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ROTHAMSTED RESEARCH

Report for 1975 - Part 1

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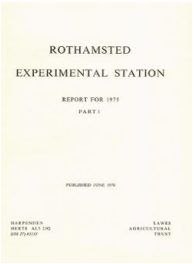

Insecticides and Fungicides Department

I. J. Graham-bryce

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Introduction

A fundamental precept underlying much of the departmental research programme is that crop protection will continue to depend on conventional chemical pesticides. There is therefore considerable incentive to improve their present very inefficient use and to discover new compounds active against pests and diseases not yet satisfactorily controlled or having advantages over existing pesticides. Current disadvantages can include adverse

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side effects, inappropriate mobility in soils or plants and decreased effectiveness against pests which have developed resistance. Of these, resistance has again been very prominent this year, particularly resistance of *Myzus persicae* on sugar beet, a problem to which we are devoting much attention.

Our survey of aphids from sugar-beet crops at many different sites, conducted in collaboration with Broom's Barn Experimental Station and the British Sugar Corporation, has confirmed that resistance is now widespread. Field experiments indicate that the levels of resistance detected in this survey could significantly diminish the control achieved by standard insecticide treatments. The potential magnitude of the practical problems posed by resistance, illustrated by these studies, underlines the need for pesticide management strategies to preserve the effectiveness of different compounds for as long as possible. Such strategies will require techniques for the early detection of resistance and knowledge of the activity of alternative compounds against resistant strains, both of which are also under investigation in the Department. Another important requirement is information about the heritability of resistance; this is a factor which is being studied as part of our work on a different practical problem, the tolerance of barley powdery mildew to fungicides. Comparison of these and other studies elsewhere emphasises the variability and complexity of resistance and the difficulties of predicting its implications for practical control in any particular situation. The general concern about the problems of resistance was demonstrated by the need to provide two sessions and a further extended discussion period for the subject at the 8th British Insecticide and Fungicide Conference to which the department made a major contribution.

The scope for substantial improvement in the efficiency of chemical control is particularly well illustrated by soil treatments. Many of the organisms not yet satisfactorily controlled are soil-borne; these include slugs, some nematodes and various diseases and insect pests attacking the underground parts of plants. A difficulty common in all these cases is to ensure that sufficient chemical reaches small target organisms which are distributed within a large mass of material in which it tends to be decomposed and through which movement is restricted. Several studies in the Department and the Chemical Liaison Unit aim to improve the use of soil-applied pesticides by learning more about the factors influencing movement and uptake and by devising more effective methods of formulation and application. A particularly attractive alternative approach to controlling soil-borne organisms is to use chemicals translocated downwards in plants following application to the foliage. It has generally been very difficult to find insecticides and fungicides with the required mobility in plants, but last year we reported two groups of compounds, certain amino acids and growth regulators, which were active against common scab of potatoes when applied to the leaves. In further work this year, these effects have been confirmed and preliminary studies on the mode of action have been started.

A more general contribution to the discovery of improved pesticides is our work on structure/activity relationships of pyrethroid insecticides. The outstandingly active pyrethroids with enhanced photostability reported during the last two years appear to be fulfilling their promise and are proving of great commercial interest. Tests to evaluate them for practical pest control throughout the world are proceeding satisfactorily. Continuing studies reported this year confirm the expanding scope of this group. Neurophysiological investigations have shown that the relative neurotoxicity of different pyrethroids does not correspond with the relative insecticidal potency, emphasising that there is still much to be learnt about the factors determining activity.

Another substantial part of our programme, complementing studies on conventional crop protection chemicals, is work on chemicals influencing insect behaviour. A wide range of chemical stimuli which influence various aspects of behaviour are being investi-

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gated. The immediate objective of much of this work is to improve the efficiency of pesticide use, for example by monitoring population movements or by using poison baits. In the longer term we hope to develop methods of control based on disruption of normal behaviour. Part of the work is undertaken in collaboration with the Behaviour Section of the Entomology Department; the success of this collaboration and the promise of the approach is illustrated by the progress made towards the development of a monitoring system for pea-moth, based on pheromone traps. Such a system would greatly improve the timing and efficiency of control measures. These investigations are described in the report of the Entomology Department.

Insecticides

Relationships between molecular structure and insecticidal activity of pyrethroids. The discovery at Rothamsted and elsewhere of several new structures of potential agricultural importance has disclosed many further possibilities for synthesis in this increasingly important group. Systematic studies are being undertaken to help clarify the full implications of these advances in relation to the structural requirements for activity.

The insecticidal activity, mammalian toxicity and photostability of pyrethroids depends to a large extent on the nature of the group R at C-3 of the cyclopropane ring (1). Recent work in the Department (*Rothamsted Reports for 1973*, Part 1, 168 and *for 1974*, Part 1, 137) showed that pyrethroids with dihalovinyl side chains *cis* and *trans* to the ester group in [1R]dimethylcyclopropane carboxylates have exceptionally favourable properties. They are very potent against insects but have low mammalian toxicity and, especially

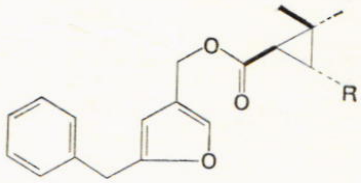

FIG. 1.

when the alcoholic component is 3-phenoxybenzyl rather than 5-benzyl-3-furylmethyl as in 1 (above), are sufficiently stable in light for insect control on agricultural crops. In view of the importance of this site in the molecule for activity, further variations have now been examined.

To simplify interpretation of insecticidal activities, all esters had 5-benzyl-3-furylmethyl as the alcoholic component with acid side chains at C-3 *trans* to the carboxyl function. Compounds were synthesised from (+)-*trans* caronic acid (Table 1, nos 1–7, 12), from (+)-*trans* caronaldehyde esters (13–28) or from other appropriate precursors (8–11), so that the chiral centre at C-1 was in the active [R] configuration in all cases. Activity against houseflies (*Musca domestica* (L.)) and mustard beetles (*Phaedon cochleariae Fab.*) was measured by topical application of measured drops of acetone solutions. Table 1 shows potencies relative to that of bioresmethrin as standard (≡1000).

Alkoxycarbonyl substituents gave relatively low potency, the most active being the ethyl ester against both houseflies and mustard beetles. The isopropyl ester was especially active against houseflies. The tertiary butyl (6), *n*-butyl (4) and phenyl esters (7) had similar low toxicities.

The polar acetoxymethyl (8) and nitrile (12) groups attached directly to C-3 conferred low toxicity, in contrast to oximinoethers, the most potent of which was the methyl compound (13), as active as bioresmethrin to houseflies and twice as active to mustard beetles. Other oxime ethers (13) with R = Et, Pr^n or allyl were less effective. The activity of (13) (R = Me) again contrasts with the almost complete lack of potency of the unsaturated ketone (14).

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TABLE 1

Effects of acid side chain on relative potency of pyrethroid esters

	R in formula (1)	Relative potency to *Musca domestica* L.	Relative potency to *Phaedon cochleariae* Fab.
bioresmethrin	CH=C.Me_2 (standard)	1000	1000
(1)	CO_2Me	19	5
(2)	CO_2Et	29	38
(3)	CO_2Pr^n	10	33
(4)	CO_2Bu^n	4	7
(5)	CO_2CHMe_2	39	8
(6)	CO_2CMe_3	5	3
(7)	CO_2Ph	5	11
(8)	$CH_2O.COMe$	3	1
(9)	$CH_2CMe_2.OMe$	230	50
(10)	$CH_2CMe_2.Cl$	67	60
(11)	CH_2CF_2Br	700	600
(12)	C≡N	20	Non-toxic
(13)	CH=N.OR (R=Me)	1000	1900
(14)	CH=CH.COMe	Non-toxic	~15
(15)	$CH=CH_2$	680	720
(16)	CH=CH.Me (E)	650	1300
(17)	CH=CH.Me (Z)	1500	1100
(18)	CH=CH.Et (>90% Z)	1600	1600
(19)	CH=CH.Pr (Z)	640	650
(20)	CH=CH.Bu^n (Z)	270	540
(21)	$CH=CH.CH=CH_2$ (E)	2000	600
(22)	$CH=CH.CH=CH_2$ (Z)	2000	390
(23)	CH=CH.CH=CH.Me (E)	1000	780
(24)	CH=CH.CH=CH.Me (Z)	2000	2000
(25)	$CH=CH.CH=CMe_2$ (E)	160	1100
(26)	$CH=CH.CH=CMe_2$ (Z)	920	1600
(27)	$CH=C(Me).CH=CH_2$ (Z)	1000	1500
(28)	CH=C(Me).CH=CH.Me (Z)	740	780

The most active compounds without unsaturated side chains (9), (10) and (11) were formed, by adding respectively methanol, hydrogen chloride and hydrogen bromide to dimethylvinyl (isobutenyl) or difluorovinyl side chains. These compounds are considerably more potent, especially the bromodifluoro derivative (11), than the parent *iso*-butylated cyclopropane but are less active than the unsaturated esters from which they are derived.

The most active compounds in Table 1, which are amongst the most potent lipophilic insecticides of any class, have mono- or di-ethylenic side chains. Most of the mono unsaturated esters, although very potent, were less active than those with two double bonds in conjugation. [A comparable situation is found in the side chains of the alcoholic constituents of the natural pyrethrins where pyrethrolone (*cis*-pentadienyl) gives more active esters than cinerolone (*cis*-but-2-enyl) and jasmololone (*cis*-pent-2-enyl)]. Activity was greatest with *trans* (E) or *cis* (Z)-butadienyl or (E) or (Z) pentadienyl substituents (21, 22, 23, 24) (4 or 5 carbon chains). The *cis*-pentadienyl compound (24) was outstandingly potent against both insect species. Further substitution of the pentadienyl unit with methyl groups (25, 26, 28) diminished potency in both *cis* and *trans* conformations. In both mono- (15–20) and di- (21–28) olefinic series, methyl substitution probably alters both the lipophilicity and the degree of steric interaction at a reaction site.

Whilst the superior potency of the unsaturated olefinic side chains at C-3 is clear, the detailed requirements for optimum activity cannot be recognised from these results; however the most active compounds in both series have 4–5 carbon atoms. It is not easy to discern common chemical, stereochemical or physical characteristics amongst the substituents giving greatest activity. The most practical basis for development remains

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synthesis and testing within a rational framework (Chemical work, Elliott, Janes and Pulman: Biological work, Farnham and Needham)

Conformation of active pyrethroids. Collaborative work is described in the report of the Molecular Structures Department (p. 187).

Mode of action of insecticides

Neurotoxicity of pyrethroids. Pyrethroids have been shown to affect nerve conduction in giant fibres of the American cockroach *Periplaneta americana* (L.) but evidence that nerve axons are the sites of lethal action is lacking. To examine the relationship between axonic neurotoxicity and kill, the effects of selected synthetic pyrethroids on conduction in giant fibres of the central nervous systems of adult male *P. americana* were compared with their toxicity to the living insects (overall toxicity).

Overall toxicity was measured by conventional bioassay, assessing knockdown and kill at intervals from 30 min to 10 days after topical application of the insecticide. Conduction in the giant fibres was measured by a sucrose gap technique that permitted measurement of resting and action potentials. This gave more reproducible results than the simpler extracellular technique previously used. Potential differences between the surface of the cord and the cut end were measured while a zone from which conducting ions were removed by perfusion with an isotonic solution of mannitol was interposed between these points, thus giving resting and action potentials approaching true values. Although this method, unlike intracellular recording, does not permit recording from single neurones, it has the advantage of being set up more quickly and easily. Insecticides dissolved in saline were perfused across an intact part of the cord and changes in response measured with an oscilloscope and camera. Of the previously reported effects of pyrethroids, increase in negative after-potential, slight depolarisation and conduction block were observed with all compounds tested, but repetitive after-discharge was rarely seen, presumably because the temperature (20°C) was too low. Percent reduction in amplitude of the maximum action potential attainable was chosen to indicate the activity of the compounds tested.

