# Report for 1974 - Part 1

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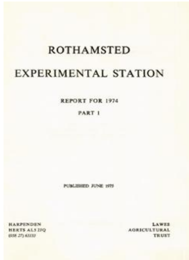

## Insecticides and Fungicides Department

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# INSECTICIDES AND FUNGICIDES DEPARTMENT

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## Introduction

This year was marked by the detection of resistance to systemic insecticides in field populations of aphids (*Myzus persicae*) on sugar beet, providing a further urgent reminder of the resilience of insect pests and the continuing need for major efforts to improve methods of pest control. A previous survey of field populations undertaken by the department in 1964 gave no evidence of resistance. A significant proportion of the



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samples collected this year by fieldmen of the British Sugar Corporation in collaboration with Broom's Barn Experimental Station were found at Rothamsted to be at least 10 times as resistant to systemic insecticides as standard susceptible strains. The exact relationship between resistance factors measured by such laboratory tests and the performance of insecticides in practice is not fully understood, so that it is not possible to assess how far resistance contributed to the severe outbreak of virus yellows on sugar beet in 1974. Other factors such as the very heavy infestations of aphids last season and the poor early growth of the sugar-beet seedlings together with unfavourable conditions at the time of spraying, which would have diminished the effective dose of insecticide in the plant, were undoubtedly of major importance. Nevertheless the development of resistance in outdoor populations of an insect previously found resistant only in the glasshouse in Britain, is disturbing. We are therefore expanding our studies on resistance to aphids to meet this new situation.

Resistance to insecticides is, of course, a long established problem throughout the world. In the past, tolerance in fungi has been much less serious, but has developed rapidly to the newer systemic fungicides in some situations. Studies on tolerance to systemic fungicides, like those on systemic insecticides, are hampered by the difficulties of devising the quantitative bioassays needed to obtain unequivocal results. These problems are particularly acute for obligate parasites which cannot be cultured or treated away from their hosts. Recent work in the department on the mode of action of the systemic fungicide, ethirimol, against powdery mildew on barley and on possible mechanisms of tolerance has therefore concentrated on the development of reliable bioassay methods. The rigorously standardized procedures now developed show that even genetically homogeneous strains of mildew can vary widely in their susceptibility, making it very difficult to interpret behaviour in the field.

The continuing spread of resistance underlines the need for new and better pesticides and emphasises the importance of the further major advances in the development of synthetic pyrethroid insecticides reported this year. 'NRDC 143' (permethrin) has shown increasing promise as a potential practical insecticide against agricultural pests. A more recent pyrethroid, 'NRDC 161', has insecticidal activity substantially greater than any previously reported compound of whatever chemical class. This discovery is of the greatest fundamental significance; 'NRDC 161' sets a new standard in the search for active compounds and provides valuable information about the mechanisms of insecticidal action. Such previously unforeseen potency opens up the prospect of finding chemicals to control insects at much smaller rates than in the past. As well as giving obvious benefits for pest control, this could greatly lessen the risks of harming the environment. The recent discoveries at Rothamsted, based on a sustained and systematic programme of fundamental work on structure-activity relationships over many years, have thus done much to establish the pyrethroids as a major class of insecticides at a time when new compounds are urgently needed to replace existing insecticides which are no longer acceptable on grounds of safety, unfavourable properties in the environment or because insects have become resistant to them. This was reflected at the 3rd International Union of Pure and Applied Chemistry (IUPAC) Congress of Pesticide Chemistry in Helsinki where a major symposium, organised by Elliott, was devoted to pyrethroids.

In addition to the need for more active and safer toxicants, better methods of application and more effective exploitation of the mechanisms for transferring the toxicant to the target organism are required. For many years a major goal of research in this field has been the discovery of insecticides and fungicides which are translocated downwards in plants following application to the foliage, as this would provide a very convenient way of protecting both the aerial and underground parts of established plants. Recent work in the department has shown that one soil-borne disease, common scab of potatoes



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(caused by *Streptomyces scabies* which attacks the developing tubers) can be controlled by treating the foliage with certain amino acids and growth regulators. The mode of action of these chemicals is not yet known; the effects could well be indirect rather than due to translocation of the applied compound and direct toxic action against the pathogen. The observations may provide an important lead, however, and will be followed up actively.

During the year there has been an increased demand for the services of the Chemical Liaison Unit, which has been reflected by the greater range of problems investigated as a service to other departments at Rothamsted or outside bodies. Some of this work is described in the reports of the appropriate departments, but for the first time other studies are reported together in a section in this report.

## Insecticides

**Relationships between molecular structure and insecticidal activity of pyrethroids.** Since last year (*Rothamsted Report for 1973*, Part 1, 168) the stability of the synthetic ester 3-phenoxybenzyl (±)-*cis,trans*-2,2-dimethyl-3(2,2-dichlorovinyl)cyclopropane carboxylate ('NRDC 143' or permethrin) has been further confirmed under practical conditions. Permethrin is therefore the first pyrethroid insecticide reported which retains the favourable properties traditionally associated with the group (great potency against insects and low toxicity to mammals) yet is sufficiently stable to be considered for agricultural and horticultural applications. These properties, in a structure simple enough for potential large scale manufacture, have stimulated considerable commercial interest.

Other esters of dihalovinylcyclopropane carboxylic acids have now been investigated. Several proved very potent, the outstanding example being 'NRDC 161' (described below) which has insecticidal activity appreciably greater than any previously described compound.

***Synthesis and insecticidal activity of dihalovinyl analogues of chrysanthemates.*** The acid component for the larger quantities of permethrin (batches >1 kg) necessary for field evaluation and other applications was made by adding ethyl diazoacetate to dichlorodimethylbutadiene(I). To provide samples of the optical and geometrical isomers of the acid, the mixture of ethyl esters (II) was first separated into (±)-*cis* and (±)-*trans* fractions by distillation through a spinning band column. The ethyl esters were hydrolysed and the acids resolved into their (+) and (−) forms with α-methylbenzylamine (*cis* acids) or *threo*-1-*p*-nitrophenyl-2-(*N,N*-dimethylamino)propane-1,3-diol (*trans* acids). Each of these four acids was esterified with 3-phenoxybenzyl alcohol ($R' = A$) or 5-benzyl-3-furylmethyl alcohol ($R^1 = B$). Bioassays established that only the (1R,*cis*) and (1R,*trans*) forms of the acids gave highly potent esters, as with the chrysanthemates (see page 139).

The corresponding (1R,*cis*)- and (1R,*trans*)-difluoro and -dibromo acids were made by Wittig syntheses from *cis* or *trans* caronaldehydes (V) and phosphoranes generated *in situ* from $CClF_2CO_2Na$ or $CBr_4$ and triphenyl phosphine, respectively. Each (1R,*cis*) and (1R,*trans*) form of the three dihalovinyl acids was esterified with the three alcohols to give 18 esters (III or IV; $R^1 = A$, B or C; X = F, Cl or Br), whose relative molar activities against houseflies and mustard beetles are shown in Table 1.

With α-cyano-3-phenoxybenzyl alcohol, the dichloro and dibromo esters (both *cis-* and *trans-*) have similar activities, four to five times greater than those of the difluoro-compounds, while with all three dihalo side chains, the *cis*-isomers are generally about twice as active as *trans-* compounds. With the 5-benzyl-3-furylmethyl and 3-phenoxybenzyl alcohols, differences in the activity of esters with the different dihalo acids were



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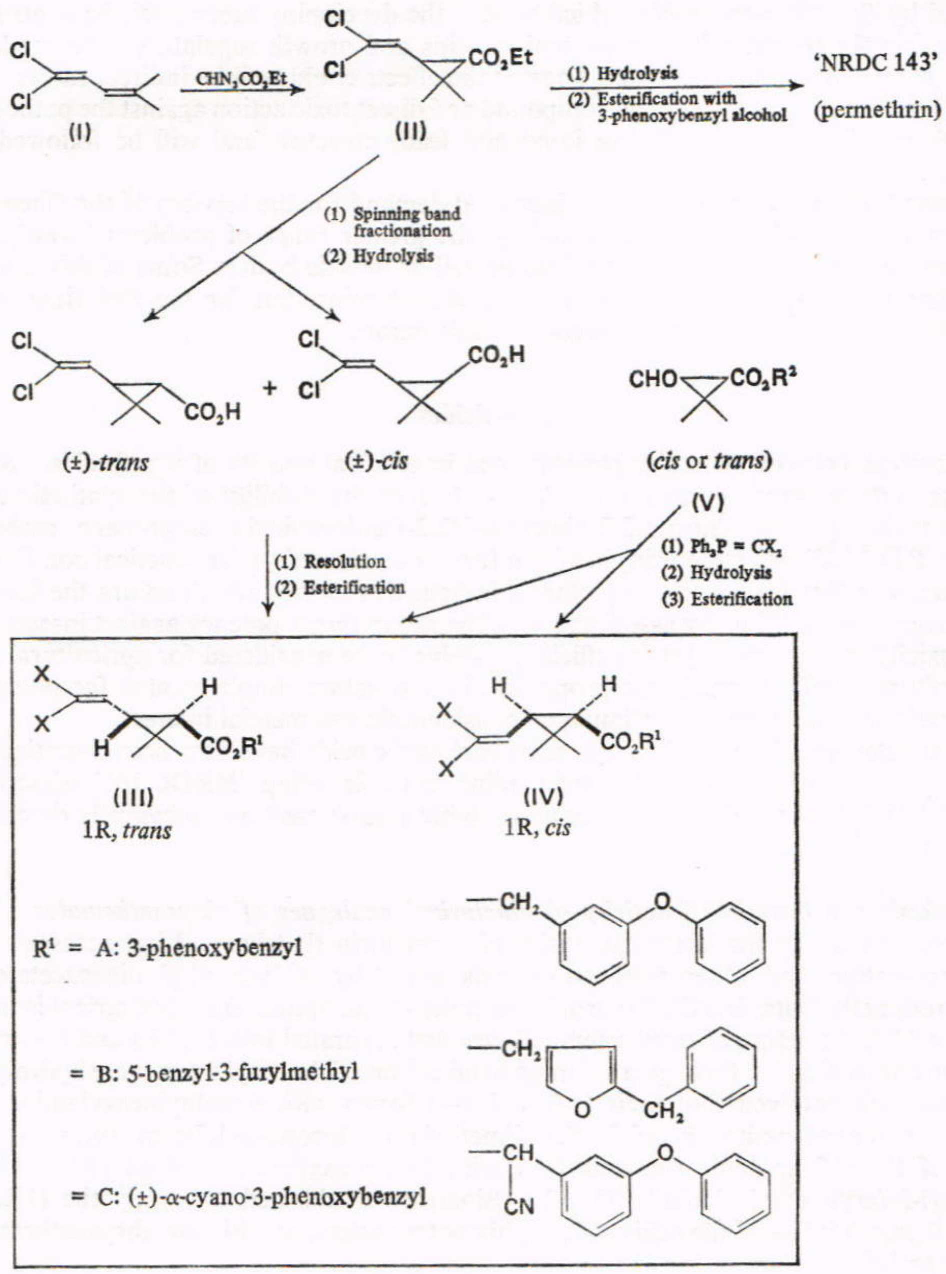


generally less, the difluoro- compounds having slightly lower activity than the dichloro- or dibromo- esters. When accessibility, ease of synthesis and activity per unit weight are considered, these results therefore suggest that the dichlorovinyl compounds are most appropriate for practical development.

