ROTHAMSTED RESEARCH

Report for 1977 - Part 1

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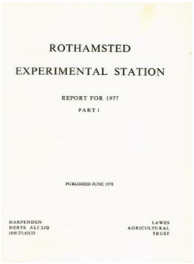

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Introduction

The two essential features of any satisfactory crop protection agent are efficacy against the target organism and freedom from adverse effects on non-target organisms. These

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characteristics depend on the intrinsic properties of the control agent and the way in which it is formulated and applied. We continue to seek improvements on both fronts.

Studies on structure-activity relationships of pyrethroid insecticides represent a major component of our programme under the first of these headings, facilitating the discovery of better toxicant structures. As the outstandingly potent and safe photostable pyrethroids recently discovered at Rothamsted begin to be introduced into practice, we have been seeking further clarification of the factors determining activity in this intriguing and important group. Toxicological properties are the outcome of three interacting processes: penetration to the site of action in the organism, metabolism and interaction at the critical site. There is now a substantial body of information on the structural requirements for activity which provides a basis for further detailed exploration by synthesis of candidate compounds. However knowledge of how activity is determined by the interplay of the three underlying processes is still extremely limited. This year we report new pharmacokinetic and physiological studies on the behaviour of pyrethroids in insects which have made good progress towards improving understanding of these factors.

Any long-term approach to crop protection should not only embrace the discovery of better control agents, but also seek to ensure that they remain effective. In practice the transience of both chemical treatments and host-plant resistance due to emergence of resistant or virulent strains of pest or pathogen has been a considerable shortcoming, to which we devote much attention. Recent work on aphids emphasises the need to evolve pesticide management strategies to prevent the proliferation of highly resistant strains on field crops; detailed studies with powdery mildew on barley illustrate the importance of competitive ability and pathogen genetics in determining whether insensitive strains become established and underline the need to consider each situation on its merits.

In seeking to improve efficacy and selectivity through formulation and method of application, the guiding principle is that the procedures adopted should ensure maximum transfer to pest organisms and minimum transfer to unintended recipients. As an essential basis for this approach, much of the work of the Department and the Chemical Liaison Unit is directed towards establishing the behaviour of pesticides in the environment and the characteristics of receiving organisms. Recently we have become increasingly concerned with foliar-spray treatments. One reason is the advent of controlled droplet application (CDA) systems based on the use of spinning-disc applicators. These provide a means of obtaining a much narrower drop size spectrum than traditional equipment which should make it possible to obtain better deposition and distribution and to reduce wastage. Preliminary studies and work on another unconventional technique, electrostatically charged sprays, are described in this year's report.

It has long been recognised that one method of improving transfer to soil-borne pests and diseases, which are often difficult to control, would be to employ compounds which were translocated downwards in plants following application to the foliage. Such a property has proved very elusive. In previous reports we have described two compounds, the growth regulator daminozide and the non-protein amino acid ethionine, which act in this way against potato common scab; work reported this year has done much to improve understanding of how they achieve their effects. More generally our capability in this and related fields has been greatly strengthened by the transfer of workers from the Unit of Developmental Botany at Cambridge and our programme is being expanded accordingly.

With regard to the parallel requirement for better information about pest incidence and movements, further progress in our work with behaviour-controlling chemicals is described this year. The use of sex attractants in traps for monitoring pest populations to improve timing of pesticide applications is illustrated by collaborative studies with the Entomology Department on pea moth. Other entries in the report demonstrate that our

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work in this field continues on a broad front. While extension of the approach to direct control by mass trapping or disruption of normal behaviour may still be some way off, this selective and environmentally acceptable method should undoubtedly be vigorously pursued.

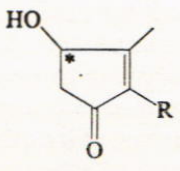
Insecticides

Relationships between molecular structure and insecticidal activity of pyrethroids

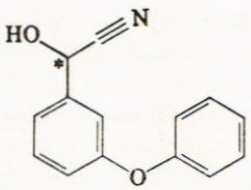
Relative activities of separate diastereoisomeric esters of chiral pyrethroid alcohols. Two important series of pyrethroid alcohols, cyclopentenolones (I) and α-substituted-benzyl alcohols (e.g. II) are asymmetric at the carbon atoms bearing the hydroxyl group. Natural and synthetic cyclopentenolones were examined before modern physical techniques were available; optical rotations were too small to give reliable indications of optical purity. The relative activities of esters from the two forms of (I) have therefore not yet been adequately characterised. Recently, esters of α-cyano-3-phenoxybenzyl alcohol (II) with unprecedented insecticidal activity have been discovered. For both theoretical and practical reasons it was important to establish with certainty the relative potencies of esters from the α-R and α-S isomers of (II). In a favourable case (α-RS-cyano-3-phenoxybenzyl 1R,*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate:NRDC 156) one isomer (decamethrin: 'NRDC 161') was obtained crystalline and identified as the S-isomer (*Rothamsted Report for 1974*, Part 1, p. 138, 169). However, neither the R-isomer, nor any of the corresponding diastereoisomers from other *cis* or *trans* dihalovinyl acids were as accessible in pure form.

More general separation methods using chromatography were therefore developed, exploiting the differences in physical properties between the two diastereoisomers in each mixture. Thin layer chromatography and high pressure liquid chromatography systems both gave satisfactory separations, the latter at loadings allowing rapid preparation of quantities (*c.* 20 mg) of adequate purity (>99%) for precise bioassay. Purities of the separated components were assessed and configurations assigned by ^{1}H NMR. Using the high-resolution 100 MHz spectrometer, signals are more completely separated than at 60 MHz, so that either the -CHCN (δ6·3–6·4), the vinylic (δ4·0–6·2), the cyclopropane (δ1·5–2·3) or the methyl (δ1·1–1·4) signals, as appropriate, gave the necessary information. Spectra from multiple accumulations, with low noise levels, detected impurities, if present, at the 1% level.

The results in Table 1 show that introducing a cyano group into the methylene of 3-phenoxybenzyl esters of this series decreases or increases the activity against houseflies, depending on whether it is in the R-configuration (1·1- to 60-fold reduction) or the S-configuration (2- to 16-fold increase). Further, in the *cis* series, the more active (α-S) component with X = chlorine has a potency very close to that of the bromo-analogue (decamethrin). Results with mustard beetles were similar. With houseflies, the fluoro compounds contrast with the other two halo series, in that the larger change in potency occurs when the cyano is introduced R (diminishing activity) whereas with chlorine and bromine, an R-cyano has a small effect, and an S-cyano increases activity considerably.



(I)



(II)

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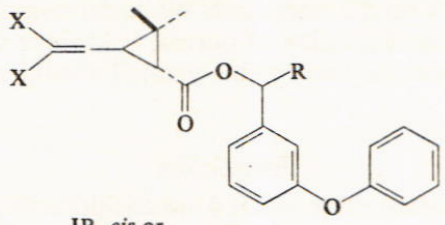


IR, *cis* or
IR, *trans*

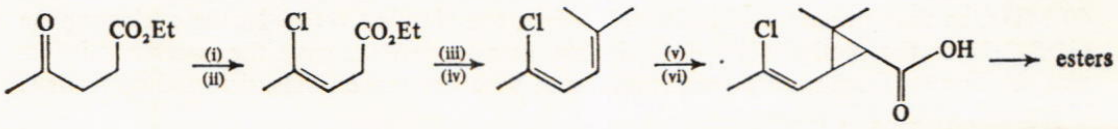
(III)

TABLE 1

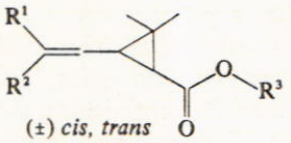
Relative potencies (molar basis) against houseflies (Musca domestica *L.) of analogues of decamethrin*

Stereochemistry in acid	X	Relative potency (bioresmethrin = 100) of compound with R = H	CN(R)	CN(S)	Factor for effect of introducing CN group R	S
1R, *cis*	F	170	3	350	x 0·017	x 2·1
	Cl	210	82	2400	0·39	11
	Br	180	48	2800 (decamethrin)	0·27	16
1R, *trans*	F	94	8	170	0·085	1·8
	Cl	88	81	1400	0·92	16
	Br	78	39	1100	0·50	15

3-(2-Chloroprop-1-enyl)-substituted pyrethroids. Chrysanthemates, with a $Me_2C{=}CH$- substituent at C-3 of the cyclopropane ring (see formula IV, above Table 2) and their dihalovinyl analogues (with, for example, a $Cl_2C{=}CH$-side chain) give highly insecticidal esters, but the intermediate compounds, with a $MeC(Cl){=}CH$-side chain, have not been reported. The new compounds were prepared by the following synthesis:



Reagents and techniques: (i) PCl_5; (ii) fractional distillation to remove two unwanted isomers; (iii) MeMgI; (iv) dehydration in formic acid; (v) t-butyl diazoacetate; (vi) H^+ (loss of isobutylene).



(±) *cis, trans*

(IV)

The diene reacted with t-butyl diazoacetate selectively at the more activated double bond, giving the cyclopropanecarboxylate as a *cis*/*trans* mixture. The insecticidal activities (Table 2) of the pyrethroid esters are intermediate between those of chrysanthemates and their dihalovinyl analogues, indicating that the response to changing substitution in this region of the molecule is consistent, as has been suggested by other results, for example, with the homologous series of 3-(Z-alk-1-enyl)-substituted esters. (Briggs *et al.*, *Pesticide Science* (1976) **7**, 236–240.)

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TABLE 2

Relative potencies of chrysanthemates and their chloro-analogues

Structure (see (IV)) R^1	R^2	R^3	Relative Toxicity (bioresmethrin = 100) to Houseflies	Mustard-beetles
Me	Me	5-benzyl-3-furylmethyl	42	38
Me	Cl	,,	44	110
Cl	Cl	,,	90	190
Me	Me	3-phenoxybenzyl	25	30
Me	Cl	,,	28	52
Cl	Cl	,,	60	100
Me	Me	α-cyano-3-phenoxybenzyl	60	50
Me	Cl	,,	78	140
Cl	Cl	,,	200	350

(Chemical work: Carson, Elliott, Janes, Johnson, Pulman and Soderlund. Biological work: Farnham, Freeman, Sandison)

Penetration and distribution of pyrethroids in cockroaches. Although understanding of relationships between molecular structure and biological activity of pyrethroids is reasonably well developed, attempts to correlate insecticidal potency against the American cockroach, *Periplaneta americana* (L.), with *in vitro* neurotoxic response as measured with axonal preparations (*Rothamsted Report for 1975*, Part 1, 155) or with giant fibre-cercal nerve synapse preparations (*Rothamsted Report for 1976*, Part 1, 164) have so far been unsuccessful. Overall structure-activity relationships reflect pharmacokinetic aspects of the intoxication process in addition to intrinsic potency, but little is known of the distribution and fate of pyrethroids in insects after application. In the only previously reported study of these factors, Burt *et al.* (*Entomologia experimentalis et Applicata* (1971), **14,** 255–269) measured the rate of penetration and total internal accumulation of pyrethrin I in *P. americana* dosed at LD90 (0·5 μg) but concentrations in the haemolymph and nerve cord of poisoned insects were below the limit of detection. The availability of optically pure ^{14}C-labelled pyrethroids of high specific activity allows a more detailed examination of penetration rates and internal distribution patterns of pyrethroids in *P. americana* after topical application.