In Table 2 the overall toxicities of seven compounds, of which all except 'NRDC 158' (see footnote (d) to Table 2) are pure isomers, are compared with their effects on giant fibre conduction. Structure-overall toxicity relationships show some consistent features.

TABLE 2

Overall toxicity and neurotoxicity of pyrethroids to P. americana

	LD50* (μg per insect)	Neurotoxicity† (μM)
'RU 15525'[a]	1·90	0·5
bioresmethrin	1·60	1·3
cismethrin	0·38	2·0
'NRDC 163'[b]	0·32	7·4
'NRDC 157'[c]	0·10	8·0
'NRDC 158'[d]	0·09	5·7
'NRDC 161'[e]	0·05	0·3

* 2 days after treatment. † Concentration decreasing amplitude of action potential by 30% in 1 h

[a] 5-benzyl-3-furylmethyl[1R, *cis*]2,2-dimethyl-3-(2-oxo-3-thiacyclopentylidenemethyl)cyclopropane carboxylate

[b] 3-phenoxybenzyl[1R, *trans*]3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

[c] 3-phenoxybenzyl[1R, *cis*]3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

[d] (±)-α-cyano-3-phenoxybenzyl[1R, *trans*]3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, i.e. a 1:1 mixture of the *trans* analogue of NRDC 161 and its epimer

[e] (S)-α-cyano-3-phenoxybenzyl[1R, *cis*]3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

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Thus *cis* isomers were always more toxic than *trans* isomers, from approximately twice ('NRDC 161' and 'NRDC 158') to three times ('NRDC 157' and 'NRDC 163') and four times (cismethrin and bioresmethrin). Also the introduction of cyano groups on the α carbon atoms of the alcoholic components of both 'NRDC 163' and 'NRDC 157', to give 'NRDC 158' and 'NRDC 161', increased toxicity. 'RU 15525', like other pyrethroids giving rapid knockdown, was a relatively poor killing agent.

Although when tested against conduction in giant fibres all compounds were strongly neurotoxic, no significant structure-activity pattern was apparent, and their neurotoxicity could not be related to their overall toxicity. Of the three *cis* and *trans* pairs tested, two showed equal neurotoxicity for the *cis* and *trans* forms but in the third the *cis* isomer ('NRDC 161') was 19 times more toxic than the α-(±)-*trans* isomer ('NRDC 158'). Substituting a cyano group in the alcoholic α carbon of the *cis* isomer 'NRDC 157' greatly increased its neurotoxicity, but the same substitution in the corresponding *trans* isomer 'NRDC 163' had very little effect. 'NRDC 163' and 'NRDC 157', whose overall toxicities are five and four times greater than those of bioresmethrin and cismethrin, are however, six and eight times less neurotoxic. 'RU 15525' is highly neurotoxic, consistent with previous tests on compounds giving rapid knockdown.

Neurotoxicity and overall toxicity may have failed to correspond either because the neurones chosen for testing neurotoxicity were not sites of lethal action or because the differential effect of processes outside the nervous system had a significant effect on the relative concentrations of the various insecticides at the site of action. However, it seems unlikely that additional processes such as penetration and detoxication would change a less ordered structure-toxicity relationship for neurotoxic action into a more ordered one for overall toxicity. Alternative sites of action within the nervous system should therefore be sought, and processes controlling the concentration of the pyrethroids at the site of action investigated in complementary studies. (Burt and Goodchild)

Neuroanatomy of the insect central nervous system. Work continued on the arrangement of nerve cell bodies and fibre pathways in the mesothoracic ganglion of the cockroach *Periplaneta americana* (L.) (*Rothamsted Report for 1974*, Part 1, 142–143) and a description was begun of the longitudinal nerve tracts, after filling them with Procion yellow or cobalt chloride.

Studies of the general neuroanatomy of the other two thoracic ganglia also continued and formed the basis for an examination of the suboesophageal ganglion, as a first step towards possible future work on the brain, to which it is closely linked by the circumoesophageal connectives. The three primitive ganglia that compose the suboesophageal ganglion were identified in its dorsal region and their more complex ventral boundaries are being investigated.

The five free abdominal ganglia were also examined to make it possible to begin interpreting the structure of the compound terminal abdominal ganglion, an important subject for electrophysiological studies of insecticide action. The terminal ganglion was found to consist of four primitive abdominal ganglia, numbers 7–10; no trace has yet been found of the final ganglion, number 11. (Gregory)

The nature and causes of resistance. The practical value of our long-term programme on resistance and of the expertise and knowledge acquired was demonstrated last year when resistance in field populations of *Myzus persicae* on sugar beet was detected. We were able to divert much of our effort to meet the urgent need for testing samples from the field and evaluating the results. This year we have again devoted much attention to this problem which continues to be of great practical concern. Our work on aphids from sugar beet has had three main objectives. First, in collaboration with Broom's Barn

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Experimental Station and the British Sugar Corporation, we have undertaken a further survey of resistance in aphids from different sites to assess the present and potential seriousness of the practical problem. Secondly, we have sought information on how far the levels of resistance detected in the laboratory are likely to affect control in the field. This has been a matter of much debate. Many attribute the recent poor control of *M. persicae* on sugar beet largely to the exceptional climatic conditions in 1973 and 1974. While this undoubtedly affected the performance of insecticide treatments, it seems certain that the large proportion of resistant individuals in some populations, found during our survey in 1974, must also have been a contributory factor. Thirdly, we have attempted to develop a simple test, capable of use by field workers, for the early detection of resistance.

The need to tackle these practical problems has inevitably limited the effort available for fundamental studies. Nevertheless, some further work on the nature of resistance mechanisms in houseflies and the way they interact, and on the characteristics of resistance to pyrethroids, has been possible. The knowledge acquired in genetical studies over several years is beginning to provide explanations for the way in which resistance in houseflies has developed in practice and thus offers hope that at least limited prediction may eventually become possible. Biochemical work on enzymes associated with resistance in aphids has also continued.

Survey of resistance to insecticides of* Myzus persicae *from sugar beet. Following last year's survey, in 1975 we examined aphids over-wintering asexually, aphids from sugar beet before spraying and those which had survived insecticide treatment. Their susceptibility to dimethoate and demeton-S-methyl was measured and the proportion in each sample with carboxylesterase activity typical of resistant aphids was determined.

Populations were classified as resistant when at least 20% of individuals had carboxylesterase activity characteristic of resistance and 10% or more survived doses of dimethoate (25 ng) or demeton-S-methyl (10 ng) which killed all of a susceptible population isolated from the field in 1974.

There was generally good agreement between the two methods of determining resistance. Where differences occurred we believe that this was due to the heterogeneity of field populations and to the insensitivity of the bioassay technique to the small differences in susceptibility.

The seven samples of aphids which had overwintered asexually on oilseed rape, sugar beet seed crops or in mangold clamps were all found to be resistant. This indicates that resistance is stable throughout the winter in the absence of selection pressure from insecticides. It is not surprising, therefore, that approximately half of the 17 samples from unsprayed sugar beet were also resistant.

Of the 38 samples of *M. persicae* which had survived up to five applications of insecticide, more than 70% were classified as resistant.

We therefore conclude that the overall situation is one of widespread resistance to certain organophosphorus compounds and that these resistant aphids overwinter asexually without losing resistance. (Needham and Devonshire)

Effects of resistance on control under field conditions. A typical resistant strain (MS1G) isolated from sugar beet in 1974, with a resistance factor to demeton-S-methyl of approximately four-fold when measured by a systemic test in the laboratory, was cultured in the glasshouse on sugar beet. A standard susceptible strain was cultured on other plants under similar conditions.

The infested plants were then planted outside, in four randomised blocks of six plots, and each was enclosed in a cage of fine mesh muslin. The number of aphids per infested

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plant was assessed in the evening seven days after planting out, and the plots were sprayed the following morning with 'Metasystox 55' (demeton-S-methyl) at the standard rate using a knapsack sprayer. The muslin cages were opened only during counting and spraying. Four days after treatment all the susceptible aphids were killed, even by the lowest rate. However, 17 and 11% of the resistant aphids were alive on plants treated with the standard and double dose respectively, although none survived treatment at four times the recommended rate.

This indicates that the low levels of resistance found in the laboratory tests can result in a failure of control by the recommended rate of demeton-S-methyl, which was chosen for this experiment because it is the most frequently used insecticide on sugar beet. (Needham and Rice)

Resistance of* M. persicae *on potatoes. A population of *M. persicae* on potatoes grown at Rothamsted, was not controlled by demeton-S-methyl in 1975 (Gibson, Plant Pathology Department, p. 271).

Survivors were shown, by topical application of demeton-S-methyl, to have a resistance factor of approximately eight, typical of resistant populations from sugar beet. (Needham and Devonshire)

Simple bioassay for discriminating between susceptible and resistant aphids. Topical application of measured drops of insecticides and systemic bioassays are generally used to determine resistance in aphids but require considerable skill, specialised equipment and are unsuitable for use outside the laboratory. We have therefore devised a simple bioassay to detect resistant aphids based on a dipping technique which will be evaluated in the field next year.

The aphids are placed in a short piece of glass tubing (25 mm high, 25 mm diameter) treated with 'Fluon' halfway up the internal wall and with the bottom end covered by a fine-mesh nylon gauze secured by an elastic band. This cage is placed in a small shallow glass dish and 2 ml of 0·03% aqueous solution of 'Metasystox 55' pipetted onto the aphids. After contact with the solution for 10 sec the insects are dried by blotting the bottom end of the cage with filter paper. The top end of the tube is then covered with nylon gauze secured by an elastic band, and the tube is inverted and tapped sharply to tip the aphids onto the clean gauze. The gauze originally at the bottom end of the tube is replaced by a fresh gauze and kill is recorded every 15 min for 1 h. Whenever possible five replicates of ten insects are used. In preliminary tests, kill was assessed 16 h after treatment, but this was decreased to 1 h when it was found that nearly all susceptible aphids died within 30–45 min of treatment, whilst many of the resistant aphids survive. Shortening the time interval between treatment and checking has the advantage that it decreases control mortality, simplifies post-treatment procedure and emphasises the differences between susceptible and resistant aphids because resistant individuals continue to die as the interval after treatment increases. This technique is designed to detect the presence of resistant individuals in heterogeneous field populations. It cannot estimate the proportion of resistant individuals because dosage/response curves overlap so that some resistant as well as all susceptible individuals die at any chosen dose level.

Factors influencing the response of aphids to this discriminating dose test are now being examined. (Rice and Sawicki)

***Electrophoretic separation of carboxylesterases from individual* M. persicae.** The total carboxylesterase activity in individual *M. persicae* has been used to characterise resistant aphids from both the glasshouse and the field. The diagnostic value of the technique can be seen from the fact that all strains known to be resistant that we have examined, even

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those with only small resistance, had greater carboxylesterase activity than susceptible aphids.