***Exceptionally potent ester of dibromovinyl acids.*** The results in Table 1 show that α-cyano-3-phenoxybenzyl alcohol, first introduced by Japanese workers (Ger. Offen. 2 231 312 to Sumitomo Chemical Co.) is an exceptionally effective alcoholic component of these insecticidal esters. The ester (IVC; X = Br) was examined further



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TABLE 1

*Relative toxicities of 18 synthetic pyrethroids (molar basis) to* Musca domestica *L. (HF) and* Phaedon cochleariae *Fab. (MB)*

| Ester structure | X = F HF | X = F MB | X = Cl HF | X = Cl MB | X = Br HF | X = Br MB |
|---|---|---|---|---|---|---|
| IIIA | 60* | 50* | 90 | 260 | 110 | 90 |
| IIIB | 390 | 180 | 350 | 320 | 210 | 270 |
| IIIC | 94 | 84 | 570 | 320 | 550 | 410 |
| IVA | 170 | 120 | 200 | 160 | 260 | 200 |
| IVB | 260 | 200 | 280 | 290 | 360 | 250 |
| IVC | 150 | 270 | 1300 | 1000 | 1200 | 1300 |

* All relative toxicities based on bioresmethrin = 100
LD50 of bioresmethrin to HF = 0·005 μg/insect; to MB = 0·007 μg/insect
Results are means of at least two tests

because, like the stereochemically related [1R,*cis*]-chrysanthemic acid, the acid (IV; $R^1$ = H, X = Br) gives crystalline esters which might be used to resolve racemic alcohols. One component (coded 'NRDC 161', VI) was obtained crystalline; this was about 10 times more active than the non-crystalline residue as shown in Table 2, which also gives

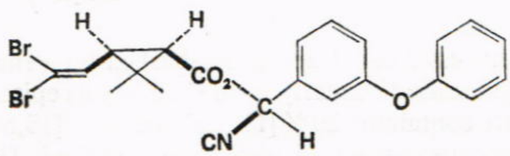
TABLE 2

*Relative toxicities of various insecticides (molar basis) to* Musca domestica *L. (HF) and* Phaedon cochleariae *Fab. (MB)*

| Compound | HF | MB |
|---|---|---|
| bioresmethrin (ester IIIB; X = Me) | 100* | 100* |
| 'NRDC 161' (crystalline isomer in ester (IVC; X = Br)) | 3400 | 2400 |
| Isomer in ester (IVC; X = Br) made by enzymic synthesis | 470 | 530 |
| DDT | 4–16 | 1·1 |
| dieldrin | 39 | 4–11 |
| parathion | 32 | 6 |

* See footnote to Table 1

activities of some standard insecticides from other classes. Comparison of the relative values emphasises the exceptional potency of 'NRDC 161'. The activity to houseflies is increased by a further 15 times by pretreatment with the synergist sesamex, giving a



(VI) 'NRDC 161'

median lethal dose of 0·002 mg $kg^{-1}$. This makes 'NRDC 161' an extremely potent poison by any standards (curare, for comparison, is active in mice at 0·5 mg $kg^{-1}$). Various lines of work are now in progress to interpret the insecticidal action of this exceptionally potent compound in molecular terms. In this connection knowledge of the absolute configuration of the active isomer is of great importance. Information about the configuration was first obtained by means of stereospecific enzymes. One optical form of the cyanohydrin from which the ester (IVC; X = Br) is derived was obtained by the action of the enzyme *D*-oxynitrilase on 3-phenoxybenzaldehyde and hydrogen cyanide; this enzyme selectively and stereospecifically cleaved one cyanohydrin in the equilibrium



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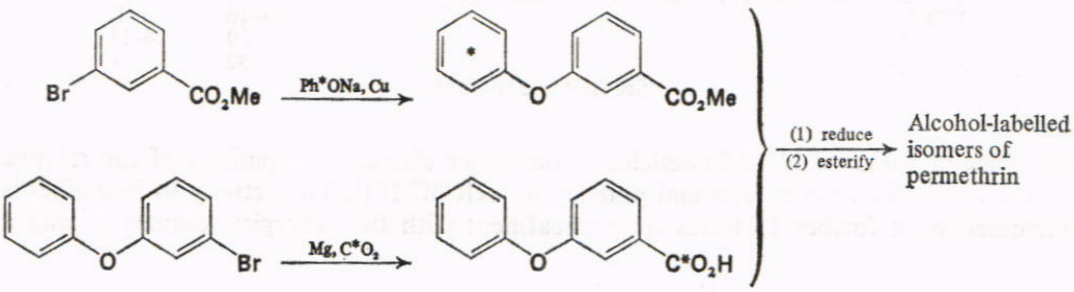
mixture, leaving the other optical isomer 95% pure. This was esterified with the 1R,*cis*-dibromovinyl acid. The cyanohydrin ester thus obtained was a liquid, less active biologically than 'NRDC 161' (Table 2), and the proton on the carbon atom bearing the nitrile group resonated at 3·72 $\tau$, in contrast to that in 'NRDC 161', at 3·64 $\tau$. This enzymically derived cyanohydrin was therefore not of the same optical series as that in the crystalline 'NRDC 161', whose absolute configuration was thus deduced to be (S) because other products from *D*-oxynitrilase (e.g. benzaldehyde cyanohydrin) can be related to acids of known (R) absolute configuration.

Because it is crystalline, and one of the few compounds with extreme biological potency which contain bromine, 'NRDC 161' is especially suitable for X-ray examination. With such molecules the conformation representing the true state of the parent molecule can be deduced without the possibility of distortion by substituents introduced only to facilitate X-ray analysis. X-ray examination of 'NRDC 161' by the Molecular Structures Department (Owen, J. D., p. 169) has confirmed the absolute configuration deduced above.

***Radiolabelled permethrin isomers.*** Routes to $^{14}C$ labelled isomers of permethrin for metabolic studies in insects and mammals and for determining residues and photodecomposition products were examined. Previous experience with pyrethroids suggested that metabolites would be derived from the intact ester and from the products of ester cleavage, so esters labelled in both the acid and alcoholic components were required.

The conditions used for reaction of the triphenyl phosphorane from carbon tetrachloride (*Rothamsted Report for 1973*, Part 1, 168) with methyl [1R,*trans*]-caronaldehyde were adopted. Starting with $^{14}CCl_4$ this gave, after processing and esterification, [1R,*trans*]-permethrin labelled at C-2 of the dichlorovinyl side chain. Satisfactory reaction




conditions for the [1R,*cis*] ester could not be established on a small scale so the chloride of the [1S,*trans*] acid, synthesised similarly from $^{14}CCl_4$, was epimerised at C-1 by heating to an equilibrium mixture containing 20% [1R,*cis*] and 80% [1S,*trans*] components. After esterification with 3-phenoxybenzyl alcohol, the required [1R,*cis*]-permethrin was separated and purified by thin layer chromatography. In a separate experiment, nmr examination of the olefinic doublets in the (—)-menthyl esters prepared from the acid chloride mixture established that the thermal inversion [1S,*trans*] → [1R,*cis*] involved only the C-1 centre and not C-3, of the cyclopropane ring.

Samples of [3-$^{14}C$]-phenoxybenzyl alcohol labelled in the phenoxy ring or at the methylene group were synthesised as shown. [U-$^{14}C$]-Phenol was converted to the sodium salt with sodium hydride dispersion in the presence of methyl *m*-bromobenzoate. After adding copper powder the Ullmann reaction was completed by heating, the ester was separated by thin-layer chromatography, reduced and esterified.

[$\alpha$-$^{14}C$]-3-Phenoxybenzyl alcohol was synthesised by a route developed from information kindly supplied by Dr. J. Miyamoto of the Sumitomo Chemical Co. The Grignard



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reagent from 3-bromodiphenyl ether was treated with $^{14}CO_2$ to give $^{14}C$-3-phenoxybenzoic acid, which after reduction, was esterified with [1R,*trans*] and [1R,*cis*] acids.

The following permethrin esters were prepared:

| | mCi/mmol |
|---|---|
| [1R,*trans*]; [—CH=$^{14}CCl_2$] | 6·9 |
| [1R,*cis*]; [—CH=$^{14}CCl_2$] | 1·7 |
| [1R,*trans*]; [PhO(U)—$^{14}$C] | 4·6 |
| [1R,*cis*]; [PhO(U)—$^{14}$C] | 4·6 |
| [1R,*trans*]; [α-$^{14}CH_2$] | 56 |
| [1R,*cis*]; [α-$^{14}CH_2$] | 56 |

These esters are being used in an examination of the photodecomposition and mammalian metabolism of permethrin in the laboratory of Professor J. E. Casida, Division of Entomology, University of California, Berkeley, U.S.A. (Chemical work, Elliott, Janes and Pulman: Biological work, Farnham and Needham)

***Solubility of synthetic pyrethroids.*** The solubility of several synthetic pyrethroids in water was determined turbidimetrically by a modification of the method of Brooker and Ellison (*Chemistry and Industry* 1974, 785). A 'Technicon AutoAnalyzer', with the plastic tubing replaced by glass wherever possible, was used to give a constant progressive dilution of methanolic solutions of the insecticides. Table 3 gives preliminary results. The value for permethrin agrees closely with results obtained by shaking the compound with water, filtration and analysis by gas–liquid chromatography. (Briggs)

**TABLE 3**

*Aqueous solubilities of synthetic pyrethroids*

| | Solubility (μg/ml) |
|---|---|
| bioresmethrin | 4·7 |
| cismethrin | 3·2 |
| permethrin | 1·9 |
| 'NRDC 161' | 3·4 |
| 'NRDC 102'[a] | 3·0 |
| 'NRDC 103'[b] | 1·7 |
| 'RU 15 525'[c] | 2·6 |
| 'RU 11 679'[d] | 1·8 |
| bioallethrin | >10 |

[a] 2,4,6-trimethylbenzyl (±)-*cis,trans*-chrysanthemate
[b] 2,3,4,5,6-pentamethylbenzyl (±)-*cis,trans*-chrysanthemate
[c] 5-benzyl-3-furylmethyl [1R,-*cis*]2,2-dimethyl-3-(2-oxo-3-thiacyclopentylidenemethyl)cyclopropane carboxylate
[d] 5-benzyl-3-furylmethyl [1R,-*trans*]2,2-dimethyl-3-(cyclopentylidenemethyl)cyclopropane carboxylate

**Mode of action of insecticides.** During 1974, electrophysiological studies were mostly concerned with recently-discovered synthetic pyrethroids. Particular attention was given to 'NRDC 161' because investigation of its outstanding insecticidal activity should contribute greatly to understanding of the fundamental poisoning process and to the rational development of further active compounds.

