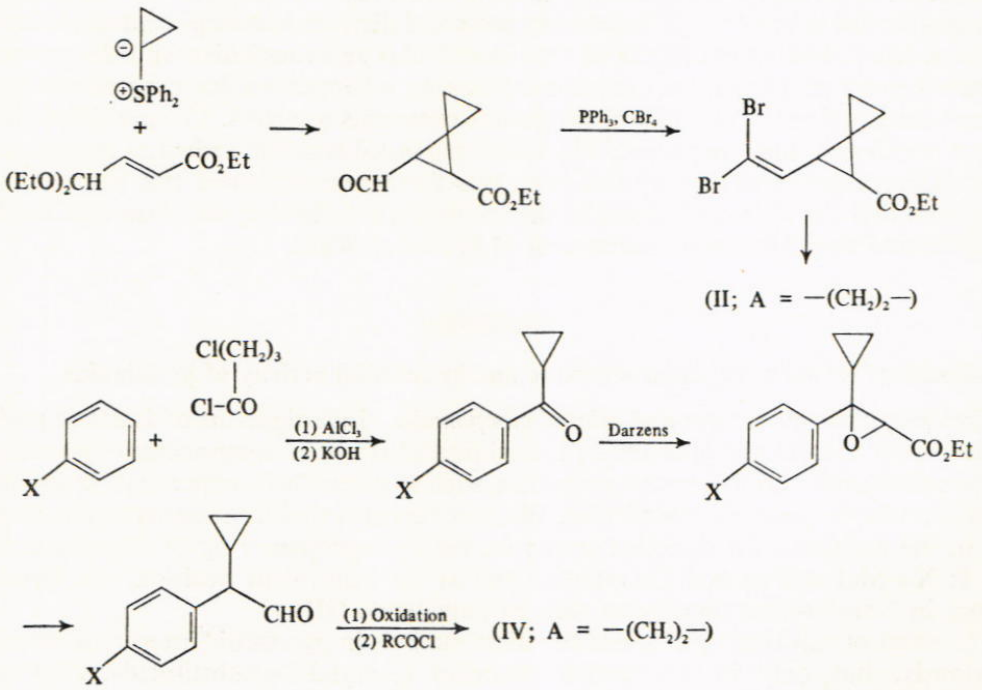

TABLE 1

Relative activities of dimethylene, dimethyl, and related compounds

Structure	Compound A	X	Relative activities to Houseflies[a]	Mustard beetles[b]
I	— (bioresmethrin)	Me	100	100
II	—$(CH_2)_2$—	Br	8	7
III	(fenvalerate)		38	60
IV	—$(CH_2)_2$— ('NRDC 181')	*p*-Cl	48	57
IV	—$(CH_2)_2$—	*p*-Br	27	61
IV	—$(CH_2)_2$—	*p*-Me	27	50
IV	—$(CH_2)_2$—	*p*-Et	20	7
IV	—$(CH_2)_2$—	*p*-F	10	77
IV	—$(CH_2)_2$—	3,4-Me_2	20	9
IV	—$(CH_2$—CHMe)—	*p*-Cl	4	10
IV	—$(CH_2)_3$—	*p*-Cl	10	10
IV	—$(CH_2)_4$—	*p*-Cl	1	<1
IV	—$(CH_2)_5$—	*p*-Cl	<1	<1

[a] *Musca domestica*, L. LD50 for bioresmethrin: 0·006 μg per insect
[b] *Phaedon cochleariae*, Fab. LD50 for bioresmethrin: 0·005 μg per insect

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the molecule as is isopropyl in the standard compound; while the chemical nature of the group at this position differs between the two series, the shape is little altered, indicating the greater importance of the latter characteristic.

Further results in Table 1 show how changing the aryl substituent X in (IV) affects activity. As in the fenvalerate series, *p*-chloro was the most generally effective substituent of those examined. Effects on activity of changing the esterifying alcohol (results not shown) were similar to those observed with esters of other pyrethroid acids i.e. α-cyano-3-phenoxybenzyl ⩾ 5-benzyl-3-furylmethyl > 3-phenoxybenzyl. Increasing the size of the ring, either by substituting the cyclopropyl group or by expanding it to cyclobutyl, cyclopentyl, or cyclohexyl diminished activity.

The spectrum of activity against a wider range of insect species was established for the most promising compound, 'NRDC 181'. The results, Table 2, suggest that this compound is more selective between different insect species and between insects and vertebrates, than is fenvalerate.

TABLE 2

Activities of 'NRDC 181' and fenvalerate compared, for various species

Species	LD50s 'NRDC 181'	LD50s Fenvalerate
Phaedon cochleariae (Coleoptera)	9 ng per insect	9 ng per insect
Musca domestica (Diptera)	16 ,,	8 ,,
Anopheles stephensi (Diptera)[a]	1·5 ,,	1·5 ,,
Glossina austeni (Diptera)[a]	30 ,,	8·5 ,,
Stomoxys calcitrans (Diptera)[a]	52 ,,	4·1 ,,
Plutella xylostella (Lepidoptera)	2·5 ,,	4·5 ,,
Rat[b]	300–450 mg per kg	50–75 mg per kg
Zebra Fish[c]	39 ppm	12 ppm

[a] Topical application; results provided by Mr. F. Barlow, Centre for Overseas Pest Research, Porton Down.
[b] Intravenous injection; results provided by Dr. R. Verschoyle, Medical Research Council Toxicology Unit, Carshalton, Surrey.
[c] Continuous flow test; results provided by Mr. R. D. Tooby, Salmon and Freshwater Fisheries Laboratory, London, SW1.

'NRDC 181' is about six-fold less toxic to rats by the intravenous route, and three-fold less toxic to fish.

^{13}C NMR Spectra: evidence for preferred conformations in iso- and cyclo-propyl isosteres. Information about preferred conformations of insecticides in solution may have a bearing on their activity. In cyclopropane based pyrethroids, ^{13}C NMR spectra indicated that an α-cyano group in the alcohol interacted weakly with the unsaturated substituent on the cyclopropane nucleus (Janes, *Journal of the Chemical Society, Perkin Transactions I* (1977), 1878). The effect is more pronounced in 2-aryl-3-methyl-butyrates and the corresponding cyclopropane substituted acetic esters now examined; Fig. 1 shows the

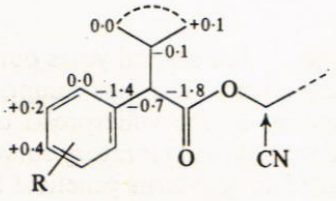

FIGURE 1

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influence on chemical shifts of the carbon atoms when an α-cyano group is introduced on to 3-phenoxybenzyl esters.

As anticipated, shifts are greatest (−1·8 to −0·7 ppm) for atoms near the site of change, and small on the aliphatic carbons. However, shifts of the aryl carbons are large, despite their distance from the cyano group, indicating that the cyano group and the aromatic ring interact, presumably through their π electrons. The directions and magnitudes of the aryl shifts together with the known anisotropic properties of the cyano group suggest that the latter is oriented linearly over the ring. The bond cannot be strong enough to prevent free rotation, but does distort rotamer populations so that the cyano group spends longer than the average period near the aromatic ring. (Chemical work: Elliott, Janes, Johnson, and Pulman; biological work: Farnham, Sandison and O'Dell)

Mode of action of insecticides on insect nervous systems

***Action of pyrethroids* in vivo.** The acquisition of new equipment has permitted development of the metathoracic leg preparation of the cockroach *Periplaneta americana* (L.) (*Rothamsted Report for 1976*, Part I, 136–7) into a useful system for studying the action of insecticides *in vivo*.

In this preparation the tips of two fine insulated copper wires (0·040 mm diameter) are inserted into the trochanter, cemented in place with melted beeswax and then attached to the proximal end of the femur with beeswax reinforced with fine copper wire. From here onward these recording wires are reinforced by a 0·065 mm diameter wire of Eureka metal, grounded at the pre-amplifier input. All three wires are bound together with quick-setting adhesive to strengthen them and prevent electrical noise generated by their relative movement. The wire bundle is looped forward, attached firmly to the pronotum with beeswax and then connected to the pre-amplifier, which is contained in a screened box together with the insect. The wires are inserted after chilling the cockroaches just enough to suppress movement. On recovery, most cockroaches move freely, run and even climb vertical surfaces, apparently with little or no residual damage.

The preparation allows action potentials, mostly from nerve 5, to be continuously recorded, the small diameter and rigidity of the trochanter favouring a high signal-to-noise ratio. A large proportion of the action potentials are undistorted and show the characteristic biphasic form of externally recorded potentials. The initial polarity of these bipolar potentials reveals the direction of their movement along the nerve and hence whether they originate centrally or peripherally.

A filter system incorporated in the pre-amplifier rejects unwanted frequencies and the amplified potentials are recorded temporarily on magnetic tape and permanently on photosensitive paper by means of a recording oscilloscope. The taped information can be analysed with a spike discriminator driving two rate-meters which count and integrate action potentials in two amplitude ranges (representing large and small axons) simultaneously.

The system is proving useful for following the development of toxic symptoms in the nervous systems of cockroaches treated with pyrethroid insecticides. (Burt and Goodchild)

The nature and causes of resistance. For several years our work on resistance to insecticides has had two principal components. The first comprises practical and basic studies with aphids, developed in response to the widespread occurrence of resistant *Myzus persicae* in the field which was detected in exploratory surveys undertaken by the Department. These studies supplemented longer-term genetical and biochemical investigation with houseflies. Although prevalent in other regions such as Scandinavia, resistance in

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houseflies has not been a problem in Britain and this insect was chosen for study largely because of its advantages as a model system which could reveal principles of general applicability. However in tests reported this year, resistance in houseflies has now been detected for the first time in Britain. Together with evidence that resistance in aphids is becoming progressively more serious, these results provide a further unwelcome reminder of the importance of this problem and the need for intensive research.

***Resistance in field populations of* Myzus persicae.** We examined relatively few samples of *Myzus persicae* from field crops this year because infestations were slight. However, we were able to confirm for the third consecutive year the presence of very resistant (R_2) aphids in Scotland indicating that this variant is now probably endemic there. Furthermore, in the west of Scotland, we detected on field crops an even more resistant variant previously only encountered in glasshouses; if this became widespread insecticidal control would undoubtedly deteriorate further.