Several different enzymes contribute to this non-specific esterase activity and these can be resolved by electrophoresis. However, a large proportion (60–70%) of the activity in aphid homogenates is associated with particulate fractions and this must be solubilised before it will enter the polyacrylamide gel used for electrophoresis. The following procedure has been found to give good results. Each aphid was homogenised in 15μl water containing (by weight) 10% sucrose and 0·5% Triton X-100. The same detergent was also incorporated (0·2%) into the polyacrylamide. The electrophoresis system was otherwise the same as that of Williams and Reisfeld (*Annals New York Academy of Sciences*, (1964), **121**, 373–381), but sample gel and spacer gel were omitted. With these modifications, it was possible to detect the esterases from individual aphids after electrophoresis, which is important when examining heterogeneous populations from the field.

Three different esterase patterns were distinguished in resistant aphids, all having much greater activity in one of the enzymes than the susceptible aphids. Most of the resistant aphids from the field were of type 1, in which two bands were more intense than in susceptible strains. One field strain was of type 2, having three bands with increased activity, and another gave a pattern similar to those of resistant aphids from glasshouses with only one band having greatly increased activity (type 3). This type also differed from the other strains in having the top band replaced by one of greater mobility. (Devonshire, Paper 11)

The technique thus makes it possible to characterise resistant aphids in more detail than the total esterase determination, and is more sensitive to the small differences in activity between susceptible and slightly resistant aphids. (Devonshire)

Analysis of the sequential development of resistance to different insecticides in houseflies in Denmark. The sequential development of resistance to different insecticides by houseflies in Denmark has been comprehensively monitored and documented by Dr. J. Keiding of the Danish Pest Infestation Laboratory. With the kind cooperation of Dr. Keiding, several of the strains used in our fundamental studies on the mechanisms of resistance and the ways they interact have been obtained from these resistant populations. The combination of detailed field observations and fundamental laboratory studies provides a very valuable model system in which attempts can be made to relate the occurrence of resistance to the mechanisms so that the pattern of development in practice can be explained.

The available evidence indicates that the genes for resistance to organophosphorus insecticides (OPs), selected by the widespread use of parathion and diazinon on Danish farms throughout the 1950s, disappeared when dimethoate became widely used for fly control in the early 1960s. These resistance genes, which were replaced by new ones selected by the contact with dimethoate, confer resistance to diazinon and many other OPs.

Although our previous genetical and biochemical studies have largely established the nature of the resistance to diazinon in the 1950s and to dimethoate in the 1960s, it was not clear how the transition between the different types of resistance occurred. We are therefore now investigating this important stage in the history of sequential resistance by examining three OP resistant strains, each with a different selection history, collected from the field by Dr. Keiding between 1963 and 1965. Bioassays and biochemical studies showed that these strains had some, but not all of the mechanisms characteristic of the pre- and post-1960 resistance.

Strains Mix 6b, selected with bromophos, retained the low carboxylesterase activity (gene a) associated with diazinon and parathion resistance in the 1950s, but unlike other

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strains with this type of resistance, it resists malathion. Malathion resistance is normally controlled by a different allele of gene a. This malathion resistance which confers cross-resistance to tetrachlorvinphos is not suppressed by either sesamex or TBTP (*S,S,S*-tributyl phosphorotrithioate) and is probably identical to that reported previously in the dimethoate-selected strain $49r_2b$ (*Rothamsted Report for 1973*, Part 1, 173). The same mechanism probably also confers resistance to these two compounds in the other two strains studied, the malathion-selected strain $153e_2b$ and the fenthion- and dichlorvos-selected strain 39åb. Resistance to parathion in 39åb is caused by a sesamex-suppressible resistance possibly controlled by gene D which is found in dimethoate resistant strains. The mechanism of resistance to malathion and tetrachlorvinphos may have been selected independently on two separate occasions, first when malathion was introduced for fly control in Denmark in 1961 and later when tetrachlorvinphos was tested in 1969. None of the strains examined had acetylcholinesterase (AChE) showing decreased sensitivity to inhibition by OPs (tests done by Devonshire). This suggests that the decreased sensitivity of the AChE in the dimethoate-resistant strains collected in the late 1960s may have been selected later, probably at the time when there was a sudden increase in dimethoate resistance on Danish farms in 1969–70 (Keiding, *Danish Pest Infestation Laboratory, Annual Report* (1971), p. 42). (Sawicki)

Side effects of pesticides on beneficial insects

Poisoning of honeybees in the field. One hundred and twelve samples of honeybees thought to be poisoned were received from beekeepers via the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food. As in previous years, these were analysed for the presence of insecticides. Eighty-six samples gave clear evidence of poisoning compared with 94 in 1974, and 63 in 1973.

Evidence to indicate how poisoning had occurred was available for about four-fifths of the cases where poisoning was confirmed. Spraying of field beans to control *Aphis fabae*, usually the most common cause of honeybee poisoning, was responsible for only six cases, presumably reflecting the low incidence of *A. fabae* and the fall in field bean acreage in 1975.

The increased acreage of oilseed rape seems to be associated with an increase in honeybee poisoning (30 samples compared with 12 in 1974). This is clearly a major cause for concern among beekeepers. We were surprised to find 22 samples associated with cereal spraying. These deaths may be attributed to foraging bees flying across cereal fields during spray application or to bees being attracted to honeydew produced by infesting aphids. Spraying carrots to control cutworm or carrot fly also caused honeybee mortalities (5 samples) presumably because of foraging on flowering weeds at the time of application. (Stevenson)

Acute laboratory toxicity of insecticides to worker honeybees. Table 3 records recent determinations of the contact and oral toxicity of unformulated pesticides to worker honeybees (*Apis mellifera*). The methods are described in *Working Document to the Pesticides Safety Precaution Scheme* No. 13; *Ministry of Agriculture, Fisheries and Food* (1966); see also *Rothamsted Report for 1972*, Part 1, 186, and *Annals of Applied Biology* (1968) **61,** 467. (Stevenson)

Diflubenzuron is a larvicide which interferes with insect cuticle formation, its low toxicity to adult bees (Table 3) is therefore not unexpected. However, a wettable powder formulation, mixed with sugar syrup and fed to small honeybee colonies, caused complete cessation of 'brood' (larva) production. This was a severe test and it does not follow that normal field applications of diflubenzuron would have this effect, but if the com-

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TABLE 3

Acute oral and contact toxicity of pesticides to worker honeybees (Apis mellifera)

	Contact			Oral		
	LD50 (μg per bee)	Fiducial limits		LD50 (μg per bee)	Fiducial limits	
'NRDC 161'*	0·047	0·034	0·062	0·079	0·034	0·13
thiofanox	0·058	0·049	0·066	0·062	0·056	0·068
permethrin	0·10	0·082	0·13	0·21	0·11	0·30
pirimiphos-methyl	0·39	0·34	0·46	0·36	0·25	0·49
ethiofencarb	2·4	1·6	2·9	1·5	1·2	1·8
fonofos	3·8	3·4	4·3	8·4	6·5	9·7
chlorfenvinphos	4·1	3·3	4·9	0·55	0·39	0·72
benomyl	>10			>10		
diflubenzuron	>30			>30		
paraquat	>48					
pirimicarb	>54			3·2	2·2	4·3

* S-α-cyano-3-phenoxybenzyl [1R, *cis*] 3-(2,2-dibromovinyl) 2,2-dimethyl-cyclopropanecarboxylate

pound is to be used in situations where bees are at risk, it would clearly be desirable to assess its toxicity by field trials. (Stevenson, with Tompkins, Entomology Department)

Effect of formulation on toxicity of insecticides to honeybees. The toxicity to worker honeybees of microencapsulated and emulsifiable concentrate formulations of fonofos was compared in preliminary laboratory tests. The results indicate that microencapsulation might substantially reduce the toxic hazard to bees, and further work will be undertaken. (Stevenson)

Aphid predators. Collaborative work on the toxicity of insecticides to aphids and beneficial insects on sugar beet at Broom's Barn is described on p. 60. (Stevenson, with Dunning and Heathcote, Broom's Barn)

Control of soil-inhabiting insect pests. We continued field studies to evaluate new chemicals as seed treatments for controlling wheat bulb fly and background laboratory studies to learn more about the factors influencing the performance of seed treatments.

Field tests of seed treatments for controlling wheat bulb fly. The insecticides permethrin and isofenphos that had shown promise as seed treatments for controlling wheat bulb fly in 1973–74 trials were examined in more detail in 1974–75 at two sites, a clay loam and a peaty loam. Liquid and powder formulations of each insecticide were compared, the powder formulations with or without a 6% aqueous emulsion of soyabean oil as a sticker. Formulations of both compounds at 0·2% a.i. to weight of seed decreased larval attack and increased the number of healthy plant shoots more than the standard insecticide chlorfenvinphos at 0·1% a.i. to weight of seed. Mixing chlorvenfinphos 1:4 with the synergist piperonyl butoxide in an attempt to increase its activity against wheat-bulb had no effect. (Griffiths, Jeffs and Scott)

Laboratory toxicity of insecticides to wheat bulb fly. A technique was developed to determine base line toxicity figures for various insecticides used against wheat bulb fly larvae. Groups of five first-instar larvae were each immersed in a 0·05 ml drop of insecticide solution placed in the centre of a cavity slide and covered with a cover slip. After 24 h, mortality was assessed by removing larvae from the cavity slides, and placing them on a line drawn across a moist filter paper; larvae that had not moved away from the

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line within 2 h were recorded as dead. By this method of assessment mixtures of chlorfenvinphos/carbophenothion (1:2) were most toxic, followed by gamma-BHC > chlorfenvinphos > carbophenothion > permethrin.

A behavioural test was used to see if the larvae were affected by permethrin even if not killed. After only 1 h immersion in 2 ppm permethrin solution, locomotion of wheat bulb fly larvae was affected. This may explain the activity of permethrin seed treatments in the field, despite its relatively low toxicity in laboratory tests. (Griffiths and C. Smith)

Release of insecticides from seed treatments. The effectiveness of different seed treatments is probably influenced considerably by the way that the chemical is released from the formulation and by its subsequent movement in soil. The rate of release of insecticide from wheat seeds treated with different formulations was therefore studied under standardised conditions. The seeds were embedded in a layer of moist sand in a sintered glass funnel through which water was passed at a constant rate (100 ml per hour). The amount of insecticide in successive fractions of the eluate was determined. Replication in successive runs appeared to be satisfactory (see below).

Release from seeds treated with 40% gamma-BHC alone (0·3 g powder per 100 g seed) was compared with that from seeds pretreated with either a 12% aqueous solution of gum arabic (1·2 ml per 100 g seed) or a 10% aqueous solution of the surfactant 'Myrj 52' (2 ml per 100 g seed) before the powder was applied. The total amounts of gamma-BHC released to 100 ml of leaching solution from the three treatments were 22, 68 and 31 μg. The amount released per unit volume reached a steady value after approximately 20 ml of leachate, the average amounts per 5 ml fraction from at least three runs for the three treatments being 1·4 $\pm$ 0·43, 4·25 $\pm$ 0·5 and 2·0 $\pm$ 0·1 μg respectively. Pretreatment with the adhesive, gum arabic, thus led to release at roughly twice the rate obtained with the surfactant, which itself had very little effect. This unexpected effect is being investigated further and other formulations are being examined. (Jeffs and Walker)

Effects of storage on the recovery of gamma-BHC from treated seeds. Analytical results obtained elsewhere for loadings on seeds treated with gamma-BHC powder have indicated that the amount of insecticide recovered decreases when the treated seed is stored for several weeks. This was attributed to sorption of the chemical into the seed.