***The action of pyrethroids on the peripheral nervous system of insects.*** It has frequently been suggested that pyrethroids may act primarily on the peripheral nervous system of insects and that their rapid knock-down action is associated with effects on sensory nerves. Previous studies at Rothamsted (*Rothamsted Report for 1971*, Part 1, 185) however, have pointed to primary sites of action within the central nervous system. To



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discriminate between these hypotheses, suitable methods for testing the action of pyrethroids on insect sensory nerves are being sought.

One preparation, chosen because of the abundance of sensory nerves, is the cercus of the cockroach *Periplaneta americana* (L.). To eliminate nerve impulses originating in the last abdominal ganglion, one of the median (larger) cercal nerves was crushed close to the ganglion and the spontaneous output arising from the cercus was monitored with silver wire electrodes applied to the nerve within the haemocoel. The signals were amplified and integrated by a rate-meter with a time constant of 10 seconds, whose output was recorded with a pen-recorder. Output from untreated cerci ranged from 480 to 1230 nerve action potentials per second (c/s) with a mean output of 920 c/s for eight experiments (S.E. $\pm$200).

The effects of insecticides on activity were investigated by applying 0·5 $\mu$g pyrethrin I (a natural pyrethroid with an LD50 of 0·3 $\mu$g/insect against adult male *P. americana* two days after treatment) or 0·1 $\mu$g 'NRDC 161', the most toxic synthetic pyrethroid so far tested against *P. americana* (LD50 0·052 $\mu$g/insect at two days) to cerci in 0·5 $\mu$l acetone. Activity from cerci treated with pyrethrin I after the abdomen had been excised was slightly increased 30 min after treatment but soon declined to the initial level, although the pattern of activity had changed. However, when the cerci of living cockroaches were treated and the abdomens subsequently excised just before measurement, the mean activity decreased progressively from 30 min after treatment onward; levels of activity also varied more than those of an untreated cercus and bursts of activity were observed. One hour after treatment there was no activity in cerci from a prostrate cockroach.

'NRDC 161' applied after excision of the abdomen acted fairly rapidly. After an initial increase, activity had ceased 1 h after treatment. Applied to cerci of living cockroaches its effects were extremely quick. Activity decreased to one-quarter of that before treatment 15–30 min after application, although at that stage the insects were affected only about as much as those treated with pyrethrin I.

The spontaneous activity of cercal nerves in cockroaches treated on their metathoracic sterna with similar doses of the same insecticides, decreased more slowly but to about the same extent as that of cercal nerves in cerci treated before excision of the abdomen.

These experiments therefore show that 'NRDC 161' is more active against cockroach cercal nerves than pyrethrin I and that the action of both insecticides on cercal nerves is faster in live than dead insects, possibly because the circulation of the haemolymph speeds penetration. (Burt and Goodchild)

***Knock-down and toxicity of new synthetic pyrethroids.*** The toxicity of nine synthetic pyrethroids against *P. americana* was measured. LD50s ranged from 5·0 $\mu$g/insect (*epoxy*-bioresmethrin) to 0·05 $\mu$g ('NRDC 161'). For comparison, the LD50s of pyrethrin I and bioresmethrin are 0·3 and 1·7 $\mu$g/insect.

Although the insects recovered little from initial knockdown by some compounds ('NRDC 157' (IVA, p 138; X = Br), 'NRDC 163' (IIIA; X = Br), biopermethrin (IVA; X = Cl), 'NRDC 167' (IIIA; X = Cl), permethrin and cismethrin), with others ('NRDC 158' (IIIC; X = Br), 'NRDC 161' and *epoxy*-bioresmethrin) there was a marked, if sometimes partly temporary, recovery. Recovery from bioresmethrin and pyrethrin I was intermediate between these groups.

A possible explanation for these results, to which electrophysiological tests lend some support, is that compounds from the effects of which there is some recovery, although highly active neurotoxins, are relatively easily detoxicated. (Burt and Goodchild)

***Neuroanatomy of insect central nervous systems.*** The neuroanatomical study of the ventral nerve cord ganglia of the cockroach *Periplaneta americana* (L.) (*Rothamsted*



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*Report for 1973*, Part 1, 172) continued with investigations of motoneuron pathways and nerve cell body distribution in the mesothoracic ganglion and of the general neuroanatomy of the other two thoracic ganglia.

A parallel study of the general neuroanatomy of the thoracic ganglia of the desert locust, *Schistocerca gregaria* (Forskål), was started, to provide structural information for work on the effects of synthetic pyrethroid insecticides injected into neuron cell bodies. First results suggest marked similarities to the arrangement of fibre tracts in the cockroach.

Work also began to identify flight motor neurons in the thoraco-abdominal ganglion of the housefly, *Musca domestica* L., to complement studies on the action of insecticides on these neurons by Dr. T. Miller (University of California, Riverside). (Gregory)

**The causes of resistance.** We continued a major programme of research on resistance to insecticides which remains one of the most serious and intractable problems facing crop protection. As our genetical and biochemical studies with resistant houseflies gradually reveal the nature of resistance mechanisms and the way they interact, we are turning increasingly to a consideration of the development of resistance in field populations and how this can be delayed or prevented, based on the fundamental knowledge previously acquired. The occurrence of resistance in field populations of *Myzus persicae* on sugar beet and its possible contribution to the virus yellows epidemic provided an unwelcome indication of the importance of our work on resistance in aphids. Our long-term programme was diverted as far as possible to meet the urgent need for testing samples collected in the field, so that the extent of the practical problem could be assessed.

***Resistance of houseflies to pyrethroid insecticides.*** Resistance to pyrethroids recently occurred on some Danish pig farms where they had been used to control houseflies following the failure of dimethoate (*Rothamsted Report for 1973*, Part 1, 174). Laboratory selection of flies from one such farm, 290, with pyrethrum extract (py. ex., giving strain 290 Py), synergised py. ex. (290 PPB), bioresmethrin (290 BIO) and synergised bioresmethrin (290 BIOPB) resulted in substrains which strongly resisted all pyrethroids. The cross-resistance of these substrains suggested that their resistance differed from that of strains NPR (selected with py. ex.) or 104 (selected with resmethrin) which were substrains of 213ab which had developed resistance to pyrethroids in the field in 1958 following the failure of DDT.

In an attempt to simulate the field situation, strain 49 which strongly resists dimethoate but which had not previously been subjected to pyrethroid pressure, was also selected with pyrethroids. Resistance, particularly to py. ex. and to synergised py. ex. developed within 10 generations, fewer than occur during one spraying season in the field, thus accurately reflecting in the laboratory what had taken place on farms like 290.

Strain 290 BIO strongly resisted the pyrethroids bioresmethrin (resistance factor, RF, 1400) and 'NRDC 161' (90% unaffected by 20 $\mu$g/♀ fly), but the progeny of the cross between this strain and a susceptible one was almost fully susceptible (RF $<5$). More detailed genetical analysis revealed that the major recessive resistance characters were located on chromosome 3 with minor ones on 2 and possibly on 1 and 5. The total resistance provided by chromosome 3 (RF 120 for bioresmethrin) was due to *pen* and *kdr* which had also been isolated from NPR, and a third factor, so far unidentified, which boosted resistance to bioresmethrin from 30 for the combination of *pen* and *kdr* to 120. There was no measurable resistance (RF $<2$) to bioresmethrin or 'NRDC 161' on the isolated chromosome 2, but combining it with the homozygous chromosome 3 gave a population which was more resistant than that with chromosome 3 alone, even when chromosome 2 was in the heterozygous state. In the substrain homozygous for both



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chromosomes, much of the original resistance of 290 BIO was recovered. The resistance factor for bioresmethrin increased to 500, and 10% were unaffected by 20 μg/♀ fly 'NRDC 161'.

These results demonstrate the very great capability of the characters on chromosome 2 for modifying other pyrethroid resistance factors. These characters normally go undetected because they only become effective in the presence of other mechanisms and therefore cannot be easily examined in isolation. However, unless these important mechanisms can be detected at an early stage in the development of resistance in the field and their potential importance in imparting cross-resistance to alternative chemical control agents assessed, rational pesticide management could be extremely difficult to achieve. (Farnham)

***Properties of acetylcholinesterase from susceptible and dimethoate-resistant houseflies.*** In further studies on the causes of resistance to organophosphorus insecticides (*Rothamsted Report for 1973*, Part 1, 174) the properties of acetylcholinesterase from houseflies selected repeatedly with dimethoate (strain $49r_2b$) and from susceptible strains were compared *in vitro*. The enzymes were solubilized with detergents and behaved as single entities both in kinetic studies and during disc electrophoresis on polyacrylamide gels.

Enzymes from the two strains differed in their affinities for both substrate and inhibitors. The $K_m$ for acetylthiocholine was greater for the enzyme from resistant flies (0·169 mM) than for that from susceptible flies (0·115 mM), and the enzyme from resistant flies was less sensitive to inhibition by high substrate concentrations, a characteristic of acetylcholinesterase. This decreased affinity for substrate was associated with a much greater decrease in affinity for organophosphorus inhibitors as measured by the bimolecular rate constants (Table 4).

**TABLE 4**

*The bimolecular rate constants ($k_i$) for inhibition of acetylcholinesterase from resistant and susceptible houseflies by a range of organophosphorus inhibitors*

| | $k_i \times 10^{-3}$ ($M^{-1}$ $min^{-1}$) | |
|---|---|---|
| | $49r_2b$ | Susceptible |
| omethoate | 2 | 21 |
| methyl paraoxon | 22 | 90 |
| paraoxon | 139 | 578 |
| isopropyl paraoxon | 9 | 76 |
| malaoxon | 48 | 870 |
| ethyl malaoxon | 94 | 932 |
| tetrachlorvinphos | 25 | 172 |

The insensitive enzyme is inhibited from one-quarter (for paraoxon) to one-twentieth (for malaoxon) as rapidly as the susceptible enzyme. It seems probable that this insensitivity contributes significantly to the great resistance of strain $49r_2b$. Although omethoate is a poor inhibitor of even the susceptible enzyme, it is very toxic to susceptible houseflies (LD50 = 0·008 μg/fly). In contrast, malaoxon is a very good inhibitor, but a poor insecticide (LD50 = 0·3 μg/fly). This suggests that malaoxon is metabolised to a much greater extent than omethoate even by susceptible houseflies. (Devonshire)

***The contribution of modified acetylcholinesterase to survival of organophosphorus-treated houseflies.*** To determine the importance of the modified acetylcholinesterase as a mechanism of resistance we measured the inhibition by omethoate of the susceptible and insensitive enzymes *in vivo* in similar environments by transplant experiments. We implanted the brains of susceptible flies into the abdomens of resistant insects (strain D,



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which has chromosome 2 derived from strain $49r_2b$) that had the modified enzyme, and the brains of resistant flies into the abdomens of susceptible insects. After topical application of omethoate, we measured inhibition of the enzyme in the head and the implant in the abdomen. For comparison brains of susceptible and resistant insects were also implanted into individuals from their own strains. The transplant technique is feasible because neither the flies nor the transplanted organs are significantly affected by the operation and there is little or no acetylcholinesterase (AChE) in a fly abdomen. When susceptible flies were fully prostrate, about 2 h after treatment with 15 ng of omethoate (equivalent to about twice the LD100) over 90% of their head AChE was inhibited compared with only 27% in apparently unaffected resistant flies. When susceptible recipients were prostrate most (90%) of the AChE in susceptible implants was also inhibited but the implanted enzyme from resistant insects was completely unaffected.