The potent insecticide 'NRDC 157' (3-phenoxybenzyl 1R,*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) and its much less active 1S,*cis* enantiomer were prepared from ^{14}C-3-phenoxybenzyl alcohol and the appropriate acid chlorides. Insects were treated topically or by injection with solutions in ethyl methyl ketone and observed for signs of intoxication for up to 48 hours after treatment. Penetration was assessed by rinsing other treated insects in pentane at various intervals after treatment and measuring radioactivity in the washings. Distribution within the insect was determined by measuring radioactivity in haemolymph, nerve cord, fat body and midgut by dissection, homogenisation (except haemolymph) solvent extraction and cochromatography (haemolymph, midgut) or direct scintillation counting (nerve cord, fat body).

When applied topically at 0·17 μg g^{-1}, 'NRDC 157' (LD50 0·085 μg g^{-1}) produced symptoms of poisoning in all insects, first apparent as muscular fibrillation and incoordination (2–4 h after treatment) followed by diuresis (4–6 h) and paralysis (6–10 h). At the same dose the 1S,*cis* ester (LD50 > 20 μg g^{-1}) had no effect.

At 0·17 μg g^{-1} penetration of both isomers during the first 14 h after treatment apparently depended on the amount of ester remaining on the outside of the insect (pseudo first order kinetics, $t_{\frac{1}{2}}$ *c.* 10·5 h). The initial distribution pattern for both isomers was also similar with rapid equilibration in the haemolymph (within 2 h) followed by slower equilibration in the nerve cord and fat body. The 1S, *cis* isomer equilibrated in the midgut

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tissue also, but with 'NRDC 157' an initial influx in the first 4 h was followed by equilibration at a much lower concentration than with the lS,*cis* enantiomer. With NRDC 157, the effect of haemolymph volume reduction between 12 and 48 h due to diuresis was evident in the lack of further accumulation by the fat body after 12 h and in a large increase in haemolymph concentration between 24 and 48 h. Pretreatment of insects by injection with a 10-fold excess of the lS,*cis* ester delayed 'NRDC 157'-induced paralysis by 2 h in 50% of treated insects (ET50) but did not influence the onset of symptoms; pretreatment did not alter the penetration rate nor the distribution pattern of 'NRDC 157'. Average steady-state levels in the haemolymph and nerve cord for 'NRDC 157' were $1{\cdot}5 \times 10^{-7}$M and $1{\cdot}2 \times 10^{-7}$M respectively. This determination of haemolymph concentration provides a valuable indication of levels at which *in vitro* studies on neurotoxic action of pyrethroids should be conducted.

These results suggest that differences in insecticidal activity between lR and lS isomers of pyrethroids result from asymmetry at the site of action rather than from differences in penetration and distribution. The procedures developed provide a practical basis for further studies on the effect of structure and stereochemistry on the pharmacokinetic behaviour of pyrethroids in insects. (Soderlund)

Action of pyrethroids at cercal nerve-giant fibre synapses. Attempts to investigate the post-synaptic action of pyrethroids (*Rothamsted Report for 1976*, Part I, 164–5) were initially frustrated by difficulties in establishing a satisfactory presynaptic block. Irrigation with salines containing relatively high concentrations of magnesium (conventionally used to block synapses pre-synaptically) sometimes failed to abolish excitatory post-synaptic potentials (e.p.s.p.) completely and often caused partial post-synaptic depolarisation. Salines with very low calcium concentrations (0·5 mM) were more effective but caused the general condition of the preparations to deteriorate. Salts of heavy metals such as lead, blocked conduction pre-synaptically but also acted post-synaptically. Antimycin A, type III, which is said to interfere with transmitter release by releasing intracellular calcium, shows promise as a pre-synaptic blocker with little post-synaptic action.

Acetylcholine is thought to be the transmitter at the synapses under investigation, so investigations were undertaken to assess the efficiency of paraoxon and isopropylparaoxon as potentiators of acetylcholine applied to test the condition of the post-synaptic membrane. Isopropylparaoxon causes least side-effects but both compounds desensitise the post-synaptic membrane to some extent.

***Action of pyrethroids* in vivo.** Recent information on the probable concentrations of pyrethroids within poisoned insects suggests that most insect neurones so far tested are insufficiently sensitive to pyrethroids to constitute critical sites of action. In an attempt to identify more susceptible neurones it is therefore proposed to observe the progress of intoxication in living insects by means of implanted electrodes. Satisfactory preparations for such studies must fulfil the following requirements:

(1) setting-up should be reasonably easy and rapid,
(2) they should last long enough to enable recording to continue throughout the course of intoxication,
(3) nerve impulses in both afferent and efferent axons of the peripheral nervous system should be accessible, and
(4) the electrodes and associated wires should not interfere unduly with the movement of the insects.

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A disposition of electrodes in the metathoracic leg of the cockroach *Periplaneta americana* L. that largely fulfils these requirements is now available. (Burt and Goodchild)

Effects of synergists on pyrethroid activity in houseflies. A feature of some pyrethroid insecticides is that they can be greatly synergised by compounds that are comparatively non-toxic. During a study of the relationships between the chemical structure of pyrethroids and the extent of their potentiation by selected synergists some anomolous results were obtained. A possible explanation was variation in the time intervals between application of synergist and insecticide, so this factor was examined further.

Normally the synergist is applied first followed 1–3 hours later by the insecticide. The effect of time between treatments was investigated for the insecticides (1,R-*trans*)-tetramethrin, S-bioallethrin and bioresmethrin with the synergist n-propyl 2-propynylphenyl phosphonate ('NIA 16388') applied to a susceptible strain of houseflies. In each test 1 μg per fly of synergist was used with doses of insecticide from which LD50s were calculated for 20 time intervals ranging from 48 h after application of synergist to 16 h before (insecticide applied first). For each insecticide potency was greatest when the insecticide was applied approximately 3 h after the synergist. From this peak the decay in synergistic activity, assessed by treating with insecticide, took about 48 h and was the same for all insecticides. The rate of decay in insecticide activity detected by later application of the synergist was more rapid than that for the synergist but some effect of the synergist could still be observed 16 h after treating the flies with insecticide. The degree of synergism at this time was much less with bioresmethrin or S-bioallethrin than with (1,R-*trans*)-tetramethrin. This implies that these three pyrethroids are not completely inactivated within 16 h. Inactivation of (1,R-*trans*)-tetramethrin takes longer than that of either S-bioallethrin, which at peak activity is equally synergised, or bioesmethrin which is much less synergised. Comparison of results from such bioassays against susceptible and resistant insects with data obtained from biochemical and neurophysiological studies should provide useful information on the importance of metabolism in the poisoning process. (Farnham)

The nature and causes of resistance. Although there were relatively few aphids on sugar beet in 1977, and our monitoring of populations was much more limited than in 1976, examination of samples from different regions showed that moderately resistant aphids (classified as R_1) were almost ubiquitous. There was evidence that the stronger resistance (R_2) although still rare is becoming more widespread. Resistance will therefore again pose serious problems when aphid numbers increase and we have continued a major programme on its nature and ways of decreasing its impact. Short term studies have been aimed at identifying possible alternative aphicides, while the objective of our longer term work has been to suggest more fundamental solutions through better understanding of the underlying process. We have also continued our comprehensive investigation using houseflies into the general characteristics of resistance, paying particular attention to processes which might affect pyrethroids as the photostable examples become widely available for use in agriculture.

***Leaf-dip bioassay for susceptibility of* Myzus persicae.** A simple leaf-dip test was developed to measure the susceptibility of *Myzus persicae* to insecticides. Potato leaves are dipped for 10 s in different dilutions of commercially formulated insecticides, and left to dry for 1 h in a current of air. Single leaflets are then removed, placed in small plastic boxes and infested with 15 adult apterae or late instar nymphs. LD50 values are calculated from assessments of kill 24 and 48 h after treatment using two replicates at each concentration.

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This leaf-dip bioassay measures the combined effect of contact, systemic or behavioural action and therefore approximates to the situation in the field following spraying when migrants or aphids surviving direct interception of the spray come into contact with treated leaves. (Direct interception of sprays of the strength recommended by manufacturers kills even R_1 aphids).

So far results obtained with this test agree well with insecticide performance in field trials on sugar beet and potatoes. The tests have also shown that although some compounds kill all the adults they do not kill progeny that emerge between infestation and death. Further, the speed of action as measured by the time between first contact with the treated surface and death is much slower in resistant aphids. This implies that the aphids may not be killed sufficiently fast to prevent transfer of virus. This important point is being investigated further.

Response of susceptible and resistant* M. persicae *to selected insecticides in leaf-dip tests. Most of the organophosphates and carbamates now being proposed for aphid control on sugar beet kill aphids classified as R_1 on the basis of topical tests with demeton-S-methyl and biochemical tests almost as well as susceptible (S) insects in leaf-dip bioassays (Table 3). Indeed the carbamate 'AC 85258' is almost one third more active against R_1 than against S aphids, and demephion (I), a well established aphicide which closely resembles demeton-S-methyl (II) is very effective against both R_1 and S insects.

demephion-S $(MeO)_2.PO.S.CH_2CH_2.S.Me$ $(MeO)_2.P.O.S.CH_2CH_2.S.Et$
demephion-O $(MeO)_2.PS.O.CH_2CH_2.S.Me$

I. demephion II. demeton-S-methyl

The compounds tested are not however as effective against R_2 aphids (Table 3). These insects are not only much more resistant than R_1 aphids to organophosphates including demephion, they also resist to a lesser extent most of the carbamate insecticides with the exception of 'AC 85258' to which they are virtually susceptible. (Table 3). It has not been established yet whether the resistance of the R_2 aphids, which we again detected in samp-

TABLE 3

Resistance levels of moderately (R_1) and strongly (R_2) resistant Myzus persicae *of field origin to selected insecticides by leaf-dip bioassay*

Insecticide	Resistance factor (susceptible=1)			
	R_1		R_2	
Organophosphates				
Acephate	0·93	(3)*	2·3	(3)
Demeton-S-methyl	9·4	(4)	94	(4)
Demephion	0·94	(2)	9·4	(2)
Carbamates				
'AC 85258'	0·69	(2)	1·3	(2)
'Aphox' (pirimicarb)	1·3	(3)	5·3	(2)
'Croneton' (ethiofencarb)	1·3	(3)	3·1	(3)
'DPX 3853'	1·1	(3)	2·5	(3)
'Hoechst 25682'	4·7	(2)	83	(2)
Pyrethroids				
Decamethrin	5·6	(4)	1250	(4)
Permethrin	6·1	(5)	87	(4)
Cypermethrin	2·1	(2)	65	(1)
Permethrin/ Demeton-S-methyl (1 : 2)	0·85	(2)	95	(2)

*figures in brackets show number of tests

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les from Scotland and for the first time in a sample from Rothamsted farm, will be sufficient to have a significant adverse effect on control when these insecticides become more widely used.