To investigate this further and to check our analytical method (Lord *et al.*, *Annals of Applied Biology* (1967), **60,** 173) we have therefore determined the effects of storage on recovery from seed treated with 40% gamma-BHC powder at two application rates, 0·3 and 0·1 g per 100 g seed. The insecticide was applied by end-over-end tumbling in screw-topped jars, and the treated seed subsequently stored in similar containers. Duplicate samples were removed for analysis immediately after treatment and at weekly intervals up to 6 weeks for the higher application rate and up to 8 weeks for the lower rate. At each time interval the seed was extracted with hexane as in the traditional method and the extracted seed was washed repeatedly with hexane to remove any remaining insecticide from the surface. To determine sorbed insecticide, the seed was then dried, crushed and extracted with acetone in a soxhlet. The total amount of gamma-BHC recovered fell slightly over the period of storage. The amount sorbed increased from about 0·2% of the total immediately after treatment to approximately 4% after 6 weeks at 0·3 g per 100 g seed and 8% after 8 weeks at 0·1 g per 100 g seed.

The results indicate that the simple analytical procedure should be satisfactory for most purposes. Where there has been extended storage more elaborate procedures may be necessary to extract sorbed insecticide. This slow sorption could affect the availability of the chemical for insect control and could increase phytotoxic effects. (Jeffs and Walker)

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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. 262) (Etheridge, with Cockbain and Bowen, Plant Pathology Department)

Control of leaf-cutting ants. Work to develop effective and durable baits for these very serious pests of crops in the tropics and sub-tropics was continued, with support from the Ministry of Overseas Development. Studies on phytochemical arrestants which would induce the ants to pick up bait particles and carry them back to their nests are described in later sections on behaviour-controlling substances. The other main objectives of work this year were to evaluate further experimental baits in the field and to devise simple and reliable liquid formulations, incorporating the toxicant, arrestant, preservative and other components in a compatible form, which would give satisfactory baits when added by farmers to local matrix materials. This could have the considerable economic advantage of avoiding the need to transport bulky bait materials over long distances.

The search for further suitable toxicants with a delayed killing action over a wide dosage range (*Rothamsted Report for 1973*, Part 1, 183–184) was continued on an opportunist basis as candidate chemicals became available. None of the compounds tested compared favourably with the standard mirex and consequently were not considered for field testing. (Etheridge)

No satisfactory gas-liquid chromatography (g.l.c.) methods were available for analysing three of the insecticides (mecarbam, dioxathion and fospirate) previously shown to be promising for incorporation in experimental baits. Quantitative g.l.c. methods involving different column packings and electron-capture or flame photometric detectors were therefore devised. (Martin)

Development of liquid formulation for locally-produced baits. With advice from Allen & Hanburys (orange juice concentrates, used as arrestants for the ants) and A.B.M. Chemicals (emulsifiers) we devised an emulsion concentrate in a 1-litre pack suitable for treating 10-kg matrix, sufficient for baiting ten large nests. The concentrate consisted of 30 g mecarbam (chosen as a suitable non-organochlorine formicide) dissolved in 370 ml soya oil and emulsified in a mixture of 400 ml orange juice concentrate (7:1) and 200 ml 10% propionic acid solution with 20 g 'Texofor FN 15' emulsifier.

Previous field trials (1974) showed that vermiculite with orange juice arrestant was a good substitute for dried citrus pulp as a matrix. The liquid formulation will therefore be tested by diluting with 7 litres water and mixing with 10 kg vermiculite (sufficient for ten nests). These trials will be done by the Entomology Division, Centro de Pesquisas do Cacau (CEPEC), Itabuna, Brazil. Subsequently the formulation can be tested with other matrices prepared from local waste products (e.g. cocoa pods ground to a suitable particle size). (Phillips and Martin)

Field trials: Effectiveness of different baits and methods of application. Previous field trials (*Rothamsted Report for 1974*, Part 1, 150) established that most of the ten non-organochlorine insecticides tested as substitutes for mirex were effective against *Atta sexdens* colonies and several showed some success against colonies of *Atta cephalotes*. Further trials, based on the results of the 1974 trials, were carried out during September–October 1975 in Bahia, Brazil, using facilities kindly provided by CEPEC. Because *Atta cephalotes* colonies are more difficult to kill, testing was concentrated on this species. Percentages quoted in the following paragraphs refer to total weight of bait. Baits were prepared as in 1974, using dried citrus pulp pretreated with propionic acid (0·3%) to prevent fungal attack. The insecticides were mecarbam, dioxathion, and fospirate (all at 0·3% concentration) dissolved in soya oil (4%) sorbed on the dried citrus pulp, and piri-

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miphosmethyl (0·2%) and permethrin (0·045%) as microencapsulated formulations (prepared by Plant Protection Ltd.) added to the dried citrus pulp together with 0·5% 'Acronal 4D' sticker. In all cases, 1·5 kg bait was applied to each nest, and either 13 or 14 replicate nests were baited with each treatment.

In addition, the effectiveness of aerial application was compared with placement of bait by hand against *Atta sexdens*, a species thought to be more appropriate for aerial baiting than *Atta cephalotes*. Aerial baiting was simulated by scattering 1 kg bait evenly over a circle, radius 15 m, centred on the nest mound, to give an application rate of 14 kg ha^{-1}.

Nest mortalities will be assessed in all tests about 3 months after application. (Phillips and Etheridge)

Weathering of baits. To extend results obtained in our 1974 weathering tests, 10 g aliquots of dried citrus pulp bait containing 0·5% insecticide in soya oil (5%) and a relatively large amount (2%) of propionic acid as fungal inhibitor were weathered on pieces of muslin spread on the ground under open sky in a cocoa plantation. The insecticides were mecarbam, dioxathion, fospirate, pirimiphos-methyl, permethrin, bendiocarb, and mirex. Three replicate samples of each bait were collected after 0, 1, 3, 7 and 16 days for subsequent g.l.c. analysis. Temperature and rainfall were recorded daily. Baits remained free of fungus during the first week but thereafter heavy rain washed the propionic acid from the baits and dense fungal growth developed, except on baits containing fospirate. This confirms the pronounced fungicidal properties of this insecticide noted in 1974. Other observations suggested that bendiocarb may have significant molluscicidal properties. (Phillips and Etheridge)

Chemicals influencing insect behaviour

Work continued on a wide range of chemicals influencing different aspects of insect behaviour with the objective of developing improved methods of controlling pests and managing beneficial insects.

Phytochemical arrestants for leaf-cutting ants. Methods of isolating non-volatile hydrophilic plant components for insect behavioural bioassays were re-examined and their limitations assessed. Several investigations of the chemical basis for insect-host relationships have shown that plant extracts which influence insect behaviour lose their activity progressively when separated into their components. This was found for the response of leaf-cutting ants to extracts of citrus pulp and their components. Such results are due largely to many compounds being responsible for the activity of the extract which therefore diminishes as components are removed. To determine whether total activity involved synergism by components which were themselves inactive, attempts were made to restore quantitatively the activity of fractions of citrus pulp extract by recombining subfractions, some of which showed no activity. These experiments were only partially successful because in some cases activity appeared to have been permanently lost. The extent of this loss was difficult to estimate because the precision of the bioassay is inevitably limited.

Experiments were conducted to determine whether the loss of arrestant activity of the citrus pulp extracts was due to the introduction or creation of repellents during fractionation or to the loss or chemical transformation of components. However no evidence was found for either the presence of repellents or for the loss of components. The apparent loss of activity was therefore attributed to chemical changes in minor constituents, which are very difficult to check analytically in a mixture containing many components but

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which nevertheless may be detectable by the ants. Such results emphasise the difficulty of obtaining quantitative results from behavioural bioassays which tend to give variable results even when the same materials are tested under the same conditions. (Mudd, with Dr. D. J. Peregrine and Dr. J. M. Cherrett, University College of North Wales, Bangor)

Further experiments on the rate of growth *in vitro* of the food fungus of the leaf-cutting ant *Atta cephalotes* on various plant substrates showed that the growth rate was affected by the plant species used, the concentration of the plant extract or plant sap and the acidity of the plant sap. However, it was not possible to correlate the rate of growth of the fungus on different plant extracts with the ants' foraging preferences which were not always consistent. There was evidence that fungal growth rate was related to the presence of growth inhibitors rather than to nutrient availability. (Mudd and Bateman)

Components of the mandibular gland secretion of the larvae of *Anagasta kuehniella* (Zeller). Difficulties were encountered in obtaining large numbers of *A. kuehniella* larvae because of disease in the cultures so methods of isolating the pheromone (*Rothamsted Report for 1972*, Part 1, 186) from whole diseased cultures were investigated. Problems arose because large quantities of material with similar chemical properties to the pheromone were present. A technique for obtaining small amounts of the secretion from larvae by placing them in contact with a heated surface was therefore used. Some of the minor components of the secretion were identified using thin-layer chromatography (t.l.c.) and mass-spectrometry. These included β-sitosterol, cholesterol and palmitic and oleic acids. The secretion also appeared to contain appreciable quantities of cholest-3,5-diene which has not previously been detected in insects. Cholest-3,5-diene can be formed from cholesterol by dehydration so this result must be viewed with some caution. However, when pure cholesterol was subjected to the same isolation and analytical procedures no detectable quantities of the 3,5-diene were produced. (Mudd)

Chemicals affecting aphid behaviour. The modified artificial diet for aphids (described in *Bulletin of Entomological Research* (1975), **65,** 349–358) has been used extensively to test the responses of aphids to plant extracts and other chemicals. Substances under test are presented in the diet or on the surface of the Parafilm membrane that covers it, and aphids are allowed to choose where to settle and lay young. Plant extracts, even those made from host plants, have so far failed to encourage settling and larviposition in the species of aphids tested (*Myzus persicae*, *Aphis fabae* and *Megoura viciae*). Compounds that deter settling and larviposition by *M. persicae* on diets, include neem extract (which also has some effect when applied to plants), the insect repellent 'GD 880' (2-(*n*-octyl-amino)-2-methylpropan-1-ol) and extracts of aphids themselves. Adults of *M. persicae* are deterred by both hydrophilic and lipid components of their own species but are less affected by extracts of other species. The components of the extracts are now being separated and examined to determine the active fraction(s). (Griffiths, Greenway and Lloyd)

Chemicals affecting the behaviour of wheat bulb fly larvae. Larvae wandering on damp filter paper make occasional, random turning movements; when a larva wanders into an area on which wheat extract has been deposited, it turns more frequently as it reaches the boundary of the treated area from within. This has the effect of retaining or 'arresting' the larva within the treated area. When oat extract is combined with wheat extract, this arrestant effect is decreased, showing that oats have an 'anti-arrestant' effect. Furthermore, larvae will positively turn away from a deposit of oat extract alone indicating that this material is repellent.

Activated charcoal absorbs wheat arrestant and box tests have shown that wheat can

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be protected from wheat bulb fly attack by growing it in a band of charcoal and compost (*Rothamsted Report for 1973*, Part 1, 181–182). Further tests have shown that protection occurs only when the box includes another row of untreated wheat so that larvae have a choice between treated and untreated plants. When both rows of wheat are growing in charcoal-treated bands or the whole box is treated, attack is similar to that in untreated boxes. Host plant location is therefore apparently facilitated by arrestant exudate, but not dependent on it. This may account for the failure of charcoal treatments under field conditions (*Rothamsted Report for 1974*, Part 1, 148–149).