The AChE of susceptible ganglia implanted into resistant recipients was inhibited much more slowly than when implanted into susceptible recipients; 2 h after treatment, when susceptible controls were prostrate, only 51% of the susceptible AChE implanted into resistant recipients was inhibited, and after 7 h 81% was inhibited. These tests showed that *in vivo* and in the same environment the susceptible enzyme is almost fully inhibited under conditions where the resistant enzyme is unaffected. However, this resistance mechanism by itself is weak and is not the only mechanism of resistance to organophosphorus insecticides in strain D. The transplant experiments showed that this strain has a further mechanism that delays the accumulation of omethoate inside the insect and that alone this mechanism is also weak because it does not prevent the eventual inhibition of the susceptible enzyme. However, the interaction of this mechanism with modified AChE can protect against concentrations lethal to flies with the normal enzyme and account for the observed resistance to omethoate. (Devonshire and Sawicki)

**Delaying the development of resistance by using insecticides in rotation.** The development of resistance can have particularly serious consequences when few effective alternative insecticides are available. In such circumstances it is essential to delay the build-up of resistance as long as possible because resistance cannot be suppressed once established. We have investigated whether the development of resistance can be delayed by using insecticides in rotation.

Laboratory populations of susceptible houseflies contaminated with a fixed ratio (1 : 300) of parathion-resistant houseflies of strain 29 were selected over several generations with one, two or three insecticides singly, sequentially or combined. The resistant insects had genes of resistance to parathion only on chromosome 2, and carried a visible recessive mutant (ocra eyes) on chromosome 5. The insecticides were: parathion, to which flies of strain 29 were resistant, and dimethoate and bioresmethrin to which all the flies were almost equally susceptible. An additional population was treated with the acetone/water solvent alone as a control. Surprisingly after only four selections spread over seven generations the ratio of parathion-resistant : susceptible insects increased from 1 : 300 to 1 : 11 in the population treated with the acetone/water mixture without insecticide, and to 1 : 5 to 1 : 3 in populations treated with the three insecticides singly, consecutively or together. Simultaneously the number of insects with ocra eyes increased, indicating that flies of the resistant strain chosen for these tests were abnormally vigorous and bred better than the susceptible insects. The reasons for this unexpected result are now being investigated. (Sawicki)

***Resistance to insecticides of* Myzus persicae *(Sulz.) from sugar beet.*** In 1973 there were some reports that systemic insecticides had failed to control aphids on sugar beet. This year we therefore undertook a survey of the susceptibility to insecticides of *Myzus*



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*persicae* from sugar beet, in collaboration with Broom's Barn Experimental Station and the British Sugar Corporation.

Before testing it was necessary to culture the small samples received from the field for two to three generations to obtain a sufficient number for bioassay. Samples were then examined by applying a discriminating dose (40 ng per aphid) of dimethoate topically. Previous experience indicated that this was the lowest dose that would always kill all individuals of a susceptible strain. In further tests a larger discriminating dose (400 ng per aphid) was applied to detect a greater level of resistance.

Of the 17 samples tested six showed resistance of ten-fold or more. Two of these had not been sprayed and four had survived one or more treatments with a systemic insecticide.

Some of the samples have been maintained in the laboratory and tested with various insecticides by a systemic technique. The aphids were allowed to feed on the leaves of Chinese cabbage seedlings grown in nutrient culture solutions containing systemic insecticides. It is possible to confine several samples on the same plant and even on the same leaf; this decreases variability and makes it possible to detect relatively small differences between the samples and the susceptible strain with confidence. Where differences are larger both strains cannot be tested on the same plants because the ranges of doses required to obtain regression lines do not overlap.

Preliminary results (Table 5) show that resistance is greatest to demeton-S-methyl and dimethoate, the two most widely used insecticides for controlling *M. persicae* on sugar beet.

TABLE 5

*Resistance factors of two field populations of* Myzus persicae *to a range of organophosphorus insecticides (systemic bioassay)*

| | Strain 1 | Strain 2 |
|---|---|---|
| demeton-S-methyl | 5 | >10 |
| dimethoate | 5 | 8 |
| pirimicarb | — | 1 |
| acephate | 1 | 1 |

The total carboxyesterase activity of these strains was compared with that of standard susceptible and very resistant strains from glasshouses. As with all resistant aphids tested previously the strains from sugar beet showed greater esterase activity than the susceptible strains. Susceptible strains from both field and laboratory hydrolysed 180–360 nmol of $\alpha$-naphthyl acetate/mg aphid/h compared with activities of 290–760 nmol/mg aphid/h in the field resistant strains, and 1250–1620 nmol/mg aphid/h for the resistant strains collected from glasshouses. The intermediate esterase activity of the aphids from sugar beet was associated with lower resistance to insecticides than that of the glasshouse-resistant strains. (Needham and Devonshire)

### Side effects of pesticides on beneficial insects

***Poisoning of honeybees in the field.*** One hundred and thirty-eight 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. Ninety-four samples gave clear evidence of poisoning compared with 43 in 1972, and 63 in 1973.

Evidence to indicate how poisoning had occurred was available for about two-thirds of the cases where poisoning was confirmed. Spraying of field beans to control *Aphis fabae* (33 samples) is still the most common cause of honeybee poisoning. Doubtless this reflects the high level of aphid infestation in 1974, but we remain disappointed that



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our recommendations for avoiding damage to bees when applying insecticides to field beans have apparently not been more widely adopted. Twelve samples were associated with spraying of oil seed rape. If the acreage of this crop continues to expand, with an associated increase in pest problems, it will be important to ensure that precautions are taken to prevent a related increase in honeybee poisoning. Treatment of fruit (11 samples) is clearly also a significant cause of trouble and must be kept under review. Poisoning due to equipment contaminated with BHC (three samples) continues to occur and may sometimes be malicious.

Poisoning due to the use of dichlorvos resin strips was not reported in 1974, we hope at least partly due to our warnings of this hazard. (Stevenson)

## Formulation of pesticides

***Microencapsulation.*** Further work was done to develop and evaluate microencapsulated formulations which offer the potential advantages of greater selectivity, controlled release and enclosure of repellent toxicants compared with conventional formulations.

Microcapsules of 250–500 $\mu$m diameter containing 5% v/v of the systemic insecticide disulfoton dissolved in toluene enclosed in gelatin/carragheenan/gum acacia walls were compared with standard 7·5% a.i. disulfoton granules as soil treatments in glasshouse experiments. Field beans grown in pots containing treated Woburn soil were tested at regular intervals for insecticidal activity by caging *A. fabae* on to the leaves. Results from both formulations were similar throughout the experiment and each gave good control even after six months.

Microencapsulated formulations of bendiocarb and permethrin were produced for incorporation in leaf-cutting ant baits for field trials in Brazil. The 100–500 $\mu$m diameter capsules contained 0·5% toxicant in soya oil enclosed in a gelatin/carragheenan/gum acacia wall and were added to the bait matrix in the proportion 200 g per 20 kg bait, together with a sticker. For effective control, good dispersal of baits within the ant colony before poisoning occurs is essential; it was hoped that this could be achieved by delaying the release of these fast-acting insecticides by microencapsulation. Interim results show that some nests were poisoned by this type of formulation, but final assessments will only be possible after several months. (Phillips and Etheridge)

## Control of soil inhabiting insect pests

***Wheat bulb fly.*** In collaboration with ADAS, previously untried toxicants and formulations were compared with standard insecticides as seed treatments for controlling wheat bulb fly in trials at four sites during 1973–74. Cyanofenphos at 0·1 and 0·5% a.i. to weight of seed was effective at only one site; 'CGA 12223' (*O,O*-diethyl-*O*-[1-isopropyl-5-chloro-1,2,4-triazolyl-(3)]-thiophosphate) at 0·2% and 'AC 92100' (*S*-(*tert*-butylthio)-methyl-*O,O*-diethylphosphorodithioate) at 0·1% a.i. to weight of seed were also relatively ineffective. Moderately effective treatments included 'AC 92100' at 0·5% a.i. to weight of seed and standard treatments with gamma-BHC. Standard treatments with dieldrin, chlorfenvinphos and carbophenothion all worked well and mixtures of the latter two gave very good protection from wheat bulb fly attack. A liquid formulation of the organophosphorus insecticide triazophos considerably decreased numbers of damaged shoots and live larvae at all sites but damaged plants at two sites. The synthetic pyrethroid, permethrin, was about as effective as triazophos against wheat bulb fly and gave no symptoms of phytotoxicity at 0·1 and 0·5% a.i. to weight of seed. This result is particularly encouraging as it demonstrates the potential of synthetic pyrethroids as soil insecticides and provides the first reported example of a seed treatment other than



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an organochlorine or organophosphate which has given good control of wheat bulb fly. However, the outstanding material was the organophosphorus insecticide isofenphos at 0·3% a.i. to weight of seed; it decreased live larvae by more than 99%, a level of control never before achieved with a seed dressing. In neighbouring ADAS plots, isofenphos at 0·05% a.i. to weight of seed gave only moderate control of wheat bulb fly. The advantage of isofenphos over standard materials is its comparative safety to plants, enabling it to be applied to seeds at high rates. In our tests the commercially available powder formulation was applied to seeds with a sticker, a practice that would not be acceptable for large scale use, so a more suitable formulation is needed.

To examine effects of depth of sowing, untreated seeds and seeds treated with chlorfenvinphos or dieldrin were sown at 2·5 cm and at 7·5–10 cm. The deep sown plants suffered much more insect attack and at 7·5–10 cm neither insecticide was as effective as at 2·5 cm.

The effects of soya bean oil sticker on insecticidal efficiency were difficult to evaluate because the insecticide used, gamma-BHC, damaged the germinating plants. Sticker with organomercury fungicide alone applied in the 'Rotostat' seed treater gave exceptionally good initial plant stands, an effect that will be studied further. (Griffiths, Jeffs and Scott)

**Control of weevils and associated viruses in field beans.** Further collaborative work on this project is described in the report of the Plant Pathology Department (p. 235). (Etheridge, with Cockbain, Plant Pathology Department)

## Behaviour-controlling substances

We continued to investigate chemicals that influence various aspects of insect behaviour, with the objective of exploiting them for pest control or management of beneficial insects.