Both R_1 and particularly R_2 aphids resist pyrethroids (Table 3). R_2 aphids appear to avoid contact with pyrethroids since many are found on the walls of plastic boxes, rather than on the treated leaf. This avoidance probably accounts for the large difference in resistance levels between leaf-dip tests (resistance factor at LD50 for permethrin : 87) and topical application tests (resistance factor for permethrin : 19).

Resistance to pyrethroids may be caused by the esterase responsible for resistance to organophosphates and carbamates. Mixtures of permethrin and demeton-S-methyl were therefore examined in the hope that the enzyme, which has an extremely high affinity for organophosphates, would bind preferentially to the demeton-S-methyl, so allowing the pyrethroid to exert its toxic action. However, no decrease in resistance was observed with either R_1 or R_2 aphids.

The role of increased carboxylesterase activity in resistance of* Myzus persicae *to insecticides. All strains of *Myzus persicae* resistant to organophosphate and carbamate insecticides examined so far have greater carboxylesterase activity (assayed with l-naphthyl acetate) than susceptible aphids. This is caused principally by changes in one of the seven esterases distinguishable by polyacrylamide gel electrophoresis. Simple esterase assays, using either crude homogenates or enzymes resolved electrophoretically from individual aphids, have therefore been used to detect the presence and degree of resistance based on this reliable correlation, although it had not been established that the enzyme was involved in resistance. Our studies have now, however, implicated the esterase in the degradation of insecticides.

The levels of resistance to insecticides in six clones of aphids parallelled their rates of hydrolysis of both l-naphthyl acetate and paraoxon (Table 4), suggesting that one enzyme hydrolyses both substrates, and causes resistance by decreasing the concentration of insecticide within the insect.

TABLE 4

Resistance, esterase activity and paraoxon degradation for different clones of Myzus persicae

Clone	Approx. resistance factor to dimethoate	Hydrolysis rate: l-naphthyl acetate (μmol h^{-1} mg^{-1} aphid)	Hydrolysis rate: paraoxon (pmol h^{-1} mg^{-1} aphid)
US1L	1	0·21	0·26
MS1G	8	0·42	1·32
French R	15	0·96	—
TIV	100	1·85	4·04
Pir R	250	3·87	7·07
G6	500	7·13	14·8

The involvement of a single enzyme is also suggested by the identical mobility on both polyacrylamide gel electrophoresis and ion-exchange chromatography of the esterase and paraoxon-degrading enzyme. The identity of these enzymes was confirmed by studying the properties of the purified esterase from resistant and susceptible aphids, and the kinetics of its reaction with the different substrates. l-Naphthyl acetate hydrolysis was strongly inhibited by very low concentrations of paraoxon (k_i, $0{\cdot}133 \pm 0{\cdot}008$ nM^{-1} min^{-1}) indicating competition between the two compounds for the active centre of the enzyme. However, the inhibited esterase recovered fully several h after removing excess paraoxon demonstrating that the phosphorylated enzyme is not stable and slowly

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hydrolyses paraoxon. Since hydrolysis of the phosphorylated enzyme is the rate-limiting reaction in its hydrolysis of paraoxon, the rate of recovery of esterase activity measures the ability of the esterase to degrade paraoxon. The first-order rate constant (0·009 min^{-1}) for this recovery was identical to the catalytic centre activity of the paraoxon-degrading enzyme (assayed with ^{14}C paraoxon as substrate) showing clearly that the hydrolysis of both l-naphthyl acetate and paraoxon is brought about by the same enzyme. This puts on a sound basis the use of carboxylesterase assays to detect and measure resistance of *M. persicae* to insecticides.

By comparing the catalytic centre activities of the enzyme purified from susceptible and resistant aphids, it was clear that the 60-fold difference between strains in its activity resulted from the presence of different amounts of the same enzyme, rather than the same amount of mutant enzymes with different substrate turnovers in each of the six strains. This must be caused by mutations of a regulatory gene, rather than a structural gene, an occurrence not observed previously in insects.

***Changes in esterase activity between generations of* M. persicae.** The esterase activity of strongly or very strongly resistant *M. persicae* (strains Pir R and G6) has been found to decrease or increase spontaneously from one generation to the next; such effects have not been observed with R_1 or R_2 aphids collected in the field. Parthenogenetically produced broods from adults with very active enzyme (40 or more times as active as that in susceptible (S) insects) often comprise individuals with enzyme activities varying from the parental level to only twice that in S insects. Decrease of esterase activity, which is much more frequent than increase, is usually step-wise and is conditioned by the activity of the parental enzyme; testing the clones of 96 aphids showed that fully resistant parents very rarely give rise to fully susceptible offspring and vice versa.

Loss of esterase activity has been attributed by Dr. R. L. Blackman (personal communication) to the variegated (V-type) positioning effect, since the highly active esterase has so far been found only in aphids with the A 1-3 chromosomal translocation. However the stability of the enzyme in strain TIV which has an elevated esterase activity and chromosome translocation suggests that other explanations, such as cytoplasmic inheritance are possible. (Devonshire, Moores, Petzing, Rice and Sawicki)

Resistance to pyrethroids in Danish houseflies. Houseflies of strain $153y^3$ kindly sent to us by Dr. J. Keiding of the Danish Pest Infestation Laboratory, are very resistant to pyrethroids, but differ in cross resistance characteristics from those used for studies on the mechanism *kdr*, described below.

The flies from strain $153y^3$ were collected in 1976 on the trial farm 153, which was treated in 1974 and 1975 with pyrethroids.

Isolation of individual chromosomes for the resistant strain showed that their strong resistance to pyrethroids is largely controlled by one or more factors on chromosome III. This resistance on chromosome III is fully recessive and resembles gene *kdr* in conferring cross-resistance to DDT and its analogues as well as to all pyrethroids tested, but is at least ten times stronger particularly to DDT. This new resistance is also unaffected by the usual synergists for DDT and pyrethroids. Since it resembles gene *kdr* so closely, it may be a more active allele of this resistance mechanism.

It seems probable that much of the strong resistance to pyrethroids recently detected by Keiding in his detailed studies on Danish trial farms may be caused by this new mechanism. (Sawicki)

Characteristics of resistance mechanisms in houseflies. The stability of the gene *kdr* that controls knock-down resistance and confers kill resistance to all pyrethroids and

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DDT, and the frequency at which it can be detected were investigated under laboratory conditions. Various substrains of strain 538ge with gene frequencies for *kdr* ranging from 0·004 to 0·5 were established. Strain 538ge is homozygous for *kdr* and for the visible markers brown body (*bwb*) and green eye (*ge*). Each factor is genetically recessive and located on chromosome III. The frequency of resistant flies in the substrains was estimated by submitting bioassay data to mixed population probit line analysis. The frequencies of *bwb* and *ge* were monitored for comparison. Resistance could not be detected with confidence once the frequency of resistant flies in the substrains fell below one in 500. This minimum detectable proportion corresponds with a gene frequency for the recessive factor *kdr* of approximately 0·05. The only satisfactory method of measuring gene frequencies for *kdr* less than this is to cross each substrain to 538ge, the strain homozygous for *kdr* and assess the proportion of resistant flies in the F_1 progeny. By this method the smallest gene frequency for *kdr* in a parent substrain that it is practicable to detect by careful bioassay technique is 0·002.

The stability of *kdr* on inbreeding the substrains without selection pressure starting from gene frequencies of 0·5, 0·25, 0·125, 0·016 and 0·004 was monitored. From an initial frequency of 0·5, the expected ratio of susceptible to resistant flies is 3 : 1. This was observed in the first two generations after establishment of the substrain, but drifted to 13·3 : 1 by F_8 which represents a gene frequency for *kdr* of 0·27, a decrease of 53%. The gene frequency in the substrain with an initial level of 0·25 was found to be the same after seven generations but in intermediate generations fluctuated between 0·21 and 0·38. After seven generations from an initial gene frequency of 0·125, the estimated level had fallen to 0·029. Thus there appeared to be no consistent pattern of increase or decrease in the gene frequency of *kdr* over seven or eight generations when the initial frequency was comparatively high. The other two substrains with much smaller initial frequencies were also inconsistent. The gene frequency was estimated after 30 generations of inbreeding. In one, it declined from 0·016 to 0·003 while in the other the change was only from 0·004 to 0·0045. These results demonstrate that although *kdr* can persist at low gene frequencies under conditions of no insecticidal selection pressure for many generations even in the comparatively small populations (up to 7000 adults) of laboratory cultures, it is difficult to predict precisely how it will behave in any particular regime.

The substrain with an estimated gene frequency for *kdr* of 0·004 (corresponding to a frequency of resistant individuals of one in 65 000) was subjected to selection pressure with DDT for five generations. Sequentially, mortality was 36%, 1%, 97·5%, 71% and 34% by which time the gene frequency for *kdr* rose to 50% showing how rapidly it may become re-established in a population. (Farnham and O'Dell)

Organophosphorus resistant acetylcholinesterase in houseflies and cattle ticks. Modified acetylcholinesterase, less sensitive to inhibition by organophosphates, is an important mechanism of resistance to these insecticides in houseflies and cattle ticks. The cholinesterase of both species has been shown to separate into multiple forms when subjected to analytical polyacrylamide gradient gel electrophoresis. In recent work this technique was used on a preparative scale to separate components of a housefly head homogenate incorporating 1% detergent ('Triton X-100'). Those parts of the gel containing acetylcholinesterase were homogenised and the enzyme extracted electrophoretically into dialysis tubes. Three forms with different molecular weights were identified, equivalent to monomer, dimer and hexamer. When re-examined on polyacrylamide gradients the monomer and dimer were stable but the hexamer reverted to a mixture of the two lower molecular weight forms. The hexamer retained its identity when 1% 'Triton X-100' was included in the extraction buffer of the second electrophoretic system. Recovery from the preparative electrophoresis was initially poor, only about 2%, but was subsequently

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improved to about 15% by reversing the polarity for the last 5 min of both the running and extraction electrophoresis steps. This procedure helped release the enzyme from the polyacrylamide matrix and the dialysis membrane. It should be possible to use this method to isolate the different forms of acetylcholinesterase in large enough quantities to study their kinetic properties.