***The biochemical nature of cross resistance to organophosphorus and carbamate insecticides in* M. persicae.** The enzyme responsible for insecticide resistance in *M. persicae* is an esterase (E-4) with a broad substrate specificity. It hydrolyses insecticidal phosphate and carbamate esters as well as the reference substrates, 1-naphthyl acetate and 4-nitrophenyl acetate. Resistant aphids have more of this enzyme than susceptible strains, and therefore detoxify insecticides faster. The hydrolysis occurs by the following mechansm:

$$EH + AB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EHAB \xrightarrow{k_2} \begin{matrix} EA \\ + \\ BH \end{matrix} \xrightarrow[H_2O]{k_3} EH + AOH$$

where EH is free enzyme, AB is the substrate with acid and alcohol represented by A and B, EHAB is a Michaelis complex between enzyme and substrate, EA is acylated enzyme, and AOH and BH are the free acid and alcohol.

Acylation occurs rapidly, and the overall reaction is governed by the rate-limiting step (k_3), the hydrolysis of the acylated enzyme. With the reference substrates, this hydrolysis is relatively fast (k_3 approx. 10^6 h^{-1}), but with the insecticidal phosphate and carbamate esters is very slow ($k_3 < 5\ h^{-1}$). Despite this extremely slow turnover, the enzyme causes resistance because it is present in aphids in molar quantity similar to the lethal dose of insecticides. Thus, the LD50 of organophosphorus insecticides to susceptible aphids is of the order of 1 ng, of which only 25%, or 1 pmol, penetrates. Susceptible aphids each contain approximately 0·3 pmol of E-4, and the most resistant aphids, 20 pmol. Because the enzyme is rapidly acylated, it can act as an efficient 'sink' for a large proportion of the lethal dose, even in susceptible aphids. Furthermore, as a result of the slow turnover by hydrolysis of the acylated enzyme, even more of the insecticide is detoxified by hydrolysis. The rate of enzyme turnover depends on the chemical nature of the acid in the ester. Hence by measuring the rate of hydrolysis of E-4 acylated by a range of acids, guidelines can be established for the types of ester to which there will be least resistance, or which may be used as synergists for other insecticides because they are bound irreversibly to the enzyme.

The purified enzyme was acylated by incubating with a large excess of ester, which was then removed by gel filtration, and the enzyme activity assayed at intervals during recovery with 1-naphthyl acetate to calculate the first order rate constant (k_3) for hydrolysis.

It is clear that the hitherto widely used dimethylphosphate esters such as dimethoate and demeton-S-methyl are hydrolysed most rapidly by the enzyme, explaining the

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TABLE 3

Rate of hydrolysis of E-4 acylated by a range of phosphate and carbamate esters

	R_1	R_2	k_3 (h^{-1})
$R_1R_2P(O)$-E4	CH_3O	CH_3O	3·8
	C_2H_5O	C_2H_5O	0·32
	nC_3H_7O	nC_3H_7O	0
	$isoC_3H_7O$	$isoC_3H_7O$	0
	CH_3O	CH_3S	0
	C_2H_5O	nC_3H_7S	0
	CH_3O	$isoC_3H_7NH$	0
$R_1R_2N.C(O)$-E4	CH_3	CH_3	0·097
	CH_3	H	0·093
	C_2H_5	H	0·034
	nC_3H_7	H	0
	$isoC_3H_7$	H	0
	nC_4H_9	H	0·79

practical observation that resistance is greatest to this class of insecticide. The more recently introduced carbamates such as pirimicarb (dimethylcarbamate) and ethiofencarb (monomethylcarbamate) are more effective against resistant aphids because they are hydrolysed at about one fortieth the rate of dimethylphosphate esters. Acephate (O,S-dimethylphosphoramidothioate) is also very effective because it phosphorylates the enzyme irreversibly. Several other classes of both phosphate and carbamate esters should have equally good potential provided insecticidal examples with the appropriate chemical and physical properties can be found.

***Factors affecting the stability of insecticide resistance in* M. persicae.** Anholocyclic *M. persicae* from the glasshouse and field can be classified into six variants, each with its characteristic carboxylesterase activity and consequent resistance to carbamate and organophosphorus insecticides. Overlapping activity distributions prevent unequivocal classification of an individual from measurements of its enzyme activity, but this may be done by cloning and measuring the frequency distribution of enzyme activity in the progeny. Although each of the four lowest esterase variants has a frequency distribution with a single peak, only the susceptible (US1L) is best described by a single normal distribution. Those of the other clones (MS1G, French R and T1V) correspond better with two normally-distributed components. However, these are not well separated and the lower component accounts for only 10–25% of the data. The very resistant clones from glasshouses have higher mean activities but with very broad distributions covering the whole range observed in this aphid. These broad distributions have peaks corresponding to those of the less resistant variants, and arise from the appearance at each generation of a small proportion of individuals with much less active enzyme than their parents. Careful monitoring of the differences in activity between parents and offspring of the most resistant variant (G6) showed that complete loss of resistance can occur either in a single step or over several generations, and that below a threshold value, reversion to higher levels is very rare. Such reversion could only be detected by selection with insecticide of large clonal populations from an individual that had lost activity. High esterase activity was not stabilised by breeding for 16 generations from only individuals with high esterase activity and is probably maintained in glasshouse populations by continuous exposure to insecticides. Spontaneous loss of esterase activity and resistance was only observed in populations from glasshouses. Measurement of the activity of E-4 in the clones examined, (rather than total esterase activity), clearly showed a doubling between each successive variant. As we have shown that this arises only from changes in the *amount* of enzyme, this doubling is most likely to be caused by a series of tandem dupli-

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cations of the structural gene for this enzyme. Loss of resistance may be attributed to the instability of the polyplicate sequence of genes in the very resistant variants. This is the first instance where such a mechanism has been identified as the cause of insecticide resistance. (Devonshire, Moores, Petzing, Rice, Sawicki and Stribley)

Detection of pyrethroid resistance in houseflies in Britain. At the request of Mr. G. Bills of ADAS we investigated reasons for poor control of houseflies in a farrowing house in the Ipswich area where synergised natural pyrethrins had been used for about 10 years.

Bioassays showed that this Ipswich strain was one-fifth to one-tenth as susceptible to natural pyrethrins and several synthetic pyrethroids as our standard susceptible strain. It also strongly resisted trichlorphon, malathion (with and without tributylphosphorotrithioate) tetrachlorvinphos and DDT, but was relatively susceptible (Resistance Factor, RF, approx. 10) to dimethoate. Its resistance to tri-butyl tin acetate, a diagnostic compound for the modifier *Pen* was exceptionally high.

Most of the resistance to pyrethroids in the Ipswich strain is controlled by an incompletely dominant factor on chromosome two, which when homozygous gives approximately eight-fold resistance to bioresmethrin, and by a very minor factor(s) on chromosome three. *Kdr*, the major factor of resistance to pyrethroids in Danish houseflies (Farnham, *Rothamsted Report for 1977*, Part 1, 141) may be present, but at very low frequencies.

Pyrethroid resistance similar to that in the Ipswich strain was also detected in samples collected from a pig-rearing farm near Harpenden, where pyrethroids were introduced only recently to control houseflies resistant to organophosphates.

These results emphasise the need to use these powerful insecticides, particularly those having residual action, with the utmost discretion to avoid the proliferation of resistance which would reduce the life of a most valuable range of products for fly control.

***Mechanisms of resistance in houseflies:* super-kdr.** Pyrethroid resistance on chromosome three in the Danish 153y^3 multi-resistant strain of houseflies (*Rothamsted Report for 1977* Part 1, 140) is most probably controlled by *super-kdr*, a more potent form of *kdr*. *Super-kdr* confers stronger resistance to pyrethroids than *kdr* (Table 4) and like *kdr* is unaffected by the usual synergists for pyrethroids or DDT. Although both *kdr* and *super-kdr* are fully recessive for resistance to cismethrin and incompletely recessive for resistance to DDT (Table 4) when crossed to the susceptible stock they interact when present together in the heterozygote to give strong resistance to DDT and to a lesser extent to cismethrin. Cross-over between *kdr* and *super-kdr* was absent in 920 flies tested, indicating that it is either absent or rare. These results, together with other evidence indicate that *kdr* and *super-kdr* are very probably allelic.

TABLE 4

Cross-resistance to DDT and cismethrin of strains 538ge (kdr), Super-kdr *and F_1 progenies of crosses between these and the susceptible Cooper strain*

Strain or cross	LD50, μg per fly Females Cismethrin		Males DDT	
		×÷SE		×÷SE
Cooper (susceptible)	0·0040	1·14	0·022	1·07
538ge	0·072	1·10	0·48	1·09
Super-kdr	1·5	1·11	15·0	1·15
F_1 (Cooper × 538ge)	0·0040	1·10	0·10	1·07
F_1 (Cooper × *Super-kdr*)	0·0040	1·11	0·11	1·06
F_1 (*kdr* × *Super-kdr*)	0·044	1·08	2·9	1·10

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Activity of carbinol analogues of DDT against DDT-resistant houseflies. Houseflies with known genetically isolated mechanisms of resistance to DDT (*DDT-ase*, *DDTmd*, *kdr*, *super-kdr*) show little or no resistance to FDMC (bis(*p*-chlorphenyl) trifluoromethyl carbinol) and dicofol, two carbinol analogues of DDT. These compounds also produce very different symptoms of poisoning compared with DDT. It is therefore likely that the carbinols act at a site different from that at which DDT exerts its primary action.