**Codling moth.** Catches of codling moth in pheromone (*trans-8*, *trans-10*-dodecadien-1-ol) and light traps at East Malling have now been compared over three years, 1972–74, in which the weather differed considerably. Pheromone traps were more efficient than light traps at the start of the season, caught moths earlier (*Rothamsted Report for 1973*, Part 1, 181), and appeared to reflect the emergence of male moths more closely. The relationship between the cumulative pheromone catch and the accumulated heat sum (above a threshold of 10°C) was similar for each year. The catch began when the heat sum was approximately 50 day degrees and the numbers caught were 10–20%, 40–50% and 80–90% of the total catch of the first generation when the heat sum was 100, 150 and 300 day degrees. In each year the numbers trapped increased considerably when the heat sum was approximately 100 day degrees which coincided with the first catches of moths in the light traps. The early season catches correlated well with the total catches.

Because the pheromone traps are very sensitive at low moth densities, they should indicate reliably whether or not insecticide treatment is necessary. However, more information must be obtained from orchards left unsprayed in order to relate the numbers trapped to fruit damage. A provisional guide is that until catches exceed 5 moths/trap/week, economic damage is insignificant. (Greenway, with Dr. J. E. Cranham, East Malling Research Station)

**Chemicals affecting the behaviour of wheat bulb fly larvae.** Laboratory box tests have shown that damage to wheat by wheat bulb fly larvae can be decreased by growing plants in various mixtures of activated charcoal and compost, possibly because of adsorption of the arrestant exuded from the wheat (*Rothamsted Report for 1973*, Part 1, 181–182). To examine this under field conditions, two charcoal treatments were included in single-



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row trials at four sites with high wheat bulb fly populations. In one treatment, seeds were pelleted with charcoal (kindly undertaken by Germain's (U.K.) Ltd); in the other, charcoal powder was mixed into the seed furrows. With both treatments seeds germinated very well. Applying charcoal to the furrows slightly decreased attack but was not as effective as in the laboratory experiments. Pelleting the seed with charcoal had no effect.

Considerable purification of the wheat 'arrestant' and the oat 'anti-arrestant' (*Rothamsted Report for 1970*, Part 1, 167–168) has now been achieved. Chemical investigations with the purified materials suggest that the arrestant is a polyphenolic compound, possibly present as a glycoside, and the anti-arrestant is a polyhydroxylated aliphatic material. (Scott and Greenway)

**Control of leaf-cutting ants, *Acromyrmex octospinosus*, *Atta cephalotes* and *Atta sexdens*.** With support from the Ministry of Overseas Development, we continued work to develop effective and durable baits for these very serious pests of crops in the New-World tropics and sub-tropics. This included the search for chemicals which would attract the ants to the bait and induce them to carry it back to the nest, the development of suitable matrix materials, the investigation of slow-acting toxicants which could be dispersed within the nest from the bait before poisoning occurred and the study of mechanisms of food sharing within the colony.

**Phytochemical arrestants.** Efforts to identify a chemostimulant responsible for the arrestant properties of grapefuit albedo and citrus pulp were discontinued when it became clear that the activity of the extract was due to a variety of common plant constituents contained in the extracts. It appears that for leaf-cutting ants, as for many phytophagous insects, an optimum balance of constituents is required for a particular plant to be chosen, rather than the presence or absence of a single stimulant or deterrent (although these can be important in the first stage of a behavioural sequence). However, for practical purposes, investigations will continue to determine those extracts, fractions, and components of fractions, e.g. carbohydrates, with sufficient activity to cause laboratory colonies of ants to retrieve inert materials such as filter paper discs or vermiculite impregnated with them, for possible inclusion in various baits to be tested in the field.

The possibility that the ants select material on the basis of its suitability as a medium for fungal growth was investigated. A method of growing the fungus of *Atta cephalotes* in sterile culture on extracts of various plant materials was devised and growth rates compared. Very significant differences in the growth rate of the fungus on different autoclaved crude plant extracts were found but these could not be related to the ants' preferences for the various plants. This is partly due to the difficulty of determining the ants' preferences, which are highly variable, in the laboratory.

Using buffered glucose–peptone growth media, pH was shown to affect the growth rate of the fungus considerably. As the pH of the plant extracts varied greatly it seemed likely that this could explain the earlier results. However, adjusting the pH of the least favourable extracts did not appear to affect the growth rate significantly.

It is likely that the availability of nutrients in the plants is greatly modified by the effect of enzyme secretions in the ants' saliva and in fecal droplets deposited on the fungus garden. Fungal growth rates were therefore compared on various extracts with added ant secretions. The most successful method of adding these secretions was to allow the ants to collect particular plant material, incorporate it into the fungus garden and afterwards remove the material for extraction. The extracts were sterilised by filtration rather than by heat to minimise chemical alteration. All the leaf material so far tested after treatment by the ants has supported good fungal growth, confirming the beneficial effect



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of the ant secretions, but again there appears to be little relation between rates of growth and the ants' preferences for different plant materials. (Mudd and Bateman)

**Pharyngeal gland components.** The suggestion that the components of this gland, which is implicated in transmission of food and insecticide through the colony, were derived directly from the diet and material gathered by the ants was confirmed by feeding specimens alternately on highly pigmented and non-pigmented substrates. The colour changes that took place in the gland were found to correspond closely with the changes in diet. (Mudd, with Dr. D. J. Peregrine, University College of North Wales, Bangor)

**Laboratory screening of insecticides for use in baits.** The search for insecticides with a delayed killing action over a wide dosage range (*Rothamsted Report for 1973*, Part 1, 183) continued. Approximately 50 compounds were tested, of which 10 gave a delayed action approaching that of the standard mirex. These were carbophenothion, dichlofenthion, chlorfenvinphos, crotoxyphos, dimetilan, dioxathion, fospirate, 'M&B 19985', mecarbam and 'Shell WL 24073'. Some of the compounds tested acted very rapidly and were effective at comparatively low concentrations. Among these were chlorpyrifos, bendiocarb and permethrin; the latter two were used in special slow release formulations in the ensuing field trials. (Etheridge and Phillips)

**Field trials with experimental baits.** Experimental baits were tested in the field in Bahia, Brazil, during September–November 1974, from a base at the Centro de Pesquisas do Cacau (CEPEC), Itabuna. Twelve types of bait were used. Mirex, chlorfenvinphos, dichlofenthion, carbophenothion, mecarbam, crotoxyphos, dioxathion and fospirate were dissolved in soya oil (with a little acetone) and sorbed on dried citrus pulp to give bait containing 5% soya oil and 0·2% insecticide. Dioxathion and fospirate baits contained 0·1% a.i. and 0·01% a.i. respectively. A microencapsulated slurry formulation of pirimiphos methyl was mixed with dried citrus pulp to give 0·1% a.i., and dry permethrin microcapsules were incorporated to give 0·005% a.i., the larger capsules in this formulation (100–500 $\mu$m diameter) being stuck to the dried citrus pulp using 0·5% of the sticker 'Acronal 4D'. A commercial slow-release formulation of bendiocarb was sprayed directly on to the dried citrus pulp at 0·02% a.i. A bait consisting of vermiculite with orange juice as an arrestant and mirex dissolved in soya oil solution as the toxicant was tested as an alternative to the dried citrus pulp carrier. All baits were made up in 20 kg batches using a cement-mixer which gave a very satisfactory uniform mix. The baits were applied against two species of leaf-cutting ants, *Atta cephalotes* which form very large nests (up to 150 m$^2$) mainly in forest areas and particularly in cocoa and rubber plantations, and *Atta sexdens* which generally form smaller nests (up to 50 m$^2$) in more open forest and scrub areas where the soil is more sandy and drier. Bait was distributed on the main active trails near the entrance holes of each nest at a rate of 1 kg per nest. Each bait was applied to at least five nests of both species. An interim assessment was made three weeks after the baiting and a second treatment with 0·5 kg of the same material applied to each nest. This first assessment already revealed signs of poisoning and many of the *Atta sexdens* nests appeared dead. A final assessment of each treatment will be made after about three months. (Etheridge, Phillips and Scott)

**Repellency and persistence trials.** Complementary trials carried out in the field in Brazil included tests of repellency by different concentrations of the insecticides (0·2, 0·1, 0·02 and 0·005%) and of the effects of weathering on the loss of insecticides from the dried citrus pulp baits. Only in one case was any repellency observed (dioxathion at the highest concentration) and even this was temporary. After three weeks weathering, which



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included high temperatures and heavy rainfall, the appearance of the different baits varied greatly. Bait containing dichlofenthion was blackened by fungus and had rotted badly, and that containing mirex was almost as poor. The bait incorporating fospirate, however, was almost unaffected and that with crotoxyphos only slightly affected, showing that these insecticides had fungicidal properties. Baits containing the different insecticides were sampled at known intervals throughout the three-week weathering period. These will be analysed by gas–liquid chromatography and the results related to the meteorological conditions. (Phillips, Etheridge and Scott)

**Material used by solitary wasps to seal their nests.** Species of the aphid-hunting genus *Passaloecus* have been proposed as agents for the biological control of aphids and thrips in glasshouses. They nest in beetle borings and other very small holes in wood using a sticky material to seal the entrances. This sticky material is of interest because, unless it is a natural secretion, it will have to be provided for wasps in culture.

The presence of α- and β-pinenes in the nest material was detected by gas chromatography–mass spectrometry. The mass spectrum of the chief component of the door material, isolated by preparative thin-layer chromatography, showed this to be a mixture of abietic and pimaric acids which are typical components of pine resins. Using a thin-layer chromatographic fingerprint technique developed for the identification of resins (Stahl, *Thin Layer Chromatography* (1969), 248, Springer-Verlag) it was possible to show that material from a nest of *Passaloecus gracilis* (Curtis), a nest of *P. insignis* (Van der Linden) and a nest of *P. corniger* (Shuckard) was similar and like the resin of *Pinus sylvestris* L.

It is of interest that larvae of the saw fly *Neodiprion sertifer* ingest this resin and use it as a defensive secretion to deter a variety of predators and entomophagous parasites. It is thus very suitable for protecting the brood of these solitary wasps. (Mudd, with Dr. S. A. Corbet, University of Cambridge)

**Pea moth.** Collaborative work is described in the report of the Entomology Department (p. 109). (Greenway, with Lewis, Macaulay and Wall, Entomology Department)

**Footprint substance of worker honeybees.** This work is described in the report of the Entomology Department (p. 114). (Greenway, with Butler and Welch, Entomology Department)

## Equipment and techniques

**Insect neuroanatomical techniques.** New work with the Bodian silver technique (*Rothamsted Report for 1969*, Part 1, 220) on the cockroach *Periplaneta americana* (L.) and house-fly, *Musca domestica* L., central nervous systems revealed that the purity of the sodium sulphite used in the silver developer greatly affects the quality of results. Details are reported in paper No. 19.

