

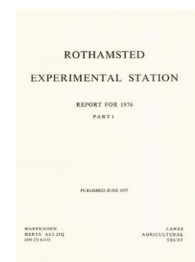
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I. J. Graham-bryce (1977) *Insecticides and Fungicides Department* ; Report For 1976 - Part 1, pp 159 - 189 - DOI: <https://doi.org/10.23637/ERADOC-1-133>

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Introduction

Crop protection against insect pests at present depends heavily on a very limited range of chemical classes. The decline of the organochlorine compounds in recent years as a result of resistance and unfavourable properties in the environment has left the organophosphates and carbamates as the only major groups from which to seek practical treatments for controlling many pests. Both groups act by inhibiting acetylcholinesterase, and the vulnerability which dependence on such a restricted range of insecticide types implies has given added impetus to our long-term studies aimed at facilitating the discovery of new insecticides and alternative methods of insect control. In this connection it is very gratifying that the synthetic pyrethroids, a group whose development has been pioneered in the Department for many years, are now receiving much attention throughout the world and are widely recognised as a very valuable and timely addition to the pesticide armoury, having great commercial promise for use in agriculture and horticulture. The photostable pyrethroids now under large-scale development all originated either at Rothamsted or from the independent approach by workers from the Sumitomo Chemical Company. The studies we report this year were directed to establishing more clearly the implications of the recent advances which led to these compounds in terms of relationships between structure and activity.

The pyrethroids are active against many pests and the practical evaluation now underway in many centres is progressively establishing their future role in crop protection. The photostable examples, like earlier members of the group, appear to be relatively rapidly degraded in metabolic systems, including soil, and so are unlikely to accumulate in the environment. Nevertheless when applied by appropriate methods they can control certain soil pests effectively, as further results on the activity of pyrethroid seed treatments against wheat bulb fly larvae, reported this year, demonstrate. The factors affecting the performance of soil-applied insecticides remain poorly understood and there is little to guide the search for new materials. The investigation of these factors therefore forms part of the wide-ranging programme of research in the Department and the Chemical Liaison Unit on the behaviour of pesticides in soil and on their availability to receiving organisms. The different aspects of this programme and of our complementary studies on seed treatments are described at various points in this report. Practical studies in this field include new work on slugs, which are increasingly important pests, particularly in direct drilled crops. In collaboration with the Entomology Department fundamental work on slug control has also been started.

The paucity of insecticidal mechanisms associated with the existing range of commercial insecticides also underlines the seriousness of resistance. We continue to devote much attention to the problems of resistance, which in the long term are possibly the most formidable facing chemical methods of crop protection. Our fundamental studies on mechanisms of resistance and the ways they interact have now been extended to include examination of organophosphorus-resistant acetylcholinesterase in different arthropods, while our practical studies have again been concentrated on aphids. There can be no doubt that resistance to organophosphorus insecticides is now well established in *Myzus persicae* in Britain; in the light of this development our work is designed to provide a sound basis for advising on control measures in the short term and to seek strategies which will avoid further problems in the long term. Parallel work on insensitivity to fungicides has emphasised the importance of understanding the population genetics and competitiveness of tolerant strains in devising such strategies. To obtain sound knowledge of the complex factors governing the development of resistance to insecticides and fungicides will require extensive investigation and different conclusions are likely to apply to each crop-pest-pesticide situation. Nevertheless it seems essential to

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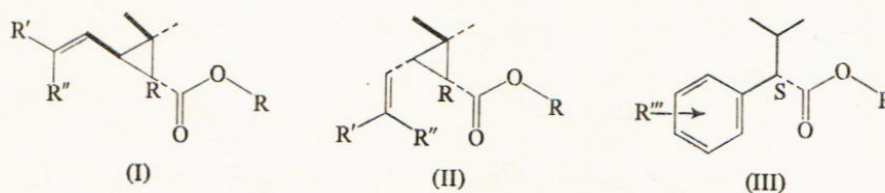
strive to obtain such knowledge if the future of these very valuable products is to be safeguarded.

Insecticides

Relationships between molecular structure and insecticidal activity of pyrethroids

Structural variations of acidic components of esters

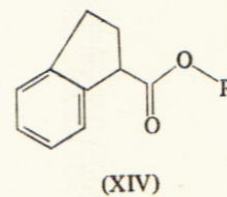
1. Non-cyclopropane esters. The most potent natural and synthetic pyrethroids are esters of (1R)-dimethylcyclopropanecarboxylic acids (I or II: $R' = R'' = \text{Me}$; or $R' = R'' = \text{F, Cl or Br}$; or $R' + R'' = (\text{CH}_2)_4$) with a 2-alkenyl or -alkadienyl-3-methylcyclopent-2-en-4-olone, 5-benzyl-3-furylmethyl alcohol, 3-phenoxybenzyl alcohol or α -cyano-3-phenoxybenzyl alcohol. Insecticidal activity is associated with methyl



groups on the cyclopropane ring held in a particular steric relation to the unsaturated side chain which is *trans* (I) or *cis* (II) to the carboxyl function. Recently, in an important advance, Ohno *et al.* (*Agricultural and Biological Chemistry* (1974) **38**, 881) showed that

TABLE 1

Compound Structure		R ¹	R ²	R ³	Relative potency (NRDC 107 = 100) to	
					HF	MB
1	(IV)	<i>p</i> -Cl	Pr ⁱ	H	12	15
2	(IV)	<i>m</i> -Cl	Pr ⁱ	H	8.5	8.0
3	(IV)	<i>p</i> -Br	Pr ⁱ	H	10	10
4	(IV)	<i>p</i> -F	Pr ⁱ	H	13	7
5	(IV)	<i>m</i> -F	Pr ⁱ	H	8.5	17
6	(IV)	<i>o</i> -F	Pr ⁱ	H	1	1
7	(IV)	<i>p</i> -Me	Pr ⁱ	H	12	6
8	(IV)	<i>m</i> -Me	Pr ⁱ	H	5.8	5.5
9	(IV)	3,5-Me ₂	Pr ⁱ	H	0.2	1.1
10	(IV)	3,4-Me ₂	Pr ⁱ	H	4.2	2.8
11	(IV)	2,4,6-Me ₃	Pr ⁱ	H	<0.1	<0.1
12	(IV)	<i>p</i> -Et	Pr ⁱ	H	2	7
13	(IV)	<i>p</i> -Pr ⁿ	Pr ⁱ	H	1	1
14	(IV)	<i>p</i> -Pr ⁱ	Pr ⁱ	H	1	2
15	(IV)	<i>p</i> -Cl	Pr ⁱ	Me	1.2	1.1
16	(IV)	<i>p</i> -Cl	Et	H	7	6
17	(IV)	<i>p</i> -Cl	Et	Et	0.8	0.2
18	(IV)	<i>p</i> -Cl	cyclopentyl	H	1	0.2
19	(IV)	<i>p</i> -Cl	cyclohexyl	H	<0.1	<0.1
		R				
20	(V)	3-phenoxybenzyl			<0.1	<0.1
21	(VI)	3-phenoxybenzyl			<0.1	0.1
22	(VII)	3-phenoxybenzyl			<0.1	0.1
23	(VIII)	3-phenoxybenzyl			0.3	0.4
24	(IX)	3-phenoxybenzyl			2	0.6
25	(IX)	5-benzyl-3-furylmethyl			<0.1	<0.1
26	(X)	5-benzyl-3-furylmethyl			0.5	<0.1
27	(XI)	3-phenoxybenzyl			<0.1	<0.1
28	(XII)	3-phenoxybenzyl			0.6	1
29	(XII)	5-benzyl-3-furylmethyl			2	2
30	(XIII)	3-phenoxybenzyl			<0.1	<0.1
31	(XIV)	3-phenoxybenzyl			<0.1	<0.1



Compounds 1-19 (Table 1) were prepared by alkylating a benzyl cyanide and then esterifying the derived acid with 3-phenoxybenzyl alcohol, a representative and accessible alcohol. The insecticidal activities of the racemic esters were compared with that of bioresmethrin (relative activity, 100) to *Musca domestica* L. (HF) and *Phaedon cochleariae* Fab. (MB) by the usual methods. The conclusions were that:

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- (i) Halogen on the ring is most effective (Cl, Br or F) in the *p*-position, slightly less *m*-, and inactive *o*-.
- (ii) A mono-methyl group in the *m*- or *p*-position is superior to the other alkyl substituents investigated (see Table 1).
- (iii) Only esters with one alkyl group at the α -position are active, with relative effectiveness in the order $\text{Pr}^1 > \text{Et} > \text{cyclopentyl} > \text{cyclohexyl}$.

Table 1, entries 20–31, shows the activities of new compounds, for which syntheses were developed, designed to explore further the relationships between structure and activity in this series. In compounds 20–23 replacement of a carbon in the active compounds 1 or 16 by an alternative isosteric atom gave compounds which were not active. Small alterations in the distance and spacing from the alcoholic component of the aromatic centre in compounds 24–27 again considerably diminished activity. In compounds 28–31 either one or both of the methyl groups of active esters are locked into a particular conformation by bridging bonds, or groups. Compounds 30 and 31 were almost inactive, but low activity remained in the esters with structure (XII).

2. Cyclopropane esters

(i) The nature of the substituent at C-3 of the cyclopropane ring strongly influences the insecticidal activities of cyclopropanecarboxylates which are, in general, greater than those of any non-cyclopropane compounds yet described. Dihaloovinyl groups are the most effective substituents in this position; a new variation ($\text{Cl}-\text{C}\equiv\text{C}-$) was provided *via* the acid obtained by dehydrochlorination of 3-(2,2-dihaloovinyl)-2,2-dimethyl cyclopropanecarboxylic acid. Esters with this modification (entries 1–3, Table 2) were less active than the parent dichloro-compounds.

(ii) Compounds with methyl groups on C-1', synthesized *via* Wittig reactions with 3-acetyl-2,2-dimethylcyclopropanecarboxylates had lower activity than the parent C-1'-H compounds (*Rothamsted Report for 1975*, Part 1, 153–4). Esters with effective side chains (e.g. $\text{Cl}_2\text{C}=\text{CH}$) but an additional methyl group on C-3 were also inactive: in both these variations the additional substituent probably inhibits attainment of the optimum conformation for activity.