Compounds acting at such different sites could be capable of overcoming certain types of resistance mechanism, and these findings therefore suggest that re-examination of well-known groups of insecticides with this in mind could be rewarding. To obtain more information about the mode of action of the carbinol analogues, their activity against the nervous systems of housefly larvae and adults is being examined. (Sawicki, O'Dell and Willott; neurophysiological work: Goodchild)

Side effects of pesticides on beneficial insects

Poisoning of honeybees in the field. Samples of honeybees thought to be poisoned were again received from beekeepers via the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food, which also collected evidence to indicate how poisoning had occurred. Of the 228 samples, 218 have been examined so far. Evidence for the presence of an insecticide or its effects was demonstrated in 187 cases, compared with totals of 71 in 1977 and 105 in 1976. Triazophos was found in 136 samples, a further 28 involved other anti-cholinesterase insecticides, while HCH was found in seven samples. In most cases where poisoning was indicated, the probable circumstances were identified.

Eleven poisoned samples were associated with the application of aphicides to field beans, and two with treatment of sugar beet. Only four involved cereals compared with 30 in both 1976 and 1977; and these were applications early in the season to control wheat bulb fly. We presume this reduction is attributable to the low incidence of cereal aphids in 1978.

Of the 136 cases where triazophos was found, three were associated with application to peas and the remainder to oilseed rape, all but four from the air. Limited clearance was given by MAFF for triazophos to be used at 'post-blossom' stage on oilseed rape, primarily to control seed weevil, but the restriction to 'post blossom' appears to have been loosely interpreted, resulting in insecticide application while bees were still in the crop. After widespread reports of heavy bee casualties the clearance was withdrawn on June 20th. We suspected poisoning by triazophos in 1977 but were unable to obtain confirmation because cholinesterase inhibition was found to be reversible with this insecticide. Our general test for anti-cholinesterase could therefore not be used. Eventually a specific method, based on extensive clean up of extracts followed by gas chromatography was developed by Miss E. Findlay of the Department of Agriculture for Scotland, and we have used this successfully. (Smart and Stevenson)

Physiological selectivity of insecticides to different insect species. To broaden our studies on the effects of pesticides on beneficial insects and provide a more fundamental basis for seeking compounds having favourable selectivity, investigations into the relative activity of systematically related structures against a representative pest and its parasite were initiated. The insect species chosen, *Ephestia kuhniella* (a lepidopterous pest of stored products) and *Venturia canescens* (its hymenopterous parasite) are readily reared in the laboratory. To assess susceptibility to insecticides, topical application techniques for the host (as adult or larvae) and the parasite, and an injection technique for *Ephestia* were developed.

Pyrethroids were chosen as the first group of insecticides for study because a wide

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range of active compounds is available in the Department. Furthermore, the recently developed photostable pyrethroids generally have wide-spectrum activity and greater selectivity would clearly be an advantage in many situations. Certain examples from both earlier and new compounds show sufficient specificity to encourage detailed investigations. Results for the first five pyrethroids examined (Table 5) indicate only minor (less than four-fold) variations in relative activity consistent with the established wide-spectrum characteristic. Nonetheless relative activity against *Venturia* compared with *Ephestia* was generally higher for bioresmethrin than for the four halogenated pyrethroids. Amplification of this effect will be sought using further compounds in an attempt to establish the underlying structure-activity relationships. (Stevenson and Walters)

TABLE 5

Relative activities of pyrethroids to different insect species

	Ephestia			*Venturia*
	Topical		Injection	Topical
	Adult	Larva	larva	adult
Bioresemthrin	100[a]	100[b]	100[c]	100[d]
Cismethrin	190	230	76	73
1R,*trans*permethrin	200	140	200	72
1R,*cis*permethrin	250	520	190	110
Decamethrin	2100	870	1900	590

LD50 values (μg per insect): [a] 0·0043; [b] 0·52; [c] 0·024; [d] 0·012.

Control of soil-borne pests

Pyrethroid seed treatments for controlling wheat bulb fly larvae. In continuing studies short-row field trials were done to test (a) permethrin with and without synergists as dry seed treatments and as infused treatments in acetone, and (b) three new experimental pyrethroids. The trials were on a peaty loam site with 6·1 million eggs ha^{-1} and on a clay loam site with 14·8 million eggs ha^{-1}.

None of the new compounds tested ('NRDC 169', 'NRDC 170' and 'NRDC 181') was consistently more effective than permethrin when tested at 0·1% a.i. to weight of seed.

Formulations containing 25% permethrin seed treatment synergised with four times the amount of piperonyl butoxide or 'FMC 16388' were applied to fungicide-dressed seeds at the rate of 0·00625% a.i. (permethrin) to weight of seed. 'FMC 16388' had no synergistic effect, but permethrin at 0·00625% plus piperonyl butoxide was at least as effective in preventing attack by wheat bulb fly larvae as the standard permethrin formulation applied at 0·1% a.i. to weight of seed.

The infusion treatments were applied by soaking seeds for 1 hour in acetone containing permethrin with or without synergists. No fungicide treatment was included. Because all infusion treatments were phytotoxic, including the control treatment where seeds were soaked in acetone alone, it was not possible to measure insecticidal or synergistic effects. (Griffiths, Jeffs and Scott)

Seed treatments for controlling slugs in cereals. Co-operative field trials with ADAS were undertaken to test the more promising materials from last year's laboratory tests. Slug damage was insufficient for reliable assessment in two of the trials but at the third site on a silty loam soil, a severe slug attack developed in December. Seed treatments with methiocarb at 0·2% a.i. to weight of seed and 'SAN 155' at 0·1% gave slightly better plant establishment than the controls in April. Treatments with ioxynil and bromoxynil were phytotoxic. (Griffiths and Scott)

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Chemicals influencing invertebrate behaviour

We continued a wide-ranging programme on behavioural responses to chemicals which might be exploited for pest control or management of beneficial insects. Much of this work is done jointly with the Entomology Department: the description of these studies is therefore divided between the reports of the two Departments.

Aphid alarm pheromones. Complete chemical characterisation of alarm pheromones continued, in conjunction with measurements of the biological activity of the synthetic components.

Chemical characterisation. (E)-β-farnesene, a component of the alarm pheromone of *Myzus persicae* and *Megoura viciae*, was present at average amounts of 1 ng and 2 ng respectively in single cornicle secretions. Other compounds detected in single cornicle secretions from *M. persicae* were identified by GC-MS analysis of the vacuum distillate from whole aphids as (Z,E)-α and (E,E)-α-farnesene. Further components of the distillate were tentatively identified as (E,Z)-α-farnesene, 2-phenyl-ethanol and 2-phenylethyl isothiocyanate. The latter two compounds were presumably derived from the food plant, Chinese cabbage. Amounts of β-pinene found only in *M. viciae* ranged up to 50 ng per single secretion but the concentration ratio with α-pinene was almost constant at 8·5:1. Another component occasionally detected in *M. viciae* was tentatively identified as limonene.

Estimation of activity. A glass apparatus was used to test responses of *M. persicae* to alarm pheromones in a more quantitative manner than previously. In this apparatus, air having passed over a known quantity of test material was directed to a leaf of Chinese cabbage bearing a colony of aphids. Responses, measured as number of aphids that moved, varied from about 5% with air alone or camphene to about 80% with (E)-β-farnesene. Amounts of 1–2 ng farnesene produced consistent, large responses at least as great as those given by single crushed aphids. Increasing the amount 1000-fold made little difference to the response; decreasing the amount below 1 ng gave smaller and unreliable responses, depending on the condition of the leaf colonies.

The apparatus was less suitable for studying responses of *M. viciae* to pheromones as leaf colonies of this species were generally less responsive. Best responses were obtained by puffing air from a glass syringe containing test material over colonies of aphids established on intact bean plant stems. Tests by this method showed that a mixture of pinenes and farnesene gave a substantial response; of the individual components, (–)-α-pinene was the most active.

Almost pure (E)-β-farnesene was obtained in poor yield from the purified precursor, (E)-nerolidol, after selective aerial oxidation and liquid chromatography on silver nitrate impregnated silica gel. However, impure (E)-β-farnesene, conveniently prepared in large amounts from the commercially available nerolidol (which includes (E) and (Z) forms), elicited similar responses in the bioassay. These findings will facilitate future investigations into use of alarm-active substances for controlling *M. persicae*. (Griffiths and Pickett)

Honeybee Nasonov pheromone. Chemical characterisation of the Nasonov pheromone continued (*Rothamsted Report for 1977*, Part 1, 146). Geranic acid was confirmed as a component and nerolic and geranic acids were estimated by GC analysis of methylated extracts from excised glands, with pelagonic acid as internal standard. A synthetic mixture with similar composition to the Nasonov secretion was prepared and bioassayed

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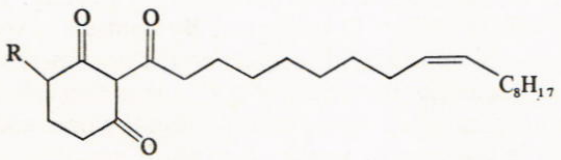
(see Report of the Entomology Department). Components of the pheromone have different volatilities and on exposure to air for 1 week the synthetic mixture, containing fixative and antioxidant, lost proportionally more (E)-citral than the less volatile component geraniol. However, analysis of residual contents of Nasonov glands after intermittent scenting, showed no reduction in the ratio of (E)-citral to geraniol. Furthermore after extensive scenting the ratio increased. It was also observed that excised Nasonov glands converted both endogenous and added geraniol to (E)-citral by what appears to be a relatively specific enzymic process. Such conversion of the more abundant geraniol to (E)-citral could enable the honeybee to maintain composition of the pheromone despite differential loss of its components. Conversion could also provide a mechanism for altering the composition of the secretion during scenting. However this was shown to be unlikely by bioassay and analysis of air from the vicinity of scenting honeybees which gave a ratio of (E)-citral to geraniol similar to that from air above the synthetic pheromonal mixture immediately after exposure. (E)-citral was further converted by excised Nasonov glands to geranic acid, which could also be used for regulating composition of the pheromone. Similar mechanisms may be employed to maintain composition of multi-component pheromones in other insects. (Pickett and Smith, with Williams and Martin, Entomology Department)

Pheromones of *Anagasta kuehniella* (Zeller). Biologically active compounds of a class hitherto unknown in animals were isolated from larvae of *A. kuehniella*. The larval mandibular gland secretion of *A. kuehniella* contains pheromones which regulate population density in three ways. Firstly, at high concentrations it inhibits egg-laying by the adult moth; secondly, it causes the larvae to disperse resulting in smaller pupae and consequently less fertile adults; thirdly it is detected by the Hymenopteran parasite *Venturia canescens* (Grav.) and elicits oviposition. The oviposition movement of the parasite was used as the bioassay to detect active material.