Two additional staining methods were successfully employed to extend the earlier studies with Procion yellow and cobalt chloride (*Rothamsted Report for 1971*, Part 1, 191 and *for 1973*, Part 1, 185–186). Toluidine blue (Altman & Bell, *Brain Research* (1973), **63**, 487–489) was used to show nerve cell body distribution in wholemounts of thoracic ganglia of cockroach and house-fly, and injection of trypan blue by atmospheric pressure (Hagmann, *Stain Technology* (1940), **15**, 115–118) to display tracheation of whole cockroach ganglia. (Gregory)

**Artificial feeding of aphids.** It was reported last year (*Rothamsted Report for 1973*, Part 1, 186) that an artificial diet lacking nine components of the Californian diet (Mittler *et al.*,



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*Journal of Insect Physiology* (1970), **16**, 2315–2326) and including some at increased concentrations was more acceptable than the Californian diet to a British strain of *Myzus persicae*, although less nutritious in long term tests. *Myzus persicae* from three other sources in Britain also settle preferentially on the modified diet as do aphids of the original laboratory strain reared on radish, bean or potato. Even aphids maintained on the Californian diet for two years settle preferentially on the modified diet when given a choice. Alate *M. persicae* behave similarly to apterous forms. Other species of aphid behave differently: adult *Aphis fabae* show a slight preference for the Californian diet but their larvae grow better in short-term tests on the modified diet. Of the aphids that are more restricted in their host plant range, adults of *Megoura viciae* settle preferentially on the Californian diet but do not feed actively: feeding is not increased by painting bean extracts on the membrane or including extracts in the diet. Larvae of *M. viciae* do not feed through the 'Parafilm' membrane. *Phorodon humuli* from hops settle on sucrose solution in preference to either diet and grow only slowly on diets. Adult *A. evonymi* from *Euonymus europaeus* will not settle on diets, sucrose or water presented via a 'Parafilm' membrane. (Griffiths and Greenway)

**Insect rearing.** The following species were reared:

PLANT FEEDERS

Homoptera
- *Acyrthosiphon pisum* (Harris)
- *Aphis fabae* (Scop.)
- *Brevicoryne brassicae* (L.)
- *Macrosiphum rosae* (L.)
- *Myzus persicae* (Sulz.)
  - Strains. Susceptible
  - Several organophosphate-resistant
- *Megoura viciae* Buckt.
- *Phorodon humuli* (Shrank)
  - Strains. Susceptible
  - Resistant

Hemiptera
- *Dysdercus intermedius* Distant

Coleoptera
- *Phaedon cochleariae* (F.)

OTHERS

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 608Q, 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
  - Several substrains of $49r_2b$ derived by selection with pyrethroids
  - 290rb—dimethoate selected strain



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| | | |
|---|---|---|
| Diptera | *Musca domestica* (L.) *continued* | |
| | Strains. | Several substrains of 290rb derived by selection with pyrethroids |
| | | NPR—pyrethrum extract selected strain |
| | | Several strains derived from NPR each with one or more factors of resistance to pyrethroids and DDT |
| | | 104—resmethrin selected strain |
| | *Calliphora erythrocephala* (Meig.) | |
| Hymenoptera | *Acromyrmex octospinosus* (Reich) | |
| | *Atta cephalotes* (L.) | |
| | *Diaretiella rapae* McIntosh | |

## Fungicides

**Control of common scab on potatoes.** Previous reports indicated that it may be possible to control common scab, caused by *Streptomyces scabies*, by haulm-sprays. This year most of our experiments were designed to explore this possibility further, in both glasshouse and field. However, in one field trial some chemicals were tested as conventional soil-treatments.

***Glasshouse tests of haulm-sprays.*** Potted Majestic plants were grown from shoots in the glasshouse. The haulms were sprayed with aqueous solutions of the test chemicals, care being taken to prevent spray material from reaching the soil directly. Two types of chemical were tested: amino acids and plant growth regulators (including some translocated herbicides at very low concentrations). Amino acids were applied several times at intervals of three to four days but growth regulators were sprayed only once. Sprays were first applied just before the tubers began to form, when they are susceptible to attack by *S. scabies*.

DL-ethionine, at 0·2%, decreased the incidence of scab (*Rothamsted Report for 1971*, Part 1, 199); at concentrations up to 1·0% it was slightly more effective, and had no effect on yield of tubers. No differences were found between the D-, L- and DL- isomers. Twenty-three other amino acids were tested, but none had any consistent effect on scab incidence.

The following growth regulators and herbicides did not affect scab incidence at the concentrations shown: chlorphonium chloride, 0·05%; $\beta$-hydroxyethyl hydrazine, 1·0%; 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride ('AMO 1618'), 0·2%; dicamba, 0·0002%; glyphosate, 0·002%; and picloram, 0·00025%.

By contrast, gibberellic acid at 0·01% decreased the incidence of scab by over 40% ($P < 0{\cdot}05$). The yield of tubers was not affected, but many of the tubers were distorted. Lower concentrations (down to 0·0025%) had less effect on scab, but still produced almost as many distorted tubers.

The effect of daminozide sprays on scab, reported last year, was confirmed. Detailed experiments with 0·15%, 0·3%, 0·6% and 1·2% aqueous solutions showed that (*a*) all concentrations decreased leaf area by about 10%; (*b*) none of the treatments affected soil moisture; (*c*) the maximum effect on plant height (a decrease of over 40%) was reached at 0·6% daminozide; (*d*) all treatments increased yield of tubers by about 8%; (*e*) scab incidence was progressively decreased by increasing concentrations, the greatest effect (a decrease of over 55%: $P \ll 0{\cdot}001$) being produced by 1·2% daminozide; (*f*) daminozide-treated plants usually produced a few distorted tubers.

Analogues of daminozide were made by combining *unsym.* dimethyl hydrazine, N-aminopiperidine or N-aminomorpholine with the anhydrides of succinic, methyl succinic, glutaric, maleic or citraconic acids. Nine such analogues were tested as single



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sprays (1·2%). There was a positive correlation between the resulting decreases in plant height and scab incidence; however, the only analogue which was comparable in effect with daminozide was dimethylamino maleamic acid ('CO-11').

***Field trials of haulm-sprays.*** DL-ethionine and daminozide, both of which decreased scab incidence in the glasshouse, were tested in two small field trials (cv. Maris Piper) at Woburn. The plants were sprayed at 125 gal/ac (1400 litres/ha) in mid-June. Scab incidence was calculated at harvest from 50 ware tubers per plot (three plots per treatment per trial). Table 6 shows the amounts of scab found, expressed as percentages of those in the corresponding unsprayed plots. Both chemicals clearly decreased the incidence of scab in the field as effectively as they had done in the glasshouse. The tendency of daminozide to cause distortion of tubers was less noticeable in the field. There was no indication that any of the treatments affected yield, but the plots were too small (one row ×4 m) for reliable estimates.

**TABLE 6**

*Effects of haulm-sprays on scab incidence in the field*

| Treatment | No. of sprays | Scab incidence Trial 1 | Scab incidence Trial 2 |
|---|---|---|---|
| DL-ethionine, 0·2% | 3 | 52 | 58 |
| DL-ethionine, 1·0% | 3 | — | 24 |
| daminozide, 1·0% | 1 | — | 47 |
| unsprayed | — | 100 | 100 |
| LSD, $P = 0{\cdot}05$ | | 40 | — |
| 0·02 | | 50 | 33 |
| 0·01 | | — | 38 |
| 0·001 | | — | 53 |

***Field trial of soil-applied chemicals.*** In a trial at Woburn, chemicals were applied to the soil on 17 April; all plots were rotavated immediately and potatoes (cv. Maris Piper) were planted on the same day. Scab incidence was calculated at harvest from 50 ware tubers per plot (four plots per treatment). Table 7 shows the yields and the amounts of scab found, expressed in the same way as in Table 6. None of the treatments with *tert.* butyl catechol, which was at least as effective as quintozene in the glasshouse (*Rothamsted Report for 1973*, Part 1, 188) gave satisfactory control in the field. (McIntosh)

**TABLE 7**

*Effects of soil-treatments on yield and scab incidence in the field*

| Treatment | Rate (kg/ha) | Yield | Scab incidence |
|---|---|---|---|
| 4-*tert*. butyl catechol, 2% solution* | 39 | 94 | 67 |
| 4-*tert*. butyl catechol, 4% solution* | 78 | 94 | 67 |
| 4-*tert*. butyl catechol, 20% dust | 78 | 86 | 76 |
| quintozene, 20% dust | 78 | 108 | 50 |
| Untreated | | 100 | 100 |
| LSD, $P = 0{\cdot}05$ | | 23 | 34 |
| 0·02 | | — | 41 |
| 0·01 | | — | 48 |

* In 30% ethanol

**Organomercury seed treatments.** Organomercury fungicides provide considerable protection to cereals from seed-borne fungal pathogens. Work on their efficiency against seed-borne *Septoria nodorum* and *Fusarium nivale*, continued and was extended to include soil-borne *Fusarium* infection.



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***Control of* Septoria nodorum.** The importance of distribution of phenyl mercuric acetate (PMA) on treated wheat seed in storage was studied using infected seed. Seed was treated with PMA at 0·5 and 0·05 $\mu$g Hg/seed (liquid treatment), mixed with untreated seed in varying proportions, and sown either immediately or after one month's storage. The plants were grown in pots in a growth room and scored for coleoptile infection after three weeks. Storage significantly improved fungicide performance only when 50 and 75% of the seed was treated with 0·5 $\mu$g PMA, but not when 100% was treated. It is suggested that all such improvements with storage were the result of an improved distribution of fungicide between seeds, and not from increased penetration into the seeds. If deep penetration of the seed is essential for good performance, then this must occur almost immediately after treatment. There was no difference in the performance of PMA on naturally infected seed compared with that on seeds which were inoculated on the surface with spores. This suggests that the infection was near the surface and easily reached by the fungicide. The use of dimethyl sulphoxide as a penetrating agent did not improve the performance of PMA.

The effectiveness of dust treatments of PMA and ethyl mercuric chloride (EMC) against *S. nodorum* was studied in a field experiment in which wheat seed (cv. Joss Cambier, 75% infected) was treated with four rates of mercury. Table 8 shows some of

**TABLE 8**

*Control of* Septoria nodorum *by organomercury seed treatments*

| Treatments | Rate of mercury $\mu$g/seed | Rate of mercury mg/kg | Emergence (plants/m) | Seedling infection (%) | Leaf 2 infection (%) | Grain yield (t/ha) |
|---|---|---|---|---|---|---|
| Nil | 0 | 0 | 57·3 | 31·7 | 29·9 | 7·47 |
| PMA | 0·016 | 0·4 | 54·9 | 15·3 | 22·4 | 7·64 |
| PMA | 0·08 | 2·0 | 61·6 | 10·0 | 21·7 | 7·34 |
| PMA | 0·4 | 10·0 | 56·3 | 1·3 | 32·7 | 7·62 |
| PMA | 2·0 | 50·0 | 33·1 | 0 | 21·8 | 6·89 |
| EMC | 0·016 | 0·4 | 59·7 | 14·0 | 30·8 | 7·32 |
| EMC | 0·08 | 2·0 | 55·2 | 8·0 | 31·0 | 7·08 |
| EMC | 0·4 | 10·0 | 65·1 | 0 | 29·0 | 7·57 |
| EMC | 2·0 | 50·0 | 13·1 | 0 | 19·9 | 5·27 |
| LSD at $P = 0{\cdot}05$ | | | 12·4 | 7·5 | 7·5 | 0·35 |

the results. Seedling infection was almost completely controlled at 0·4 $\mu$g Hg/seed, a rate below the normal commercial target of 0·5–1·5 $\mu$g. Seedling infection was low and did not result in damage, but seedling establishment and consequently yield were reduced by the highest rate of mercury. Leaf disease developed late in the season and did not correlate with the level of seedling infection, suggesting either that the spread of disease between plots was very efficient or that the seed-borne infection was not responsible for the leaf disease.

