ROTHAMSTED RESEARCH

Report for 1972 - Part 1

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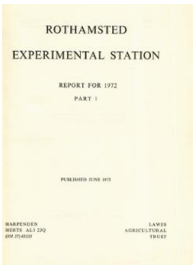

Insecticides and Fungicides Department

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INSECTICIDES AND FUNGICIDES DEPARTMENT

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It is increasingly recognised that pesticides should be used more discerningly. Much of our work is directed towards achieving this by understanding the influence of environmental factors and formulation on pesticide behaviour. In practice only a very small fraction of the pesticide which is applied reaches the intended target, the remainder entering the environment without contributing to pest control. For example our work on persistence of insecticides on leaf surfaces and in ant baits shows that most of the toxicant rapidly evaporates into the atmosphere, particularly under tropical conditions, while our work with systemic compounds shows that less than 2% of the applied dose is likely to enter the plant after being applied to soil. There is therefore considerable scope for using pesticides more efficiently; large improvements should be possible by developing formulations and methods of application which relate the release of the toxicant and its pattern of distribution more accurately to the behaviour of the pest. The work on microcapsules and granular formulations illustrates this approach while also emphasising that the properties of the toxicant and the environment ultimately limit the extent to which pesticide behaviour can be controlled.

Favourable properties in the environment have no doubt contributed to the increasing use of the synthetic pyrethroids which we and others have developed. This group provides a range of different insecticidal and knockdown properties, but as a whole is characterised by very great toxicity to insects with extremely small toxicity to mammals and by short persistence. So far they have not found much use on field crops because they are relatively expensive and do not persist. However their rapid action, very great toxicity, the absence of residues and indications that resistance will be slow to develop give them considerable flexibility which may prove very valuable in future integrated control programmes, for example by enabling selectivity to be obtained by timing. The increasing interest in the pyrethroids should lead to new developments, widening the scope of the group still further. We are therefore maintaining our efforts to understand their mode of action and metabolism and the mechanisms whereby insects become resistant to them.

While conventional treatments with chemical pesticides must be the backbone of pest control for the foreseeable future, improvements coming mainly from making their use more efficient, our work on behaviour-controlling substances continues to suggest that more sophisticated methods may ultimately become practicable in suitable cases. At present the factors which determine how insects respond to most pheromones are not well understood and initially these compounds will probably find most use in environments such as food stores where conditions are reasonably predictable. The isolation and characterisation of the crowding pheromone from *A. kuehniella* and the demonstration that it occurs in other related stored-products pests is therefore particularly interesting. However, the broad potential of manipulating responses to behaviour controlling substances is shown by the observations that adding charcoal or extracts of oat plants to soil can decrease the ability of wheat bulb fly larvae to respond to exudates from wheat plants.

Work on the growth and health of crops in other departments at Rothamsted also frequently involves the use of pesticides and related chemicals. In the past, such studies

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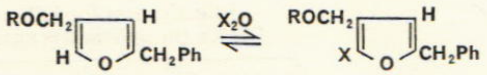
have sometimes been hampered by lack of information about the distribution, movement and stability of the chemicals used. To provide this information, workers contributed by Chemistry, Insecticides and Fungicides, Nematology and Plant Pathology Departments now form a group of chemists, associated with the Insecticides and Fungicides Department, who will collaborate with the biologists concerned with effects of these compounds. Examples of existing collaborative projects include work on the use of chemicals to control potato cyst nematodes in soil, the application of systemic fungicides to potato seed tubers and the reasons for differences in response of wheat and barley to foliar sprays of CCC (cycocel). These studies are reported with the work of the appropriate departments.

Insecticides

The natural pyrethrins and related synthetic compounds

Radiolabelled bioresmethrin (NRDC 107) and related compounds. The synthetic pyrethroids developed here (*Rothamsted Reports for 1966–1971*) are finding more and more practical uses in horticulture, and for aircraft disinsection, as well as to control domestic insects. It is therefore increasingly important to be able to detect small quantities of the compounds and to determine their mode of action and the structures of metabolites formed in insects and mammals. Such studies are considerably facilitated if the compounds are available in radiolabelled form. In previous work (*Rothamsted Report for 1970*, Part 1, 172) mammalian metabolites of pyrethrins I and II and of allethrin were identified using esters labelled with tritium in the alcoholic component, and a method has now been developed for incorporating tritium into the furan ring of 5-benzyl-3-furylmethyl esters. The proton at the 2-position of the furan ring is exchanged under acid-catalysed conditions; bioresmethrin [R = (+)-*trans*-chrysanthemate] and deuterium oxide in dioxan with a trace of hydrochloric acid, gives the product (X = D) containing more



than 80% D at the 2-position of the furan ring, and none elsewhere. The position of exchange was established from the nmr spectrum of the exchanged product, which had changed only at 2·7 τ, and the purity of the product was demonstrated by nmr, mass spectra, tlc, glc, refractive index and optical rotation. These results with deuterium oxide indicated that with tritium oxide under similar conditions, only the proton at the 2-position would be replaced with tritium. An exchange reaction with six related esters was therefore performed at the Radiochemical Centre, Amersham, with tritium oxide of very high specific activity. We purified the six preparations here by chromatography through a short column of alumina to give products with the properties shown in Table 1. Radio-