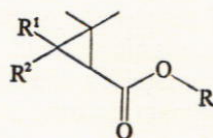


TABLE 2

Compound	R ¹	R ²	R	House flies	Mustard beetles
1	$\text{Cl}_2\text{C}\equiv\text{C}-$	H	3-phenoxybenzyl	15	10
2	$\text{Cl}_2\text{C}\equiv\text{C}-$	H	α -cyano-3-phenoxybenzyl	70	50
3	$\text{Cl}_2\text{C}\equiv\text{C}-$	H	5-benzyl-3-furylmethyl	32	19
4	$\text{H}_2\text{C}=\text{C}(\text{CH}_3)-$	H	5-benzyl-3-furylmethyl	2	—
5	$\text{CH}_3\text{CH}=\text{C}(\text{CH}_3)-$	H	5-benzyl-3-furylmethyl	2	—
6	$\text{CH}_3\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)-$	H	5-benzyl-3-furylmethyl	<1	1
7	$\text{CH}_3(\text{CH}_2)_2\text{CH}=\text{C}(\text{CH}_3)-$	H	5-benzyl-3-furylmethyl	<1	<1
8	$\text{Cl}_2\text{C}=\text{CH}-$	CH_3	5-benzyl-3-furylmethyl	NT	<1
9	$\text{Cl}_2\text{C}=\text{CH}-$	CH_3	3-phenoxybenzyl	NT	<1
10	$\text{Cl}_2\text{C}=\text{CH}-$	CH_3	α -cyano-3-phenoxybenzyl	NT	<1

Structural variations of alcoholic components of esters. α -Cyano-3-phenoxybenzyl esters are more active insecticides than the parent esters without this substituent, as illustrated by the relative potencies (*ca.* 1:10) of esters of [1R, *cis*]-3-(2,2-dibromovinyl)-

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2,2-dimethylcyclopropanecarboxylic acid with 3-phenoxybenzyl and [S]- α -cyano-3-phenoxybenzyl alcohols. To investigate the effect of placing this substituent in the same region of the molecule, but attached at a different site, 2- and 6-cyano-3-phenoxybenzyl esters were synthesised. Both compounds were almost inactive, so that in neither did the location and/or the influence on conformation of the cyano group simulate that in the α -methylene substituted compound.

Preparation of larger samples of pyrethroids developed at Rothamsted. In work since September 1975 supported jointly by the NRDC and its licensees, large samples of various compounds have been prepared. The work has involved detailed examination of the methods originally used in the laboratory so that procedures and yields could be improved to the standard needed for large-scale syntheses. (Chemical work: Carson, Coxon, Elliott, Janes, Johnson and Pulman. Biological work: Farnham and Freeman)

Mode of action of insecticides

Neurotoxicity of pyrethroids. Although certain consistent relationships have been established between the structures of synthetic pyrethroids and their toxicities to whole insects (overall toxicity), previous studies (*Rothamsted Report for 1975*, Part 1, 155–6) failed to reveal any such relationships between structure and neurotoxicity. The giant fibre axons examined proved relatively insensitive to the action of pyrethroids. It was concluded that these axons are unlikely to be the critical sites of action for pyrethroids and in work this year effects on synapses in the cercal nerve/giant fibre pathway within the terminal abdominal ganglion were investigated. The test insects were again adult male *Periplaneta americana* (L.) and a sucrose gap technique (Callec & Sattelle, *Journal of Experimental Biology* (1973), **59**, 725–38) similar to that used for the giant fibre axons was employed. Stimulation was applied to one or both cercal nerves XI and the response recorded from the abdominal nerve cord while the terminal abdominal ganglion was irrigated with solutions of the test compounds in saline containing 0.5% acetone.

All pyrethroids decreased the post-synaptic response, eventually blocking the synapse. Pharmacological tests indicated that failure was post-synaptic and non-depolarising. Thus synapses treated with pyrethroids respond progressively less strongly to nicotine and acetylcholine (which act specifically post-synaptically); eventually spikes can no longer be initiated, and there remains only a small excitatory post-synaptic potential which is abolished by nicotine. The possibility of additional pre-synaptic effects cannot be excluded, but conduction in pre-synaptic axons is not significantly affected at block because in many preparations with blocked synapses pre-synaptic action potentials from the cercal nerves are still detectable.

Relationships between molecular structure and synaptic neurotoxicity were no more apparent than for axonic neurotoxicity (Table 3). However, the results do provide further evidence that the action of pyrethroids at synapses is different from their effect on axons. In general synapses are more susceptible than axons but not equally so to all compounds. Thus, although decamethrin ('NRDC 161') was 20 times more toxic than 'NRDC 158' to giant fibre axons, the two compounds are almost equally toxic to synapses. 'NRDC 163', cismethrin ('NRDC 119'), bioresmethrin ('NRDC 107'), 'NRDC 103' and 'Kadethrin' were all more toxic to synapses than to giant fibres, but 'biotetramethrin' and 'NRDC 157' were more toxic to giant fibres. Most compounds became comparatively more effective against synapses the longer they were applied to the preparations. Desheathing ganglia before treatment increased the toxicity of the compounds tested (bioresmethrin, 'NRDC 158' and decamethrin) up to ten times.

The discrepancy between overall and neurotoxicities may be associated with processes outside the nervous systems such as differential penetration and detoxication; if so,

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neurotoxicities should parallel overall toxicities obtained by injection more closely than those obtained by topical application. Table 3 shows, however, that LD50s recorded two days after injection resembled those for topical application very closely, with the sole exception of 'biotetramethrin'. Table 3 also indicates that although overall toxicity of pyrethroids is unrelated to their polarity neurotoxicity increases with increasing polarity.

TABLE 3
Overall toxicity and neurotoxicity of pyrethroids to *P. americana*

	LD50(μ g)*		Neurotoxicity (μ M) ⁺	
	Topical	Injected	To giant fibres	To synapses
'Kadethrin' ('RU15525')	1.90	1.30	0.5	0.3
Bioresmethrin	1.60	1.10	1.4	0.9
Cismethrin	0.38	0.19	2.0	0.8
'NRDC 163' ^a	0.32	0.25	7.4	4.4
'NRDC 157' ^b	0.10	0.09	8.0	9.7
'NRDC 158' ^c	0.09	0.10	5.7	1.0
Decamethrin ^d	0.05	0.08	0.3	0.8
'NRDC 103' ^e	65.00	9.00	100.0	7.0
'Biotetramethrin' ^f	36.00	47.00	1.1	4.8

* 2 days after treatment

⁺ concentration decreasing amplitude of response by 30% in 1 h

^a 3-phenoxybenzyl (IR, *trans*)3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

^b 3-phenoxybenzyl (IR, *cis*)3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

^c ($\pm$)- α -cyano-3-phenoxybenzyl (IR, *trans*)3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, i.e. a 1:1 mixture of the *trans* analogue of decamethrin and its epimer

^d provisional common name for 'NRDC 161' or (S)- α -cyano-3-phenoxybenzyl (IR, *cis*)3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

^e pentamethylbenzyl (IRS, *cis, trans*) chrysanthemate

^f 3,4,5,6-tetrahydrophthalimidomethyl (IR, *trans*) chrysanthemate

Although cercal nerve-giant fibre synapses are somewhat more susceptible to many pyrethroids than are giant fibres, the toxic concentrations are still high relative to the probable concentrations in the haemolymph of poisoned insects and this, together with the inconsistent structure-toxicity relationships, suggests that these synapses are not critical sites of action. (Burt and Goodchild)

Neuroanatomy of the insect central nervous system. The description of the nerve cell body groups and their fibre pathways in the mesothoracic ganglion of the cockroach *Periplaneta americana* (L.) (Rothamsted Report for 1975, Part 1, 156) was continued. Detailed work on the seven unpaired midline cell groups, comprising some 200 neurons, mostly apparently interneurons, was almost completed and the detailed description of the other, paired, cell groups begun. Other aspects of ganglion neuroanatomy, closely involved with the cell groups, also received detailed attention: identification of interganglionic interneurons was started; it became possible to begin unravelling the fibre composition of the complex commissural tracts that link the two halves of the ganglion; and a small nerve, now termed the recurrent nerve, which branches from the posterior interganglionic connectives and was excluded from the earlier account of the peripheral nerve roots (Gregory, *Philosophical Transactions of the Royal Society of London B* (1974), 267, 421-465), was described.

Work on the neuroanatomy of other ventral nerve cord ganglia continued with studies of the longitudinal tracts of the suboesophageal and pro- and metathoracic ganglia, for comparison with those of the mesothoracic ganglion. A preliminary investigation of the suboesophageal ganglion of the desert locust, *Schistocerca gregaria* (Forskål), was also undertaken, to help in interpreting the corresponding but more highly modified ganglion of the cockroach. (Gregory)

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The nature and causes of resistance. The practical problems of resistance in aphids (*Myzus persicae*) on sugar beet again occupied much of our attention this year. Work aimed at providing a basis for advising on control measures in the short term and at anticipating future resistance problems continued. Longer term fundamental studies on the mechanisms of resistance were also maintained.

Survey of resistance in *Myzus persicae*. Approximately 4000 aphids from 340 samples were examined biochemically for resistance during the sugar beet growing season. Aphids from 90 samples were also assayed by the simple discriminating bioassay (dip test) described last year (*Rothamsted Report for 1975*, Part 1, 158). The samples were either collected by Rothamsted Staff, or were sent by workers from Broom's Barn Experimental Station, the British Sugar Corporation, ADAS or the Scottish Agricultural Colleges. Most came from sugar beet or potatoes.

Even with this very large effort, our data are not fully representative because samples could not be obtained from all regions, many had to be collected on an opportunistic basis and often contained few individuals. Nevertheless, certain general conclusions are clear. Almost all the samples examined contained resistant (R) aphids and in 70% of these samples at least 75% of the individuals were resistant. Aphids were all susceptible in only six samples. The proportion of resistant aphids varied considerably in the different regions examined (Table 4). In the South East and East Anglia almost all aphids examined were resistant; resistant aphids were also predominant in Northern England and Western Scotland but susceptible (S) aphids were fairly frequent in South Western England, Wales and in Derbyshire (Shardlow) where *M. persicae* were very numerous during early summer.

TABLE 4
Geographical distribution of resistant aphids

Region	Numbers of samples with specified percentage of resistant individuals			
	0-25	26-50	51-75	76-100
S. Wales and S.W. England	1	3	1	5
N. Wales and W. Midlands	4	7	5	3
S.E. England and E. Anglia	0	1	6	101
Derbyshire (Shardlow)	5	14	6	1
N. England	1	4	2	20
W. Scotland	1	0	0	9

Some samples from Scotland and Northern England contained individuals with greater esterase activity and greater resistance than the moderate levels typical in other regions. These more resistant aphids resembled strain T1V found last year in Bedfordshire. They strongly resist organophosphorus insecticides, for example they show 100-fold resistance to dimethoate compared with only 8 to 10-fold in the standard MS1G type, and they also resist pirimicarb ($\times 4-9$).

The proportions of resistant aphids in samples collected from the different regions changed little throughout the season. This apparent stability would be expected where resistance was already well established as in South East England and East Anglia where almost all aphids collected by G. D. Heathcote (Broom's Barn) from overwintering sites between early March and mid-May were resistant: during the remainder of the season 54/60 samples of alates and 48/49 samples of apterae contained 75% or more R individuals. However R/S ratios also remained constant throughout the season where susceptible individuals were more numerous as at Shardlow. This suggests that the proportions of R and S aphids are affected relatively little by insecticide spraying during late spring and early summer and are governed more by the genotypes of the successfully overwintering parthenogenetic females. If it can be confirmed that this applies generally,

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evaluation of R/S ratios early in the year should give a good indication of the incidence of resistance to be expected during the remainder of the season.

Further evaluation of the dip test for discriminating between resistant and susceptible aphids. Laboratory studies showed that the dip test is sensitive to temperature and discriminates best and fastest between R and S aphids at about 20°C. Where plants have been treated with 'Metasystox', at least two days should elapse before aphids are collected for the test.