Several gland components were previously isolated by thin layer chromatography (TLC) and identified by mass-spectroscopy but none was active. Additional spectroscopic information was necessary to determine the structures of the active compounds and to obtain this information very much larger quantities of material were required. Material from 2500 dissected pairs of larval mandibular glands was separated by high pressure liquid chromatography (HPLC) giving two active compounds.

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR), ultra-violet, infra-red and mass-spectra of the two compounds were determined and also their circular dichroism.

The chemical and spectroscopic data on the compounds are consistent with enol forms of 2-oleoylcyclohexane-1, 3-dione (I, R=H) and 4-hydroxy-2-oleoylcyclohexane-1, 3-dione (II, R=OH):



(I) R = H
(II) R = OH

These β-triketones were previously unknown in animals; a few have been identified in plants but with different patterns of substitution. By analogy with the triketones found in plants it seems possible that the compounds will have antibiotic activity. (Mudd)

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Sex attractant lures for pea moth. Rubber lures containing (E)-10-dodecenyl acetate, a sex attractant for the pea moth (*Cydia nigricana*) discovered in previous work, have now been in use for three seasons to monitor moth populations in pea-growing areas of Britain (*Rothamsted Report for 1977*, Part 1, 94). The information from this monitoring survey (undertaken in collaboration with ADAS and PGRO) has replaced the previously used egg-counting system as the basis for advising growers on a regional basis about the date to apply insecticides. In 1978 a moth trapping kit using (E)-10-dodecenyl acetate as attractant became commercially available (Oecos Ltd., Kimpton, Herts.) for growers to monitor pea moth in their own crops.

Ideally, the lure should be equally attractive to moths throughout a single flight season (up to 3 months) so that growers do not need to replace weathered lures during this period. Field tests showed that lures containing 1–10 mg are equally attractive and that 1 mg lures maintain constant attraction for about 1 month. The release of (E)-10-dodecenyl acetate from the rubber lures was investigated in the light of these results to determine a suitable initial loading.

Lures dosed with 1, 3 and 10 mg of (E)-10-dodecenyl acetate were exposed in the field and samples analysed at intervals. Loss of attractant followed a simple exponential course for all three loadings with half-lives ($t_{\frac{1}{2}}$) of 64, 65 and 67 days respectively. Thus, lures with an initial dose of 3 mg should contain approximately 1 mg after 3 months field exposure and therefore maintain constant attractiveness during this period. (Greenway and Davis)

Fungicides

Soil-borne diseases

Control of soil-borne diseases by foliar sprays. Work described in previous reports showed that the incidence of potato common scab or clubroot of cabbage, both caused by soil-borne organisms, can be decreased by foliar sprays of daminozide (scab only), flurecol (clubroot only) or ethionine (both diseases). Work this year was directed towards clarifying how these chemicals exert their effects, and at finding others with similar action.

Laboratory tests with ethionine. Previous evidence indicated that ethionine may decrease scab incidence by acting directly on the pathogen (*Streptomyces scabies*).

Studies this year showed that exposing *S. scabies* for short periods (up to 1 h) to ethionine in liquid medium had the following effects. At 0·2 mM, ethionine increased incorporation of radio-labelled methionine into protein, and decreased conversion of ^{35}S-methionine to S-adenosyl methionine (SAM) and to cysteic acid. At 2·0 mM, ethionine decreased incorporation of methionine into protein in addition to decreasing the amounts of label in SAM and cysteic acid. Long term exposure (24h) to ethionine (0·2 and 2·0 mM) led to a general decrease in metabolism, as indicated by incorporation of lysine into protein, and by the uptake of lysine and homoserine; at 2·0 mM, ethionine markedly decreased the amount of methionine incorporated into protein. In these experiments, L-ethionine was more effective than D-ethionine. By contrast, experiments with potato tuber tissue showed that ethionine had little effect on the host metabolism.

The results suggest that ethionine selectively disrupts the metabolism of *S. scabies* because the pathogen accumulates it more readily than the host and has a higher rate of metabolism. Ethionine is toxic to *S. scabies* because it specifically inhibits methionine metabolism.

Experiments with the model system of coltsfoot infected with *Puccinia poarum* showed that the pathogen absorbed ethionine from the host, and that ethionine inhibited the absorption of methionine and its incorporation into protein by the pathogen.

Other work showed that ethionine is very toxic to *Phytophthora infestans* in culture and as a potato foliage protectant, and to *P. erythroseptica* in culture. (Burrell)

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Field investigations of scab control. Control of potato common scab by daminozide was investigated in a field trial (cv. Maris Piper) at Woburn. The plants were sprayed once or three times with 1% solutions at about 1400 litres ha^{-1} in June. Scab indexes calculated at harvest from 50 ware tubers per plot (three plots per treatment) were: after single sprays on 7, 11 and 19 June, 8; after three sprays on 7, 11 and 19 June, 6; from unsprayed plots, 15. (LSDs: 7 (P=0·02) and 8 (P=0·01)). Effects on scab incidence were not related to the timing of the single sprays, nor to the amount of daminozide sprayed. (McIntosh)

Glasshouse tests against clubroot of cabbage. Soil in which potted 3-week old cabbage plants were growing was inoculated with spores of *Plasmodiophora brassicae*. The leaves were sprayed with solutions of experimental chemicals 14 and 21 days later. Spray mixtures contained enough surfactant to wet the leaves thoroughly, but the soil was protected from spray solution. Fresh weights of tops and clubs (five plants per treatment) were measured when the plants were about 10 weeks old. Effects on tops were less marked than on clubs.

The action of ethionine sprays in decreasing club weight was largely reversed by providing the plants with methionine, the natural amino acid to which ethionine is closely related. In two experiments with DL-isomers, mean fresh weights (g) of clubs were: (i) from plants sprayed twice with 0·5% ethionine, 3·5; (ii) from plants sprayed twice with 0·5% ethionine plus 0·46% methionine (i.e. equimolar concentrations), 6·5; (iii) from plants sprayed as (i) but with three methionine soil drenches (0·1 g per 50 ml per pot) before, between and after the sprays, 11·4; (iv) from plants sprayed as (ii) and drenched as (iii), 12·8; (v) from unsprayed undrenched plants, 15·8. (LSDs: 2·5 (P=0·05), 3·3 (P=0·01) and 4·3 (P=0·001)).

Tests of the morphactins flurecol and chlorflurecol confirmed that chlorflurecol was the more active. In three experiments, mean fresh weights (g) of clubs after sprays at M/5000 were: flurecol, 10·6; chlorflurecol, 7·9; unsprayed, 14·0 (LSDs: 2·7 (P=0·01) and 3·4 (P=0·001)). The flurecol sprays decreased club weight less than ethionine, but were much more dilute; their effects did not increase with increasing concentration (McIntosh, with Macfarlane, Plant Pathology Department)

Growth room tests against clubroot of swede. A relatively rapid bio-assay was developed for testing the activity of foliar-applied fungicides and growth regulators against clubroot of swede. Seeds were sown in a sand:soil mixture (3:1, pH 4·5) saturated with a spore suspension of *Plasmodiophora brassicae* (Ingram's strain S, 3×10^6 spores ml^{-1}). Controls were similar except that the spore suspension was replaced by distilled water. Plants were kept in a growth room at 16°C lit by fluorescent tubes (1400 lux). Ten days after sowing the infected seedlings were transferred to EFF compost. At 17 days solutions of the test compounds were applied to the first pair of leaves with a small camel-hair brush. The treatments were repeated at 20 and 23 days. At 30 days the plants were harvested and disease severity assessed. So far the following compounds have been tested: the fungicide, aluminium tris (ethyl phosphonate) 'LS 74783' and the growth regulators, maleic hydrazide (MH), 2-chloro-ethyltrimethyl ammonium chloride and 2,3,5-triiodobenzoic acid. Of these, only MH had a marked effect on disease symptoms. At 5 g $litre^{-1}$ the clubbing was very much reduced compared to that of untreated plants. However, at this concentration MH caused root discoloration and reduced the number of leaves. (Butcher, Searle and Sogeke)

Studies on the host's response to clubroot disease. The characteristic symptom of clubroot disease, the abnormal tissue proliferation of the roots, suggests that *Plasmodio-*

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phora brassicae interferes with hormonal regulation in the host. Furthermore it has been established that most crucifers susceptible to clubroot accumulate large amounts of certain glucosinolates, (eg. 3-indolylmethylglucosinolate (IMG) and benzylglucosinolate) which can be converted under certain conditions to compounds with high auxin activity (eg. 3-indolylacetonitrile (IAN) and phenylacetonitrile (PAN)). Such observations and evidence from earlier investigations led to a hypothesis implicating these compounds in the host's response to *P. brassicae*. It was suggested that when the pathogen infects the tissues of the host, it disturbs cellular organisation in such a way that the glucosinolates are enzymatically transformed to the products with auxin activity. It was supposed that high levels of these products caused abnormal proliferation of the root tissues. This hypothesis has been investigated further by monitoring the changes in the concentrations of IAN 3-indolylacetic acid (IAA) and IMG during the early stages of club-root development. Infected and non-infected swede plants were grown by the methods described in the previous section and samples taken at intervals from 19 to 33 days after sowing. The IAA and IAN concentrations in the tissues were measured by the α-pyrone spectrophotofluorimetric method and IMG determined indirectly from the thiocyanate ions liberated during enzymatic degradation. The results showed that before visible symptoms developed (19-day sample) IAN concentrations in infected tissues were about three times those in non-infected tissues. This difference was maintained until 26 days, but thereafter declined. The concentration of IMG was also increased in the infected tissues, but the increase occurred after visible symptoms developed and was maintained for the remainder of the experiment. No significant differences in IAA levels between infected and non-infected tissues were detected. The findings that the concentration of IAN, a probable degradation product of IMG, was very high in infected tissues at the very early stages of clubroot development is consistent with the proposed hypothesis. (Butcher, Searle and Sogeke)

Fungicides for soil-borne diseases of wheat. Effective measures to control take-all would be of great value but attempts to find satisfactory chemical treatments have so far been unsuccessful. We are therefore undertaking a systematic search for effective fungicides and examining the basic requirements for chemical control.