***Control of seed-borne* Fusarium nivale.** Liquid and powder treatments were applied to seed of winter wheat and spring barley, 30% infected with *F. nivale* and effectiveness evaluated in pot experiments. PMA and EMC were less effective than against *S. nodorum* although storage of treated seed for six weeks at 15°C improved performance. PMA controlled *F. nivale* better on wheat than on barley. These results may be explained by the location of infection. *Fusarium nivale* is probably deep-seated in the seed, thus largely escaping the effects of the fungicide, apparently unlike *S. nodorum* which is more easily controlled. *Fusarium nivale* was not completely controlled even by fungicide rates two to



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three times those applied commercially. The presence of a hull on barley seed probably further decreases the performance of the fungicides.

Agar plate tests with two isolates of *F. nivale* and four of *S. nodorum* showed both fungi to be sensitive to PMA, *F. nivale* being only slightly less sensitive than *S. nodorum*. This supports the hypothesis that differences in the site of infection are mainly responsible for the differences in the susceptibility of the two diseases on wheat seed.

***Control of soil-borne Fusarium.*** There is some controversy about the importance of mercury seed treatments for controlling soil-borne seedling blights. Some reports suggest that control may be either partial or negligible. We therefore started experiments using inoculated soil and PMA-treated wheat seed to determine the extent of control that can be achieved in the early stages of plant growth. Using soil inoculated with *F. culmorum* more than 50% control of coleoptile infection was achieved by PMA at 2 $\mu$g Hg/seed. Control was not improved at 3 $\mu$g ,but even this level of control may have significant effects in the field. The 3 $\mu$g dose was slightly phytotoxic, causing a reduction in mean shoot length.

***Relationships between fungi on seed.*** *Fusarium nivale* frequently fails to respond consistently to increasing rates of PMA applied to the seed. In general, increasing rates up to 0·125 $\mu$g Hg/seed does not improve control. This could be explained by differences in the depth of infection or by differential toxicity of PMA to other seed-borne organisms which inhibit or promote infection. To gain more knowledge about such interactions between seed-borne pathogenic fungi and other organisms inhabiting the seed, wheat seed was inoculated with *F. nivale* or *S. nodorum* mixed with common seed-borne saprophytic fungi (*Alternaria*, *Epicoccum* and *Cladosporium* spp.) and with other pathogenic *Fusaria* (*F. culmorum* and *F. avenaceum*). None of the saprophytes significantly affected disease and mixtures of pathogens were usually less damaging than expected from results with single species, although no definite interactions were observed. After inoculating barley seed, already naturally infected with *F. nivale*, with saprophytes, one isolate of *Epicoccum* and two of bacteria significantly reduced seedling disease. There was no evidence that organisms other than bacteria can consistently influence seedling disease. Microbial relationships in the presence of mercury are now being investigated. (Bateman)

**Mode of action of systemic fungicides.** The effect of ethirimol on successive steps in the infection of barley by powdery mildew (*Erysiphe graminis* f.sp. *hordei* Marchal) was examined with the aim of developing a quantitative bioassay for the fungicide, and of providing material suitable for biochemical studies on its mode of action. This led to a detailed examination of two assay techniques; one involving the *in vitro* effect of ethirimol on germ-tube growth, the other its inhibition of appressorial formation *in vivo*. Despite

**TABLE 9**

*Response of two mildew strains to ethirimol*

| | Tolerant | | Sensitive | | |
|---|---|---|---|---|---|
| | ED50 | 95% confidence limits | ED50 | 95% confidence limits | Ratio T : S |
| In vitro assay (a) | 2·00 | 0·75–5·10 | 0·00048 | 0·00018–0·0012 | 4166 |
| In vivo assay (b) | 1·23 | 0·78–2·10 | 0·017 | 0·011–0·025 | 72 |

(a) Conidia deposited on cellophane membrane overlying 0·5% (w/v) agar; ED50 values expressed as $\mu$g/ml ethirimol present in agar
(b) ED50 values given as ppm (fresh weight) within leaf segment at inoculation



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standardized procedures, and the use of genetically homogeneous material, considerable variation was encountered in both assays. Nevertheless, a strain (tolerant) isolated from ethirimol-treated barley where mildew control was poor, and a strain (sensitive) not previously exposed to the fungicide, clearly differed in their response to ethirimol (Table 9). Examination of six single pustule progeny of each strain gave information about the stability of this tolerance. In both bioassay procedures, ED50 values for progeny differed widely from those of their respective parents, and only the *in vitro* assay gave clearly different results for tolerant and sensitive progeny. For individual progeny the results of the two assays did not correlate well suggesting that separate aspects of ethirimol action were being measured. These results indicate that field populations of mildew are likely to vary widely in their response to ethirimol. (Hollomon)

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

Previous results (*Rothamsted Report for 1973*, Part 1, 190) indicated that benomyl and 'NF48' controlled mildew (*Erisyphe graminis*) and smut (*Ustilago nuda*) on barley more effectively than thiophanate-methyl. Effectiveness appeared to depend partly on relative stability, but other factors were also involved. In further field experiments this year, a wide range of benzimidazoles was compared. The compounds tested were benomyl, cypendazole, carbendazim, thiophanate methyl and 'R28921' (2-[(3′-methoxycarbonyl)-thioureido]-*O*,*O*-diethyl phosphoranilide). All treatments except 'R28921' significantly decreased smut, but were less effective than in 1973, possibly because the initial level of infection in the seed was greater in 1974. Average values from four replicate plots were 8·1 smutted heads per row for benomyl, 18·4 for thiophanate-methyl, 21·9 for cypendazole, 33·6 for carbendazim and 71·7 for 'R28921' compared with 74·5 for untreated controls. Relative effectiveness against mildew was broadly similar to that against smut but differences between treatments were smaller and less consistent. All treatments except 'R28921' increased yield of grain significantly. However, the increases were not all consistent with the relative effectiveness of the different treatments against the diseases assessed, average yields being 4·86 t/ha for benomyl, 5·02 t/ha for thiophanate methyl, 4·86 for cypendazole, 4·53 for carbendazim, 4·04 for 'R28921' and 3·81 for untreated controls. It is of interest that benomyl gave better yields than thiophanate methyl in 1973, whereas their effectiveness was reversed in 1974.

To obtain more information about the factors determining performance, the uptake and translocation of carbendazim and the most effective precursors from the field experiments in 1973 and 1974, were studied in detail in pot experiments using radiotracers. Seeds treated with $^{14}C$-labelled benomyl, thiophanate-methyl, 'NF48' and carbendazim were planted in Woburn soil in controlled environment rooms. At intervals from 2 to 28 days after planting, seeds and seedlings were harvested and radioactivity in seeds, roots, stems and leaves determined by scintillation counting following freeze-drying and oxygen flask combustion. Preliminary results indicate that the pattern of uptake is generally similar for all compounds except 'NF48'. Amounts present in the seed decreased after the first sampling at two days in all cases, although the decrease was least marked for 'NF48'. With benomyl, thiophanate-methyl and carbendazim amounts in the roots changed little after an initial uptake, but there was a continuing accumulation of 'NF48'. In the aerial parts of the plants, the amounts of radioactivity increased progressively for all compounds except 'NF48' which appeared to level off after 14 days, at which stage uptake was much greater than for the other compounds. There was a steady accumulation of radioactivity in the leaves for all compounds. Total uptake by the whole plant was thus much greater for 'NF48' than for the other compounds, particularly in the initial stages



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of growth. This substantial early uptake could explain why 'NF48' was particularly effective against the seed-borne disease, smut, in the field trials. Further experiments are under way to investigate the patterns of uptake and translocation in more detail. Physical and chemical properties of the different compounds, such as solubility, octanol/water partition coefficients and pK values are also being measured with the objective of defining the properties required for efficient uptake. (Graham-Bryce and Nicholls)

### The Chemical Liaison Unit

As the Unit has become better established, increasing use has been made of its analytical services. The work done during the year has thus included many short-term investigations to solve urgent problems or to check pesticide treatments, as well as longer term investigations mostly planned in conjunction with biological departments at Rothamsted.

Examples of short-term studies include many analyses of seeds treated with BHC, ethirimol or mercury to help with investigations into the performance of seed-treating machinery or to check seed used in experiments. Many soil and crop samples have also been analysed. Thus unexpectedly small populations of wheat bulb fly in an experiment on its behaviour were shown to be due to the unintended use of dieldrin-dressed seed. Regular patches of virus-infected potatoes in an otherwise healthy crop were found to be caused by unsatisfactory application of phorate, while failure of diazinon to control lettuce root aphids was attributable to rapid metabolism in the soil. Other problems investigated have involved the analysis of aldicarb, benzimidazole fungicides, endosulfan, formothion and amine fungicides in soils, crops and pesticide formulations, the identification of carbaryl and MCPA in poisoned bees, the examination of deposits following a fire in a pesticides warehouse, and the analysis of oxygen and ethylene concentrations over stored potatoes.

Activities undertaken to facilitate the work of other departments include the purification of pesticides (e.g. quintozene for use in isolating strains of fungi) and the synthesis of $^{14}C$-labelled benzimidazole fungicides. The Unit has thus had to deploy a wide range of synthetic and analytical techniques in providing a service to those interested in the fate of pesticides. Projects forming part of the longer-term programme of the Unit are described below.

**Uptake of pesticides from soils by worms.** Previous work suggested that it might be possible to describe the distribution of pesticides between worms and moist soils using similar equations to those developed for partition between soils and water (*Rothamsted Report for 1973*, Part 1, 180). This was investigated further by keeping individual worms in jars of pesticide-treated soil and measuring the pesticide content of worms and soil at intervals.

Equilibration between soils and worms is slow, taking four to eight weeks, in contrast to the relatively rapid equilibration between living worms and water (4–8 h) or between worm solids and water, which is complete within minutes.

The slow equilibration in soil may be ascribed to the slow and only partial mixing of soil in the tests, which was accentuated by the formation of 'permanent' burrows by the test species (*Lumbricus terrestris*). When the soil was stirred at intervals equilibration was no faster although the concentration of the stable pesticide dieldrin in the worm was increased.