Investigations of the wheat 'arrestant' and the oat 'anti-arrestant' have led to the development of fractionation schemes for purifying these chemicals (*Rothamsted Report for 1974*, Part 1, 148–149). The active fractions thus obtained contain several major components and larger quantities of these purified materials are now being produced to isolate the biologically active compound(s). (Scott and Greenway)

Codling moth and other fruit tortricids. Attractive traps baited with synthetic sex pheromones are now being used by East Malling Research Station and ADAS to monitor populations of codling moth, *Adoxophyes orana* and *Archips podana* in various orchard situations. Results for codling moth from the 1975 season at East Malling show a good correlation between trap catches and fruit damage. These observations, together with previous results (*Rothamsted Report for 1974*, Part 1, 148), offer good prospects for a spray warning system. However, preliminary results with *Adoxophyes orana* and *Archips podana* are less encouraging. (Greenway, with Dr. J. E. Cranham, East Malling Research Station)

Sex attractants for pea moth. Further collaborative work is described in the report of the Entomology Department (p. 123). (Greenway, with Lewis, Macaulay and Wall, Entomology Department)

Footprint substance of the worker honeybee and substitutes for the honeybee pheromone, 9-oxodec-2-enoic acid. These investigations are described in the report of the Entomology Department (p. 131). (Greenway, with Butler and Welch, Entomology Department)

Equipment and techniques

Insect neuroanatomical techniques: effect of ageing on alcoholic Bouin fixative. The picro-form-acetic-alcohol fixative ('alcoholic Bouin') used to preserve insect nerve tissue for neuroanatomical study using the Bodian silver stain was previously found to give better preservation when aged for about 40 days at 60°C. (Gregory, *Acta Zoologica, Stockholm* (1970), **51,** 169–178). The chemical changes brought about by ageing are now being analysed, using thin-layer and gas chromatography and ultra-violet and mass spectrometry. During ageing the concentrations of picric acid, formaldehyde and probably also the alcohol change little. Acetic acid concentration first falls and then increases slightly. About 5% ethyl acetate and 1% methyl acetate are also present in the matured solution, as well as diethoxymethane and smaller amounts of dimethoxymethane and methoxyethoxymethane (formals). The concentrations of all these reaction products continue to increase slowly as solutions age beyond the optimum time, accompanied by a decline in the quality of fixation. Histological testing of these compounds is being undertaken to determine which, if any, are responsible for improved fixation and to see whether an effective mixture that needs no ageing can be formulated. (Gregory, Lord and Greenway)

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Spraying equipment. An electrostatic liquid spraying system, operating at 50 kV and 13 790 kN m^{-2} was evaluated in field trials on sugar beet and potatoes, in collaboration with NRDC and Sheffield University. Results failed to support laboratory work at Sheffield which had indicated that charging the spray particles greatly improved their deposition on plant surfaces. Technical limitations imposed by using the laboratory equipment in the field may have been partly responsible for the discrepancy. Further experiments will therefore be done next year with modified equipment using lower pressures and improved power supplies. (Arnold)

Equipment for mass pollination of coconuts. In collaboration with the Coconut Industry Board of Jamaica and with support from the Ministry of Overseas Development, improved equipment for the extraction and processing of coconut pollen has been designed and constructed as part of the mass pollination programme. The equipment includes a flower cracking apparatus and fluid bed dryer, now being evaluated in Jamaica after satisfactory preliminary trials. Consideration is now being given to the development of a coconut flower stripper and pollen applicator. (Arnold)

Laboratory seed treater. Seed treated with experimental and commercial formulations is needed for many experiments undertaken by members of the Department, others at Rothamsted and collaborators elsewhere. A machine capable of treating appropriate quantities for field experiments was therefore required. With permission and advice from ICI Plant Protection Division, a laboratory treater based on the 'Rotostat' principle (Elsworth & Harris *Proceedings 7th British Insecticide and Fungicide Conference*, 1973) **1,** 349–355) was designed and constructed. In this machine, weighed quantities of seed and liquid or powder formulations are mixed by the centrifugal action of a rotor spinning inside a stationary cylinder, against which the moving seed is thrown.

Initial tests have given promising results. Loadings very close to the target (250 μg g^{-1} seed) have been achieved using commercial liquid formulations of gamma-BHC. With dry powder formulations of the same insecticide, loadings were only about 75% of the target, but pretreatment with an adhesive increased this to 95–100%. This is similar to loadings obtained on a much smaller scale with adhesives using careful laboratory techniques (*Rothamsted Report for 1973*, Part 1, 177–178).

Seed for the 1975 ADAS wheat bulb fly trials was treated successfully with this machine. Seventy 7-kg batches of seed were treated with a variety of chemicals in one day. (Jeffs and Lazarides, with Edwards, Hobbs and Turnpenny, Instrument Workshops)

Nuclear magnetic resonance spectrometer. With financial support from NRDC a JEOL PMX-60 and JEOL PFT-100 NMR spectrometer system was acquired, installed and commissioned during the year. Both instruments are working satisfactorily. The larger instrument in particular, with its facilities for various elements, especially ^{13}C, and its greater sensitivity to protons than our previous instrument which will allow us to measure spectra on quantities of 100 μg or less, will greatly improve our ability to determine molecular structures. (Janes)

Insect rearing. The following species were reared:

Homoptera	*Acyrthosiphon pisum* (Harris)	
	Aphis fabae (Scop.)	
	Myzus persicae (Sulz.)	
	Strains.	Susceptible
		Several organophosphate-resistant
	Megoura viciae Buckt.	

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Hemiptera	*Dysdercus intermedius* Distant
Coleoptera	*Phaedon cochleariae* (F.)
Orthoptera	*Blaberus discoidalis* (L.)
	Periplaneta americana (L.)
Diptera	*Drosophila melanogaster* (Meig.)
	Strains. Normal
	Vestigial wings
	Musca domestica (L.)
	Strains. A wild-type susceptible strain
	ac; ar; bwb; ocra—called 608, a multi-marker susceptible strain.
	SKA-diazinon selected, very resistant to many organophosphorus insecticides
	Several strains derived from SKA, each with one or more factors of resistance to organophosphorus insecticides or DDT
	Several strains derived from 49r_2b each with one or more factors of resistance to dimethoate and other organophosphorus insecticides
	49PPB, a substrain of 49r_2b derived by selection with pyrethrum extract and piperonyl butoxide
	290BIO, a substrain of 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
	Calliphora erythrocephala (Meig.)
Hymenoptera	*Acromyrmex octospinosus* (Reich)
	Atta cephalotes (L.)
	Aphidius matricariae Haliday
Lepidoptera	*Plutella xylostella* (L.)

Fungicides

Control of soil-borne diseases by foliar sprays. Previously we reported that the incidence of potato common scab, caused by soil-borne *Streptomyces scabies*, can be decreased by foliar sprays of either the plant growth regulator daminozide or the synthetic amino acid DL-ethionine. Work this year was directed at clarifying the mode of action of these chemicals, and at extending the investigations to other growth regulators and to other diseases caused by soil-borne organisms.

Laboratory tests. The *in vitro* activity of quintozene (widely used as a soil-treatment for practical scab control), daminozide and DL-ethionine against three isolates of *S. scabies* was assessed in 'poisoned agar' plate tests. In each test, 15 colony diameters were measured after incubation on treated potato dextrose agar for 3 weeks at 25°C.

Preliminary figures show that daminozide and DL-ethionine, although not inactive, are considerably less effective than quintozene. However, it proved difficult to obtain unequivocal values for relative potencies from these tests. Responses varied with different isolates, and dose-response curves were not always parallel, making comparisons complicated. (Bateman)

Growth-room tests. Translocation of daminozide was studied in potato plants (cv. Majestic) grown in hydroponic culture, using full-strength Long Ashton mineral nutrient solution. For one week before applying daminozide, the plants were subjected to a short (8 h) day to induce tuberisation. A standard quantity (1·5 mg) of daminozide

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was then applied to the first expanded compound leaf. After 24 h the residual daminozide was washed off. The distribution of daminozide in the plants was determined at various intervals after application by a modification of the method used by Dicks (*Pesticide Science* (1971), **2,** 176). Results obtained so far indicate that daminozide moved into stems and into other leaves within a week. By the third week after treatment, significant quantities could be detected in stolons, tubers and roots, and some daminozide had leaked into the culture medium. The total amount of daminozide in the system remained constant during the period of study. (Sadler)

Glasshouse tests. Chemicals were applied by various routes to young potato plants (cv. Majestic or Epicure) grown in pots containing scab-infected soil. Scab indexes (estimates of the percentage of tuber surfaces disfigured by scabs) were calculated at harvest. In routine tests, the foliage was sprayed with aqueous solutions or dispersions of the test chemicals about two weeks after potting, care being taken to prevent spray from reaching the soil directly. In a few tests, daminozide was also applied either as a soil-treatment before potting, or as a root-treatment two weeks after potting by temporarily substituting daminozide solutions for the water given daily to the plants *via* saucers.

Many of the tests were done during the unusually hot summer months this year, when the temperature of the soil in the glasshouse probably exceeded the optimum for infection by *S. scabies* (about 20°C). As a result there was relatively little scab, and some tests were inconclusive.

In foliar spray tests, analogues of ethionine and other chemicals (mostly growth regulators) were applied once or several times. The following either failed to decrease the incidence of scab, or damaged the plants, or both, at the concentrations shown: DL-homocysteine thiolactone, 1·0%; DL-methionine, 1·0%; L-cysteine, 0·5%; *S*-methyl-L-cysteine, 0·5%; *S*-ethyl-L-cysteine, 0·5%; ancymidol, 0·1%; *N,N*-dimethyl morpholinium chloride, 0·5%; *N,N*-dimethyl piperidinium iodide, 0·5%; ethyl 5-(4-chlorophenyl)-2*H*-tetrazol-2-ylacetate ('PP 528'), 0·1%; griseofulvin, 0·2%; orthonil, 0·2%; phenylacetic acid, 0·1%.

Table 4 shows the combined results of three identical tests in which the different methods of applying daminozide were compared. The soil-treatment significantly decreased ($P \sim 0{\cdot}01$) scab incidence, although it was less effective than the root- and foliar-treatments applied later. Thus daminozide or an active metabolite seems to be reasonably stable in the soil used. The Table also shows the amounts of daminozide in tubers taken from one of the tests 9 weeks after potting. Unchanged daminozide was found in tubers after soil-

TABLE 4

Effects of daminozide, applied by various routes, on scab incidence and daminozide content of tubers in the glasshouse

Treatment	Application: Route	Application: Time	Dose per pot (g)	Mean scab index	Daminozide in tubers (μg g^{-1} fresh wt: replicate analyses)
nil	—	—	—	22	0, 0
daminozide	soil-mix	(1)	0·1	15	1·8, 2·3
daminozide	roots *via* saucer	(2)	0·1	11	2·1, 2·6
daminozide	foliar spray	(2)	~0·1	10	6·2, 7·0
quintozene	soil-mix	(1)	~0·04	3	—
LSD, P=0·05				5	
0·01				7	
0·001				9	

(1) at potting
(2) 2 weeks after potting

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or root-treatments, although in smaller amounts than after foliar-treatment. While the results indicate that daminozide itself was reasonably stable in the soil, the figures were obtained from tubers on senescent plants, and thus do not necessarily indicate the amounts of daminozide present in the stolons at the time of infection (about 2–3 weeks after potting).