The reliability of the test for detecting resistance was assessed by estimating biochemically the proportion of R aphids present in each of the 90 samples tested, which were collected throughout the season in different parts of Britain and from different crops as discussed above. The dip test failed to detect resistance in only 4 of 75 samples estimated biochemically to contain 50% or more R aphids. This efficiency of detection (about 95%) may be regarded as satisfactory in view of the low resistance levels of field populations which are such that between 60 and 90% of the insects in resistant populations are killed by the dose which kills all S aphids in every test.

The help of British Sugar Corporation trials officers was enlisted to evaluate the dip test under practical conditions. The test was demonstrated during a one-day course held at Rothamsted, and each officer was provided with a dip test kit.

Due mainly to shortages of adult apterae, only three officers were able to complete enough tests with sufficient accuracy to allow proper evaluation. They obtained fair to good correlations between dip test results and the degree of control, and their results agreed reasonably well with tests done on the same aphids at Rothamsted. It was clear that field workers would often find it difficult to perform dip tests in addition to other urgent work during the busy period of the year in the growing season. It may therefore be best to use the test mainly for forecasting the likelihood of resistance early in the season (see above), which would be best undertaken by a limited number of trials officers thoroughly trained and experienced in its use.

Relationships between the presence of resistance and effectiveness of control sprays. To determine how the presence of R aphids affects the performance of control sprays, aphids were counted and tested biochemically and by dip test before and after spraying with demeton-S-methyl ('Metasystox') or pirimicarb ('Aphox') at recommended rates on seven farms. The results are difficult to interpret because the infestations were relatively slight and very localised in most of the fields inspected. However, the observations suggest that Aphox gave very good to excellent control of the aphids and its effect lasted up to 11 days after treatment, even though this period coincided with trapping of very large numbers of aphids at Broom's Barn and Rothamsted. 'Metasystox' at 420 ml in 450 or 675 litres water ha⁻¹ gave less effective, in some cases unsatisfactory, control of apterae in all but one of the fields examined. Differences between the proportion of R aphids pre and post spray were slight because most of the pre-spray samples already contained 80% or more R aphids.

In a carefully monitored test at Rothamsted, 'Metasystox' failed to control adequately a heavy infestation of *M. persicae* on potatoes. Spraying with the recommended dose of 'Metasystox', 420 ml in 450 litres water ha⁻¹, decreased the infestation from eight to four apterae per leaf (two days after spraying) but increased the proportion of R aphids in the samples from 75 to 100%. An inspection of the field eight hours after treatment showed that all but the apterae on the underside of the bottom-most leaves were killed by 'Metasystox'. The survivors reinfested the tops within two days of treatment even though laboratory experiments showed that the leaves from all parts of the potato plant were lethal to susceptible laboratory-reared aphids by systemic tests. Parallel experiments done with laboratory-reared R aphids showed that many survived on these 'Metasystox'-

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treated leaves. However, both S and R aphids died in similar tests on leaves treated in the field with 'Aphox'. This confirmed field experience at Rothamsted, where 'Aphox' successfully cleared heavy infestations of *M. persicae* on potatoes, close to fields where 'Metasystox' had failed.

These tests showed clearly that R aphids survive 'Metasystox' treatment because they can withstand systemic doses lethal to S aphids. The resistance is however insufficient to prevent kill by the direct contact of the sprays; hence improving the efficiency of spraying could improve control of R aphids by 'Metasystox'. (Devonshire, Moore, Petzing, Rice and Sawicki)

Effects of inhibitors on response of susceptible and resistant houseflies to insecticides. 1-naphthyl N-propyl carbamate (NPC) has been shown to inhibit selectively carboxyl-esterases which detoxify pyrethroids and some organophosphate insecticides (Jao & Casida, *Pesticide Biochemistry and Physiology* (1974), **4**, 465-72). As part of our investigations into the nature of resistance mechanisms we have therefore investigated the effects of NPC on the activity of parathion, paraoxon and malathion against susceptible houseflies and against two strains with different mechanisms of resistance to organophosphorus insecticides.

The effects of NPC and NPC plus piperonyl butoxide on the activity of paraoxon and parathion (Table 5) were unexpected, particularly the antagonism of paraoxon by NPC in all three strains, including strain 29 in which a major mechanism of resistance is an esterase mutated into a phosphatase. The strong synergism of parathion by NPC in strain 29 is also puzzling. These results cannot be explained by the known properties of NPC which is a good substrate for monooxygenases and associates with acetylcholinesterase in addition to selectively inhibiting esterases. It appears therefore that the action of NPC with organophosphorus insecticides is considerably more complex than with pyrethroid esters of primary alcohols, which it synergises because of its esterase-inhibiting activity.

TABLE 5

Effect of NPC alone and with piperonyl butoxide on the activity of parathion and paraoxon against different housefly strains

Insecticide	Additive µg per fly	Response of strain indicated to insecticide plus additive(s)		
		susceptible	29 ^a	53 ^b
Parathion	NPC (5.2.5 and 1.25 µg)	slight synergism	strong synergism	no effect
	piperonyl butoxide (2µg)	slight synergism	strong antagonism	strong synergism
	NPC (2.5µg) + piperonyl butoxide (2µg)	moderate synergism	very strong synergism	strong synergism
Paraoxon	NPC (5.2.5 and 1.25µg)	antagonism	antagonism	antagonism
	piperonyl butoxide (2µg)	synergism	synergism	synergism
	NPC (2.5µg) + piperonyl butoxide (2µg)	greater synergism than with piperonyl butoxide alone		

^a known mechanisms of resistance to organophosphates: phosphatase, ethyl glutathione transferase, monooxygenase

^b known mechanisms of resistance to organophosphates: monooxygenase (different from that in strain 29), ethyl glutathione transferase and acetylcholinesterase with decreased sensitivity to organophosphates

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NPC resembles other additives, notably *S,S,S*-tributyl-phosphorotrithioate in having no effect on malathion resistance controlled by gene *M*. (Sawicki)

Resistance of houseflies to pyrethroids. The most important mechanism of resistance to pyrethroids in houseflies is the knock-down resistance factor, *kdr*. It conveys cross-resistance to a wide range of compounds including DDT and all pyrethroids, and is unaffected by pretreatment with either the methylenedioxyphenyl synergists such as sesamex or the phosphonate esters such as NIA 16388. Genetically the mechanism is recessive and has been located on chromosome III at approximately 40 units from the marker, green eye. It has been isolated from strain NPR, which originally developed resistance to pyrethroids in the field in 1958 in Sweden when a mixture of natural pyrethrins and piperonyl butoxide (py/pb) was used following the failure of DDT. Since 1967, NPR has been subjected to prolonged selection in the laboratory with natural pyrethrins, resulting in a level of resistance to the selecting agent of greater than 300-fold, and to bioresmethrin of 200-fold. These levels declined very little when selection was subsequently withdrawn. The mechanism *kdr* has also been detected in 290 BIO, a strain which originally developed resistance to bioresmethrin in the field in Denmark when this insecticide was used following the failure of dimethoate. Further selection in the laboratory with bioresmethrin increased this resistance to 1000-fold, but on removal of selection pressure it fell back to approximately 250-fold. In an attempt to mimic the development of field resistance to pyrethroids in a dimethoate resistant strain of flies in the laboratory, strain 49 r₂b was selected with py/pb giving strain 49 PPB. Resistance to the selecting agent developed rapidly for about ten generations, but then remained steady for a further 40 generations in spite of strong selection pressure (approximately 90 % mortality) at alternate generations. The resistance factors for natural pyrethrins and bioresmethrin were approximately 10, but reached 120 for synergised natural pyrethrins. Genetical analysis of this strain showed that *kdr* was absent, and that the main resistance system was located on chromosome II. This demonstrates firstly that pyrethroid resistance can develop without *kdr*, although the levels of resistance are less spectacular than for strains with *kdr* such as NPR or 290 BIO; secondly that the previous history of pesticide usage influences the mechanism that will be selected initially in the development of resistance to a new toxicant; and thirdly that well established pyrethroid resistance systems such as those in NPR may become stabilised in a population by extensive selection pressure, even when they are genetically recessive, whereas newer systems such as those of 290 BIO may be less stable. (Farnham)

Organophosphorus-resistant acetylcholinesterase in houseflies and cattle ticks. Further biochemical work was done on the less sensitive acetylcholinesterase which is an important cause of resistance in several species. Bimolecular rate constants for inhibition by malaoxon of the enzyme from resistant and susceptible houseflies were compared. Values of $950 \times 10^{-3} \text{ l mol}^{-1} \text{ min}^{-1}$ for susceptible insects and $41.9 \times 10^{-3} \text{ l mol}^{-1} \text{ min}^{-1}$ for the resistant strain were similar for acetylcholinesterase from the head and from the thorax, demonstrating that the resistant enzyme is present in both sources. Differences between resistant and susceptible forms were also similar for the naturally soluble fraction (about 12 %) and that solubilised by detergent.

In an attempt to identify further differences between resistant and susceptible enzymes, they were examined by gel electrophoresis using a range of buffer systems and gel concentrations with and without incorporation of the detergent 'Triton X-100' in the gel. Pretreatment of the enzyme with neuraminidase to remove any bound sialic acids had no effect on electrophoretic mobilities. No differences between resistant and susceptible

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enzyme from either houseflies or cattle ticks could be detected by these electrophoretic methods.

The different molecular forms of acetylcholinesterase were resolved best by electrophoresis on polyacrylamide gradients between 4 and 26%. Three molecular forms were observed in both resistant and susceptible houseflies and cattle ticks. Their molecular weights were estimated by gradient gel electrophoresis. In the naturally soluble fraction from the housefly, forms corresponding to mol. wt. of approximately 8×10^4 and 1.6×10^5 were evident. A further band with a mol. wt. of about 5×10^5 was present in enzyme extracted with Triton X-100. Corresponding forms were obtained from cattle ticks but with mol. wt. of approximately 6.6×10^4 , 1.3×10^5 and 4×10^5 . These molecular weights were confirmed by measuring the relative mobilities of the bands on disc gels over a range of gel concentrations.

These results suggest that differences between resistant and susceptible forms of acetylcholinesterase involve relatively subtle changes in the active centre with little change in the properties of the molecule as a whole. (Stokes and Devonshire)

Side effects of pesticides on beneficial insects

Poisoning of honeybees in the field. One hundred and thirty-five samples of honeybees thought to be poisoned were received from beekeepers via the Bee Advisory Service of MAFF, which also collected evidence to indicate how poisoning had occurred. In about two-thirds of the 105 cases where the presence of an insecticide or its effects were demonstrated (86 in 1975 and 94 in 1974) the probable circumstances of the application were identified.

The association between the increased area of oilseed rape and honeybee poisoning was again apparent (24 samples, compared with 30 in 1975 and 12 in 1974). In ten incidents HCH (BHC), sometimes with an anticholinesterase insecticide, was implicated, causing particularly heavy casualties; the remaining incidents were ascribed to anticholinesterase insecticides. We have cooperated with ADAS in planning field experiments to seek insecticide treatments for this crop which are safer for honeybees than some of those now widely practised.