TABLE 1

Tritium-labelled esters of 5-benzyl-3-furylmethyl alcohol ('5B3FA')

Compound	Chemical name	Yield (mg) from 100 mg of unlabelled ester	Total activity (mCi)	Specific activity (mCi/mmole)	Radiochemical purity (%)
NRDC 107	5B3F (+)-*trans*-chrysanthemate	71	135	641	98
NRDC 119	5B3F (+)-*cis*-chrysanthemate	32	51	537	95
RU 11,679	5B3F (+)-*trans*-ethanochrysanthemate	35	72	740	98
NRDC 108	5B3F 2,2,3,3-tetramethylcyclopropane carboxylate	48	78	551	92
RU 11,934	5B3F (−)-*trans*-chrysanthemate	29	28	325	96
RU 12,065	5B3F (−)-*cis*-chrysanthemate	39	27	230	96

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chemical purity was determined by scanning a thin layer chromatogram (silica gel; ether-hexane 2 : 1), a technique possible because of the high specific activity of the product. Although only one proton per molecule was exchangeable, the specific activity attained was comparable to that obtained previously in pyrethrin I, pyrethrin II and allethrin by exchange of five protons because the tritium oxide used had greater specific activity.

Structural requirements for knockdown activity in pyrethroids. The work of Burt and Goodchild (*Rothamsted Report for 1971*, Part 1, 185) indicated that knockdown and kill by pyrethroids are probably progressive stages at one site in the central nervous system rather than separate actions, one peripheral and one central, and that differences in speed of knockdown are associated with different rates of penetration and detoxification. This agrees well with the observation that more polar compounds (pyrethrin II, tetramethrin, 5-benzyl-3-furylmethyl pyrethrate) which may have physical properties giving more rapid penetration are faster knockdown agents than related less polar compounds that are good killing agents (pyrethrin I, 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate). We have recently found that simply by introducing a polar group (oxygen, to form an epoxide, as shown) on to the side chain of the acid component of insecticidal esters, killing power is diminished but knockdown remarkably increased.

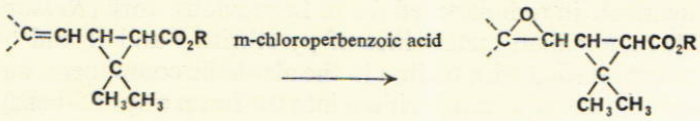


TABLE 2

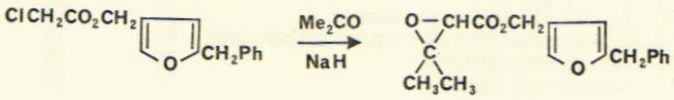
Kill and knockdown by some synthetic pyrethroids and their epoxides

Compound	Effectiveness for kill, as LD50s (in μg/insect)[a] against Houseflies		Effectiveness for kill, as LD50s (in μg/insect)[a] against Mustard beetles		Effectiveness for knockdown[b] against Houseflies	
	Parent	Epoxide	Parent	Epoxide	Parent	Epoxide
NRDC 107	0·005	0·11	0·004	0·018	poor	good
NRDC 119	0·012	0·40	0·008	0·042	moderate	good
RU 11 679[c]	0·004	0·045	0·0024	0·11	poor	good
3-Benzylbenzyl (+)-*trans*-chrysanthemate	0·033	0·44	0·008	0·34	poor	moderate
Neopynamin (= tetramethrin)	0·20	>2	0·18	>2	good	very poor
bioallethrin	0·08	—	0·22	—	good	—

(a) By topical application of 1 μl drops in acetone to adult female *Musca domestica* L. and male and female *Phaedon cochleariae* Fab.
(b) By spray test in a Kearns-March chamber (*Rothamsted Report for 1970*, Part 1, 176).
(c) 5-Benzyl-3-furylmethyl (+)-*trans*-ethanochrysanthemate, a gift from Roussel/Uclaf, S.A.