The observations that daminozide is found in the underground parts of potato plants after foliar application, that it is fairly stable in the soil used, and that it has some inhibitory action *in vitro* on growth of *S. scabies* support the hypothesis that it acts directly as a downward moving systemic fungicide. (McIntosh and Sadler)

Control of club root of cabbage. Soil in which young potted cabbage plants were growing was inoculated with spores of *Plasmodiophora brassicae*. Within the following few weeks, the leaves were sprayed twice with aqueous solutions or dispersions of candidate chemicals; all spray mixtures contained sufficient 'Manoxol OT' or 'Tween 20' to ensure thorough wetting of the leaves. The club root organism, unlike *S. scabies*, has a high optimum temperature for infection, and clubs formed satisfactorily in unsprayed plants.

In one unconfirmed test, sprays of DL-ethionine or β-hydroxyethyl hydrazine, both in the range 0·1–1·0%, slightly decreased the weights of clubs and slightly increased the weights of tops. In contrast, the following either failed to affect the disease, or damaged the plants, or both, at the concentrations shown: ancymidol, 0·05%; chlormequat chloride, 0·2%; chlorphonium chloride, 0·1%; daminozide, 1·0%; *N*,*N*-dimethyl morpholinium chloride, 0·2%; *N*,*N*-dimethyl piperidinium iodide, 0·2%; ethephon, 0·3%; ethyl 5-(4-chlorophenyl)-2*H*-tetrazol-2-ylacetate ('PP 528'), 0·1%; flurecol, *n*-butyl ester, 0·01%; gibberellic acid (GA_3), 0·02%; maleic hydrazide, 0·1%; naphthylacetic acid, 0·03%; orthonil, 0·2%; phenylacetic acid, 0·1%; prothiocarb, 1·0%; pyroxychlor, 0·3%; tri-iodobenzoic acid, 0·01%. (McIntosh, with Macfarlane, Plant Pathology Department)

Field trials. Daminozide and DL-ethionine, both of which decreased the incidence of potato scab when applied as foliar sprays in the glasshouse, were tested in a field trial (cv. Maris Piper) at Woburn. The plants were sprayed once or several times at about 1400 litres ha^{-1} in June. The DL-ethionine sprays caused a characteristic temporary chlorosis on the haulms; this was more severe than in previous years, when the summers were cooler, and most obvious in the plots that were sprayed three times. At harvest, scab indexes were calculated from 40 ware tubers per plot (three plots per treatment). Table 5 shows the results. In the exceptionally dry summer, tubers from unsprayed plants were very badly scabbed. All treatments with daminozide clearly decreased scab incidence, but the degree of control was not related to the concentration sprayed or to the timing of the sprays. Both treatments with DL-ethionine also decreased the amounts

TABLE 5

Effects of foliar sprays on scab incidence in the field

Treatment	Dates of spraying in June	Scab index
daminozide, 1·0%	17 — —	31
daminozide, 1·0%	— — 26	25
daminozide, 1·0%	17 — 26	38
daminozide, 0·5%	17 — 26	26
DL-ethionine, 1·0%	— 20 —	31
DL-ethionine, 1·0%	17 20 26	8
unsprayed		61
LSD, P=0·05		13
0·001		23

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of scab; the three applications almost eliminated it. The plots were too small (one row × 4 m) for reliable estimates of yield, but there was no indication of any effect except for the three sprays with DL-ethionine, which approximately halved the yield.

S. scabies causes scab on red beet as well as on potatoes. In a small trial alongside the potato trial the foliage of young beet plants was sprayed at various times in June. Daminozide (1·0%) slightly decreased the amount of scab but DL-ethionine (0·5%) decreased its incidence by about half. (McIntosh, with Lapwood and Adams, Plant Pathology Department)

Organomercury seed treatments. Work on organomercury cereal seed treatments continued. Several experiments included comparisons with a variety of different non-mercury fungicides.

Susceptibility of* Septoria *isolates. Isolates of *S. nodorum* from a range of infected seed samples used in previous experiments (*Rothamsted Reports for 1973*, Part 1, 189, and *for 1974*, Part 1, 155) were compared for pathogenicity and susceptibility to phenyl mercuric acetate (PMA). By inoculating wheat seeds with conidial suspensions of *S. nodorum*, five isolates from wheat seed were all found to be similar in pathogenicity to seedlings and in sensitivity to PMA seed treatment. They were also equally sensitive to PMA *in vitro* and their growth and appearance on agar was similar. One isolate from infected barley seed was also tested; this differed from the others in appearance and in being non-pathogenic to wheat seedlings. It was also much more sensitive to PMA *in vitro*. None of the isolates caused seedling disease on barley. A short period of repeated subculturing onto agar with PMA did not increase mercury tolerance in any isolates.

***Soil-borne* Fusarium.** PMA seed treatments significantly reduced the severity of seedling blight of wheat in soil inoculated with *Fusarium culmorum*, *F. nivale* and *F. avenaceum* in growth room experiments. High inoculum level and dry soil increased the severity of infection by *F. culmorum* without reducing the performance of the fungicide. Disease severity was also increased by deep sowing, which delayed emergence and reduced the effectiveness of PMA.

Barley leaf stripe and wheat bunt. Glasshouse experiments were done to determine the minimum rates of mercury (as PMA) which would control heavy seed infections of barley leaf stripe (*Drechslera graminea*) and wheat bunt (*Tilletia caries*). Leaf stripe was completely controlled by PMA at 1·0 μg Hg per seed but not by 0·25 μg. However, disease incidence was always low at rates of 0·05 μg Hg per seed or greater. Bunt was usually well controlled at 0·5 and 1·0 μg Hg per seed but some bunted ears subsequently appeared. Any reduction in the rates of mercury normally used for commercial treatment (0·5–1·5 μg Hg per seed), which at present apparently keep these two potentially serious diseases in check, would therefore probably be unsafe.

Comparison of seed treatment fungicides. In a field experiment the performance of a range of fungicides (including mercury as PMA) was compared on winter wheat seed, cv. Chalk, heavily infected with *S. nodorum*. Table 6 shows that all treatments controlled seedling disease by *S. nodorum* well and none affected emergence. Although subsequent development of leaf infection was slight, it was similar for all treatments including the control. This supports previous results (*Rothamsted Report for 1974*, Part 1, 155), indicating that treatment of infected seed is likely to have little effect on the incidence of the potentially more damaging leaf disease phase. Mildew, eyespot and sharp eyespot infections were slight, with no consistent differences between treatments.

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A pot method was used to compare the control of a range of seedling blight fungi by nine seed treatment fungicides including PMA, carboxin, maneb, guazatine, quinacetol sulphate, mixed formulations with maneb, and two undisclosed experimental formulations. Most treatments controlled seed-borne *S. nodorum* on wheat and *F. nivale* on barley as well as PMA, but none of the treatments of known chemical composition was as consistently effective as PMA against soil-borne *F. culmorum*. Increases in severity of

TABLE 6

Effects of seed treatments on infection of wheat by seed-borne Septoria nodorum

Treatments	Rate (mg a.i. kg^{-1})	Emergence (plants m^{-1})	Seedling infection (%)	Leaf 2 infection: Incidence (%)	Leaf 2 infection: Severity (% leaf area)	Grain yield (t ha^{-1})
nil	—	42	40·0	57·5	1·3	7·27
PMA	1 (Hg)	45	5·5	58·7	1·1	7·40
PMA	5 (Hg)	44	2·5	61·2	1·4	7·25
PMA	25 (Hg)	51	2·5	58·7	1·3	7·08
carboxin	4500	49	1·0	60·0	1·5	7·16
maneb	3200	40	0·0	50·0	1·3	7·08
guazatine	800	45	2·0	45·0	0·8	7·01
guazatine+maneb	600+600	46	3·0	65·0	1·7	7·11
quinacetol sulphate +maneb	268+451	43	0·0	50·0	1·4	7·30
thiabendazole	2400	50	0·0	60·0	2·2	7·36
LSD at $P=0·05$	—	N.S.	6·1	N.S.	N.S.	N.S.

seedling disease from seed-borne *F. nivale* after treatment with carboxin, and from soil-borne *F. culmorum* with maneb, indicated that control cannot always be expected and may depend to some extent on the composition of the seed and soil microflora. Agar plate and germination tests generally indicated good control with all treatments. These quick and simple laboratory tests of seed treatments are evidently less reliable and sensitive than the pot tests.

Relationships between fungi on seed. Investigations into the effects of saprophytes on seed-borne pathogenic fungi continued. Seed naturally infected with *S. nodorum* and *F. nivale* was inoculated with several isolates of *Epicoccum*, *Alternaria* and bacteria. All bacteria, but only some isolates of the fungi, significantly reduced disease. The variation between the isolates of fungal seed-borne saprophytes confirms earlier results (*Rothamsted Report for 1974*, Part 1, 156).

The relative occurrences of *F. nivale* and saprophytes on PMA-treated and untreated seed samples, determined by agar plate tests, indicated a degree of mutual antagonism between *F. nivale* and *Alternaria* and possibly also between *F. nivale* and *Epicoccum*, but not between *F. nivale*, *Cladosporium* and yeasts. (Bateman)

Tolerance of barley powdery mildew to fungicides. Studies on the nature of tolerance to fungicides by barley powdery mildew continued, in association with work on the mode of action of ethirimol.

Monitoring for ethirimol tolerance. Present methods for monitoring barley powdery mildew (*Erysiphe graminis*) populations for tolerance to the fungicide ethirimol, are time consuming and only semi-quantitative. An *in vitro* bioassay was developed which provides quantitative dose-response data for ethirimol. This was adapted for monitoring purposes as a discriminating dose assay (2 ppm ethirimol) and used in a survey of mildew tolerance in 1975. Two genetically homogeneous mildew strains, one

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able to tolerate 10–100 times more ethirimol than the other, were used as standards in this survey. Samples were collected, in collaboration with ADAS, at several farms from mid-June onwards, when mildew first became common. This was only 6 weeks after many of the sampled crops had been sown. By then many of the samples were as tolerant as the standard tolerant strain. Generally, mildew from ethirimol-treated crops was more tolerant than that from neighbouring untreated crops, but exceptions were observed. Tolerance was not related to disease control. The *in vitro* measurements provided a rapid indication of the level of field tolerance, which correlated well with observations made on whole plants grown from ethirimol-treated seed, and exposed at some sites.

Samples showing tolerance were maintained in the absence of fungicide and tested again by the same techniques. Tolerance declined in all samples kept in this way, and in some cases after only one generation, they appeared to be little different from the sensitive standard. Experiments with whole plants confirmed that the tolerance observed in the field was not maintained after a period of culture in the absence of ethirimol. If the level of field tolerance to ethirimol is to be monitored, samples must therefore be tested directly, and not propagated on ethirimol-free leaves beforehand. Some measure of the stability of any tolerance is also required if its implications are to be properly assessed.

The characteristics of stable tolerance. The response to ethirimol of the tolerant strain used as a standard in the survey has remained unaltered after two years in the absence of fungicide. This type of tolerance is not yet common in the field. If such strains were to become widespread the effectiveness of ethirimol, especially in delaying the onset of mildew epidemics, might be reduced to such an extent that yield increases were no longer obtained. Factors likely to affect the spread of such tolerance within a population have therefore been examined in laboratory experiments. In a mixture the tolerant strain competed poorly with a sensitive one. Tolerance was not transferred during vegetative growth to a sensitive strain, as might be expected if some form of heterokaryosis occurred. The tolerant strain has been successfully crossed with a sensitive one; analysis of progeny indicates that tolerance is not controlled only by a single gene. Insufficient progeny were available to determine if tolerance was polygenic or if cytoplasmic factors were involved.