In pot experiments benomyl was the most effective systemic fungicide applied as a soil mix treatment against take-all. 'KWG 0599' also gave some control. Benomyl, 'KWG 0599' and iprodione inhibited disease when applied to pots as pre-emergence drenches; nuarimol was slightly effective with both application methods. Benomyl soil treatment controlled eyespot in some pot experiments. Several other fungicides ineffective as soil treatments against take-all were as active as benomyl against *Gaeumannomyces graminis* (the take-all fungus) on agar plates. Foliar sprays with several chemicals reported to act as downward translocated fungicides in some plant species had no effect on take-all in pots.

Field plots treated by placing benomyl powder beneath the seed rows at 35 kg ha^{-1} had significantly less take-all than untreated plots up to growth stage (G.S.) 39. Benomyl incorporated in gelatin fragments (as a slow release formulation) and applied at the same rate controlled take-all to a similar extent. There was also a significant reduction in incidence and severity of eyespot with gelatin treatments, which may be partly attributable to the nitrogen contained in the gelatin. Hardening the gelatin by treatment with formaldehyde did not seem to prolong the fungicidal action. The effects of gelatin alone and of a lower rate of benomyl are being investigated. (Bateman)

Foliar disease of cereals

Attempts to grow* Erysiphe graminis *f. sp.* hordei *in axenic culture. Our biochemical studies on host-pathogen interactions of *E. graminis* and on mode of action of fungicides

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would be greatly facilitated by techniques for growing the pathogen in axenic culture. This is very difficult to achieve with such an obligate parasite, but some encouraging results have been obtained in investigations into the requirements for suitable media.

As a pre-requisite for attempts to grow the pathogen in axenic culture, two methods for isolating and maintaining uncontaminated cultures were developed. The first depends on the regeneration of uncontaminated fungus from infected barley leaves whose surfaces had been sterilised by treatment with 6% w/v sodium hypochlorite for 5 min. The other method involves the germination of ascospores liberated from cleistothecia. Single spore isolates from uncontaminated cultures were tested for virulence against a series of barley varieties. The infection types produced were very similar to those obtained from the original contaminated strain.

The uncontaminated fungal material has been used in preliminary experiments to test the effect of various complex organic supplements on germ-tube growth. The 20 longest germ tubes on each of five agar plates were measured for each treatment after 7 days incubation of conidia. Additions of peptone and yeast extract at 2·5 g litre^{-1} have resulted in two-to-three-fold increases in germ tube length but so far indefinite growth has not been achieved. (Sogeke, Butcher and Searle)

Mode of action of ethirimol. Previous work (*Rothamsted Report for 1977*, Part 1, 154) suggested that ethirimol inhibited barley powdery mildew through some effect on adenine metabolism. Detailed information on purine metabolism during primary infection has now been obtained using 8-^{3}H adenine as the precursor. Acid soluble metabolites were separated by ion-exchange HPLC, and nucleic acids by polyacrylamide gel electrophoresis after phenol-sodium dodecyl sulphate extraction. Six hours after inoculation 70% of the incorporated radioactivity was recovered as adenine. Much of the remainder was present as adenosine (9%) and inosine (7%), with smaller amounts in hypoxanthine (3%), inosinate (3%), adenylate (3%), NAD (1%) and NADP (1%). All classes of RNA were labelled. If ethirimol was present during this period appressoria failed to form, but ^{3}H-adenine uptake was unaltered. Incorporation into inosine, nucleotides and RNA was reduced. The amount of radioactivity in all adenine metabolites had increased after 24 h incorporation, when appressoria were fully formed. At this stage inosine was the most heavily labelled intermediate. This incorporation was reduced by 64% if ethimirol was present. Incorporation into nucleotides and RNA was even more severely inhibited by ethirimol.

Because insufficient developing appressoria were available, the *in vitro* effect of ethirimol on several enzymes involved in purine metabolism and interconversion was examined using dialysed extracts from ungerminated conidia. Radiotracer assays were used throughout and the products separated by TLC. Adenine phosphoribosyl transferase (E.C. 2.4.2.7), adenosine phosphorylase (E.C. 2.4.2.1) and adenosine kinase (E.C. 2.7.1.20) were all detected, indicating that conidia may well synthesise adenylates from adenine by at least two distinct routes. None of these was inhibited by ethirimol. Although extracts did not deaminate AMP directly, it was rapidly converted first to adenosine (5′ AMP nucleotidase E.C. 3.1.3.5 – or possibly alkaline phosphatase), and then to inosine (adenosine deaminase E.C. 3.5.4.4.)

Conidial adenosine deaminase is a soluble enzyme which also catalysed the deamination of deoxyadenosine. Adenine, AMP, guanosine and cytidine were not deaminated. Activity of the deaminase from both ethirimol-resistant and sensitive conidia was inhibited non-competitively by ethirimol (k_I 4 × 10^{-4}M).

Deaminase activity in heavily diseased barley plants 8 days after inoculation was two to three times that in healthy plants. However, whereas the activity in healthy plants was not inhibited by ethirimol, that in diseased plants was. Dimethirimol, and 6-methyl

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purine, an adenine analogue which affects mildew development in ways similar to ethirimol (*Rothamsted Report for 1976*, Part 1, 181), both inhibited adenosine deaminase activity in conidial extracts.

Ethirimol acts specifically against powdery mildews. It inhibited the deamination of adenosine in extracts from *Erysiphe polygoni* conidia, but not in extracts from the spores of *Puccinia obtegens*, *Uromyces phaseoli*, and *Penicillium* sp. Purified adenosine deaminase from calf-intestinal mucosa was not affected by the fungicide, whilst in yeast (*Saccharomyces cerevisiae*) little or no deaminase activity could be detected. It seems that the mode of action of ethirimol may involve the inhibition of adenosine deaminase. However, the reason why failure to form inosine, or deoxyinosine, should restrict mildew development is as yet not clear. (Hollomon)

Competitive ability of ethirimol resistant mildew strains in the field. Cold weather during April and May delayed the appearance of mildew in field plots of Proctor barley, grown from ethirimol ('Milstem') treated seed. By G.S. 31 (May 25th), 14% of the first leaf was infected; but only 7% in plots which received 3·2 g ethirimol kg^{-1} seed, and 1% in plots receiving 14·0 g kg^{-1} seed. At this stage, only the mildew from plots treated at this high ethirimol rate showed any significant resistance to ethirimol, as indicated by bioassay (Table 6).

TABLE 6

Ethirimol sensitivity in field plots

Ethirimol treatment (g kg^{-1} seed)	ED50 ($\mu g\ ml^{-1}$ ethirimol) G.S. 31	G.S. 39	G.S. 46
None	0·61	0·91	1·00
0·93	0·80	0·82	0·90
3·20	1·60	0·97	1·30
14·04	10·10	5·25	1·25
SE	∓2·90	∓1·30	∓0·40

Mildew control was not maintained, for by G.S. 39, when the epidemic was at its peak, the infection levels on the second leaf was 23% regardless of whether plots had been treated with ethirimol or not. Where resistance to ethirimol had been present it declined, and by G.S. 46 sensitivity was uniform throughout. Furthermore, the range of variation observed in bioassays of genotypes from treated and untreated plots was identical. Thus, mildew may be present in ethirimol treated plots at G.S. 46, or later, but this provides no indication of how sensitive it is to ethirimol.

These results are consistent with earlier laboratory and field studies (*Rothamsted Report for 1977*, Part 1, 153), for although ethirimol clearly selected for resistance at the start of the epidemic, the resistant strains did not compete well and their frequency declined as the epidemic gathered momentum. Despite control of mildew, ethirimol did not increase yields. Reasonable yields (5·35 t ha^{-1}) were, however, obtained from control plots, suggesting that mildew levels were never sufficiently severe at those stages in crop development when yield is likely to be affected. (Hollomon)

Improving the efficiency of pesticide use

The broad philosophy underlying our studies on improving the efficiency of pesticide use is to determine the factors governing pesticide movement, degradation and availability in the crop environment and to establish the characteristics of target and non-target organisms as recipients for pesticides. This information can then be related to studies on application methods to suggest improved procedures.

Our programme includes soil and aerial systems. Various insecticides and fungicides and some herbicides are studied: the principles revealed by such studies should be of

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general application and in some cases compounds are therefore selected for investigation more on the basis of their physico-chemical properties than because of the type of biological activity they show.

Fundamental work on behaviour of pesticides in soil. Much has been learnt about the general features of pesticide movement and availability in soils and useful progress has been made in developing simulation models based on this knowledge (see p. 149). Even in favourable cases with uniform soils of simple structure, however, such models are not yet fully satisfactory and our understanding of behaviour in well-aggregated soils is much less advanced.

To evaluate simulation models more fully (particularly that devised by Dr. M. Leistra, Laboratory for Research on Insecticides, Wageningen (see *Rothamsted Report for 1977*, Part 1, 156) and to obtain more information about behaviour in soils of different structure, a comprehensive field experiment was started in collaboration with the Weed Research Organisation and the ARC Letcombe Laboratory. The movement of the herbicides fluometuron and simazine in comparison with tritiated water and $^{36}Cl^{-}$ions was investigated in cultivated and uncultivated plots at Begbroke and Compton Beauchamp. In complementary investigations designed to extend our previous laboratory studies of the kinetics of adsorption and desorption by natural soil aggregates, we examined the distribution of fluometuron in aggregates from the field experiment. The site at Compton Beauchamp has a well-structured soil suitable for this purpose. Sleeves, 45 cm square, were inserted into cultivated and uncultivated plots to a depth of 20 cm. The soil surface within the sleeves was treated with fluometuron mixed with a small amount of sand as carrier at 100 kg a.i. ha^{-1} in April. Sleeves containing the undisturbed soil blocks were removed for analysis after 1 and 5 months. Soil from the cultivated plots consisted of a surface layer (1 cm deep) of well developed crumbs overlying a more massive structure. This characteristic surface of loose crumbs was absent in the uncultivated plots. The top 1 cm from the cultivated soil was separated by dry sieving into different aggregate size fractions which were extracted with methanol and analysed for fluometuron by HPLC.