DEAE cellulose columns used with various buffer systems and elution gradients, failed to resolve more than a single peak from either cattle tick or housefly homogenates whether or not detergent was present. A similar result was obtained with starch block electrophoresis which separates proteins on the basis of charge alone, in contrast to polyacrylamide gel electrophoresis which is influenced by molecular size. This indicates that molecular size provides the best basis for separating the different forms and supports the hypothesis that they represent aggregates of a basic catalytic unit. (Stokes and Devonshire)

Side effects of pesticides on beneficial insects

Poisoning of honeybees in the field. One hundred and nineteen samples of honeybees thought to be poisoned were received from beekeepers via the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food (MAFF), which also collected evidence to indicate how poisoning had occurred. Of the 71 cases where the presence of an insecticide or its effects were demonstrated (105 in 1976 and 86 in 1975), at least 43 samples involved anti-cholinesterase insecticides; dieldrin, HCH and DDT were also found. In about four-fifths of the poisoning cases, the probable circumstances of the application were identified.

The eight samples (two HCH, six anti-cholinesterase) associated with oilseed rape represent a substantial reduction compared with 1976 and 1975 (24 and 30 samples respectively), but there were also incidents where poisoning by triazophos applied to rape was suspected, but could not be substantiated.

Thirty samples were associated with cereal spraying compared with the same number in 1976 and 22 in 1975; again we suspect that these resulted from bees being attracted to aphid honeydew or flowering weeds, or flying over the crop during or after treatment.

As in 1976, there were small aphid infestations on field beans and a low incidence of poisoning (five samples).

Of the other incidents, malicious poisoning was suspected in four (2 HCH, 2 anti-cholinesterase) and poisoning due to use of insecticides on wooden hive parts or during comb storage in a further four (dieldrin and anticholinesterase). Three cases occurred because foraging bees flew across potato fields during spraying and one was associated with soft fruit. (Stevenson and Smart)

Detection of starvation in honeybees. The symptoms of poisoning and of starvation in honeybee colonies are often similar, namely accumulation of dead bees in front of the hive entrance. Early in the flight season, a large proportion of the samples of allegedly poisoned bees which we receive show no evidence of poisoning and we have suspected that many of the deaths were due to starvation. A method of distinguishing positively between these causes of death would therefore be of considerable interest.

Previous work showed that bees which are deliberately starved have very little glucose or fructose is their thoraces compared with healthy bees, while bees poisoned in the laboratory have intermediate amounts (*Rothamsted Report for 1976*, Part 1, 133). This year, we therefore measured levels of these sugars in the 21 samples of bees received before July 4th showing no evidence of poisoning; 14 of these samples had levels suggesting starvation. (Greenway, Smart and Stevenson)

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Behaviour of pesticides in soil and control of soil-borne pests. Some proportion of almost all the pesticide applied for crop protection reaches the soil, either by deliberate application as soil or seed treatments, or unintentionally by rainwashing, leaf fall or because the applied chemical misses the intended target. Knowledge of behaviour in soil can therefore make an important contribution to the two general objectives, efficacy and freedom from adverse side effects, underlying the Department's programme. We therefore continued to investigate both fundamental aspects of pesticide interactions with soil and practical treatments for controlling soil-borne pests.

Fundamental work on behaviour of pesticides in soil. Most pesticides are significantly adsorbed by the solid phase in soils. The detailed effects of such adsorption on biological availability and mobility depend to a considerable extent on how far the process is reversible, that is on whether the solid acts as a permanent sink for the pesticide or a temporary reservoir from which the compound will be released again when the concentration in the soil solution falls because of degradation, movement or uptake by soil inhabiting organisms. Quantitative information about rates of adsorption and desorption is needed for computer models currently being developed to simulate behaviour in soil. We have therefore examined the kinetics of these processes for the herbicide linuron.

Adsorption and desorption isotherms were determined at a range of time intervals for soil from Fosters fallow by the slurry method using radiotracer and u.v. analysis. Amounts adsorbed increased significantly between 30 min and 3 h and more slowly up to 24 h by which time equilibration appeared complete, giving a distribution coefficient of 4·5. For desorption there was no measurable change after 3 h when the distribution coefficient reached 6·7. The apparent hysteresis therefore depended on the equilibration time.

The observations also indicated that desorption was faster than adsorption which is contrary to most currently held views. However rates of equilibration can only be rigorously compared under strictly standardised conditions of concentration range and surface occupancy, requirements not met in previous investigations. We therefore undertook more detailed studies in which adsorption and desorption were compared over the same range of surface coverage and under the same driving force, as reflected by solution concentration differences. Pretreatment of the soils was also standardised and the experiments conducted at 5°C to minimise degradation. The results confirmed that for material partitioning readily between solid and solution, desorption is faster than adsorption, while also giving evidence of much slower adsorption processes. The reversibility results are difficult to explain on simple diffusion theory and the causes are being investigated further. (Graham-Bryce and Nicholls)

Permethrin seed treatments for controlling wheat bulb fly larvae. In further field trials undertaken this year, the principal objectives were to determine

(1) if use of stickers would increase the effectiveness of permethrin seed treatments against wheat bulb fly,
(2) whether permethrin doses could be decreased below those traditionally applied (about 0·1% a.i. to weight of seed) without significantly decreasing activity and
(3) whether the effectiveness of permethrin seed treatments would be greatly decreased by deep sowing.

The results of a short row trial on a sandy clay loam site with 17·3 million eggs ha^{-1} showed that none of the stickers tested (methyl cellulose, gum arabic, soya bean oil emulsion or 'Myrj 52') improved the effectiveness of permethrin seed treatment. However permethrin proved effective at low dosages: attack by wheat bulb fly was significantly decreased even when applied at the lowest rate of 0·025% a.i. to weight of seed. Also,

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although the activity of non-systemic seed treatment chemicals against wheat bulb fly is normally decreased by deep sowing of the seed, permethrin prevented larvae entering the plant even when the seeds were sown 10 cm deep. (Griffiths, Jeffs and Scott)

Seed treatments for controlling slugs in cereals. In laboratory tests to find treatments effective against slugs, individual grey field slugs (*Derocerus reticulatum*) were confined in glass tubes with seeds treated at 2 g a.i. kg^{-1} on damp cotton wool for 10 days. Of 31 compounds tested, only thiocarboxime gave less than half as many live slugs as the untreated controls at the end of the test. Methiocarb, γ-HCH, permethrin, copper oxychloride and copper sulphate gave 50–80% live slugs compared with controls. Several compounds prevented damage to seeds. On the 3rd day of the test, thiocarboxime, methiocarb, chlorfenvinphos, the copper salts, 'San 155', capsaicin, ioxynil and bromoxynil all gave less than half as many damaged seeds as the controls. However the effect persisted to the end of the test only with thiocarboxime, methiocarb, ioxynil and bromoxynil.

In tests with the garden slug (*Arion hortensis*) none of the compounds tested killed many slugs. On the 3rd day of the test, seed damage was less than 50% of that in the controls for seeds treated with copper sulphate, sodium pentachlorophenolate, 'SAN 155' and ioxynil, but the level of protection subsequently declined. (Scott)

Analysis of microencapsulated seed treatments. Microencapsulated formulations have potential advantages as seed treatments in reducing phytotoxicity and delaying release of the active ingredient; there is consequently increasing interest in their use for this purpose. For studying their performance appropriate analytical methods are needed. In the course of investigating seed treatment with suspensions of polyurea microcapsules containing dyfonate (nominal 48% a.i.) a procedure for rapid determination of the active ingredient was sought.

Comparison of a wide range of solvent systems showed that effectively complete extraction of the toxicant, assayed by GLC, could be achieved by shaking the formulation for 30 min with methanol, ethyl acetate or chloroform or for 24 h with acetone. The time required for extraction by acetone could be greatly decreased by procedures which assisted disruption of the microcapsules. Only 2 h was required with continuous mechanical shaking and the addition of glass beads, cereal seeds or anydrous sodium sulphate while blending in a Silverson homogeniser reduced this to 5 min plus 15 min further soaking. In all cases efficiency of extraction was assessed by comparison with standards obtained by grinding the formulation with sand and exhaustive extraction with hot acetone in a 'Soxhlet'.

Analytical methods for seeds treated with the microencapsulated formulation were investigated using wheat treated in the laboratory to give a nominal loading of 960 μg g^{-1} seed. Complete extraction was achieved by shaking 10 g portions of treated seed in stoppered flasks with 10 ml acetone for 2 h on a mechanical shaker. (Jeffs)

Control of leaf-cutting ants. Work on these important pests of Central and South America was maintained, with support from ODM.

Laboratory screening of insecticides for use in baits. The search for suitable insecticides with a delayed killing action over a wide dosage range continued on an opportunist basis as candidate compounds, mainly experimental, became available. None of those tested acted especially slowly but some had a very effective rapid toxic action at low doses. These included two novel compounds, 'San 197' and 'San 279' from Sandoz, Basle. (Etheridge)

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Development of liquid formulations for locally produced baits. Further laboratory work was done on emulsion concentrates for addition to locally produced matrices (see *Rothamsted Report for 1976*, Part 1, 172). Of the many emulsifiers tested for the preparation of these mayonnaise-like emulsion concentrates, 'Agral LN' gave the most stable emulsions. However, it was found possible to prepare stable emulsion concentrates of some insecticides such as dioxathion without the use of conventional commercial emulsifiers, provided the concentration of orange juice was sufficiently high. When mirex was the insecticide, benzene was required as solvent, and it was found that a small amount of polyvinyl alcohol as a thickening agent gave more stable emulsion concentrates than any emulsifier tested. Examples of such formulations are shown in Table 5.

TABLE 5

Formulations of emulsion concentrates for use in leaf-cutting ant baits

	Dioxathion	Mirex
Insecticide	30 g	25 g
Benzene	—	150 ml
Soya oil	400 ml	250 ml
Orange juice concentrate (7:1)	400 ml	400 ml
Propionic acid	20 ml	20 ml
Polyvinyl alcohol 9% suspension	—	20 ml

These laboratory studies have now reached the stage where thorough evaluation of the improved formulations in the field in South America is required. Comprehensive testing has not yet been possible, but results from a small scale trial by Dr. S. W. Robinson in Paraguay have been encouraging. An emulsion concentrate containing mirex, prepared at Rothamsted and mixed some months later with the locally available soya meal as matrix, eradicated *Atta sexdens* colonies completely in the test area. (Phillips, Etheridge and Martin)

Chemicals influencing invertebrate behaviour

The appointment of a senior chemist allowed some expansion in our broadly based programme on chemicals influencing invertebrate behaviour which seeks to find improved methods for controlling pests or managing beneficial insects. New work on aphids and bees is reported this year; we have also begun studies on slugs which are particularly difficult to control with conventional pesticide treatments and which are increasingly important pests, particularly where reduced cultivation methods are adopted. Studies on behaviour controlling chemicals are essentially multidisciplinary and much of our work is done in close collaboration with the Entomology Department. The description of these collaborative studies is divided between the reports of the two Departments.