These results suggest that this stable form of tolerance is unlikely to spread rapidly, providing sufficient sensitive individuals can be maintained within the population. (Hollomon)

Factors influencing the effectiveness of systemic fungicides applied as seed treatments. Work on factors influencing the relative effectiveness of different precursors of carbendazim (MBC) following application as seed treatments continued.

Carbendazim, benomyl, thiophanate methyl, 'NF 48' and ethirimol were compared in a further field trial using smutted spring barley seed (cv. Sultan). Carbendazim and 'NF 48' had not previously been compared in the same year. Technical grade compounds were applied to seed in equimolar proportions following pretreatment with a gum acacia sticker. All compounds except ethirimol significantly reduced smut (*Ustilago nuda*). Average values from four replicate plots were 0·04 smutted heads per metre row for 'NF 48', 0·44 for benomyl, 1·9 for thiophanate methyl and 3·8 for carbendazim compared with 4·6 for untreated controls. This confirms the relative effectiveness indicated in previous trials. Mildew (*Erysiphe graminis*) was assessed three times during the season. The order of effectiveness in late June was 'NF 48', ethirimol, thiophanate-methyl, carbendazim, benomyl. By mid-July this had changed to 'NF 48', thiophanate-methyl, benomyl, carbendazim, ethirimol, possibly reflecting the differential effect of the very dry season on the uptake of the various chemicals.

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To complete our studies on uptake, a further pot experiment was undertaken using the smutted (Sultan) seed treated for the 1975 field experiment. Amounts of fungicide in roots, leaves and coleoptiles were determined at 4, 8, 16 and 32 days after planting using the bioautographic technique of Homans and Fuchs (*Journal of Chromatography* (1970), **51,** 327–329). The plant material was extracted with methanol and components in the extract separated on silica t.l.c. plates. Fungicidal components were located by spraying the chromatograms with suspensions of *Penicillium expansum* spores in nutrient solution. The plates were maintained in humid chambers and two days after spraying inhibition zones became visible. The areas of these zones were measured using the Quantimet Image Analyser and compared with those produced by standard amounts of carbendazim.

Preliminary results indicate a pattern of uptake consistent with relative effectiveness in the field trials. To determine the factors governing performance, absorption and translocation of different compounds by plants will be compared with their physical properties and behaviour in soil. (Graham-Bryce and Nicholls)

Chemical Liaison Unit

During the past year the Unit has again collaborated with several departments at Rothamsted and with outside bodies on a diverse range of topics reported in following sections. The common theme of these studies is the investigation of the initial distribution of crop protection chemicals and of their subsequent redistribution and persistence. This work is supported by the development of techniques such as high speed liquid chromatography and novel analytical methods, for example, the assay of oxamyl.

Various shorter investigations related to the main theme but not reported in detail were also undertaken; these included: the analysis of wheat crops to test for suspected contamination by insecticides when wheat bulb fly followed an unusual pattern of development; testing seeds used for field experiments to ensure correct treatment with pesticides, and assay of soils from various sources for residues of fenamiphos, oxamyl, dazomet, dichloropropane-dichloropropene, and benomyl.

Analytical techniques

Clean-up of soil extracts by ultrafiltration. The assay of pesticides at part per million levels in crops and soils requires sensitive methods but these often also detect degradation products and much larger quantities of naturally occurring compounds. These interfering substances must be separated from the pesticide before it can be measured. In an attempt to extend the use of u.v. spectrophotometry for assaying pesticide residues, directly or following high pressure liquid chromatography, we examined the use of ultrafiltration membranes for separating pesticides from other u.v. absorbing materials extracted from soils.

A range of membrane filters such as Millipore PASC, Nuclepore N010 and Amicon PM30, removed u.v. absorbing substances from methanolic extracts of soil when these were diluted with water. The amounts retained by the filter increased with the proportion of water, and up to 80% was retained from solutions in 30% aqueous methanol. The permeability of Amicon PM30 filters to a range of pesticides was examined. Using 30% aqueous methanol only the highly hydrophilic substances aldicarb and oxamyl passed freely through the membranes. Ethirimol and carbendazim were partly restrained but others including chlortoluron, metobromuron, carbaryl and diazinon were almost completely retained. All compounds could be recovered by washing the filters with more concentrated methanol (60–100%). The mechanism by which these pesticides are retained by the filters is unknown as their molecular weights are many times smaller than the molecular exclusion limits quoted by the manufacturers of the membranes (for 'Amicon

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PM30' = 30 000). Possibly some form of sorption by the membrane material is involved. However our results indicate that the method warrants further investigation as a possible way of separating hydrophilic pesticides from u.v. absorbing materials extracted from soil. (Lord and Tinkler)

High speed liquid chromatography (HSLC). To establish patterns of behaviour during reverse phase chromatography, 81 substances including 70 pesticides were examined using 'Permaphase' ODS as stationary phase with aqueous buffers or methanol buffer mixtures as mobile phase in a Dupont 830 Liquid Chromatograph fitted with a u.v. absorption detector. The chromatographic process involved, distribution between the stationary lipid phase (octadecyl groups bonded to silica spheres) and a mobile aqueous phase, is essentially similar to partition between two solvents. Partition between octanol and water is commonly used to predict the distribution of chemicals between the aqueous and lipoidal phases in naturally occurring systems such as soils or living organisms. With 54 substances tested systematically, which included groups of phenylureas, acetanilides, oximecarbamates and organic solvents, octanol/water partition coefficients correlated well with net-retention volumes during chromatography in aqueous methanolic buffer mixtures of constant composition. In such cases mobility in HSLC systems can be used in place of partition measurements.

Because increasing the methanol content of the mobile phase decreases retention volumes, HSLC mobility can be measured for substances of widely differing properties and therefore promises to be especially valuable for chemicals too lipophilic for distribution between octanol and water to be reliably measured directly. Chromatography has the additional advantage that measurements can be made on individual components in mixtures, so that highly purified materials are not essential.

There was no simple relationship between the net-retention volumes and octanol/water distribution coefficients of the four benzimadazoles and three thiophanates tested. These compounds were generally retained more strongly on the chromatographic column than anticipated from their octanol/water partition coefficients. It is of interest that they are also immobile and strongly sorbed on soil, suggesting that the chromatographic method may be better for predicting mobility in soil. (Austin, Briggs, Bromilow, Lord and Tinkler)

Gas-liquid chromatography (g.l.c.) of carbamates. There is no easy and generally applicable method for assaying carbamate pesticides. The instability and low volatility of these compounds makes their determination by direct g.l.c. difficult or impossible. We found that injecting carbamates into the gas chromatograph with trimethylphenylammonium hydroxide (0·1M in methanol), gives derivatives with good g.l.c. properties. This procedure yields methoximes from oximecarbamates and anisoles from substituted-phenyl-*N*-methylcarbamates. Yields over a wide range of operating conditions were generally better than 90%, except from aldoximecarbamates where competing reactions intervened. Using this method, derivatives from eight sulphur-containing carbamate insecticides/nematicides were formed and successfully separated and determined by g.l.c. with flame-photometric detection in the sulphur mode. (Bromilow and Lord)

Oxamyl residues. We devised the following simple method for assaying the oximecarbamate nematicide oxamyl. Oxamyl is extracted with dichloromethane: acetone (1:1) from the soil or macerated crop sample mixed with an equal weight of sodium sulphate. It is then separated by column chromatography on florisil from co-extracted contaminants, including the oxime formed from oxamyl by hydrolysis, which would interfere with the subsequent assay of oxamyl by the g.l.c. method described above.

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Recovery of oxamyl from fortified samples of crops and soils was generally better than 90%. Potatoes, peas and tomatoes grown in soils treated before planting with up to 10 kg a.i. oxamyl ha^{-1} contained less than 0·01 ppm at harvest (the detection limit of the analytical method), this level being toxicologically insignificant. (Bromilow and Jabbar)

Studies of the movement and persistence of chemicals in soil

Leaching and degradation of oxamyl in Woburn soil. Oxamyl, radio-labelled with ^{14}C in the carbamate methyl (specific activity 0·22 mCi $mmol^{-1}$) was applied to the surface of soil contained in plastic tubes (20 cm deep × 5·5 cm in diameter) buried level with the soil surface in Stackyard, Woburn and exposed to natural weathering. In the first week following application on 12 May there was heavy rainfall (4·78 cm) which leached much of the oxamyl in the sandy clay loam soil to a depth of 7·5–15 cm. In a plot where the soil was modified by addition of peat, leaching was less and most of the oxamyl was found at depths between 2·5 and 7·5 cm. Little further leaching occurred in the subsequent dry weather. Oxamyl degraded rapidly in both the unmodified and peat amended soils, losses following approximately first-order kinetics with a half-life of 1–2 weeks. At all sampling dates over 90% of the radioactivity extracted from the soils by acetone chromatographed with oxamyl on t.l.c. (Bromilow and Edmondson)

Chlortoluron degradation in soil. Degradation of chlortoluron and its demethylation products in three soils was followed using HSLC analysis. Half lives were four weeks for chlortoluron and eight weeks for desmethyl chlortoluron and 3-chloro-4-methylphenyl urea. The parent 3-chloro-4-methylaniline was lost very rapidly from soils and could not be detected as a product of chlortoluron degradation. Aniline coupling products were detected at concentrations above 5 ppm but not at lower concentrations. The main product from the aniline was 3(or 5)-chloro-4-methylbenzoquinone-2-(3-chloro-4-methyl) anil with 3,3′-dichloro-4,4′-dimethylazobenzene and an unidentified trimer as minor products.

The results are thus similar to those found with other phenyl ureas where breakdown to the parent anilines is too slow to give concentrations large enough for self polymerisation. (Briggs and Smith)

Carbendazim decomposition in glasshouse soils. To help interpret observations of the relative effectiveness of 'Benlate' fungicide at different glasshouse sites on the island of Jersey, glasshouse soils, variously treated with 'Benlate' were incubated aerobically at 25°C with aqueous solutions of carbendazim at pH 6. The degree of breakdown of carbendazim observed in each case was closely related to the extent of previous soil treatment with 'Benlate'. In one week, the extent of breakdown ranged from 98% for a soil which had been heavily treated to 75% for a previously untreated standard Woburn soil from which 50% of a carbendazim dose would normally be recovered after about 3·6 months under field conditions. (Austin, Davies and Rolfe, with Mr. S. Hill, States of Jersey)

Benomyl residues in Woburn soil. Further measurements of benomyl residues, expressed as carbendazim, in soils from Broadmead Field, Woburn, were made 48 months after 17·8 kg a.i. ha^{-1} was applied in April 1971 (*Rothamsted Report for 1974*, Part 1, 240). As predicted, liming (29 months after the original treatment) increased the rate of loss progressively as the soil pH gradually increased. Extrapolation from the present values for recovered carbendazim (13–2% of the amount applied) suggests that the residue levels will be below 5% on all plots five years after the 'Benlate' was applied. (Austin)

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Identification and assay of thiophanate methyl residues in soil. In an extension of previous work (*Rothamsted Report for 1974*, Part 1, 240), (2,2′-^{14}C)-thiophanate methyl was applied to four soil tubes in each of two plots on Stackyard, Woburn, with high (4·5%) and low (0·7%) organic matter contents. After one week the top inch of soil from each of the two plots was extracted with acetone: M-ammonium chloride (1:1). After removing acetone each extract was acidified and extracted with ethyl acetate (acidic plus neutral fraction) and then made alkaline and re-extracted (basic fraction). The ethyl acetate solutions were analysed by thin layer chromatography (t.l.c.) and radioactivity scanning. The basic fractions contained carbendazim as the predominant radioactive component with a small proportion of 2-aminobenzimidazole. The acidic plus neutral fractions contained unchanged thiophanate methyl together with two further radioactive compounds, 'X' and 'Y' which were characterised by their t.l.c. mobilities and colour reactions. 'X' may be a sulphur-containing benzimidazole. Synthesis of an authentic sample confirmed that 'Y' was a thiophanate methyl analogue, in which one thioureido sulphur atom has been replaced by oxygen. Thiophanate methyl, 'X' and 'Y' do not persist in these Woburn soils for longer than two weeks, and can therefore contribute to disease control for a short time only.