Thirty samples were associated with cereal spraying (22 in 1975). These poisonings seem to occur mostly where bees are flying over a crop during or soon after treatment, or when honeydew is being collected from aphids infesting the crop.

The forecast of light aphid infestations on field beans proved correct. This, and the smaller area of beans probably account for the continuing lower incidence of poisoning (7 samples, compared with 6 in 1975, 33 in 1974) associated with this crop.

The earliest casualties (4 samples) were caused by the use of carbaryl on pears and there is some evidence to suggest that this chemical may become an increasing hazard in orchard areas. (Stevenson and Smart)

Laboratory bioassay of insecticide toxicity to an aphid parasite. Knowledge of the relative toxicities of different pesticides to natural enemies of pests is an essential component of any approach to minimising harmful side effects of pesticide treatments. A contact toxicity bioassay for adult *Aphidius matricarius*, a fragile parasite of *Myzus persicae*, has therefore been devised. The principal problems were to remove the insects from their cultures without damage and to maintain them in a healthy state for at least 24 h. A satisfactory handling and test procedure has now been evolved and will be used to compare toxicities of commonly used aphicides.

Field work on the effects of aphicide applications on aphid parasites and predators continued. (Stevenson)

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Behaviour of pesticides in soil and control of soil-borne organisms. Knowledge of the behaviour of pesticides in soil is required both to improve efficiency of utilisation and devise more effective treatments for soil-borne pests and diseases, which are particularly difficult to control, and to minimise any adverse effects of pesticides reaching the soil adventitiously or by deliberate application. The Department continued a major programme of fundamental work on behaviour in soil and on seed treatments, and of practical studies on the control of soil-borne pests. Increased attention is being given to control of slugs.

Fundamental work on behaviour of pesticides in soil. Investigations into the interactions of pesticides with soil have done much to characterise adsorption mechanisms and equilibria, and behaviour under carefully controlled conditions. This has enabled broad patterns of behaviour in the field to be explained and predicted. However, it has become increasingly apparent that for more detailed understanding and prediction, it is necessary to consider not only equilibrium properties, but also non-equilibrium factors such as rates of adsorption and the accessibility of pores and surfaces in structured soils. To assess the magnitude of some of these factors, rates of adsorption and desorption of linuron by different size fractions of intact natural soil aggregates were measured. Soil from fallow land on Fosters field was separated into different aggregate sizes by sieving. To minimise disintegration, samples of aggregates were first wetted carefully with a fine Arnold spray and linuron solutions then added very slowly. The solution was gently stirred and amounts of linuron adsorbed determined from the change in solution concentration after various time intervals. Rates of adsorption for soil with an equilibrium distribution coefficient (K_d) of 2.97 were fastest for the 1–3 mm aggregate size fraction (half time, $t_{1/2} = 16$ min) and slowest for the largest 7–9 mm fraction ($t_{1/2} = 98$ min) compared with $t_{1/2} < 1$ min for a tumbled suspension of the same soil.

Rates of desorption were measured for soil with K_d for adsorption of 3.51. For the 1–2 mm fraction the half time for desorption was 26 min compared with 60 min for the 7–9 mm fraction. The time course of desorption for the 7–9 mm fraction gave evidence of a two-stage process with a change of slope when approximately half the herbicide had been desorbed.

These results indicate that rates of adsorption and desorption could have substantial effects on movement of pesticides in structured soils. (Graham-Bryce and Nicholls)

Seed treatments. Work on factors other than adhesives influencing effectiveness of insecticide application to cereal seed continued.

The effects of duration of mixing were investigated by tumbling 40% gamma-HCH (gamma-BHC) powder (0.3 g) with wheat seed (100 g) in glass jars at 90 rpm for varying periods. Treatments were assessed by the retention test described previously (*Rothamsted Report for 1973*, Part 1, 177). Initial adherence, before the retention test, increased from 50% of the theoretical maximum after 15 s to 70–100% after 5 min and 90–100% after 60 min. Amounts remaining on the seed after the retention test increased steeply from 22% of the maximum after 15 s mixing to 71% after 2 min, followed by a more gradual increase to 90% at 5 min and approaching 100% after 30 min. The treated seeds were examined by scanning electron microscopy which showed that the particles of powder were increasingly impacted into the seed surface as mixing time increased. Similar results were obtained using a 40% isofenphos powder applied at four times the rate of the gamma-HCH.

The effects of particle size on adhesion were investigated using a formulation of gamma-HCH and kaolinite each separated into different particle size fractions by means of an 'Alpine zig-zag classifier' kindly made available by the London School of Phar-

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macy. The fractionated insecticide and filler were mixed to give a series of 40% gamma-HCH mixtures with size ranges of <5, 5–10, 10–12, 20–50 and >50 μm . The adhesion and retention by wheat seed of each mixture, and of similar size ranges of undiluted gamma-HCH were determined using the methods described above. With all particle sizes retention increased with the duration of mixing. As expected, with undiluted gamma-HCH retention after 2 min mixing decreased with increasing particle size from 86 and 75% with the <5 μm fraction for rates of 0.15 and 0.5 g insecticides per 100 g seed to 64% and 70% with the corresponding rates of the 20–50 μm fraction. With the 40% powder, however, retention increased from 30% with the <5 μm fraction applied at 0.3 g per 100 g seed to 50% for the 20–50 μm range and then decreased again to 32% for the >50 μm particles. Percentage retention was significantly less for all size fractions at an application rate of 0.5 g per 100 g seed. The tendency for a maximum retention at intermediate sizes between 10 and 50 μm was evident for all mixing times. The results also show that the addition of a diluent powder significantly decreases retention.

Poor adhesion and retention were shown previously to be major shortcomings of existing commercial powder seed treatments. These studies indicate several factors which could be adjusted to optimise the efficiency of application. (Jeffs)

Field tests of seed treatments for controlling wheat bulb fly. Further short row trials to evaluate candidate insecticides on a peaty loam and a clay loam, during 1975–76, showed that seed treatments with diflubenzuron, 'SAN 155' (5-dimethylamino-1,2,3-trithiane-hydrogenoxalate) tazimcarb ('PP 505') or the insect repellent 'GD 880' were relatively ineffective at protecting wheat from attack by wheat bulb fly larvae. Of the synthetic pyrethroids tested 'NRDC 149' (proposed common name cypermethrin), 'NRDC 161' (proposed common name decamethrin), 'WL 41706' (: 'S-3206') and 'WL 43775' (: 'S-5602 proposed common name fenvalerate'), all significantly decreased the numbers of plants attacked by wheat bulb fly larvae but none was more effective than permethrin. (Griffiths and Scott)

Chemical control of slugs in cereals. Damage to cereal seed by slugs is becoming an increasing problem particularly in direct drilled crops. Work has started to find a suitable toxic or repellent chemical which can be applied as a seed treatment. A site for experimentation has been reserved at Rothamsted with cultivation, crop sequence and irrigation designed to build up large numbers of slugs.

A test for screening seed treatments in the laboratory has been developed in which a single treated seed is placed on damp cotton wool in a glass tube. A slug (*A. reticulatus*) previously starved for three days, is put into the tube and observed for up to nine days. Preliminary results have shown that seeds treated at the rate of 0.2% a.i. with thio-carboxime, methiocarb or pentachlorophenol, decreased feeding and killed over 50% slugs tested. Seeds treated with 0.2% of ioxynil or 'SAN 155' failed to kill slugs but dramatically decreased feeding. (Scott and Griffiths, with Stephenson, Entomology Department)

Control of leaf-cutting ants. Work on the development of more effective baits for use against these important pests of Central and South America continued, with support from the Ministry of Overseas Development.

Development of liquid formulations for locally produced baits. The emulsion concentrate formulated at Rothamsted for addition to locally produced baits (*Rothamsted Report for 1975, Part 1, 163*) is being evaluated in several countries. Samples containing mirex as toxicant have been requested from Cuba, Guyana and Paraguay for small-scale field

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trials. Mercabam bait prepared from the concentrate by workers in the Entomology Division, Centro de Pesquisas do Cacau (CEPEC), killed only 30% of *Atta sexdens* nests in trials in Brazil. Greater efficacy against this species would have been expected from previous results (Phillips, Etheridge and Scott, *Bulletin of Entomological Research* (1976), 66, 579–85) suggesting that the bait may not have been dried sufficiently before application.

Pick-up of paper discs impregnated with the emulsion concentrate, but without insecticide by three ant species was investigated by Dr. A. R. Jutsum (UCNW, Bangor). The soya oil contained in the formulation was shown to attract ants to bait, optimum pick-up being obtained with concentrations of about 4%, confirming the composition previously chosen for all our field baits. Tests with several different matrices also showed that pick-up was most effective when the concentrate was diluted 1:8 (v/v) with water. This lies within the dilution range used in our strongly sorbent vermiculite baits, giving further support for the formulation specification recommended from our previous studies.

In further work this year, attempts have been made to find better emulsifiers which will give more stable emulsions at smaller concentrations. Several good emulsifiers have been found for insecticides which dissolve easily in soya oil, but benzene must be added to dissolve mirex and different emulsifiers are required for the soya oil–benzene solvent. (Phillips, Etheridge and Martin)

Effectiveness of different baits in the field. With the help of workers from CEPEC, further results have been obtained from field trials in Bahia, Brazil (*Rothamsted Report for 1975*, Part 1, 163) to evaluate the effectiveness of different baits against *Atta cephalotes*, previously shown to be the most difficult species to control. Nest mortalities were assessed three, six and nine months after baits were applied. Five insecticides in dried citrus pulp baits were each compared, using 15 replicate nests. Final mortalities were: pirimiphos-methyl (microencapsulated) 36%; fospirate 54%; permethrin (microencapsulated) 64%; dioxathion 61%; mecarbam 15%. The encapsulated permethrin was applied at only 0.045% in the bait compared with 0.3% for fospirate and dioxathion. Fospirate was also found to be an excellent fungal inhibitor. The permethrin formulation thus appears to have great potential as a formicide against this intractable species of leaf-cutting ant. (Phillips and Etheridge)

Weathering of baits. Samples of bait from the trials in Brazil containing five different insecticides on dried citrus pulp were allowed to weather in the field and sampled at intervals for analysis by gas–liquid chromatography. Very little insecticide was lost after one week of weathering at temperatures in the range 18–30°C when rainfall was negligible. However, subsequent very heavy rain removed the propionic acid fungicide from the baits, leading to rapid deterioration and dense fungal growth on all samples except those containing fospirate. (Phillips, Etheridge and Martin)

Effects of insecticides on virus spread and yield of field beans. Further collaborative work on this project is described in the report of the Plant Pathology Department (p. 268). (Etheridge, with Cockbain and Bowen, Plant Pathology Department)

Chemicals influencing insect behaviour

Work continued on a wide range of insect responses to behaviour – modifying chemicals with the objective of developing improved methods of controlling pests or managing beneficial insects. These studies require a multi-disciplinary approach and much of the work is done in association with the Entomology Department, a collaboration to which

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we attach much importance. These collaborative projects are described in the report of the Entomology Department.