It appears that knockdown agents need not be stable compounds (the epoxides decomposed slowly even when stored at −20°) *in vitro* or *in vivo*. On the other hand, the ability to survive detoxification long enough at the site of action to produce an irreversible lesion is essential for good kill.

Tetramethrin (see Table 2) is a good knockdown agent for houseflies, although not very toxic, and it is therefore very interesting that its epoxide shows neither knockdown nor kill. We also synthesised by the Darzens condensation a 5-benzyl-3-furylmethyl dimethyl-



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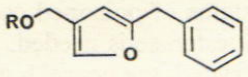
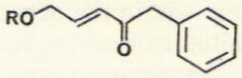
glycidate which incorporates the epoxy ring in a simpler structure. However, this compound also lacked both knockdown and killing power.

Acyclic esters of chrysanthemic acid related to bioresmethrin. In the previous report (*Rothamsted Report for 1971*, Part 1, 187) we discussed open chain esters synthesised to determine the structural features responsible for the exceptional insecticidal activity of bioresmethrin (1, R = (+)-*trans*-chrysanthemate). A further compound (2) has now



(1)



(2)

been synthesised in which the stereochemical features make it possible for the side chain phenyl group to take up similar relative positions to the chrysanthemate component RO as in bioresmethrin when the oxygen functions (—O— or C=O) are also in comparable positions.

The compound (2, R=H) required to prepare the ester is a γ-hydroxy-α-β-unsaturated ketone, of which class there are few acyclic examples. An efficient synthesis, as follows, was eventually developed:

$$Ph.CH_2CHO + BrMgCH_2.CH{=}CH_2 \rightarrow Ph.CH_2CH(OH).CH_2CH{=}CH_2$$

$$Ph.CH_2CH(OH).CH_2CH{=}CH_2 \xrightarrow[\text{benzoic acid}]{\text{m-chloroper-}} Ph.CH_2.CH(OH)CH_2.\underset{\diagdown\,O\,\diagup}{CH{-}CH_2} \xrightarrow[\text{Pyridine}]{CrO_3} Ph.CH_2CO.CH_2.\underset{\diagdown\,O\,\diagup}{CH{-}CH_2}$$

$$Ph.CH_2CH(OH).CH_2.\underset{\diagdown\,O\,\diagup}{CH{-}CH_2} \xrightarrow[1\text{ minute}]{Et_3N/20^\circ} Ph.CH_2CO.CH{=}CH.CH_2OH$$

The chrysanthemate of this alcohol was almost inactive against houseflies and mustard beetles, and thus gives further evidence for the steric requirements necessary for insecticidal action. (Chemical work: Elliott, Janes and Pulman. Biological work: Farnham and Needham)

Mode of action of insecticides

Neurotoxic action of omethoate. Devonshire found surprisingly large concentrations of omethoate in the haemolymph of houseflies poisoned with dimethoate (*Rothamsted Report for 1972*, Part 1, 182) and concluded that omethoate (the more toxic phosphate analogue of dimethoate) was probably less neurotoxic than paraoxon or diazoxon. We therefore investigated the neurotoxicity of omethoate directly, using the cockroach *Periplaneta americana* as a test insect, as its nervous system is more suitable for electrophysiological studies than that of the housefly.

Both dimethoate and omethoate kill cockroaches more slowly than most other organophosphorus compounds. An LD95 of diazoxon (2·6 μg), topically applied, took about 2 hours to affect cockroaches severely, but an LD95 of omethoate (3·5 μg) took more than four times as long to produce a similar effect. When tested on the nervous system, a 10 μ*M* solution of omethoate in saline took 260 minutes to block conduction of nerve impulses through the giant fibre pathways of the metathoracic ganglion, 30 times longer than a similar concentration of diazoxon, which suggests that the slow action of omethoate on cockroaches is associated with its slow action on their nervous systems.

Possible explanations for the slow action on the nervous system are (1) penetration of omethoate into the nervous system is restricted in some way, (2) omethoate is relatively rapidly detoxified inside the nervous system, or (3) omethoate is an inferior anticholines-

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terase. The second explanation is unlikely because comparisons over a period of 16 hours showed that dimethoate and omethoate persist much longer than diazinon in the haemolymph of cockroaches following topical application; the nervous system is unlikely to detoxify these compounds more rapidly than other tissues. Although the concentrations of dimethoate and omethoate in the nervous tissues were similar to those in the haemolymph, the concentration of metabolites in the nervous tissue was only 5% of that in the haemolymph.