After 1 month the concentration of herbicide was greatest (375 ppm) in the $<$ 1 mm fraction. This fell steeply to 140 ppm in the 1–2 mm fraction and then declined steadily to 32 ppm in the $>$ 11·2 mm crumbs. After 5 months, amounts of fluometuron in the surface had declined largely due to leaching and degradation. Concentrations in the different aggregate fractions expressed as percentages of those after 1 month were: $<$ 1 mm, 18%; 1–2 mm, 16%; 2–4 mm, 8%; 4–5·6 mm, 66%; 5·6–8 mm, 75%; 8–11·2 mm, 69%, $>$ 11·2 mm, 100%.

Changes in relative concentration were largest in aggregates smaller than 4 mm, particularly the 2–4 mm fraction. These results are consistent with the previously reported observations that adsorption and desorption of herbicide is most rapid in the smallest aggregates. The weight of crumbs smaller than 4 mm was greater than that in larger fractions (2·7 : 1 in the sample taken at 1 month). The total amount of herbicide in each fraction was calculated from the concentration measurements and aggregate weight distributions. After one month, 88% of the fluometuron was associated with the $<$ 4 mm aggregates and 12% with the $>$ 4 mm aggregates; at 5 months the corresponding figures were 61 and 39%. This shift in favour of larger aggregates is likely to be associated with a decrease in availability for redistribution or biological action. (Graham-Bryce and Nicholls)

Factors influencing the adhesion of powders to seeds. Seed treatment is an increasingly important method for applying both residual and systemic insecticides and fungicides.

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Previous work in the Department established that existing methods of applying seed treatments have important limitations such as poor retention or uneven distribution between seeds. It was also shown that retention could be improved by the use of adhesives. Little is known of the principles determining adhesion to seeds, however, and we are therefore investigating the factors involved to provide a more informed basis for devising better treatments.

Location of powder retained on seeds. Seeds were treated at rates of 0·1, 0·3 and 0·5 g per 100 g with pure or 40% HCH powders of different particle size. Treated seeds were dissected into three parts: beard, body and scutellum, for measurements of initial adhesion. Further samples were examined similarly after a standard retention test. (*Rothamsted Report for 1973*, Part 1, 177). The separated parts from ten seeds were bulked and insecticide in each fraction determined by extraction and G.L.C.

Distribution of powder over the seed surface was not significantly affected by particle size, rate of application, formulation or by application of the retention test. In all cases the beard, a relatively small part of the seed carried about 30% of the powder. The proportion on the body of the seed was about 60% with roughly 5% on the scutellum. These results are consistent with previous electron micrographs of treated seeds which showed relatively large masses of powder entrained in the beard.

Amounts of pure γ-HCH retained by the seeds increased with rate of application over the range studied from approximately 400 μg per ten seeds at 0·1 g per 100 g seed to 1350 μg at 0·5 g per 100 g seed. Loadings of 40% powder were much less and appeared to reach a maximum at the intermediate rate of application. (Jeffs)

Factors influencing the efficacy of insecticidal seed treatments. To complement radiotracer studies by I.C.I. Plant Protection Division on persistence and movement in plants and soil of permethrin applied as seed treatment to wheat, the influence of seed depth and location of wheat bulb fly eggs on the effectiveness of permethrin seed treatments was examined in boxes of soil. Seeds sown 2 cm deep typically gave rise to a rhizome about 1 cm long beneath the bulb; 90% of the larvae that successfully entered the plant bored into the internal tissues within 2 mm of the junction of rhizome and bulb. Seeds sown at 6·5 cm depth typically resulted in plants with rhizomes about 4 cm long and about 75% of larval entry holes were within 2 mm of the junction of rhizome and bulb. In boxes with no insecticide, slightly more plants were attacked when wheat bulb fly eggs were placed near the surface than when placed deep (89 *v* 70 attacked plants) probably because larvae had less distance to travel in the soil. Permethrin seed treatments applied to shallow-sown seeds halved plant attack but were ineffective on deep-sown seeds, no matter where the wheat bulb fly eggs were placed, presumably because of the greater separation between insecticide on the seed and point of larval entry. (Griffiths and Scott)

Foliar sprays

Controlled drop application: placement spraying. Studies on the use of controlled drop sprays at relatively low volume rates against powdery mildew on barley were continued with modified equipment.

Field experiments in 1978 compared conventional hydraulic sprays with CDA placement spraying of tridemorph at rates of 700, 350 and 70 ml 'Calixin' ha^{-1} ('Calixin' contains 75% a.i. tridemorph).

The hydraulic sprays were applied with standard equipment at a spray volume rate of 335 litres ha^{-1} whilst Micron 'Battleship' spinning disc atomisers (Micron Sprayers Ltd) mounted on a boom were used for controlled drop application at a spray volume rate

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of 18 litres ha^{-1}. With the CDA treatments only, the effect of doubling the drop density was investigated by making two passes on certain plots. Permethrin was added to all treatments at a rate of 100 ml a.i. ha^{-1} as a tracer for subsequent analysis.

Leaf samples were taken throughout the crop at different levels for measurements of the gross deposition of chemical and its distribution and penetration within the crop. Disease was assessed at two intervals after application.

Results indicated that the hydraulic sprays at the standard rate of tridemorph (700 ml 'Calixin' ha^{-1}) controlled powdery mildew most effectively, but that similar results were obtained with the CDA sprays at half this rate. Doubling the drop density with the CDA treatments for the same rate of fungicide had no significant effect.

Yields were similar for both conventional and CDA treatments, being related more to the amount of a.i. per unit area than to the method of application. (Arnold, Etheridge, Phillips and Pye)

Controlled drop application: drift spraying. There is increasing interest in drift methods of crop spraying particularly for applying pesticides to cereals. The method was evaluated in field trials using the 'Ulvamast Mark II' sprayer fitted with a multi-disc 'Aerial' atomiser. This tractor-mounted unit delivered 600 ml min^{-1} of oil-based tridemorph dispersed as 60 μm drops. Permethrin was added to the formulation as a tracer for measurements of spray penetration and distribution within the crop. Tridemorph was applied at 526, 263 and 132 ml a.i. ha^{-1} to 50 × 250 m plots, the highest rate being calculated to give an estimated effective 'drift' swath width of 25 m and a total spray application rate of 1·8 litres ha^{-1}. After spraying, leaf samples were taken at distances up to 200 m downwind from the spray base line. The GLC analysis for these samples has yet to be completed.

Assessment of disease incidence 14 days after application indicated that at the full and half rate of tridemorph, mildew was controlled effectively up to 25 m from the spray source. These results will be compared with the spray deposition measurements. (Arnold, Etheridge, Phillips and Pye)

Bird repellents

Effect of formulation on the persistence of deposits. Collaborative work with Long Ashton Research Station has continued (*Rothamsted Report for 1977*, Part 1, 147) using the experimental acaricide 'PP 199' (I.C.I. Plant Protection Division) which is being evaluated as a bird repellent. The primary objective of our contribution was to investigate ways of prolonging the life of the spray deposit on the bud surface, mainly by altering the formulation.

In a small trial at Long Ashton the following formulations of 'PP 199' prepared at Rothamsted were compared:

1. A dispersable grain formulation (12·5% a.i.; I.C.I. Ltd.).
2. The same suspension plus 'Acronal 4D', a polybutyl acrylate (B.A.S.F. Ltd.).
3. A colloidal suspension (25% a.i.; I.C.I. Ltd.) to which methyl dibutylamine stearate was added.
4. A microencapsulated formulation (5% a.i.) with the a.i. dissolved in acetophenone and enclosed in polyurea walls.
5. An oil formulation (6·25% a.i.) with the a.i. dissolved in a mixture of 'Risella 33' oil (75%) and butyl methyl ketone (25%).

Treatments 1–4 were applied as 0·5% a.i. aqueous suspensions ('Acronal 4D' and methyl dibutylamine stearate were present at a concentration of 5% v/v) using a pressurised knapsack sprayer. Treatment 5 was applied by a spinning disc applicator (Micron

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Ulva 8') giving an approximate drop diameter of 80 μm. Each treatment was applied to both redcurrant and gooseberry bushes.

Bud damage by birds was assessed at weekly intervals after treatment. All treatments reduced bud damage compared with untreated controls, but damage incidence was disappointingly low (only 5% in the controls) so that differences were not significant.

Twelve weeks after application, twig samples were taken and 'PP 199' extracted by shaking with hexane for subsequent analysis by GLC. Amounts remaining were similar for all treatments (approximately $1{\cdot}2 \times 10^{-3}$ mg cm^{-2} surface area of twig) with the exception of the microencapsulated treatment which had significantly higher residues ($8{\cdot}6 \times 10^{-3}$ mg cm^{-2}). (Phillips and Etheridge, with Dr. B. D. Smith and Dr. D. A. Kendall, Long Ashton Research Station)

Equipment and techniques

Insect neuroanatomical techniques: modified synthetic fixatives for silver staining. Investigations continued into the action of the modified fixatives derived from synthetic aged alcoholic Bouin (*Rothamsted Report for 1976*, Part 1, 175) on insect nerve tissue to be stained by the Bodian protargol technique. It was found that one of the reaction products of ageing, the expensive diethoxymethane, could be replaced by increasing ethyl acetate concentration from the original 5 to 25%. The only obvious disadvantage was some increase in staining intensity, particularly of the neural lamella of ganglia, which necessitated more washing out or differentiation of sections after silver impregnation. Further experiments on other constituents of the fixative revealed that although picric acid concentration was not critical (*Rothamsted Report for 1977*, Part 1, 148) its omission markedly reduced the quality of both preservation and staining, and led to much undesirable staining of the glial tissue that surrounds the nerve cells. Omission of formaldehyde produce an opposite effect, greatly lessening or preventing altogether glial staining, without apparent detriment to the fixation or staining of the neurons. This modification seems to offer a promising new approach to the problem of staining neurons without simultaneously staining the glial cells that tend to obscure them, and is being examined further. (Gregory)

Equipment for mass pollination of coconuts. Work in support of the mass pollination programme continued, sponsored by ODM. A coconut flower stripper based on contra-rotating cylinders fitted with rubber fingers was developed and evaluated under field conditions. This apparatus proved effective and capable of stripping both immature and grossly distorted inflorescence stalks. The feed opening is restricted so that although several stalks can be stripped simultaneously there is negligible risk of an operator accidentally contacting the rotating components. The design evaluated is portable, simple and therefore reasonably inexpensive to produce.