Chemicals affecting aphid behaviour

Factors influencing settling and feeding. Adult *M. persicae* respond to various substances applied to the surface of a Parafilm membrane through which they can probe into an artificial diet. Extracts of aphid bodies applied to the membrane deter settling and larviposition. Fractionation of these extracts showed that the neutral and acidic lipids were active. Aphid fatty acids and related compounds were therefore tested for biological activity: the normal acids with chain lengths of C_8 to C_{13} were found to be deterrent while C_{17} and longer acids stimulated settling. Introduction of a second (ω) carboxylic acid group generally enhanced settling while unsaturation made little difference to the deterrent compounds but made the stimulatory ones less attractive.

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In tests where one half of the undersurface of a chinese cabbage leaf was treated with an emulsion of carboxylic acid (C_{11} to C_{13}) and the other half left untreated more aphids settled on the untreated half. However when whole plants at different stages of growth were sprayed with carboxylic acids and then infested with 5 aphids per plant there were only minor differences in the degree of colonisation. (Greenway and Griffiths)

Alarm pheromones. A volatile alarm pheromone is released from the defensive secretion produced at the cornicles in many species of aphid. The pheromone has been reported to consist of (E)-β-farnesene in several cases. However, by direct application of the cornicle secretion to gas-chromatography coupled with mass-spectrometry (GC-MS) several other compounds have been detected. Species investigated were *Myzus persicae*, *Aphis fabae* (two cultures), *Phorodon humuli*, *Megoura viciae* (two cultures), *Acyrthosiphon pisum* and *Sitobion avenae* (two strains). In the case of *Megoura viciae* the major volatile components of the secretion were found to be α-pinene, β-pinene and (E)-β-farnesene. Other sampling techniques were used to demonstrate that the α-pinene was not formed from β-pinene by thermal re-arrangement during analysis.

Responses to alarm substances were assessed by counting the proportions of *M. persicae* or *M. viciae* that left an infested piece of plant tissue (Table 6) when test material on filter paper was introduced nearby. Each species responded to the release of chemicals from crushed bodies of its own and the other aphid species and to a vacuum distillate of *M. viciae*. However only *M. viciae* responded to α-pinene or β-pinene, which occur in *M. viciae* but not *M. persicae*. Although the chirality of the pinenes in *M. viciae* has not been established chemically, results from the bioassay suggest that α-pinene is present as the (–) form. (Pickett and Griffiths)

TABLE 6

Responses of aphids to alarm substances

	Myzus persicae	*Megoura viciae*
Crushed aphids		
M. viciae	+	+
M. persicae	+	+
Distillate of *M. viciae*	+	+
(−)-β-pinene	−	+
(±)-α-pinene	−	−
(−)-α-pinene	−	+

Honeybee Nasonov pheromone. The secretion of the Nasonov gland in the honeybee *Apis mellifera* contains a volatile pheromone that is implicated in foraging and clustering behaviour. Major volatile components of the secretion were identified using a new technique involving analysis, by GC and GC-MS, of hexane injected into the unexposed gland of a single insect. The presence of (Z)-citral, (E)-citral, geraniol and nerolic acid as major components was confirmed and nerol and (E,E)-farnesol were identified for the first time. The relative amounts of the citrals, nerol and nerolic acid were estimated and the absolute amounts per honeybee of the most abundant components geraniol and (E,E)-farnesol determined by extraction of excised glands followed by GC analysis using camphor as internal standard. Synthetic mixtures were prepared on the basis of this information using compounds isolated from commercial products; preliminary biological studies demonstrated that these mixtures have pheromonal activity. (Pickett, with Williams, Entomology Department)

Pheromones of *Anagasta kuehniella* (Zeller). The observation that escapees from cultures of the hymenopteran parasite *Venturia canescens* (Grav) are able to locate

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cultures of their host *A. kuehniella* very rapidly suggested that the parasite responds to volatile chemicals emanating from cultures of its host. To investigate this further the volatile components from both a culture of *A. kuehniella* larvae and an equal quantity of culture medium without larvae were isolated. These components were obtained by pumping them from the cultures under vacuum at room temperature through a trap containing 'Tenax' in series with two empty glass traps cooled to −70°C. The volatile material absorbed on the Tenax was recovered by elution with a small quantity of hexane and that from the other two traps by extraction with ether. In preliminary bioassays only volatile material trapped by Tenax from culture medium containing larvae arrested the normally rapid locomotion of the parasite. This material is clearly different from the less volatile oviposition stimulant reported earlier (*Rothamsted Report for 1975*, Part 1, 165) in that it did not elicit an oviposition response or antennal searching of treated filter paper. Comparison by capillary gas chromatography showed that the volatiles from culture media with and without *A. kuehniella* larvae differ in composition indicating that the feeding larvae produce additional volatile chemicals which may be detected by their parasite.

Work on the identification of the less volatile oviposition stimulant continued. (Mudd)

Slug behaviour. Slugs may find food by means of olfactory cues provided by volatile food components. The response by slugs to food plant volatiles was investigated using a glass tubular Y-maze with and without air flow. The field slug *Deroceras reticulatum* appeared to respond positively to the vapour from crushed carrot root when the air flow was employed. However in many experiments no choice was made. An alternative technique was devised in which volatile components were first isolated from the test material by vacuum distillation. The aqueous distillate was then presented to the slug as a trail on moist filter paper. Response to test material was expressed in terms of distance followed along the trail compared with total length of trail. Plant material examined so far is listed in order of increasing response elicited with *D. reticulatum*; pea plant, maize meal, carrot root, lettuce leaf.

A major volatile component from lettuce was identified as (Z)-3-hexen-l-ol. At optimum concentration this compound elicited a response corresponding approximately to half the activity of the total lettuce volatiles as measured in the trail-following bioassay. (Pickett, with Stephenson, Entomology Department)

Equipment and techniques

Formulation: Bird repellents. In a continuation of the collaborative work with Long Ashton Research Station (*Rothamsted Report for 1976*, Part 1, 174), a further small trial was done using an experimental compound, 'PP. 199' (I.C.I. Ltd) thought to be more effective than either thiram or anthraquinone as a bird repellent. Five experimental formulations were used in the form of aqueous sprays applied by knapsack sprayer. These were: (i) a colloidal suspension (prepared by I.C.I.), (ii) the same suspension with 'Acronal 4D' added as a sticker, (iii) the same suspension with methyl dibutylamine stearate added to prevent losses by rainwashing, (iv) a microcapsule formulation, with the active ingredient dissolved in acetophenone and encapsulated within polyurea walls (prepared at Rothamsted), (v) the same microcapsule formulation with Acronal 4D as sticker. Each treatment was applied to young Victoria plum trees, redcurrant and blackcurrant bushes growing in large bins which were distributed, together with untreated controls, over an area where bird damage could be expected. All treatments showed 50% reduction in bud loss during the first week after application and there was about 20% reduction in bud loss over 6 weeks with the first two treatments which were situated in a

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part of the area where most damage to controls occurred. (Phillips and Etheridge, with Drs. B. D. Smith and D. A. Kendall, Long Ashton Research Station)

Insect neuroanatomical techniques: improved synthetic substitute for aged alcoholic Bouin fixative. Continued histological testing of the synthetic substitute for aged alcoholic Bouin fixative (*Rothamsted Report for 1976*, Part 1, p. 175) confirmed that it gave results in conjunction with the Bodian silver stain fully as satisfactory as the normal fixative aged for 40 days at 60°C. Other experiments showed the improved fixation brought about by ageing the normal fixative to be due chiefly to the changes in the concentrations of its primary components, ethanol, acetic acid, formaldehyde and picric acid, rather than to the presence of reaction products. Analysis of the effects of each primary component revealed that, within limits, decrease in ethanol concentration and increase in acetic acid improved preservation and staining of the nerve cells, while decrease in formaldehyde reduced staining of the glial tissue surrounding them; alterations in picric acid concentration had little effect. From these results a modified synthetic fixative was developed, containing 25% ethanol:5% acetic acid in place of the 35:3·4% in the original synthetic mixture. This modified fixative has so far given much improved preservation and staining of both cockroach and locust nerve tissue, permitting some structures to be interpreted clearly for the first time. It is now being tested to determine its keeping properties and effectiveness with other insect species. (Gregory)

Pesticide spraying techniques. Traditional hydraulic sprays for applying pesticides produce a wide range of drop-sizes which may waste material in unnecessarily large drops and create a danger of environmental contamination by drift of excessively small drops. There is therefore much interest in the development of improved methods which will provide a drop size more closely related to the intended objective and ensure better delivery to the target.

Electrostatically charged sprays. In further development work with the electrostatic spraying system described last year (*Rothamsted Report for 1976*, Part 1, 175) the penetration and distribution of electrostatically charged drops in crop canopies was examined in field trials with field beans and sugar beet. The effect of charging was determined using tractor mounted rotary atomisers delivering water-based sprays at 31 litres ha^{-1}, and results were compared with those obtained with a conventional hydraulic atomiser system delivering 560 litres ha^{-1} as a standard for reference. Deposition of permethrin, used as a tracer, was determined by GLC.

Charging increased gross deposition by 50 to 100% compared with similar uncharged sprays. Gross deposition of the charged spray was further improved when only a single atomiser was used, indicating some interaction between spray heads. Deposition on the underside of the upper leaves of beans was also greater, but this effect was only significant for the comparison between a single charged atomiser and the conventional farm sprayer.

When the field beans were approximately 25 cm high deposition of the charged spray was almost equally divided between the upper and lower leaves, but when the crop had reached a height of 1 m the increased deposit was almost entirely located on the top leaves. (Arnold and Pye)

Controlled droplet application. The disadvantages of conventional hydraulic spraying are greatly decreased by the use of recently-developed controlled droplet application (CDA) systems based on rotary atomisers which allow much greater control of drop size. These systems will undoubtedly be used extensively for applying insecticides and fungicides to agricultural crops but at present there is little knowledge of the optimum drop

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size, composition and density for different crop protection problems, in relation to pesticide properties and formulation which would enable the technique to be exploited most effectively. As a contribution to obtaining such information we have begun investigations into the control of powdery mildew on barley by foliar sprays of systemic fungicides, currently applied at conventional medium-volume rates.

In preliminary field experiments a spinning disc applicator (Micron Herbi by Micronsprayers Ltd.) fixed to a tractor mounted boom was evaluated. Oil-based formulations consisting of 70% Risella oil and 30% 'Shellsol A' by volume and containing either 40 or 80 ml technical tridemorph l^{-1} (giving spraying solutions of viscosities 15–20 centistokes) were applied at 7·8 litres ha^{-1} with droplet size approximately 250 μm. These treatments were compared with a conventional hydraulic spray containing 'Calixin' (75% tridemorph) applied at 330 litres ha^{-1}. Mildew levels were low and differences between treated and untreated plots were small, but useful experience of the technique was gained. Future work will include detailed assessment of both placement and drift methods of application using the CDA system. (Etheridge, Phillips, Arnold and Pye)

Equipment for mass pollination of coconuts. Further development of equipment was undertaken with support from ODM.