Electroantennogram studies with leaf-cutting ants and stored products pests. The application of electroantennogram (EAG) techniques to the study of pheromones of leaf-cutting ants and lepidopterous stored products pests was investigated by measuring responses of the ants and of *Venturia canescens* (Grav.), a hymenopteran parasite of stored products Lepidoptera, to applied chemicals.

Extracts of the head, abdomen and thorax of virgin queen *Acromyrmex octospinosus* (Reich) were presented in the vapour phase to antennae of *A. octospinosus* workers. The EAG responses to the extracts or components of extracts were significantly greater than the EAG responses to air, water or solvent controls indicating that behaviour-influencing chemicals may be present in the extracts.

Similarly, EAG responses were obtained from antennae of the parasite *Venturia canescens* (Grav.) to the pheromones of its host, *Anagasta kuehniella* (Zeller) larvae. The EAG response of both these insects is much smaller (<1 mV) than that normally observed for Lepidoptera in response (up to 8 mV) to their sex pheromones; also their antennae are tougher than lepidopteran antennae and more difficult to penetrate directly with glass electrodes so that somewhat different techniques to those normally employed in EAG measurements are required.

To facilitate the identification of the larval pheromone of *A. kuehniella* by spectroscopic methods, syntheses of model compounds similar to the pheromone were begun. (Mudd)

Sex attractants for pea moth. Further collaborative work is described in the report of the Entomology Department (p. 125). (Greenway, with Wall, Entomology Department)

Detection of starvation in honeybees. Collaborative studies are described in the report of the Entomology Department (p. 133). (Greenway and Stevenson, with Simpson, Entomology Department)

Equipment and techniques

Formulation

Microencapsulation. Previous work concentrated on the production and evaluation of dry free-flowing microcapsules of 200–500 μm diameter with gelatin/gum acacia/carrageenan double walls made by a coacervation process, based on that developed by the National Cash Register Co. However, very small (1–5 μm diameter) plastic walled microcapsules produced in the form of an aqueous slurry by interfacial polymerisation condensation, based on methods used by Pennwalt Inc., offer potential advantages as readily made agricultural sprays, and work is now proceeding with this process. Several compounds have been successfully encapsulated, including permethrin for use in leaf-cutting ant baits. When contacted by the leaf-cutting ant *Acromyrmex octospinosus* in laboratory tests, these permethrin formulations were completely without the immediate toxic effect normally associated with this very potent insecticide, demonstrating the efficiency of the encapsulation process. (Etheridge, Phillips and Martin)

Bird repellents. In collaboration with Drs. B. D. Smith and D. Kendall (Long Ashton Research Station) investigation into methods of improving the persistence of bird repellents applied to fruit trees and bushes, particularly against rainwashing, were started.

The effects of additives were investigated in a small field trial by comparing commer-

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cial 80% wettable powder formulations of both thiram and anthraquinone with similar formulations containing 5% v/v of either 'Acronal 4D' (a commercial polybutyl acrylate sticker manufactured by BASF Ltd.) or methyl dibutylamine stearate, synthesised by us. Sprays containing 20 g litre⁻¹ of the bird repellent were applied in winter by knapsack sprayer to plum trees, gooseberry and red currant bushes, ensuring good coverage of the buds. Visual assessments a few months later showed that deposits from formulations containing either 'Acronal 4D' or methyl dibutylamine stearate still remained, whereas the normal wettable powder deposits had been washed off by the winter rain. Despite the greatly increased persistence conferred by these additives, however, there were no significant differences in levels of bird damage between treatments, including controls. This emphasises the need for more effective repellents than thiram and anthraquinone. (Phillips and Etheridge, with Dr. B. D. Smith and Dr. D. A. Kendall, Long Ashton Research Station)

Insect neuroanatomical techniques: synthetic substitute for aged alcoholic Bouin fixative.

The study of the chemical changes brought about by ageing alcoholic Bouin fixative for about 40 days at 60°C (*Rothamsted Report for 1975*, Part 1, 166), which gives improved preservation of insect nerve tissue for staining by the Bodian silver method, continued. Further analysis by gas chromatography gave the final percentages of the volatile constituents in the optimally aged solution as approximately: ethanol 35, diethoxymethane 15, formaldehyde 6, ethyl acetate 5, acetic acid 3.4 and methyl acetate 0.4; picric acid (0.46%) and water are also present. Histological tests of a mixture with this composition freshly made from the individual reagents have so far yielded fixation indistinguishable in quality from that given by optimally aged alcoholic Bouin. Further tests are in progress to discover whether this synthetic substitute is fully as good as the normally aged fixative and whether modification of the synthetic mixture will improve the quality of fixation still further. (Gregory, Lord and Greenway)

Equipment for mass pollination of coconuts. Development of apparatus for extracting and processing coconut pollen continued in collaboration with the Coconut Industry Board of Jamaica and with support from the Ministry of Overseas Development.

Evaluation of the equipment developed during 1975 gave satisfactory results but showed the need for apparatus that would strip the flowers from the inflorescence stems. Based on this study and field work in Jamaica an entirely new device incorporating a novel feed system is being designed to strip and crack the male flowers. Preliminary tests have proved encouraging; feed problems caused largely by the high sucrose content of the flower have been almost entirely eliminated.

A liquid spray pollen applicator has also been developed, based on an atomiser designed at Rothamsted. This unit enables a known volume of pollen suspension to be sprayed so that a calculated number of pollen grains can be discharged over a given area. Tests of performance in the field are now required to evaluate both the carrier liquid and the mechanical design. (Arnold)

Pesticide spraying equipment. The electrostatic spraying system developed at Sheffield University with support from NRDC (*Rothamsted Report for 1975*, Part 1, 167) was evaluated in field trials with sugar beet.

Analysis by gas-liquid chromatography showed that the electrostatic system increased the gross deposition of permethrin applied as water-based droplets (diameter <50 µm) at ultra low volume by 55%. Bioassay using aphids supported the chemical measurements. Electrostatic charging had no significant effect on the distribution of the deposits: there was no increase in deposition on the undersides of the leaves, possibly because of the

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broad leaf shape and the low profile of the sugar beet plants at the time of spraying. (Arnold)

Insect rearing. The following species were reared:

Homoptera	<i>Acyrtosiphon pisum</i> (Harris) <i>Aphis fabae</i> (Scop.) <i>Myzus persicae</i> (Sulz.) Strains. Susceptible Several organophosphate-resistant <i>Megoura viciae</i> Buckt.
Hemiptera	<i>Dysdercus intermedius</i> Distant
Coleoptera	<i>Phaedon cochleariae</i> (F.)
Orthoptera	<i>Blaberus discoidalis</i> (L.) <i>Periplaneta americana</i> (L.)
Diptera	<i>Drosophila melanogaster</i> (Meig.) Strains. Normal Vestigial wings <i>Musca domestica</i> (L.) Strains. A wild-type susceptible strain <i>ac</i> ; <i>ar</i> ; <i>bwb</i> ; <i>ocra</i> —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 ₂ b each with one or more factors of resistance to dimethoate and other organophosphorus insecticides. 49PPB, a substrain of 49r ₂ b 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. Several strains derived from 290BIO each with one or more factors of resistance to pyrethroids and DDT. <i>Calliphora erythrocephala</i> (Meig.) <i>Delia antiqua</i> (Meig.)
Hymenoptera	<i>Acromyrmex octospinosus</i> (Reich) <i>Atta cephalotes</i> (L.) <i>Aphidius matricariae</i> Haliday <i>Venturia canescens</i> (Grav.)
Lepidoptera	<i>Plutella xylostella</i> (L.) <i>Anagasta kuehniella</i> (Zeller)

Fungicides

Control of soil-borne diseases by foliar sprays. Work reported in the last few years has established that the incidence of potato common scab, caused by soil-borne *Streptomyces scabies*, can be decreased by foliar sprays of daminozide or ethionine. Further studies this year were directed at discovering how these chemicals exert their action and investigating the effects of foliar sprays on other diseases caused by soil-borne organisms.

Laboratory studies. *In vitro* toxicities were measured against three isolates of *S. scabies* on two media in 'poisoned agar' plate tests. In each test, 15 colony diameters were measured after incubation for three weeks at 25°C. Tables 6 and 7 show weighted mean EC₅₀s from two tests per chemical.

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TABLE 6

EC50s (ppm) of quintozone, daminozide and DL-ethionine against three isolates of S. scabies on two media

Isolate	Czapek-Dox agar			Potato dextrose agar		
	Quintozone	Daminozide	DL-ethionine	Quintozone	Daminozide	DL-ethionine
1	20 ± 5	400 ± 1	4 ± 0.1	14 ± 2	200 ± 8	660 ± 150
2	10 ± 1	1200 ± 70	270 ± 50	14 ± 5	560 ± 50	1000 ± 520
3	∞	560 ± 20	3 ± 0.1	9 ± 1	230 ± 5	100 ± 30

Table 6 compares daminozide and DL-ethionine with quintozone, which is widely used as a soil-treatment for scab control. The three isolates differed greatly in their susceptibility. Strain 3 was remarkably resistant to quintozone on Czapek-Dox agar, and strain 2 was most resistant to daminozide and DL-ethionine. There were also large differences between the two media. Potato dextrose agar contains methionine, which is known to antagonise the action of ethionine on several microorganisms, including *S. scabies*, so that it is not surprising that DL-ethionine was less toxic on potato dextrose than on Czapek-Dox agar. By contrast, daminozide was more toxic on potato dextrose agar.

The great variation in the EC50s of both daminozide and DL-ethionine indicates that (1) if they exert their effects by direct systemic fungicidal action, their effectiveness in the field where different strains of *S. scabies* co-exist is likely to vary from soil to soil; and (2) drawing conclusions from data on the amounts of these chemicals found in potato tubers (see below) requires caution.

TABLE 7

EC50s (ppm) of DL-, D- and L-ethionine against three isolates of S. scabies on Czapek-Dox agar

Isolate	Ethionine isomer		
	DL-	D-	L-
1	38 ± 6	200 ± 100	36 ± 2
2	280 ± 40	∞	130 ± 50
3	6 ± 0.6	18 ± 4	5 ± 0.6

In similar tests, DL-ethionine was compared with the separate D- and L-isomers against the same isolates of *S. scabies*. Table 7 shows that the L-isomer was more toxic than the D-isomer but that, as before, the absolute and relative toxicities of the isomers varied amongst isolates.