A liquid pollen application system was constructed and tested. The applicator consists of a back-pack pressure vessel supplying air, via pressure reducing valve, to a twin-fluid atomiser mounted on the end of a set of light alloy telescopic poles. The effectiveness of this system and of liquid pollen application will not be known until adequate information on fruit-set can be obtained from the field. (Arnold and Pye)

Electrostatically charged spray application systems. An electrostatically charged spraying system using an inductively charged rotary atomiser was evaluated further in limited field trials. The most serious problem with the inductively charged atomiser was extensive wetting of the atomiser body caused by attraction of the oppositely charged particles to the atomiser.

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Laboratory work indicated the effect on spray distribution of high voltage charging of the liquid, and the influence of charge and distance from the atomiser on deposition. Work on the effect of air movement on the deposition and distribution of the charged spray is now in progress. (Arnold and Pye)

Insect activity. During insect-behaviour studies it was found that the flight activity of houseflies (*Musca domestica*) was related to pre-emergence conditions and the age of the flies, and that the initial level of activity when released into an aktograph area was related to the size of the rearing cages, population density and method of release.

To reduce or eliminate these sources of variation a compact apparatus for obtaining flies of a known age was developed. The apparatus operates in the principle of rotating a dish of pupae under a circle of cages so that emergence into the cages can be precisely controlled. With this equipment the emergence containers are large enough to permit continued rearing in controlled environment rooms until required for direct release into the aktograph cage. (Pye).

Seed treatment apparatus. The performance of a commercially produced laboratory seed treatment machine based on the 'Rotostat' principle and designed to treat 50–500 g of seed, was tested with liquid and powder formulations of permetrin.

By using a hypodermic syringe with a nylon canula extension it was possible to introduce accurately as little as 0·6 ml of liquid, via a spinning disc, to small batches of seed. Loadings achieved by this technique were 80–90% of the target dose which is good compared with traditional procedures. With powders only 54% of the target dose was achieved, unless the seeds were pretreated in the machine with a sticker, when more than 80% adhered to the seeds.

The machine was used to prepare seed for short row trials of seed treatment chemicals against wheat bulb fly. (Jeffs and Woodcock)

Insect rearing. The following species were reared:

Homoptera	*Aphis fabae* (Scop.)	
	Myzus persicae (Sulz.)	
	Strains.	Susceptible
		Resistant (several)
	Megoura viciae (Buckt.)	
Coleoptera	*Phaedon cochleariae* (F.)	
Orthoptera	*Blaberus cranifer* (L.)	
	Periplaneta americana (L.)	
Diptera	*Drosophila melanagaster* (Meig.)	
	Strain.	Vestigial wings
	Musca domestica (L.)	
	Strains.	A wild-type susceptible strain
		ac; ar; bwb; ocra—called 608, a multi-marker susceptible strain.
		SKA-diazinon selected, very resistant to many organophosphorus insecticides.
		Several strains derived from SKA, each with one or more factors of resistance to organophosphorus insecticides or DDT.
		Several strains derived from the dimethoate resistant $49r_2b$ each with one or more factors of resistance to dimethoate and other organophosphorus insecticides.
		49PPB, a substrain of $49r_2b$ derived by selection with pyrethrum extract and piperonyl butoxide.
		290BIO, a substrain of the dimethoate/bioresmethrin resistant 290rb derived by selection with bioresmethrin.
		NPR—pyrethrum extract selected strain.
		Several strains derived from NPR each with one or more factors of resistance to pyrethroids and DDT.

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		Several strains derived from 290BIO each with one or more factors of resistance to pyrethroids and DDT.
		IPSWICH pyrethroid-resistant strain. Several strains derived from IPSWICH each with one or more factors of resistance to pyrethroids.
	Delia antigua (Meig.)	
Hymenoptera	*Acromyrmex octospinosus* (Reich)	
	Atta cephalotes (L.)	
	Aphidius matricariae (Haliday)	
	Venturia canescens (Grav.)	
Lepidoptera	*Plutella xylostella* (L.)	
	Ephestia kuehniella (Zeller)	

Technique for rearing cereal aphids. A technique has been developed for rearing cereal aphids on excised wheat leaves. The aphids are maintained in 5 cm lengths of young leaves resting in contact with a layer of 0·5% agar in transparent plastic boxes (60 mm × 40 mm × 20 mm). The leaves are changed at weekly intervals.

This method is particularly suitable for rearing very large numbers of clones of these aphids for studies on susceptibility to insecticides and resistance. (Sawicki and Stribley)

THE CHEMICAL LIAISON UNIT

Much of the work of the unit continues to be done in collaboration with various departments at Rothamsted or with other organisations in Britain and overseas. Investigations into the fate and behaviour of pesticides in the crop environment in relation to biological effects comprise the major theme of the Unit's programme. This includes short-term projects to evaluate methods of application, determine distribution and persistence or monitor residues. Longer term studies on underlying principles underpin these projects. The development of methods for analysing trace amounts of chemicals is a continuing requirement in this work; because of the nature of its activities the Unit receives many requests for assays of naturally-occurring biologically active substances and for assistance, advice or training in methods of residue analysis.

Fate of chemicals in soil

Degradation of oximecarbamate nematicides in Woburn sandy loam soil. Degradation of oxamyl, aldicarb and aldicarb sulphone, ^{14}C-labelled in the carbamate methyl group, was studied in two soils in laboratory incubations at a range of temperatures (5°–15°C) and moisture contents (5–15%). The soils were the sandy loam and the same soil modified by the addition of peat, used for the earlier tests of leaching and degradation (*Rothamsted Report for 1977*, Part 1, 155). Aldicarb was degraded by the expected pathway of oxidation to the sulphoxide and sulphone, with concurrent hydrolysis of these compounds to the non-toxic oximes. Between 67 and 92% of the added aldicarb was degraded via its sulphoxide, the lower values occurring at 5% moisture content where oxidation of aldicarb was markedly slower. The proportion of aldicarb sulphoxide oxidised to sulphone decreased with increasing temperature and moisture content; between 50 and 73% of applied aldicarb was degraded via the sulphone. Rather more oxidation occurred in these tests than observed by other workers in similar experiments, suggesting that the Woburn soils and/or the conditions in the incubation experiments strongly favoured oxidative reactions. Oxamyl was degraded by hydrolysis to the oximino compound; the thioether group was not oxidised. Degradation was faster in the unmodified soil than in that amended with peat.

All reactions followed first-order kinetics. Rates were slower at low moisture contents, and decreased substantially when the temperature was reduced from 10° to 5°, but less

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steeply between 15° and 10°C. At 15°, half-lives for oxamyl ranged from 15 to 28 days and for aldicarb sulphone from 33 to 129 days depending on soil type and moisture content. (Bromilow and Freeman, with Baker, Statistics Department)

Computer simulation of pesticide degradation and movement in soil. Development of the model (*Rothamsted Report for 1977*, Part 1, 156) for simulating pesticide behaviour in soil continued. On the basis of laboratory measurements of adsorption and degradation in soil together with the meteorological data, the model described reasonably well the behaviour of aldicarb and oxamyl applied to fallow sandy loam soils at Woburn in 1975 and 1977.

Some weaknesses remain in the simulation procedure: redistribution of water following rainfall in the field was somewhat faster than predicted by the model, reflecting the difficulty of characterising the hydraulic properties of the soils. Consequently the predicted leaching of pesticide was often slightly lower than that observed. During long drying periods, the model predicted a large accumulation of chemical at the soil surface; this did not occur in practice presumably because of undescribed loss processes such as volatilisation or photodecomposition. (Bromilow and Freeman, with Baker, Statistics Department and Dr. M. Leistra, Laboratory for Research on Insecticides, Wageningen, The Netherlands)

Relationship between physicochemical properties of pesticides and their adsorption by soil. The relationship between adsorption of organic chemicals by soil organic matter and their octanol/water partition coefficients derived from measurements with Rothamsted soils (*Rothamsted Report for 1973*, Part 1, 62) was shown to describe adsorption by soils from Eastern and Western Australia well. This provides further evidence for the view that adsorbing properties of organic matter are similar for soils of widely different origin.

In other studies based on McGowan's treatment of parachor (*Journal of Applied Chemistry* (1954) **4,** 41) theoretical relationships between soil adsorption, octanol/water partition, water solubility and bioconcentration factors were derived. These relationships were similar to those computed by Kenaga and Goring (4th International Congress of Pesticide Chemistry, 1978) using data assembled from the literature. (Briggs)

Microbiological studies

Nitrifying bacteria. Following collaborative work on nitrification and nitrifying bacteria with Dr. K. N. Wickramasinghe (*Rothamsted Report for 1977*, Part 1, 156) in which *Nitrosospira* spp. were identified in acid tea soils from Bangladesh and Sri Lanka, a similar species was isolated in pure culture from a mull soil sample (pH *c.* 4·2) from Geescroft wilderness and identified by electron microscopy. This is the first authenticated isolation of a *Nitrosospira* sp. from a Rothamsted soil and adds to evidence that this genus of nitrifier may be typical of acid soils.