The new apparatus developed for the cracking of the male flowers prior to drying was evaluated and found satisfactory in all respects. All feed problems have been eliminated and the improved cracking effected by this system increases the drying rate by approximately 10%.

Evaluation and development of the coconut flower stripper and liquid pollen application system continues. (Arnold and Nixon)

Mass spectrometry. During the year the department acquired a new double focussing mass spectrometer; the 'MM 70–70 F' by VG Micromass, Altrincham, Cheshire. This instrument has many advantages over the spectrometer it replaces and incorporates some of the most significant recent advances in the technique. In addition to allowing mass spectra to be obtained conventionally with ease, the instrument is quickly converted for on-line analysis of gas chromatographic effluent. A fast pumping system enables glass capillary columns to be linked directly to the mass spectrometer source, when compounds in sub-nanogram quantities can be isolated and detected by use of an integrating ion monitor. A mass marker provides unit mass marking on spectra obtained with a u.v. oscillograph. The instrument is also equipped for chemical ionisation mass spectrometry which can give relative enhancement of parent ions. A new field ionisation and desorption source is at present being fitted which will enable mass spectra of certain non-volatile compounds to be obtained. A mestatable scan unit allows linked scanning of the magnetic and electrostatic fields to provide parent ion and daughter ion spectra. (Pickett, Greenway and Mudd)

Insect rearing. The following species were reared:

Homoptera	*Aphis fabae* (Scop.)	
	Macrosiphum euphorbiae (Thos.)	
	Myzus persicae (Sulz.)	
	Strains.	Susceptible
		Resistant (several)
	Megoura viciae Buckt.	
Coleoptera	*Phaedon cochleariae* (F.)	
Orthoptera	*Blaberus cranifer* (L.)	
	Periplaneta americana (L.)	
Diptera	*Drosophila melanogaster* (Meig.)	
	Strains.	Normal
		Vestigial wings

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	Musca domestica (L.)	
	Strains.	A wild-type susceptible strain
		ac; ar; bwb; ocra—called 608, a multi-marker susceptible strain.
		SKA-diazinon selected, very resistant to many organophosphorus insecticides.
		Several strains derived from SKA, each with one or more factors of resistance to organophosphorus insecticides or DDT.
		Several strains derived from the dimethoate resistant $49r_2b$ each with one or more factors of resistance to dimethoate and other organophosphorus insecticides.
		49PPB, a substrain of $49r_2b$ derived by selection with pyrethrum extract and piperonyl butoxide.
		290BIO, a substrain of the dimethoate/bioresmethrin resistant 290rb derived by selection with bioresmethrin.
		NPR—pyrethrum extract selected strain.
		Several strains derived from NPR each with one or more factors of resistance to pyrethroids and DDT.
		Several strains derived from 290BIO each with one or more factors of resistance to pyrethroids and DDT.
	Delia antiqua (Meig.)	
Hymenoptera	*Acromyrmex octospinosus* (Reich)	
	Atta cephalotes (L.)	
	Aphidius matricariae Haliday	
	Venturia canescens (Grav.)	
Lepidoptera	*Plutella xylostella* (L.)	
	Anagasta kuehniella (Zeller)	

Fungicides

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

Plate tests. *In vitro* toxicities against *Streptomyces scabies* (the causal organism of potato scab) were measured in plate tests using 'poisoned' Czapek-Dox agar. In each test, 15 colony diameters were measured after incubation for 3 weeks at 25°C.

DL-homoserine and the following analogues and homologues of ethionine failed to decrease growth of one isolate of *S. scabies* at the molar equivalent of 100 ppm of ethionine (S-ethyl-homocysteine): L-cysteine, S-methyl- and S-ethyl-L-cysteine, DL-methionine-S-methylsulphonium chloride ('methyl methionine'), DL-2-hydroxy-4-methylthio butyric acid ('DL-methionine hydroxy analogue'), DL-ethionine sulphone, DL-methionine (S-methyl-DL-homocysteine), S-allyl-, S-isopropyl-, S-n-butyl-, S-hexyl-, S-phenyl- and S-benzyl-DL-homocysteine. DL-Homocysteine (as thiolactone hydrochloride) had a very slight effect. In these tests, DL-ethionine at 100 ppm restricted colony diameter to less than 4% of that on untreated agar.

In other similar tests the effects of DL-ethionine on growth of *S. scabies* were measured in the presence of a range of concentrations of DL-methionine, DL-homocysteine or DL-homoserine. Three isolates of *S. scabies* all gave similar results: at each concentration of DL-ethionine (20, 100 and 500 ppm), the added acid decreased its toxic action. DL-methionine was the most effective antagonist; when present at the molar equivalent of DL-ethionine, it restored growth to about 90% of that on untreated agar. DL-homocysteine and DL-homoserine which (in their L-forms) are intermediates in the synthetic pathway to methionine, also antagonised the action of DL-ethionine, but to a lesser extent. (McIntosh, Bateman and Burrell)

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Other laboratory tests. Laboratory studies on the modes of action of ethionine and daminozide against potato scab, using plants (cv. Majestic) from growth rooms when necessary, gave the following results.

Ethionine, applied as a foliar spray, accumulated in tubers in concentrations sufficient to inhibit the growth of *S. scabies* in culture. The *n*-propyl, *n*-pentyl and *n*-octyl esters of ethionine penetrated potato leaf cuticle much more easily than ethionine itself, and were rapidly hydrolysed to ethionine after penetration.

Measurements on the metabolism of U-^{14}C-homoserine by *S. scabies* indicated that ethionine inhibits the synthesis of methionine. However, it is not clear whether this is due to a general inhibition of metabolism, or a specific effect on methionine biosynthesis. A general inhibition could occur if ethionine replaced methionine in protein synthesis, with formation of abnormal proteins.

The results of these various studies suggest that ethionine behaves as a systemic fungicide, acting by interfering with methionine metabolism by *S. scabies*.

Daminozide did not appear to have any effect on primary metabolism of *S. scabies* at concentrations that could occur in tubers after foliar application; nor did it affect the wound response of tubers. The available evidence suggests that its action against scab is indirect, its primary effect being to increase the resistance of the plant to infection by *S. scabies* or to prevent the formation of scabs. (Burrell)

Glasshouse tests against potato scab. Chemicals were applied as foliar sprays to young potato plants (cv. Majestic) in pots containing scab-infected soil. In some tests, heights of plants were measured after spraying and in all cases scab indexes were measured at harvest.

The action of daminozide in decreasing stem extension is thought to involve inhibition of gibberellin synthesis in the plant. Consistent with this, adding small amounts of gibberellic acid (GA_3) to daminozide sprays counteracted the dwarfing effect of daminozide. Thus, in two tests, mean heights (cm) of plants 3 weeks after the spray treatments indicated were: (*a*) unsprayed, 52; (*b*) 0·6% daminozide alone, 31; (*c*) 0·6% daminozide plus 0·005% GA_3, 49; (*d*) 0·005% GA_3 alone, 58 (LSDs : 3 at $P = 0{\cdot}02$ and 5 at $P = 0{\cdot}001$). However, the figures for scab incidence in the same two tests did not give such a clear picture of antagonism; the corresponding mean scab indexes were: (*a*) 7·7; (*b*) 4·2; (*c*) 5·7; (*d*) 4·2 (LSDs 2·2 at $P = 0{\cdot}05$ and 2·9 at $P = 0{\cdot}01$). Each chemical separately significantly decreased the scab index, but the mixture did not.

In two tests, foliar sprays of the systemic fungicide triadimefon (0·5%) did not decrease scab incidence. (McIntosh)

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

The results confirmed earlier work with DL-ethionine, which was applied at three concentrations in each of four tests. Table 7 shows mean fresh weights of tops and clubs at harvest. All concentrations of DL-ethionine decreased club weight; the 0·25% sprays also increased top weight, but this effect on tops was masked by slight damage (chlorosis) at 0·5%.

Tests of isomers and analogues gave the following results. L-ethionine was more effective than D-ethionine; the *n*-propyl and *n*-pentyl esters of DL-ethionine were no more effective than the acid itself on a molar basis; and the *n*-octyl ester was very phytotoxic.

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TABLE 7

Effects of two foliar sprays of DL-ethionine on cabbage plants in soil inoculated with P. brassicae

Treatment	Fresh weight (g) of tops	clubs
0·5% DL-ethionine sprays	43	3·7
0·25% DL-ethionine sprays	54	6·4
0·125% DL-ethionine sprays	47	7·8
Inoculated, unsprayed	42	13·4
Uninoculated, unsprayed	57	—
LSD, $P = 0·05$	5	1·7
$0·001$	8	2·9

By contrast, the analogues used in the plate tests described above (excluding DL-homoserine and S-hexyl-, S-phenyl- and S-benzyl-DL-homocysteine) did not damage the plants or affect the disease as 0·5% or 0·25% sprays.

In tests of compounds with known systemic action against other diseases, triadimefon (0·25%) decreased club weight but injured the plants and 2-pyridinethiol-l-oxide (0·1%) and triforine (0·05%) were ineffective.

In a previous report (*Rothamsted Report for 1975*, Part 1, 170) several growth regulators were recorded as being inactive against clubroot as foliar sprays. However, re-testing showed that the morphactin flurecol (as *n*-butyl ester) was in fact quite active. In four tests with 0·01% sprays, mean fresh weights (g) at harvest from sprayed and unsprayed plants were, for tops, 36 and 35 (LSD : 4 at $P = 0·05$) and, for clubs, 12·1 and 16·9 (LSD : 3·4 at $P = 0·001$). The sprays caused a characteristic change in the pattern of leaf growth, but did not affect their weight. The decrease in club weight was less than from ethionine sprays, but the flurecol sprays were much more dilute (0·01% compared with about 0·25% ethionine for optimum effect). As with ethionine, stronger sprays of flurecol injured the plants. Preliminary tests indicated that the closely related chlorflurecol was more active than flurecol itself. (McIntosh, with Macfarlane, Plant Pathology Department)

Soil and seed treatments for soil-borne diseases of wheat. Soil treatment with systemic fungicides is being investigated as a means of controlling soil-borne diseases of wheat. The main problem is likely to be the maintenance of a sufficient concentration of fungicide in the root or shoot base throughout the long period in which the plant is susceptible to infection. Preliminary work was done using powder formulations mixed with soil in pots; fungicide availability and systemic uptake were measured by a bioassay in which infection was assessed after localised application of *Fusarium culmorum* to the shoots of wheat seedlings. Using this method thiabendazole was found to be still effective after 24 weeks in the soil, but benomyl began to lose effectiveness after 16 weeks and thiophanate methyl after 6 weeks. Thiabendazole and benomyl, the most persistent of the fungicides studied, were also incorporated into gelatine granules. Both powder and granule treatments significantly reduced infection in the *Fusarium* bioassay and in pot experiments with *F. culmorum* footrot (developed from soil inoculum) and eyespot. Effectiveness against take-all is now being investigated. The persistence of granular formulations of benomyl is also being studied further in pots and in the field.