The effects of daminozide and ethionine on respiration of *S. scabies* were also measured. Daminozide completely halted oxygen uptake at 11 mM but ethionine increased oxygen uptake slightly at concentrations above 5 mM. If the concentrations of ethionine and daminozide found in tubers are used as a guide, it seems unlikely that their effects on respiration could explain their activity against scab. (Bateman, Burrell and McIntosh)

Growth room tests. To help elucidate how the foliar treatments reduce the amount of potato common scab, translocation of ethionine was studied by measuring its distribution in the roots, tubers, stems and leaves after application as a foliar spray or as discrete drops of 1% solution to the leaves of potato plants (cv. Majestic) grown in controlled environment rooms. The results (Table 8), taken from one of two experiments, indicate that ethionine was translocated to the roots and tubers but that a large proportion of the chemical applied failed to penetrate the leaf surface after two weeks. In addition the plants treated with ethionine had an increased level of amino acids in the roots and stems. A

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comparison of the amounts of ethionine and of daminozide in the tubers (*Rothamsted Report for 1975*, Part 1, 168–169) suggests that ethionine accumulates in the tubers to a greater extent than daminozide. (Burrell)

TABLE 8
Distribution of ethionine in the various parts of potato plants two weeks after application as drops of 1% solution

	Weight of tissue (g)	Weight of ethionine (μg)
Apex	0.67	0.0042
Stem	4.39	1.4755
Leaves	23.99	7.0411
Roots	3.46	0.0065
Tubers	12.96	0.0467
Washed from leaves before extraction		25.60
Total recovered		34.17
Total applied		60.00

Glasshouse tests against potato scab. Chemicals were applied as foliar sprays to young potato plants (cv. Majestic) growing in pots containing scab-infected soil, and the incidence of scab was measured at harvest. In the extremely hot weather last summer the plants grew very badly, and the tubers were often only lightly scabbed; some tests had to be abandoned, and many were inconclusive.

However, tests of daminozide analogues (*Rothamsted Report for 1974*, Part 1, 153–154) were completed; they confirmed that of nine analogues tested, only dimethylamino maleamic acid ('CO-11') had an effect on scab comparable to that of daminozide.

Some tests were designed to detect movement of daminozide (a growth retardant) into soil from sprayed plants by measuring the effects on plant height. Two young plants were grown in each pot instead of the usual single plant, and one of each pair was sprayed with 1.2% daminozide solution. During spraying the second plant and the soil were very carefully shielded from spray solution, and afterwards contact between the foliage of the sprayed and the unsprayed plants was prevented by plastic sheets. The roots were, of course, in close contact. Three weeks after spraying, the sprayed plants were almost exactly half the height of unsprayed plants in untreated pots, but the height of the unsprayed plants in treated pots was unaffected. As growth of potato plants can be retarded by daminozide deliberately applied to soil, and as daminozide is known to be translocated from foliage into roots, the result suggests that little or no daminozide passes into soil from roots or stolons. Its action on *S. scabies* may therefore be within the plant rather than in the rhizosphere. (McIntosh)

Glasshouse tests against clubroot of cabbage. Soil in which potted, three-week-old cabbage plants were growing was inoculated with spores of *Plasmodiophora brassicae*. The leaves were sprayed with solutions of experimental chemicals at various intervals from one week before to four weeks after inoculation. Spray mixtures contained sufficient 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 ten weeks old.

Daminozide, which decreases the incidence of potato scab as a foliar spray, had no effect on clubroot. By contrast, the results of six tests with DL-ethionine showed that, although 0.5% sprays applied before or up to ten days after inoculation had no effect, sprays applied 2–4 weeks after inoculation decreased the weights of clubs. The weights of tops were unaffected, but they sometimes became slightly chlorotic. Single sprays were most effective when applied two or three weeks after inoculation and, when up to three sprays

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were applied, club weight decreased with increasing frequency of spraying. For example, in one experiment the mean fresh weights (g) of tops and clubs were: from plants sprayed three weeks after inoculation, 37.3 and 15.2; from plants sprayed two, three and four weeks after inoculation, 36.0 and 10.2; and from unsprayed plants, 31.8 and 22.5 (LSDs: 11.0 ($P = 0.05$) for tops, and 6.2 ($P = 0.05$) and 10.7 ($P = 0.001$) for clubs). Soil drenches of DL-ethionine before inoculation were ineffective against clubroot and damaged the plants.

Thus, foliar sprays of DL-ethionine, seem to have a downward-moving systemic action against *P. brassicae*, similar to that against *S. scabies*, provided they are applied 2–3 weeks after inoculation when the clubs are beginning to grow rapidly. (McIntosh, with Macfarlane, Plant Pathology Department)

Glasshouse tests against other soil-borne diseases. As a result of improvements in method, satisfactory pot experiments can now be done on control of scab on red beet, caused by soil-borne *Streptomyces* spp. In one unconfirmed experiment, roots were inoculated at the decortication stage, and the plants were sprayed 11 days or one day before or nine days after inoculation with three concentrations of daminozide or DL-ethionine, plus suitable wetter, the soil being protected from spray solution. Daminozide had no effect on scab incidence, but DL-ethionine, applied one day before inoculation, significantly decreased scab to about half (0.5% spray) or one quarter (1.0%) of the amount on unsprayed plants.

The possibility of controlling violet root rot of carrots, caused by soil-borne *Helicobasidium purpureum*, was investigated in a similar experiment. Carrots growing in infected soil were sprayed in August, or in July, August and September, with either 1.0% daminozide or 0.2% DL-ethionine, plus suitable wetter. However, it was clear that the sprays did not decrease the amount of disease found at harvest in November; carrots from all treatments were severely infected. (McIntosh, with Adams and Lapwood, Plant Pathology Department, and Byford, Broom's Barn)

Control of potato scab in the field. Daminozide and DL-ethionine were again tested for scab control in a small field trial on potatoes (cv. Maris Piper) in Schoolfield, Woburn. Each chemical was sprayed at two concentrations on each of three dates in late May and early June, or on all three dates (*Rothamsted Report for 1975*, Part 1, 170). In the very hot dry summer the tubers from the unsprayed plots were very badly scabbed. The effects of the treatments were generally similar to those in last year's trial in Great Hill Bottom, Woburn; however, scab was not so well controlled, possibly because the strains of *S. scabies* in Schoolfield are less susceptible. The most favourable time for application was 7 June, when the haulms were just touching along the drills but only occasionally across them. (McIntosh)

Fungicidal seed treatments

Seed treatment and soil-borne disease. Previous experiments (*Rothamsted Report for 1975*, Part 1, 171) showed that treating wheat seed with phenyl mercuric acetate could partially protect the young shoot from invasion by *Fusarium culmorum* in inoculated soil. In further experiments to examine the action of mercury fungicides in more detail, inoculation of untreated plants grown adjacent to treated plants gave no evidence for the existence of an effective protective zone in the soil around the treated seed. Other inoculations showed however that the aerial parts of shoot bases were protected by seed treatment indicating either that fungicide was present in the shoot or that treatment induced resistance in the shoot. In contrast ethyl mercuric chloride, a more volatile fungicide,

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gave a more effective protective zone in the soil but less effective protection of the aerial part of the young shoot.

Fusarium culmorum was selected as a suitable pathogen for studies on the behaviour of other fungicides applied as seed and soil treatments. Shoots of wheat seedlings grown from seed treated with a range of systemic and non-systemic fungicides were inoculated 12 or 28 days after planting. The performance of carbendazim-producing compounds was of interest. Against the earlier inoculations effectiveness decreased in the order benomyl, 'NF48', carbendazim, thiophanate methyl. Carbendazim gave only moderate control and thiophanate methyl was barely effective. *In vitro* tests against *F. culmorum* on agar plates showed carbendazim and benomyl to be very effective and thiophanate methyl and 'NF48' to be ineffective. These results can probably be explained in terms of relative rates of release of carbendazim. Following application to seed, carbendazim itself would be translocated rapidly to the leaf edges, outside the area of inoculation, while thiophanate methyl is converted slowly in the plant or soil to carbendazim, its fungitoxic product. Thiophanate methyl was more effective against the later shoot inoculations and also against root inoculation, giving further evidence for retention in the root region with a gradual release of carbendazim. Such behaviour suggests that thiophanate methyl could be of value against more serious foot and root diseases, notably take-all, which have a long period of infectivity in the field; this is being investigated in further studies. (Bateman)

Phytotoxic effects of 'NF48' seed treatments. Barley seed, cv. Sultan, infected with loose smut, was treated in the laboratory 'Rotostat' machine with carbendazim, 'NF48' and carboxin, for a field demonstration plot. Although all fungicides were applied at rates similar to those used in previous experiments (*Rothamsted Report for 1975*, Part 1, 173), emergence from seed treated with 'NF48' was poor. Unused seed was analysed for fungicide, and germination in soil determined in further laboratory tests. The analyses showed that 'Rotostat' treatment resulted in a higher dose of fungicide on the seed than previous methods of application which involved mixing seed with fungicide in rotating or shaken containers. In all cases technical grade fungicide powder was applied after pretreating the seed with gum arabic sticker. Phytotoxic symptoms appeared at doses between 3.5 and 6.0 g kg⁻¹ depending on growing conditions. The phytotoxic effect was greatest in wet soil. (Nicholls and Bateman)

Relationships among seed-borne fungi. Some interactions of mixtures of micro-organisms on wheat and barley seed at the germination stage were examined previously, particularly in relation to the development of seedling disease by *Fusarium nivale* (*Rothamsted Report for 1974*, Part 1, 156 and for 1975, Part 1, 172). Relationships during the colonisation of seed after inoculating flowering ears of wheat have now been studied in a glasshouse experiment. Agar plating of the ripened seed suggested that *F. nivale* and saprophytic fungi (*Alternaria*, *Cladosporium* and *Sporobolomyces*) were mutually inhibiting, but the first fungus to colonise the ear remained dominant. This effect was probably influenced, however, by the physiological state of the ear, early inoculations being more successful than later inoculations made towards the end of the flowering period. (Bateman)

Mode of action of pyrimidine fungicides. Hydroxypyrimidine fungicides act selectively on mildews only. They inhibit appressorial formation and have other, smaller, effects on later stages of development. Although mildews cannot, as yet, be grown in culture, some limited germ-tube growth does occur *in vitro*, and this is also inhibited. In an attempt to determine the mode of action of this group of fungicides, the influence of various metabolites on the fungitoxicity of ethirimol was studied using barley powdery mildew (*Erysiphe graminis* f.sp. *hordei*). The effects of several purine and pyrimidine analogues,

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and of some unrelated inhibitors, on mildew were also examined to determine if any had similar action to that of ethirimol.

Although pyrimidine analogues (2-thiouracil, 6-azauracil, 5-fluorouracil) inhibited some stages of mildew development, they did not affect appressorial formation. Pyrimidines did not reverse the action of ethirimol, whereas adenine did to some extent. Several adenine analogues inhibited both appressorial formation and growth *in vitro* (Table 9), but had less pronounced effects on subsequent disease development. Mildew strains less susceptible to ethirimol were cross-tolerant to both kinetin and 6-(γ,γ -dimethylallyl-amino)-purine. Ethirimol action was not reversed by guanine and 8-azaguanine was inactive. All analogues were less active than ethirimol in these tests.