Autotrophic NH_3-oxidising nitrifiers in a series of Woburn and Rothamsted soils used in trials of nitrification inhibitors were isolated in pure culture and identified. The organisms found in the Rothamsted soils were *Nitrosolobus* spp. whereas most of those in the Woburn soils were *Nitrosomonas* spp. (Walker, in collaboration with Ashworth and Rogers, Soils and Plant Nutrition Department)

Degradation of pesticides. Work started on factors influencing the degradation of insecticides in soils with examination of the degradation of *cis* and *trans*-permethrin and decamethrin in three soils differing mainly in organic matter content. The degradation rate of permethrin in soil is greatly influenced by the water content; thus at a moisture

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content of about two-thirds field capacity in Kettering loam at 25°C the half life of permethrin was about 40–50 days, whereas at field capacity this was reduced to approximately 20 days. Adding small amounts of inorganic N, P or K increased degradation somewhat but small supplements of organic nutrients such as cellulose or protein substances either slowed the rate of degradation or had no effect.

The enhanced capacity of soils to degrade diazinon (a substituted pyrimidylorganophosphorus compound) following repeated applications, first observed in 1974 following failure of diazinon to control lettuce root aphids, was confirmed using soils from plots treated with diazinon by Mr. Woodville of ADAS, Cambridge. Aqueous suspensions of these soils (2 g in 100 ml) decomposed up to 2 mg diazinon in 1–2 days, whereas no significant degradation of diazinon occurred in similar suspensions of untreated soil over the same period. Supernatant from the active soil suspensions added to aqueous diazinon solutions containing mineral salts and buffered at pH 7 also caused breakdown of diazinon in 1–2 days. Decomposition occurs under both aerobic and anaerobic conditions. This is the first demonstration that micro-organisms capable of degrading diazinon can develop under British agricultural conditions, a phenomenon previously recognised only in rice cultivation. (Walker, Lord and Forrest)

Uptake and movement of chemicals in plants

Behaviour of chlormequat chloride in barley and wheat. The growth retardant chlormequat chloride (2-chloroethyl-trimethylammonium chloride; CCC) shortens the stems of wheat plants but has different and less obvious effects on barley as reported previously (*Rothamsted Report for 1975*, Part 1, 41). Preliminary investigations with detached leaves indicated that uptake and translocation of ^{14}C-chlormequat chloride was slower in barley than wheat (*Rothamsted Report for 1972*, Part 1, 193–4). These results have now been confirmed in tests with growing plants using chromatographically purified ^{14}C-chlormequat chloride.

About 40 μg ^{14}C-labelled chlormequat chloride in 50% aqueous acetone solution was applied to the centre of the fully elongated lamina of the fourth main-stem leaf of wheat and barley plants. A week after treatment autoradiography showed both basipetal and acropetal movement in both cereals, but more in wheat than barley. At this stage, some ^{14}C-chlormequat chloride could be washed from the leaf near the point of application with water and thus had presumably remained on or near the surface. However the majority of the chemical disappeared rapidly from the leaf surfaces in the first 24 h, when recovery in the leaf washings was about 18% for barley and about 11% for wheat. Subsequently the amounts on the surface diminished slowly and after 8 weeks about 4% was recovered from barley and 3% from wheat.

Additional radio-labelled material was recovered by grinding leaves in water; aliquots of the extracts were assayed by liquid scintillation counting after removing chlorophyll by shaking with ethyl acetate to eliminate quenching. The total amount of radio-activity recovered from treated leaves diminished with time. Loss from wheat leaves was about twice as fast as from barley: from wheat approximately 20% activity was lost in 1 day, 30% in a week and 60% in 8 weeks. Some activity was recovered from other parts of the plants, most being found in the main stem and its other leaves, and in the tillers. The roots contained little radioactivity. Amounts of tracer in the mainstem leaves decreased as they senesced and simultaneously accumulated in main-stem ears. More radio-activity was always recovered from barley plants than wheat.

Greater proportions of larger doses of chlormequat chloride (100 and 1000 μg) were taken into leaves and more moved towards the tip of the leaf. (Lord, with Wheeler, Botany Department)

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Systemic properties of iprodione. Despite reports that the fungicide iprodione is non-systemic, preliminary studies indicated that soil applications controlled stem inoculations of *Phoma exigua* var. *foveata* in potato plants (*Rothamsted Report for 1977*, Part 1, 226).

Further evidence for systemic action was provided by the demonstration that stems of potatoes growing in soil infected with *Rhizoctonia solani* were protected by dust applied to the sprouts of seed tubers. (*Rothamsted Report for 1978*, Part 1, 225).

We therefore examined the uptake of iprodione into potato plants growing in pots containing a range of soils. The soils were watered with iprodione solutions three times per week and after 6 weeks the aerial parts of the plants were analysed. Some iprodione was found in all treated plants, but most in plants from soils which adsorbed least chemical from water.

Other tests showed that iprodione is absorbed by and translocated in tomato plants growing in nutrient solution containing the fungicide. (Cayley and Tillotson, with Hide, Plant Pathology Department)

Distribution and movement of tecnazene in potato stores. Previously developed extraction and analytical procedures for the potato sprout suppressant tecnazene (*Rothamsted Report for 1977*, Part 1, 154) were used to investigate the effect of application method on the distribution of suppressant in potato clamps. The traditional method of shovelling tecnazene on to a bulk of potatoes just before loading into store resulted in doses on individual tubers ranging from 0 to 1071 mg kg^{-1} with a mean of 157 mg kg^{-1}. Applying the chemical continuously to potatoes as they passed along the store loading elevator using a mechanical 'Fusarex' applicator gave doses ranging from 18 to 322 mg kg^{-1} with a mean of 94 mg kg^{-1}. Although the mechanical distributor gave a more uniform distribution at the time of application, after 6 months storage there was no significant difference between the methods. The mean residue levels were 43 mg kg^{-1} (range 0–218) from the shovel method and 44 mg kg^{-1} (range 0–185) from the applicator. (Cayley, Lord and Manlove)

Collaborative work described in the reports of other departments

With Plant Pathology Department. The use of fungicides against tuber pathogens. (Cayley with Hide)
Chocolate Spot on winter beans. (Cayley with Bainbridge)
Chemical preservation of damp hay. (Lord, Cayley and Manlove with Lacey)

With Nematology Department. Trials with nematicides. (Bromilow with Whitehead)

Staff of the Department and the Chemical Liaison Unit

We were delighted when Dr. C. Potter accepted our invitation to become the first Honorary Scientist associated with the Department, in accordance with the new scheme approved by the Lawes Trust Committee. We are very glad to be able to recognise in this way his great contribution to our work, formerly as Head of Department and fortunately continuing as a senior colleague.

It was also with great pride and pleasure that we learnt of the award of the UNESCO Science Prize to seven members of the Department, M. Elliott, A. W. Farnham, N. F. Janes, P. H. Needham, C. Potter, D. A. Pulman and R. M. Sawicki, for their work on pyrethroid insecticides. We greatly value this important award which represents international recognition of a sustained multidisciplinary project that has led to major practical advances. For his work on pyrethroids M. Elliott also received the Holroyd

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medal of the Society of Chemical Industry and presented the medal address; in addition he was nominated as the second Jeyes medallist and lecturer of the Chemical Society.

During the year we welcomed K. Chamberlain and G. W. Dawson transferred from the ARC Unit of Systemic Fungicides. They will provide valuable additional chemical expertise for our studies on fungicides and translocation of pesticides in plants. Stephanie Petzing transferred to the Biochemistry Department to help with the genetic manipulation programme. Dr. R. Botham continued on secondment from Wellcome, Berkhamsted Ltd., investigating neurophysiological approaches to the discovery of new insecticides using electron microscopy and micro-autoradiographic methods. G. G. Briggs returned to the Chemical Liaison Unit after spending a year at the Department of Soils and Plant Nutrition, University of Western Australia; A. W. Farnham began a 7-month visit to the same country, attached to the Department of Zoology, University of New South Wales.

Professor L. C. Terriere from Oregon State University spent 3 months in the Department studying insecticide metabolism and resistance to insecticides. Mrs. Bonnie Wright from the Department of Agriculture Zambia visited us for a similar period working on tolerance of pathogens to fungicides. Dr. Katrina Gorog from the Research Institute for Plant Protection, Budapest completed a 6-month secondment to the Chemical Liaison Unit. Other visitors who spent shorter periods in the Unit included Dr. G. Perez da Silva from the Institut Edafologia y Biologia Vegetal, Madrid; Dr. M. Leistra and Mr. J. Boesten from the Laboratory for Research on Insecticides, Wageningen and Dr. E. Flores Ruegg from the Instituto Biologico, Sao Paulo. J. Gibson, Janine Mills and R. Stokes worked as postgraduate students in the Department under the CASE scheme. Sandwich course students who worked in the Department or the Chemical Liaison Unit were Susan L. Cox, S. A. Davis, Susan E. Day, C. J. Easton, Margaret E. Forrest, M. Jeffrey, Sandra Naish, M. R. Slaughter, Mary Thompson and Sandra L. Turner. M. Omasta joined the Chemical Liaison Unit for a 1-year appointment in the absence of Avis Evans in the USA.

The Department made a major contribution to the 4th International Congress of Pesticide Chemistry (IUPAC) in Zurich. M. Elliott and I. J. Graham-Bryce acted as Chairmen and Organisers of symposia and presented invited papers. A. L. Devonshire was chairman of a Discussion Session, P. E. Burt and K. A. Lord presented papers and N. F. Janes was co-author of an invited paper. We were also strongly represented at the International Congress of Plant Pathology in Munich where M. M. Burrell, D. W. Hollomon and A. H. McIntosh presented papers. At the invitation of the organisers I. J. Graham-Bryce contributed to an advanced course on pest, disease and weed control in sugar beet at Saragossa, sponsored by OECD. M. Elliott and N.F. Janes were invited to present a paper at a meeting on pyrethroids of the Societé Francaise de Phytiatrie et de Phytopharmacie in January at Versailles. A. J. Arnold spent one month in Jamaica completing the ODM-sponsored project to design and develop specialised equipment for use in the coconut mass pollination scheme. K. A. Lord made a further visit to the Radioisotopes Centre, Instituto Biologica, São Paulo to continue collaborative work on the behaviour of pesticides in soils and plants. While in Brazil he contributed to the third meeting of Pesticide Residue Analysts. R. H. Bromilow visited the same Institute for a three month period. He also continued collaborative work on computer simulation of pesticide behaviour in soils with the Laboratory for Research on Insecticides, Wageningen.

Publications

General Papers

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