After one week there were major differences between the recovery of radioactivity from the two soil types. Thus, 42% of the ^{14}C applied was recovered from that with high organic matter content, compared with 21% from the soil containing low organic matter. Similar amounts of carbendazim, 2-aminobenzimidazole, 'X', and 'Y' were extracted from both soils, but 14% of unchanged thiophanate methyl was recovered from the soil containing high organic matter, some 20 times more than from that with low organic matter, implying that thiophanate methyl decomposes much faster when organic matter content is low.

The t.l.c. behaviour of 'Y' is similar to that of carbendazim, normally regarded as the major fungitoxic derivative from several commercial fungicides and the two substances could be confused in t.l.c. bioassays following treatments with thiophanate methyl. The extraction scheme used separates substance 'Y' and carbendazim and the specific colour reagents permits the two substances to be distinguished. (Austin, with Whitby, Mutwakil and Reynolds)

Aldicarb residues in crops. Aldicarb residues in crops from field trials have been monitored since 1972. Usually potatoes, peas and onions grown in soil having aldicarb incorporated at rates of up to 5 kg a.i. ha^{-1} (about twice the recommended rate) before planting have contained residues of aldicarb plus its sulphoxide and sulphone of less than 0·1 ppm. However, in 1975 crops grown in sandy soil were examined for the first time and much larger residues were found in peas (0·85 ppm from 4 kg ha^{-1} treatment) and potatoes (0·31 ppm from 6 kg ha^{-1}).

At Rothamsted, onions grown in rows given a side-dressing of as little as 1 kg aldicarb ha^{-1} in late spring contained residues of about 0·2 ppm. Field beans treated with up to 4 kg aldicarb ha^{-1} in the rows before sowing in spring contained less than 0·03 ppm residues in the beans at harvest. (Bromilow, Jabbar and Middleton)

Inhibition of nitrification. Carbon disulphide (CS_2) was recently recognised as a very potent nitrification inhibitor in laboratory experiments using closed systems which prevent its rapid volatilisation. We considered injection of anhydrous or aqueous ammonia about 10 cm below the soil surface would give a sufficiently contained system for CS_2 to be effective as an inhibitor under field conditions. In laboratory tests aqueous ammonia and inhibitors were injected 7·5 cm below the surface of soil in open test tubes; CS_2 prevented nitrification completely for three weeks at 25°C and was as effective as nitrapyrin ('N-Serve').

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Aqueous emulsions of carbon disulphide and nitrapyrin were injected under grass with aqueous ammonia using modified commercial injection equipment (see Chemistry Department report p. 87 for agronomic results), and the distributions of the two inhibitors around the injection point measured. One week after injection about 80% of the nitrapyrin was localised within 2 cm of the injection point. After injection in November it diffused little further during the following ten months, while residues at the centre of the injection band decayed from 20 mg kg^{-1} to 0·3 mg kg^{-1}. CS_2 diffused rapidly and was found at least 10 cm away from the injection point after one week in very wet conditions and was barely detectable after three weeks. Inhibitory effects lasted 3–4 months after November injection of both chemicals. (Briggs and Evans, with Ashworth, Chemistry Department)

Pyroxychlor (2-chloro-4-trichloromethyl-6-methoxypyridine), a new fungicide translocated from leaves to roots, is similar in structure to nitrapyrin (2-chloro-6-trichloromethylpyridine). In soil pyroxychlor was as powerful a nitrification inhibitor as nitrapyrin; 0·5 mg kg^{-1} inhibited nitrification completely for 14 days at 25°C. Pyroxychlor was lost rapidly from three soils, the time for 50% loss ranging from four days in an unmodified Woburn soil with 1% organic matter, to 15 days in a Woburn soil amended with peat to give 5% organic matter. Nitrapyrin persisted longer, having half lives of one and two months in the same soils. (Briggs and Evans)

Chemical reference plots. Last year we reported the establishment at Rothamsted of chemical reference plots treated annually with representative crop protection chemicals with the objective of evaluating long term effects on soils and crops. This year yields of spring barley (mean 5·18 t ha^{-1}) were a tonne lower than in 1974, probably because of late sowing followed by a dry season and early harvest.

Benomyl applied before sowing reduced mildew throughout the season and aldicarb controlled aphids on leaves and ears well. However in contrast to the previous year, benomyl had little effect on yields whereas aldicarb increased yields by about 10%.

Soil residues of benomyl, assayed as carbendazim, varied with soil pH. On most plots more than half of that applied was still present in late July but less remained on the most alkaline plots. Aldicarb residues, measured as sulphone, were about 0·5 mg kg^{-1} in late July, consistent with a half-life in soil of about one month. Residues in whole plants from treated plots were 0·8 mg kg^{-1} 3 weeks before harvest. (Briggs and Osafo-Kroffah)

Redistribution of tecnazene in a potato store. The Potato Marketing Board compares the sprouting characteristics of new potato varieties at their Sutton Bridge Experimental Station, using the commercial sprout suppressant 'Bygran S'. Assays for the active ingredient (tecnazene) in the peel of 'treated' and 'untreated' tubers from opposite sides of a divided store showed that the volatile chemical had become widely distributed throughout the store, consistent with the anomalous sprouting behaviour observed for the 'untreated' tubers. (Austin, Reynolds and Rolfe, with T. Dent, Sutton Bridge Experimental Station)

Collaborative work with other departments

The following collaborative work is described in the reports of other departments:

With the Plant Pathology Department

Residues of endosulfan. (Austin and Manlove, with Plumb)
Effects of herbicides on disease. (Austin with Rawlinson)
Thiabendazole uptake by inactive potato tubers and its distribution following storage. (Austin and Rolfe, with Adams)

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Absorption and movement of thiabendazole in tubers and cuttings. (Austin, with Hide)
Screening of compounds for fungitoxicity. (Austin and Rolfe, with Adams)
Prevention of moulding in damp hay. (Lord and Manlove, with Lacey)

With the Botany Department

Phenylacetonitrile, a new plant-growth regulator. (Tinkler, with Wheeler)
Dwarfing of barley by chlormequat chloride (CCC). (Lord, with Wheeler)

Staff

It was with very great pleasure that we learnt during the year that M. Elliott had been awarded the American Chemical Society's 1975 Burdick and Jackson International Award for Research in Pesticide Chemistry, in recognition of his outstanding research on pyrethroid insecticides.

D. Carson and A. Coxon were appointed on two-year grants provided by NRDC to augment the team working on synthetic pyrethroids. R. F. Sadler joined us on a temporary grant, financed 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. A. D. Rice was appointed on a grant provided by the Sugar Beet Research and Education Committee to help with studies on the resistance of aphids to insecticides. Dr. A. E. Smith from the Canada Department of Agriculture, Regina, completed a year in the Department as a visiting worker. Other visiting workers included Mr. S. Osafo-Kroffah from the Cocoa Research Institute, Ghana, Mr. M. Mohmed Ahmed from the Ministry of Health Chemical Laboratories, Sudan, Mr. Ali Sadoughi from the Ministry of Agriculture and Natural Resources, Iran, and Miss E. Beascoa from the University of Barcelona, Spain. Sandwich course students who worked in the Department and the Chemical Liaison Unit were C. Adams, Jane Bowes, Angela Coates, C. Davis, R. Edmondson, C. Lazarides, Carol Livesey, Padzilah Othman, Carol Reynolds, I. Rumble, Pauline A. Ruggles and J. J. Tinkler.

At the invitation of the Ministry of Overseas Development and the Coconut Industry Board, A. J. Arnold spent a further month in Jamaica advising on methods of processing and storing coconut pollen as part of a mass pollination programme to increase production of hybrids resistant to lethal yellowing disease. P. Etheridge and F. T. Phillips spent a further six weeks in Brazil conducting field trials on baits for leaf-cutting ants. They were again based at the Centro de Pesquisas do Cacau (CEPEC) Itabuna, by kind permission of the Director Dr. P. Alvim. M. Elliott presented an invited paper to a conference on Human Health Effects of New Approaches to Insect Pest Control, organised by the U.S. Department of Health, Education and Welfare and the Environmental Protection Agency, in Durham, North Carolina. He and N. F. Janes were also invited by the organisers to present papers to the meeting of CILDA (Comité pour les Applications des Insecticides dans les locaux et la Protection des Denrees Alimentaires) in Paris. K. A. Lord again spent short periods in Tehran and Rome, acting as a consultant to the FAO in connection with a project to examine the fate of chemicals used to control locusts. He also contributed to a joint FAO/IAEA research coordination meeting on Isotopic Tracer-aided Studies of Foreign Chemical Residues in Food, Agriculture and Fisheries in Vienna. At the invitation of the organisers, K. A. Jeffs presented a report on seed treatments at the CIPAC meeting at Oeiras, Portugal. Several members of the Department participated in an International Symposium on the Evaluation of Biological Activity organised by the Pesticide Group and the Physicochemical and Biophysical Panel of the Society of Chemical Industry in Wageningen. I. J. Graham-Bryce acted as Chairman, and M. Elliott, A. R. Greenway, D. W. Hollomon, A. Mudd and P. H.

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Needham contributed papers. R. M. Sawicki visited institutes in Egypt under the auspices of the Royal Society–Egyptian Academy of Science Exchange programme to discuss resistance to insecticides. With financial support from Bayer A.G. he also visited the Company's research laboratories and other institutes in Belgium and the Netherlands with P. H. Needham to discuss resistance in aphids. A. W. Farnham extended a private visit to Australia to discuss resistance to insecticides with workers at various institutes; funds to support these visits were provided by CSIRO. J. H. Stevenson participated in a further meeting of the OILB Working Group on the Effects of Pesticides on Beneficial Insects in Colmar, France. A. R. Greenway presented a paper at the inaugural meeting of the OILB Working Group on Pheromones in Wageningen.

The Department made a major contribution to the 8th British Insecticide and Fungicide Conference. I. J. Graham-Bryce was Vice-Chairman of the Programme Committee, Chairman of a Session and presented an invited plenary paper. P. H. Needham acted as a Session Organiser and presented a research report. R. M. Sawicki gave an invited paper and a research report; other research reports were presented by A. L. Devonshire, M. Elliott, D. C. Griffiths, D. W. Hollomon, K. A. Lord, A. H. McIntosh and J. H Stevenson.

P. H. Needham organised exhibitions of the Department's work on pyrethroid insecticides at the Chelsea Flower Show and the British Growers Look Ahead Exhibition in Harrogate. Several other members of the Department helped to prepare and man the exhibits.

Publications

General Papers

1 Devonshire, A. L., Needham, P. H., Rice, A. & Sawicki, R. M. (1975) Monitoring for resistance to organophosphorus insecticides in *Myzus persicae* from sugar beet. *Proceedings 8th British Insecticide and Fungicide Conference* **1**, 21–24.

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Research Papers

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CHEMICAL LIAISON UNIT

RESEARCH PAPERS

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