TABLE 9

Effect of purine analogues on appressorial formation and growth in vitro

	<i>In vitro</i> EC ₅₀ ($\mu\text{g ml}^{-1}$ in nutrient agar)	Appressorial formation EC ₅₀ ($\mu\text{g ml}^{-1}$ in solution on which leaf segments were floated)
Ethirimol	0.02	0.233
6-furfurylaminopurine (kinetin)	3.92	0.37
6-(γ,γ -dimethylallylamino)purine	1.51	10.00
8-azaadenine	2.00	above 200
6-methylpurine	30.90	166.00
2-amino-6-mercaptopurine	57.5	above 200
6-mercaptopurine	184.1	above 200

Inhibitors of purine biosynthesis (azaserine) and tetrahydrofolate-directed C-1 transfer reactions (trimethoprim, pyrimethamine, methotrexate) had no effect on mildew. The effects of ethirimol were not antagonised by folate, N⁵-formyl tetrahydrofolate (leucovorin), *p*-aminobenzoic acid, or metabolites expected to donate C-1 units (methionine, serine, choline). This suggests that although ethirimol may interfere with adenine metabolism, it is unlikely to act by inhibiting purine biosynthesis. Both cycloheximide and griseofulvin affect appressorial formation and growth *in vitro*, but there is no cross-tolerance in strains less sensitive to ethirimol. (Hollomon)

Insensitivity to ethirimol. Twenty-five single pustule isolates of barley powdery mildew from winter and spring sown crops were bioassayed this year. Many were obtained by selection but the range of ethirimol sensitivity was no greater than that encountered previously. No strain was significantly less sensitive than that used as a standard insensitive strain, which was originally isolated in 1973.

In laboratory experiments using detached leaves, all insensitive strains examined so far have competed poorly with more sensitive ones in the absence of ethirimol. However, insensitive strains were not eliminated and increased again in frequency when a selection pressure, equivalent to the rate of ethirimol used commercially, was applied. When only two strains are mixed, the one sensitive to ethirimol may be lost at these levels of selection, and insensitivity does not then decline when the fungicide is removed. The extent to which susceptibility may shift in these simple mixtures seems to depend on the magnitude of the difference between the two strains used. In more complex mixtures using six strains sensitivity fluctuated less, and strains of intermediate sensitivity dominated both in the presence and absence of ethirimol.

In a field experiment, plots of barley (cv. Proctor) grown from seed treated with a range of ethirimol rates up to four times that applied commercially were inoculated with an ethirimol-sensitive strain of mildew. This strain did not infect treated plots but did establish an early mildew epidemic on untreated plots. It was soon replaced, however, by

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the natural population which was less sensitive and which provided inoculum for all treated plots. At first sensitivity was less on all treated plots than on untreated ones, although sensitivity was not related to the ethirimol level employed. Once the sensitive strain was displaced insensitivity increased on untreated plots and became uniform over the whole experiment. Nevertheless, control of early mildew was significant and yield increases of the order of 20% were recorded on the plots receiving four times the commercial rate.

These results show that as the complexity of the mildew population increases, changes in sensitivity in response to selection become less pronounced. Strains at either extreme of the range of natural variation in ethirimol sensitivity compete poorly in the natural population, which is dominated by strains of intermediate sensitivity. Even at ethirimol levels four times the commercial rate insensitive strains lack fitness. Until such strains acquire the necessary fitness, sensitivity levels are likely to remain fairly stable within field populations. (Hollomon)

THE CHEMICAL LIAISON UNIT

The Unit provides chemical support for work with crop protection chemicals by several departments at Rothamsted and various outside bodies. This involves both short investigations, usually analyses of initial treatments such as seed dressings or of residues in soils or plants at harvest, and longer studies of the behaviour of specific chemicals in soils and crops.

The Unit also has its own programme on the factors governing the behaviour of chemicals in soils and plants. There is much interest in systemic chemicals and we have begun to investigate relationships between physical properties and root uptake and translocation by plants.

Analytical techniques

Identification of benzimidazole and thiophanate fungicides and related compounds after thin-layer chromatography. The work on thiophanate methyl residues described last year (*Rothamsted Report for 1975, Part 1, 177*) was greatly facilitated by the use of specific colour reagents sprayed on TLC plates for identifying the components present in extracts. The reagents were vanadium pentoxide, Wood's reagent (bromophenol blue/silver nitrate), *N*-1-naphthylethylenediamine dihydrochloride, and 2,6-dichloro-*p*-benzoquinone-4-chlorimine. By eluting several spots of a mixture or soil extract and spraying each chromatogram with a different reagent, it proved easy to distinguish among twenty benzimidazoles and related compounds on the basis of their different colour reactions, together with their mobilities on the TLC plate. (Austin, with Mutwakil and Davies)

Extraction and clean-up of thiabendazole residues in soil. Thiabendazole (TBZ) is finding increasing use for the protection of potato tubers from fungal pathogens (*Rothamsted Report for 1975, Part 1, 271*) and a method for determining thiabendazole residues in soils was required. The method for analysing thiabendazole residues in plant material was unsuitable for soils and the acetone/ammonium chloride extraction procedure for the related benzimidazole carbendazim (*Pesticide Science* (1976), **7**, 201) gave only 50% recovery of freshly-added thiabendazole from Woburn soils and 20% recovery from the highly organic Mepal soil.

A new extraction procedure using a mixture of ethanol, 2M-NH₄Cl adjusted to pH 10 with ammonia (sp. gr. 0.880) and chloroform in the ratio 1:3:4 gave greater than 90% recovery of thiabendazole from Mepal soil. Much organic matter is also extracted and extensive clean-up is needed for the analysis of thiabendazole concentrations less

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than 5 ppm. Preliminary results indicate that the thiabendazole is present in the chloroform layer which can be separated by centrifugation. The thiabendazole in an aliquot of this layer, concentrated if necessary, is separated from soil organic material on a silica gel column by elution with a mixture of acetic acid, hexane and isopropanol (1:8:11), the isopropanol containing 1 mg ml⁻¹ of ferric chloride to reduce movement of thiabendazole which is then eluted with ethanol/hexane (60:40) containing 1% phosphoric acid. The eluate is colourless and thiabendazole can be determined by ultra-violet spectrometry or fluorimetry. Work is in progress using metal ion impregnated silica gel in an attempt to simplify clean-up. (Cayley, Davies, Lord and Nasim)

Behaviour of chemicals in soil

Adsorption of thiabendazole by soils. Thiabendazole is much more difficult to extract from soil than carbendazim, probably because it is more strongly adsorbed. Soil/water distribution coefficients (K_d) of 15 ± 3 and 130 ± 15 for soils from Woburn and Mepal are considerably larger than those for carbendazim. Preliminary measurements indicate that, as for carbendazim, K_d increases greatly as soil pH decreases because the thiabendazole cation is strongly adsorbed. Thus, treatment at pH 10 is necessary for efficient extraction of thiabendazole residues from soil, as discussed above. (Camp, Cayley and Lord)

Incorporation of nematicide granules into soil. Methods used to incorporate nematicide granules in soil can influence both crop yield and nematode multiplication, and the reasons for this have been investigated.

In trials on peaty loam soils at the Arthur Rickwood EHF, Mepal, rotavators (L-bladed or spiked) incorporated granules to the working depth (usually 15–20 cm) whereas harrows (rotary, reciprocating and spring-tined) gave a shallower distribution generally leaving most granules in the top 5 cm. Similar distributions were obtained in clay loam and sandy loam soils in tests comparing the L-bladed rotavator and rotary harrow (Roterra).

Control of potato cyst-nematode (*Globodera rostochiensis*) by aldicarb or oxamyl in the peaty loam soils in each of the years 1973–75 was better following incorporation by rotavation than by harrowing, and use of the rotavators also gave more reliable increases in potato yields. However, in 1974 and 1975 on a sandy loam soil at Woburn, the method of incorporation of aldicarb had little effect on control of potato cyst-nematode or on tuber yield.

Thus the more reliable yield increases and nematode control achieved by nematicides following incorporation by the rotavators can be attributed to the deeper distributions of granules achieved by these implements. In soils of low organic matter content, redistribution of the chemical by leaching may compensate for shallow initial distribution. The spiked rotavator was shown to be as effective as the L-bladed rotavator on peaty loam soils and may prove useful on soils that are easily 'glazed' by the action of the L-bladed rotavator. (Bromilow, Jabbar, Lord and Middleton, with Whitehead, Nematology Department and Mr. J. Smith, ADAS)

Aldicarb and oxamyl residues in crops. Monitoring of nematicide residues in crops continued in 1976, and samples of all potatoes grown at Woburn and onions at Rothamsted treated with oximecarbamate nematicides were examined at the request of the Subcommittee for Agricultural Chemicals. Some peas were also analysed.

Oxamyl residues in peas and potatoes grown in soil having up to 11.2 kg a.i. ha⁻¹ incorporated at planting did not exceed 0.014 µg g⁻¹, a toxicologically insignificant level.

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Larger residues of aldicarb plus its sulfoxide and sulphone were detected; potatoes grown in sandy loam soil treated with $11.2 \text{ kg aldicarb ha}^{-1}$ at planting contained up to $0.37 \mu\text{g g}^{-1}$ aldicarb residues at harvest. However, potatoes and winter onions grown in soils treated with the commercial rates of aldicarb (3.36 and 1.68 kg ha^{-1} respectively) contained less than $0.15 \mu\text{g g}^{-1}$ at harvest; human consumption of these crops would result in the intake of at least 70 times less chemical than that ingested by rats at the highest feeding level which produced no ill effects. As cooking reduces residues further, for example, by 30–70% in potatoes, residue levels in these crops grown in soil treated with the recommended rates of aldicarb at planting are most unlikely to exceed those considered safe by the presently accepted standards. (Bromilow, Jabbar and Middleton)

Shortening of barley stems by chlormequat chloride (CCC). Studies on the effects of times and methods of applying chlormequat to barley and wheat continued in glasshouse experiments designed to clarify how these plants respond to the chemical. It was confirmed that soil treatment with chlormequat shortened barley stems only when applied over a limited growth period (*Rothamsted Report for 1975*, Part 1, 41). Localised application to leaves showed that doses of chlormequat that shortened main stems of barley (5 mg per plant) tended to be more toxic, especially on tillers, than similar applications to leaves of wheat.

In an attempt to elucidate why chlormequat shortens wheat stems more than barley, main-stem ears were removed from within the flag-leaf sheath of chlormequat-treated and untreated plants. Removing the ears shortened the main stem of wheat, particularly the uppermost internodes, but had little effect on barley as the main stem had by then finished elongating. Chlormequat, which mainly shortens lower internodes, still affected these earless stems, but as before shortened wheat more than barley.

Methods of separating chlormequat from choline chloride and other impurities and possible metabolic products are under investigation. (Lord and Wheeler)

Uptake of pesticides by plants. Many pesticides including pre-emergence herbicides and systemic insecticides and fungicides are taken up by plants via roots or leaves and then translocated to the site where they exert their biological action. The early work of Collander (*Physiologia Plantarum* (1954), 7, 420) indicated that uptake of different chemicals by plant cells increased with increasing diethyl ether/water partition coefficient. Collander worked with compounds which were relatively polar compared with many pesticides and other studies have suggested that there is an optimum lipophilicity for passage across cell membranes. Octanol/water partition coefficients (P) provide a good estimate of relative lipophilicity and our measured or calculated values for chemicals having useful systemic activity mostly lie in the range $\log P = -1$ to $+4$.