The slow equilibration between worms and soils makes the prediction of anything but the upper limits of concentration in worms (or other soil-inhabiting organisms) difficult because the pesticides may be metabolised as rapidly as they are taken up from the soil, which was the case with chlorfenvinphos. Equally, the concentration attained in the organism following application of a given amount of pesticide to the soil may be limited by slow degradation in the soil as with diazinon; in this case the concentration in soil decreases continually but the concentration in the worm rises to a maximum and then slowly decreases. (Lord)

**Prevention of moulding in damp hay.** Previous work (*Rothamsted Report 1973*, Part 1, 122) emphasised the need to improve the distribution of propionic acid during baling but did not account for the erratic distribution of mould through bales or the almost complete absence of acid from portions of individual grass stems, where moulds had grown.

To discover whether this was the result of metabolism of propionic acid, samples of hay containing 40% water were treated with 0·2 or 0·4% of propionic acid and placed in Dewar flasks. Concentrations of propionic acid were assayed at intervals.

Hay treated with 0·4% propionic acid did not mould and the concentration of the acid remained constant. However, hay treated with 0·2% propionic acid heated spontaneously with the development of moulding which was preceded by the almost complete disappearance of propionic acid. Heating and moulding were delayed by about five days compared with untreated hay.

In a parallel test, damp hay treated with propionic acid was incubated in loosely closed jars at 25°C. At 0·2%, propionic acid did not prevent uninoculated hay from moulding following degradation of the acid. Degradation was accelerated by inoculating the hay with spores of *Paecilomyces varioti* but not with *Fusarium culmorum* or an unidentified yeast. At 0·4%, propionic acid was not metabolised and prevented the hay from moulding whether or not it was inoculated. (Lord, with Lacey, Plant Pathology Department)

**Effects of repeated applications of pesticides on soil properties.** The intensive use of combinations of crop protection chemicals seems likely to increase, even on traditional low-value farm crops. The effects of such repeated treatments are not known and a long-term field experiment was therefore started at Rothamsted to create chemical reference plots treated annually with representative agricultural chemicals, singly and in all combinations. The chemicals chosen were benomyl, chlorfenvinphos and aldicarb incorporated into the seed bed and chlortoluron applied as a pre-emergence spray to continuous spring barley sown with untreated seed and given optimum fertiliser and a hormone weedkiller. Disease, residues and yield will be measured annually and effects on the soil microflora assessed by the Soil Microbiology Department.

The first year's results were much as expected although aldicarb (50% loss in six weeks) was rather more persistent than found previously and benomyl, determined as carbendazim, with an apparent half-life of about three months, was much less persistent than at Woburn. Benomyl reduced mildew by about 50% in June and by slightly less in early July. Aphids were virtually eliminated by aldicarb, although numbers were small even on untreated plots.

Benomyl increased yield by 15%; the best yields (6·9 t/ha) being given by combinations of benomyl with aldicarb or chlorfenvinphos. The herbicide, chlortoluron, which is recommended only for winter cereals, but has been used experimentally in spring cereals, reduced yield by about 10%, although there were no obvious signs of damage. As one of the objects of this experiment is to investigate whether repeated annual treatments affect degradation rates and whether several chemicals together have additional effects, we shall continue to use chlortoluron and determine any changes in its effects on yield. (Briggs)



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**Behaviour of 'N-Serve' in relation to its effects on nitrification.** 'N-Serve' has been extensively used as a nitrification inhibitor but no work seems to have been published on the behaviour of the chemical itself in the field. 'N-Serve' treated ammonium sulphate was either broadcast or placed 3 cm deep in bare soil at Woburn in May on two plots of the Soil Structure enclosure, with organic matter contents of 1·4 and 5·9%. More than 80% of the inhibitor volatilised overnight from the broadcast treatment, after which subsequent loss was slow. The small amount of inhibitor remaining caused no inhibition of nitrification. Nitrification was inhibited for at least nine weeks by the incorporated treatment. 'N-Serve' decayed steadily by an apparently first order process, 50% being lost after 28 days in the soil low in organic matter and after 50 days in the soil high in organic matter. 'N-Serve' is hydrolysed in soil to 6-chloropicolinic acid and other chemicals that are hydrolysed in soil are known to persist longer in soils with high organic matter contents.

Upward movement of ammonium N and 'N-Serve' over short distances was observed during the first week after incorporation when it was dry. Ammonium ions were more mobile than 'N-Serve' in the more strongly adsorbing soil high in organic matter. 'N-Serve' was leached more readily in the soil low in organic matter but in both soils the maximum 'N-Serve' concentration was within 2 cm of the application depth even after 140 mm of rain.

The usual method for treating small fertiliser samples with 'N-Serve' is to add 'N-Serve' solutions in an organic solvent to the fertiliser and evaporate the solvent in air or in a rotary evaporator. Tests showed that up to 25% of the 'N-Serve' applied in this way was lost overnight when treated fertiliser was left in a tray covered with a plastic sheet and up to 80% was lost when the rotary evaporator was used. Because 'N-Serve' is so volatile, it is therefore not possible to prepare treated fertiliser containing accurately known amounts of inhibitor by the solvent evaporation method and analysis of the product before use is essential. (Briggs)

**Incorporation of nematicide granules into soils.** Nematicide granules should be uniformly distributed in soil to a depth of at least 10 cm to minimise multiplication of potato cyst-nematode (*Rothamsted Report for 1973*, Part 1, 164). Of the methods used to incorporate nematicide granules into soil in the field, rotavation has usually reduced multiplication most, although increases in potato yields are often little affected by the method of granule incorporation. To determine the distribution of nematicide in a peat soil following incorporation by four different implements of 50 kg/ha 'Temik' 10 GC granules (containing 10% a.i. aldicarb), 5 cm diameter soil cores taken immediately after treatment were divided into horizontal sections and the concentration of aldicarb in each section measured.

The spiked rotavator incorporated granules to the nominal working depth of 15 cm, and the Roterra and reciprocating harrow to just below 5 cm. The distribution of granules between cores was similar for these three implements. In contrast, the spring-tine harrow gave a less uniform distribution to a depth of about 10 cm. (Bromilow and Lord)

## Development of analytical methods

***Chlortoluron.*** Gas-chromatography of chlortoluron proved difficult and was not considered a satisfactory basis for a residue assay method. High pressure liquid chromatography (HPLC) was more successful and a method for measuring residues of chlortoluron in soils using this technique has been developed, capable of measuring 0·2 ppm chlortoluron in Rothamsted and Woburn soils with very little clean-up.



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Chlortoluron is extracted from soil with methanol. The extract is evaporated to dryness and the remaining solids extracted with hexane. The hexane extract is then assayed by HPLC using a 0·5 m × 2 mm column with silica packing (10 $\mu$m particle size), 15% propan-2-ol in hexane as solvent and a u.v. detector at a wavelength of 240 nm. (Lord and Smith)

### Collaborative work with the Plant Pathology Department

The following collaborative work is described in the report of the Plant Pathology Department:

*The absorption and movement of benzimidazole fungicides in potato tubers and soil* (p. 240). (Austin)
*Top-roll of potato plants* (p. 241). (Austin, Gibson and Simkins)
*Assessing the bacterial contamination of tubers* (p. 238). (Austin, Lapwood and Legg)
*Sclerotinia rot of clover* (p. 234). (Austin and Jenkyn)
*Chemical control of ryegrass pathogens* (p. 233). (Austin and Plumb)

### Staff

P. H. Nicholls was appointed to help with work on physicochemical aspects of the behaviour of pesticides. S. Dickinson was appointed to a temporary post provided by the Ministry of Overseas Development to help with work on the storage and processing of coconut pollen. I. H. Williams from the Canada Department of Agriculture, Vancouver, completed a year in the department as a visiting worker and Dr. A. E. Smith from the Canada Department of Agriculture, Regina, arrived for a similar visit. Other visiting workers included Mr. Theodoros Broumas from the Ministry of Agriculture, Athens, and Dr. Iskandar Ajjan from the College of Agriculture, Lattakia, Syria. Sandwich course students who worked in the department were Angela Coates, Brenda O'Connor, Mrs. Valerie Rhenius, Pauline Ruggles, Dilys Seig, J. Hunt, J. J. Tinkler and I. P. Walker.

At the invitation of the Ministry of Overseas Development and the Coconut Industry Board, A. J. Arnold spent one 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. K. A. Lord spent four weeks in Tehran and shorter periods in Rome acting as a consultant to the Food and Agriculture Organisation in connection with a project to examine the fate of chemicals used to control locusts. K. A. Jeffs participated in the CIPAC symposium on seed treatment at the invitation of the organisers and visited research institutes in Wageningen. J. H. Stevenson was invited by the OILB Working Group on Beneficial Arthropods to discussions at Darmstadt on minimising harmful effects of pesticides. Several members of the department participated in the Third IUPAC Congress of Pesticide Chemistry in Helsinki: M. Elliott organised a symposium on pyrethroid insecticides; A. W. Farnham, N. F. Janes, and R. M. Sawicki presented papers and I. J. Graham-Bryce acted as Chairman of a session. With support from NRDC, M. Elliott spent three months working on the synthesis and metabolism of radio-labelled pyrethroids in the laboratory of Professor J. E. Casida, Division of Entomology, University of California, Berkeley, USA. He also contributed a paper to the American Chemical Society Symposium on Mechanisms of Pesticide Action in Los Angeles and was invited to present a paper on pyrethroid insecticides at a symposium on Insecticides for the Future organised by the Rockefeller Foundation in Bellagio, Italy. At the invitation of the organisers, I. J. Graham-Bryce participated in



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discussions of the EURATOM Contact Group Radioentomology in Wageningen and presented a paper at the 5th International Symposium on Integrated Control in Orchards at Bolzano, Italy. He also acted as a UK representative to the CENTO Conference on Toxicology of Pesticides in Tehran, contributing an invited paper. With financial support from the Ministry of Overseas Development, P. Etheridge, F. T. Phillips and G. C. Scott spent three months in Brazil conducting field trials on baits for leaf-cutting ants. They were based at the Centro de Pesquisas do Cacau (CEPEC), Itabuna, by kind permission of the Director, Dr. P. Alvim.

In collaboration with T. Lewis (Entomology Department) and Dr. S. A. Corbet (Westfield College), A. Mudd, F. T. Phillips, P. Etheridge and I. J. Graham-Bryce presented an exhibit on the use of behaviour-controlling chemicals for pest control at a Royal Society Conversazione. P. H. Needham organised an exhibit of the department's work on control of aphids at the Chelsea Flower Show. Several other members of the department helped to prepare and man the exhibit.

## Publications

### Thesis


1 Farnham, A. W. (1974) Genetics of resistance to insecticides in a pyrethrin resistant strain of houseflies. Ph.D. Thesis. University of London.


### General Papers


2 (Crane, E.) & Stevenson, J. H. (1974) Apicultural Abstracts. *Bee Research Association, 1949–74, A history of the first 25 years*, Chapter 7, 78–83. (Suppl. to *Bee World*, Vol. 55.)

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