Uptake of fungicides from seed treatments was determined by a bioautographic method (Graham-Bryce and Nicholls, *Rothamsted Report for 1975*, Part 1, 174). Uptake was greater from thiabendazole and benomyl treatments than from carbendazim and

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thiophanate methyl, in agreement with results from the *Fusarium* bioassay method (*Rothamsted Report for 1976*, Part 1, 180). (Bateman)

Studies on insensitivity to fungicides

Monitoring of sensitivity to ethirimol. Thirty single pustule isolates were bioassayed during the year for sensitivity to ethirimol ('Milstem') and tridemorph ('Calixin'). Many isolates were from 'Milstem' treated crops, but the range of ethirimol sensitivity was similar to that encountered in previous years. Isolates were not routinely bioassayed against tridemorph until this year, and data available prior to 1977 are limited. However, none of the isolates examined this year was significantly less sensitive to tridemorph than isolates available from previous years.

Competitive ability of an ethirimol insensitive mildew strain in the field. Field plots of barley grown from seed treated with 'Milstem' at up to four times the commercial rate were inoculated with six mildew strains having a range of ethirimol sensitivities. The weather in early spring was not favourable for mildew development, and the epidemic was not established until the beginning of June, when ethirimol levels within the plants had declined. Although the intensity of the epidemic was not severe, only the highest rate of 'Milstem' gave significant mildew control. This control was accompanied by a decrease in ethirimol sensitivity in the mildew population. When characterised on a series of barley differentials, 14% of the single pustule isolates from these plots resembled the most insensitive strain used in the inoculum. In early July, when it was no longer possible to detect differences in ethirimol sensitivity between plots, 400 single pustule isolates from treated and untreated plots were characterised. Less than 1% of the isolates from plots receiving the highest 'Milstem' level then resembled the most insensitive strain, a frequency no different from that in untreated plots, or in the natural mildew population. Clearly, the selection pressures used in this experiment were not sufficient to maintain this particular ethirimol insensitive strain for long after it was introduced into the plots.

Genetics of ethirimol insensitivity. Twenty eight progeny were obtained from a cross between a sensitive ($V_{as}+$) and an insensitive ($+V_g$) strain of mildew. Each virulence gene segregated independently but the progeny could not be separated into classes on the basis of ethirimol sensitivity. Instead sensitivity was normally distributed, with a mean close to the mid-point between the ED50's of the two parental types, and extremes which exceeded the sensitivity of either parent. An analysis of variance from replicate bioassays of each progeny showed that ethirimol sensitivity was genetically determined, and that its heritability was 60%. A similar analysis of progeny from a second cross (sensitive; $V_{as}+$x insensitive; V_{as} V_g) gave almost identical results. Thus, ethirimol sensitivity seems to be controlled by a complex heritable system, and not simply by one or two major genes. The high level of heritability indicates that insensitivity might increase rapidly if selection is applied when the sexual spores (ascospores) germinate. However insensitivity has not increased dramatically in the field since the introduction of ethirimol, perhaps because sexual reproduction is not important in natural populations in Britain where most reproduction is probably clonal.

Table 8 relates ethirimol sensitivity to the virulence genes segregating in the progeny. From this data there is no evidence for an association between ethirimol sensitivity and ability to grow on the barley varieties Julia or Sultan. If such an association exists in field populations, then some measure of restricted gene flow must operate.

Mode of action of ethirimol. In addition to those analogues listed in last year's report (*Rothamsted Report for 1976*, Part 1, p. 181), 6-methylaminopurine, 6-dimethylpurine,

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TABLE 8

Ethirimol sensitivity and virulence

			No progeny	Mean ED50 log $\mu g\ ml^{-1} \times 10^8$	LSD 5%
Avirulent on Julia	(+)	Cross 1	14	2·615	0·469
Virulent on Julia	(V_g)	Cross 1	14	2·430	
Avirulent on Julia	(+)	Cross 2	16	2·258	0·432
Virulent on Julia	(V_g)	Cross 2	12	2·217	
Avirulent on Sultan	(+)	Cross 1	16	2·654	0·475
Virulent on Sultan	(V_g)	Cross 1	12	2·359	

6-methylmercapto purine and 4-aminopyrazolo (3,4-d)-pyrimidine (an adenine anti-metabolite) also showed similarities with ethirimol in the way that they inhibited mildew. Ethirimol resistant strains were cross-resistant to these analogues.

Incorporation of ^{3}H-adenine into RNA by an ethirimol sensitive strain during appressorial formation was almost totally inhibited by ethimirol. A similar degree of inhibition in resistant strains was only obtained if higher fungicide levels were used. Incorporation of 2-^{3}H glycine or U-^{14}C glycine into acid insoluble material was also inhibited. However whereas glycine enters acid insoluble protein largely unaltered, both hydrogens on carbon-2 are lost during purine biosynthesis and so are not incorporated into nucleic acids. Furthermore, incorporation of both glycine isotopes was prevented by the protein synthesis inhibitor cycloheximide, but was unaffected by azaserine which inhibits pure biosynthesis. It seems that purine biosynthesis is not essential during appressorial formation and that ethirimol acts at some later stage of adenine metabolism. (Hollomon)

THE CHEMICAL LIAISON UNIT

The unit continued to investigate the efficiency of methods of applying pesticides and to measure residues in crops and soils in support of the work of several departments at Rothamsted and other organisations. A developing area of collaboration is the investigation of fungal metabolites as a means of detecting decay, identifying organisms and monitoring their toxic products. These investigations depend on the availability of suitable analytical methods, the development of which comprises an essential part of the Unit's programme.

The studies on practical problems are underpinned by a longer term programme on the behaviour of crop protection chemicals in soils and plants. Understanding of leaching and degradation is now sufficient to allow the development of computer simulation models for predicting behaviour in soils from basic information about pesticide properties, soil conditions and weather; results collected by the Unit are now being used to evaluate such models. The Unit has also been directing more attention to the degradation and metabolism of chemicals, especially in soils.

Analytical techniques

Assay of tecnazene on potatoes. A method for analysing the sprout suppressant tecnazene, on potatoes was developed. The chemical is extracted from the surface of individual potatoes by shaking with ethanol for 3 h. Tecnazene in the extract is then assayed by high performance liquid chromatography (HPLC) using a 0·5 m column packed with 'Permaphase ODS', and 45% ethanol-water as the mobile phase; the eluent is monitored at 210 nm using a Cecil 212 u.v. detector. (Cayley, Lord and Manlove)

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Analysis of anthraquinones and identification of fungi. Anthraquinone pigments often occur as metabolic products of fungi, frequently giving rise to bright colours in the mycelium. One fungus producing such pigments, *Phoma exigua* var. *foveata* is responsible for the potato tuber rot, 'gangrene'. It is sometimes difficult to distinguish gangrene rots from 'dry rot' caused by *Fusarium* spp. Mosch and Mooi (*Netherlands Journal of Plant Pathology* (1975) **81,** 86–88) developed a thin layer chromatographic procedure to separate the anthraquinone pigments produced by *P. exigua* var. *foveata* in rotted potatoes and reported that the major component of the pigment was chrysophanol. However, their method does not distinguish between the anthraquinones chrysophanol and pachybasin. We have used a liquid chromatographic method to show that pachybasin is the major component of the pigment produced by 20 different isolates of *P. exigua* var. *foveata* even though the amount of pigment produced by each isolate differs. Rotted tissue or malt extract agar plates were macerated with ethyl acetate which was then concentrated and chromatographed on a 0·5 m column packed with 'Permaphase ODS' using mixtures of methanol/pH3 buffer as the mobile phase.

As pachybasin is less common than chrysophanol in fungal pigments, monitoring for its presence provides a possible means of detecting the gangrene fungus. We are attempting to use this method to measure the amount of inoculum in infected soils rapidly and quantitatively. (Camp and Cayley, with Adams, Plant Pathology Department)

Fate of pesticides in soil

Leaching and degradation of oximecarbamate nematicides in Woburn soil. Oxamyl, aldicarb and aldicarb sulphone, ^{14}C-labelled in the carbamate methyl group, were mixed individually into the top 5·0 cm of soil contained in plastic tubes (20 cm deep and 5·5 cm diameter) buried level with the soil surface and exposed to natural weathering at Woburn. Two soils were compared: a sandy loam (pH 7·0, organic matter content 1·4%) and the same soil modified by the addition of peat over several years. (pH 6·0, organic matter content 6·0%).

Aldicarb was degraded by the anticipated pathways, being rapidly oxidised to the sulphoxide which in turn was slowly oxidised to the sulphone, with concurrent hydrolysis of these compounds to yield non-toxic oximes. Fourteen days after application in April, 75–85% of the ^{14}C-containing compound extracted by acetone was aldicarb sulphoxide, the remainder being mostly aldicarb sulphone. By 85 days after application, about 90% of the residues extracted from the top 20 cm soil consisted of aldicarb sulphone. Breakdown of oxamyl and aldicarb sulphone proceeded by loss of the carbamate group, no ^{14}C-containing compounds other than the parent chemicals being observed when acetone extracts of soils were examined by thin-layer chromatography.

All these oximecarbamates are only weakly sorbed by soil, especially soils containing little organic matter. Thus the chemicals are readily available for leaching. In the unmodified sandy loam, 3·2 cm of rain transported most of the oxamyl to depths of 5 – 10 cm 14 days after application. Aldicarb sulphoxide and aldicarb sulphone, formed in soil following application of aldicarb, were leached slightly further than oxamyl in this time, being found mostly at 5 to 15 cm. After 29 days and 5·6 cm rain, all these chemicals were distributed throughout the 20 cm of topsoil in which measurements were made, the highest concentration occurring between depths of 7·5–15 cm. After 86 days and 13·64 cm rain, no oxamyl could be detected and only about 4% of the applied aldicarb was recovered, mostly as aldicarb sulphone and all below 5 cm.

In the soil modified by addition of peat, there was much less leaching and none of the chemicals moved out of the top 20 cm. Hydrolysis was slower than in the unmodified soil. After 85 days, 4·6% of the applied oxamyl remained, and aldicarb residues comprised 25% of the applied dose. Increased persistence in this soil was also observed in laboratory

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incubations, and may be due to lower pH and/or increased adsorption. (Bromilow and Freeman)

Computer simulation of pesticide degradation and movement in soil. Our measurements of the degradation and movement of oximecarbamate nematicides were used in a collaborative project to test the computer model designed by Dr. M. Leistra (Laboratory for Research on Insecticides, Wageningen) to simulate the behaviour of pesticides in soil. Rate constants for the oxidation and hydrolysis reactions as a function of soil temperature and moisture content were obtained for the model by laboratory measurement.