Two series of ^{14}C -labelled compounds, oximecarbarnates (insecticides/nematicides) and phenylureas (herbicide metabolites), were prepared with lipophilicity spanning this range. The oximecarbarnates were synthesised by reaction of the oxime precursor with ^{14}C -methyl isocyanate generated by the reaction of ^{14}C -methylamine hydrochloride with N,N' -carbonyl di-imidazole. The phenyl ureas were prepared from reaction of substituted anilines with potassium ^{14}C -cyanate in aqueous acetic acid solution.

Uptake of these compounds from nutrient solution by 9-day-old barley plants was measured. Accumulation in the roots increased with increasing lipophilicity. The most lipophilic compounds reached concentrations in the roots up to 10 times that of the external solution. As they were sorbed equally strongly by macerated root tissue, the predominant process appears to be partition into lipids in or on the root.

Translocation, expressed as the transpiration stream concentration factor TSCF (concentration in transpiration stream divided by concentration in the external solution), was

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generally greater for the oximecarbarnates than for the ureas of similar lipophilicity but a similar pattern of uptake was found for both series. The TSCF increased sharply above $\log P = -0.5$ to a broad optimum at about $\log P = 2$ followed by a sharp decline above $\log P = 3$.

There are no comparable published results for series of compounds but the TSCFs of individual compounds taken from the literature fit into this general picture well. Lipophilicity is much more important than water solubility in determining translocation, oxamyl (TSCF 0.2, 6% soluble in water) being translocated much less effectively than other chemicals with appropriate lipophilicity but with water solubility 1000 times less.

Published work shows that the TSCF for uptake of a chemical from soil solution is the same as that in nutrient solution. However, not all the chemical applied to soil is in solution because of adsorption on to the solid phase. Adsorption increases, and hence soil solution concentration from a given rate of application decreases, as $\log P$ increases so that the optimum $\log P$ for root uptake from soil (estimated as 0.7) is smaller than that from nutrient solution. (Briggs, Bromilow, Edmondson and Johnston)

Prediction of relative soil activity. The results of the uptake experiments described in the preceding section may be related to attempts some years ago to predict relative soil activity of urea herbicides. The assumption made, and now supported by the uptake experiments, was that the amount of chemical reaching a plant from a soil application is inversely proportional to the logarithm of the soil organic matter/water distribution Q . From adsorption measurements with several ring-substituted *N,N*-dimethyl-*N'*-phenyl ureas it was found that $\log Q$ could be related to the Hammett sigma constant and Hansch pi constant for the substituent by the equation

$$\log Q = 1.29\sigma + 0.1\pi + 1.1$$

For a similar group of ureas activity as inhibitors of the Hill reaction can be correlated with the same two constants (Hansch & Deutsch, *Biochimica et Biophysica Acta* (1976), 112, 381) by the equation

$$\log 1/C = 0.544\sigma + 1.29\pi + 4.18$$

where C is the concentration for 50% inhibition.

Relative activity by soil application is a function of the activity of the herbicide and its availability. An estimate of this is given by $\log(1/C)/\log Q$.

A computer programme was written (Ashworth, Chemistry Department) to calculate this ratio for all possible compounds (about 1300) formed by any combination in the 3, 4, and 5 positions of the ring of 10 substituents with known pi and sigma values. Despite the assumptions in the treatment, the highest 100 values printed out by the computer include most of the compounds of this class, or obvious relatives, introduced commercially since this work was done (1969). Similar exercises using published data for other herbicides and systemic fungicides also indicate those with greatest activity following soil application. (Briggs)

Chemical reference plots. In the third crop of spring barley yields were lower (mean 3.96 t ha⁻¹) than in the two previous years. Some of this decrease in yield was probably due to the exceptionally dry season. The nitrogen fertiliser was applied to the soil surface and remained there throughout the season, an effect observed in other experiments this year, and the crop was noticeably deficient in nitrogen. Aldicarb again increased yields by about 10% and controlled aphids well. Chlortoluron did not decrease yield in contrast to the wetter season in 1974 when it decreased yields by about 10%.

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Collaborative work described in the reports of other Departments

With the Plant Pathology Department. Prevention of moulding in damp hay (Lord, with Lacey). Uptake and distribution of thiabendazole in potatoes (Cayley and Lord, with Hide). Treatment of ware tubers (Austin, Cayley, Davies and Lord, with Hide).

With the Chemistry Department. Nitrification inhibitors (Briggs and Evans, with Ashworth).

With the Field Experiments Section. Uptake of aldicarb by field beans (Briggs, Bromilow and Jabbar).

With the Nematology Department. Control of the pea cyst-nematode (Bromilow and Middleton, with Whitehead).

Staff

(Department and the Chemical Liaison Unit)

It was with very great pleasure that we learnt during the year that Rothamsted had won a Queen's Award to Industry for the Department's work on highly active safe insecticides. We also took very great pride in the presentation to M. Elliott of the American Chemical Society's Burdick and Jackson International Award for Research in Pesticide Chemistry. Later in the year his outstanding contribution to research on pyrethroid insecticides received further recognition with his appointment as the Holroyd Memorial Lecturer of the Society of Chemical Industry.

I. J. Graham-Bryce was appointed a Visiting Professor in the Department of Zoology and Applied Entomology, Imperial College, London. J. A. Pickett, previously with the Brewing Research Foundation, was appointed to a new post in the Department to lead and coordinate chemical work on substances influencing pest behaviour. D. J. Austin left the Chemical Liaison Unit to become Head of the Plant Protective Chemistry Section, East Malling Research Station. G. R. Cayley was appointed in his place. N. Walker was transferred from the Soil Microbiology Department to the Chemical Liaison Unit. M. M. Burrell joined the Department on a grant provided by the Potato Marketing Board to study the mode of action and translocation of chemicals controlling tuber diseases of potatoes when applied to the foliage. R. Botham has joined the Department on secondment from Wellcome, Berkhamsted Ltd., to study neurophysiological action of insecticides using electron microscopy techniques. Dr. D. M. Soderlund, formerly of the Department of Entomological Sciences, University of California, Berkeley was awarded a Rockefeller Foundation Fellowship to work in the Department on pyrethroid insecticides. Mrs. Pauline Damant was appointed as personal secretary when Mrs. Wyn Parker became the Director's personal secretary.

Miss N. Khan and Mr. A. I. Nasim from the Federal Pesticide Laboratory, Pakistan and Mrs. G. Önal from the Turkish Atomic Energy Commission came as visiting workers to the Chemical Liaison Unit. R. Stokes, J. Gibson and M. Inwood worked as post-graduate students in the Department under the CASE scheme. Sandwich course students who worked in the Department or the Chemical Liaison Unit were C. Caligari, A. Camp, D. Clarke, R. Edmonson, F. Gudyanga, B. Heck, Catharine Ingham, Margaret Johnston, C. Lazarides, Carol Livesey, Padzilah Othmon and A. Plant.

M. Elliott delivered an address to the American Chemical Society in San Francisco on the occasion of the Burdick and Jackson Award presentation. N. F. Janes also contributed to a symposium on pyrethroid insecticides arranged in association with the

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presentation. With financial support from NRDC visits were made to companies in the USA concerned with the commercial development of pyrethroids discovered at Rothamsted. M. Elliott also presented invited papers to the Third Pest Control Conference in Cairo and at a study week organised by the Academia Pontificia Scientiarum, Vatican City, Rome. In addition he was appointed vice-President of CILDA (Comité pour les Applications des Insecticides dans les locaux et la Protection des Denrées Alimentaires) and presented a paper to its meeting in Paris. Both he and R. M. Sawicki were invited to contribute papers to the XV International Congress of Entomology in Washington. R. M. Sawicki also acted as convenor of a session on resistance to insecticides. K. A. Lord made a short visit to the Biological Institute, São Paulo, Brazil, as a preliminary to a six-month period of secondment. I. J. Graham-Bryce presented an invited keynote lecture to the meeting of the International Soil Science Society on Agrochemicals in Soils, in Jerusalem. He was also invited to contribute papers to the Annual Meeting of the Society of Chemical Industry in Stirling and to the Meeting of the Royal Society on Managing Agricultural Inputs.

Publications

GENERAL PAPERS

- 1 BURT, P. E., ELLIOTT, M., FARNHAM, A. W., JANES, N. F., NEEDHAM, P. H. & STEVENSON, J. H. (1977) Evaluation of pyrethroids for insect control. In: *Crop protection agents: their biological evaluation*. Ed. N. R. McFarlane. London: Academic Press, pp. 384-402.
- 2 ELLIOTT, M. (1976) Mammalian toxicity of pyrethroids. *Feuilles d'information du CILDA*, No. 7, 4-13.
- 3 ELLIOTT, M. (1976) Future use of natural and synthetic pyrethroids. In: *The future for insecticides: needs and prospects*. Ed. R. L. Metcalf & J. J. McKelvey, Jr. John Wiley & Sons Inc.
- 4 ELLIOTT, M. (1976) Properties and applications of pyrethroids. *Environmental Health Perspectives* **14**, 3-13.
- 5 ELLIOTT, M. (1976) Chemistry, biochemistry and insecticidal action of natural and synthetic pyrethroids. *Pesticide Science* **7**, 223-224.
- 6 GRAHAM-BRYCE, I. J. (1976) Crop protection: present achievement and future challenge. *Chemistry and Industry* 545-553.
- 7 GREENWAY, A. R., LEWIS, T., MUDD, A., SCOTT, G. C. & WALL, C. (1977) Some chemical and entomological problems in the investigation and use of behaviour-controlling chemicals. In: *Crop protection agents: their biological evaluation*. Ed. N. R. McFarlane. London: Academic Press, pp. 162-180.
- 8 PHILLIPS, F. T. (1976) Controlling the persistence of insecticides with special reference to microencapsulation. *Proceedings of Symposium on 'Persistence of Insecticides and Herbicides'*, British Crop Protection Council, Reading, March 1976, 217-227.

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- 9 ARNOLD, A. J. (1976) New technique for the pollination of coconuts. *Kenya Farmer* (Kenya) 11 also *Coconut Bulletin* **7** (4), 1-3.
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- 11 BATEMAN, G. L. (1976) Factors affecting the control of seed-borne *Septoria nodorum* by laboratory application of phenyl mercuric acetate to wheat seed. *Annals of Applied Biology* **83**, 479–483.
- 12 BRIGGS, G. G., ELLIOTT, M., FARNHAM, A. W., JANES, N. F., NEEDHAM, P. H., PULMAN, D. A. & YOUNG, S. R. (1976) Insecticidal activity of the pyrethrins and related compounds. VIII. Relation of polarity with activity in pyrethroids. *Pesticide Science* **7**, 236–240.
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- 27 AUSTIN, D. J., LORD, K. A. & WILLIAMS, I. H. (1976) High pressure liquid chromatography of benzimidazoles. *Pesticide Science* **7**, 211–222.
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- 29 BROMILOW, R. H. (1976) The determination of residues of oxamyl in crops and soils by gas-liquid chromatography. *The Analyst* **101**, 982–985.
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