Initial problems were encountered in obtaining an adequate simulation of water flow through the soil, due to uncertainties about the water retention and hydraulic conductivity properties of the soil profile. However, by dividing the top soil into several compartments with a gradation in hydraulic characteristics dependent on the compaction, a good description of water flow was obtained. Preliminary simulation runs described the movement and degradation of oxamyl and aldicarb reasonably well. Further work is in progress to refine and simplify the modelling procedures. (Bromilow and Freeman, with Baker, Statistics Department and Dr. M. Leistra, Laboratory for Research on Insecticides, Wageningen, The Netherlands)

Soil microbial studies

Degradation of asulam and sulphanilamide. Work on the asulam and sulphanilamide degrading soil bacterium was concluded with identification of the organism as a member of the group of *Flavobacterium* sp. containing DNA with a high molar percentage of guanine and cytosine.

Nitrification of ammonium fertilisers. Nitrification and denitrification have an important bearing on the economical use of ammonium fertilisers. Work on identifying the autotrophic nitrifying bacteria in very acid tea soils from Sri Lanka and Bangladesh continued with the examination of soils having pH values between 4 and 4·3.

The only ammonia-oxidising organisms found in the Bangladesh soils were *Nitrosospira* species, in constrast to British soils where *Nitrosolobus* species are dominant. A greater variety of species was isolated from the Sri Lanka soils including *Nitrosomonas*, *Nitrosolobus*, *Nitrosospira* and a member of the newly discovered genus *Nitrosovibrio* previously found in a Welsh mountain soil and isolated by German workers from Hawaiian and Dolomite soils.

Altogether 11 new isolates were obtained in pure culture and examined by electron microscopy. (Walker, with Wikramasinghe, Soils and Plant Nutrition Department)

Chemical Reference plots. In the fourth year of this long term experiment to evaluate the effects of repeated applications of crop protection chemicals on soil fertility, spring barley yields were higher (mean 4·7 t ha^{-1}) than in 1976. Bird damage to some plots made interpretation of yields difficult. Aldicarb increased yield by about 8% although it did not control a small aphid infestation. Benomyl neither controlled mildew nor affected yield.

Plant growth regulators. Investigation of the behaviour of growth regulators in wheat and barley continued. Two additional growth regulators 'DPX 1840' (2-(4-methoxyphenyl)-3,3a-dihydro-8H pyrazolo (5,1-a) isoindol-8-one) and 'F4' (an active metabolite of 'DPX 1840') showed similar effects to chlormequat (*Rothamsted Report for 1976*,

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Part I, 184) although both are more toxic to cereals than chloremequat. (Lord, with Wheeler, Botany Department)

Chemical preservation of damp hay. Work on the prevention of moulding of damp hay by chemicals, especially propionic acid and its salts, continued. Previous laboratory tests were done by rewetting hay with water containing the preservative; we have now developed a method for testing concentrated chemical formulations. The equipment simulates the method of application used in the field. Chemicals are applied through a single vibrating jet on to chopped part-dried hay stirred in the rotating drum of a concrete mixer. It was necessary to modify and adjust the internal paddles to disperse and mix the hay satisfactorily and ensure distribution of the chemical throughout the hay. The small volumes (0·5 – 10 ml) of formulations needed to treat 0·5 – 1 kg samples of hay are accurately applied to the jet under pressure from all-glass syringes.

No differences in the moulding of hay in Dewar flasks were observed when this method was used to treat part dried or dried and rewetted samples with propionic acid half neutralised with ammonia, confirming deductions from previous laboratory and field tests. Preliminary experiments suggest that the method may be scaled down further to allow tests on 50–100 g portions of hay.

A technique was also developed for laboratory studies on the spread of moulding into treated hay from foci formed by pockets of untreated or poorly treated hay. Chopped hay is uniformly treated with preservative and weighed quantities are packed in glass tubes (internal diameter approximately 23 mm) at a density about the same as that in the normal hay bale. The focus of moulding is formed by including a weighed plug of untreated hay sandwiched between treated hay. Mould can be seen developing first in the untreated plugs and then spreading into the treated sections. Mould advanced along tubes of hay (30% moisture content) treated with either 0·6% or 0·46% propionic acid at similar rates (about 3 mm day^{-1}). The rate was independent of the size of the plug of untreated hay although the onset of spread from the smallest plug (1 g) was delayed longer than for plugs of 1·5, 2 and 4 g.

In hay packed to half the standard density mould advanced 2–2·5 mm day^{-1} from a 1 g plug but twice as fast from 2 and 4 g plugs, indicating a greater sensitivity to the weight of plug and more rapid linear development than in hay packed to standard density. However the weight of hay moulded in a day was less in more loosely packed conditions.

The rate that mould progressed through hay treated with a range of concentrations of propionic acid applied as free acid, acid half neutralised with ammonia (ABP) or as ammonium propionate was examined using 1 and 2 g untreated plugs. Moulding began in both 1 and 2 g plugs if insufficient acid was present in the treated hay and then advanced through the treated sections. Once moulding had started, the rate of advance (2–3 mm day^{-1}) appeared independent of the concentration or form in which the acid was applied to the hay. Free acid was twice as effective as its ammonium salt in preventing the onset of mould growth in the untreated plugs and the efficacy of the half neutralised acid was intermediate. About double the concentration of chemical needed to prevent the moulding of 1 g plugs was required to protect 2 g plugs of untreated hay. Concentrations of chemical which failed to prevent moulding sometimes delayed the moulding of untreated plugs, the delay increasing with the concentration. (Lord and Cayley, with Lacey, Plant Pathology Department)

Collaborative work described in the reports of other departments.

With Nematology Department. Leaching and degradation of oxamyl in the Witham

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pea trial. (Bromilow with Whitehead). Distribution of 'Vydate' oxamyl granules achieved by the tine injector in Mepal peat soil. (Bromilow with Whitehead).

With Plant Pathology Department. The use of fungicides against tuber pathogens. (Cayley with Hide, Bell and Mayne). Testing of potential bactericides. (Cayley with Lapwood and Harris).

Staff of the Department and Chemical Liaison Unit

In recognition of his outstanding contribution to work on insecticide toxicology and resistance to insecticides, R. M. Sawicki was awarded an individual merit promotion to Senior Principal Scientific Officer. We were also very pleased to learn that, as a result of their discovery of the pyrethroid insecticides resmethrin and bioresmethrin, M. Elliott and N. F. Janes would be the first scientists to benefit from a new scheme of awards to inventors introduced by the ARC.

During the year we welcomed four staff transferred from the Unit of Developmental Botany to the Department: D. N. Butcher, Linda M. Searle, A. K. Sogeke and J. H. H. Walters. They will be working on a range of projects concerned with control of fungal diseases, translocation in plants, and selectivity of insecticides. At the end of 2-year contracts provided by NRDC, A. Coxon and D. Carson left; S. J. Edge from Sheffield University and B. Khambay from Queen Mary College, London, were appointed to similar new posts. D. R. Nixon completed a short term appointment financed by the Ministry of Overseas Development (ODM) to help with the development of equipment for mass pollination of coconuts. Dr. R. Botham continued on secondment in the Department from Wellcome, Berkhamsted Ltd., studying neurophysiological action of insecticides using electron microscopy methods. Dr. D. M. Soderlund completed a period of fifteen months as a Rockefeller Foundation Fellow and returned to the USA to take up an appointment in the Department of Entomology, University of Cornell.

Mrs. Katrina Gorog from Plant Protection Centre, Budapest, Hungary, came as a visiting worker to the Chemical Liaison Unit; Mrs. Guler Onal returned to Turkey after fifteen months on a similar visit. Dr. A. Leal from C.S.I.C., Institute of Immunology and Microbiology, Madrid, spent two months in the Department studying the control of foliar diseases of cereals. J. Gibson, Sheena Lauckner and R. Stokes worked as post-graduate students in the Department under the CASE scheme. Sandwich course students who worked in the Department or the Chemical Liaison Unit were Heather Anderson, S. A. Ballard, Alison Bradley, A. Camp, F. W. Gudyanga, M. I. Jeffrey, I. M. Laing, C. J. Morgan, M. H. Sherwood and Mary Thompson.

I. J. Graham-Bryce was appointed as an Honorary Lecturer in the Department of Applied Biology, University of Strathclyde and was elected as Honorary Secretary (Home) of the Society of Chemical Industry. He was invited to present the opening Plenary Lecture to the 29th International Crop Protection Conference in Ghent at which R. M. Sawicki also contributed a paper. At the invitation of I.C.I. (Australia) Ltd. and the Wellcome Foundation, M. Elliott visited organisations concerned with the development of synthetic pyrethroids in Australia, New Zealand and the Far East. He and D. Soderlund contributed invited papers to a meeting of the Entomological Society of America in Washington. R. M. Sawicki visited the Danish Pest Infestation Laboratory on a grant from the Danish Authorities to discuss problems of resistance with Dr. J. Keiding. J. H. Stevenson attended a meeting of the IOBC Working Group on the effects of pesticides on beneficial insects in Darmstadt, Germany, and K. A. Jeffs participated in the 21st Annual Meeting of CIPAC at Braunschweig, Germany, as an observer and expert witness. Under the auspices of ODM, A. J. Arnold visited the Coconut Industry

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Board in Jamaica to discuss and commission specialised equipment developed at Rothamsted. He was also invited to visit the Cocoa Research Centre (CEPEC) in Brazil to evaluate and make recommendations on laboratory technical support services.

K. A. Lord returned from a 6-month visit to the Radioisotopes Centre, Instituto Biologico, Sao Paulo, Brazil, where he advised on methods for studying the behaviour of pesticides in soils and plants. N. Walker visited Hamburg University and Göttingen University with a grant from the Royal Society's European Fund to lecture and discuss work on autotrophic nitrifiers. R. H. Bromilow spent 1 month in the Laboratory for Research on Insecticides, Wageningen, partly financed by the Laboratory, for a collaborative project on simulation modelling of nematicide behaviour in soils. G. G. Briggs began a 1 year secondment at the Department of Soils and Plant Nutrition, University of Western Australia on a grant from the University.

The Department and Unit again made a major contribution to the British Crop Protection Council's Conference on Pests and Diseases at Brighton. I. J. Graham-Bryce was Vice-Chairman of the Programme Committee and A. L. Devonshire and P. H. Needham were Session Organisers. D. C. Griffiths was co-author of an invited Plenary paper and several other staff contributed research reports. Together with the Entomology Department, we acted as hosts to the second meeting of the IOBC Working Group on Insect Pheromones which was held at Rothamsted: participants attended from many European countries and from North America and Australia. A. R.Greenway, A. Mudd and J. A. Pickett were principally involved in the organisation. A. R. Greenway also collaborated with the Entomology Department in staging an exhibition on 'Sex attractants for monitoring pea moth' at the British Growers' Look Ahead exhibition in Harrogate and the Chelsea Flower Show.

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