To distinguish between the other two explanations, more information about the concentration of omethoate within the nervous systems of poisoned cockroaches and about its efficiency as an inhibitor of cockroach cholinesterase is needed. However, if penetration of omethoate into the nervous system is restricted, the neural lamella contributes little to this effect, as after its removal (desheathing), omethoate at the concentration found in the haemolymph of poisoned cockroaches blocks conduction only 15% faster.

During these experiments differences in the method of applying anti-cholinesterases to the nervous system were found to affect the blocking time. When 10 μM concentrations of omethoate were applied to nerve ganglia intermittently, conduction was blocked twice as quickly as when the insecticide was applied continuously, but at larger concentrations (1 mM) the method of application had no effect. Continuous irrigation with 10 μM concentration may remove accumulating neurotransmitter, so postponing block, but at larger concentrations, when transmitter is produced rapidly and irrigation time is short, differences in the method of application are less likely to affect the rate at which it accumulates. These results emphasise that the conditions under which toxicants are applied can influence their action in complex ways, so that conditions must be standardised carefully for valid comparisons. (Burt, Devonshire and Goodchild)

The causes of resistance. Resistance remains one of the most important problems associated with the use of insecticides. We continued to study resistance to organophosphates and pyrethroids in houseflies, where genetical and biochemical work is steadily establishing the nature of the complicated mechanisms involved. Much less is known about resistance in aphids and genetical studies are not practicable at present. In our work with aphids we have therefore concentrated on physiological and biochemical studies.

Resistance of houseflies to dimethoate. The two factors of resistance to dimethoate on chromosome II in dimethoate-resistant strains of houseflies postulated last year (*Rothamsted Report for 1971*, Part 1, 179) have now been successfully separated and bred in a homozygous condition.

Because resistance in the parent resistant strains (49 r_2b and 239 fb) was heterogeneous, the strains isolated previously with individual chromosomes derived from the parents were also heterogeneous for resistance. The resistance factors given previously (*Rothamsted Report for 1971*, Part 1, 179–180) were therefore probably too small.

Mechanism *a*, which is controlled by a gene about 20 cross-over units from the marker *ar*, contributes most of the resistance to dimethoate and its analogues, and is much more important than previously believed. It confers strong resistance to dimethoate (R.F. about ×50) and very strong resistance to omethoate (no kill at 0·6 μg/female; LD50 susceptible females about 0·015 μg/fly) and 0,0-dimethyl S(N-carbamoyl-methyl) phosphorothiolothionate. It gives weak resistance to parathion (R.F. about ×5) moderate resistance to malathion (R.F. 10) and strong resistance to malaoxon (60% kill at 11 μg/fly). The factor is incompletely recessive and is inhibited greatly by pretreatment with sesamex. This suggests that it may inhibit mixed function oxidases.

The other mechanism, *b*, which is controlled by a factor close to the marker *ar*, confers

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relatively weak resistance to dimethoate, omethoate and 0,0-dimethyl S(N-carbamoylmethyl) phosphorothiolothionate (R.F. about ×10) but very strong resistance to malathion (no kill at 12 μg/female) and malaoxon. Resistance to malathion is unaffected by TBTP (S,S,S,-tributyl phosphorotrithioate) a carboxyesterase inhibitor, or by sesamex, the mixed function oxidase inhibitor. Resistance to malaoxon also seems to be unaffected by sesamex. The properties of this mechanism do not correspond to any of the recognised mechanisms conferring resistance to malathion and its nature is unknown. Unfortunately, this strain was recently contaminated by other stray flies and must be reselected for homogeneity before further studies are possible. (Sawicki)

Genetics of resistance to pyrethroids in houseflies. The genetical analysis of the factors causing resistance to pyrethroids in houseflies continued with further studies to identify the factor located on chromosome III. This factor, now called *kdr-NPR* had been isolated genetically in strain *348* from *NPR* which resists natural pyrethrins, and gave resistance to both DDT and pyrethroids (*Rothamsted Report for 1971*, Part 1, 180). The standard resistant strains *bwb kdr* and *kdr-0* which have known mechanisms on chromosome III proved unsuitable for identifying this factor and further genetical manipulation was required. Strain *bwb kdr* contains an additional resistance mechanism, DDT dehydrochlorinase, on chromosome II which would interfere with the analysis and this factor was therefore removed by substituting the marked chromosome II from the susceptible quadruple marker strain *608Q*. In the resulting strain *507*, resistance to DDT and pyrethroids was due only to the factor *kdr*. Strain *kdr-0* was heterozygous for DDT resistance so was selected once with DDT which purified the strain. Its resistance to DDT and pyrethroids remained constant over 30 generations.

The two factors *kdr* and *kdr-0* are recessive like *kdr-NPR*. Crossing strain *348* (with factor *kdr-NPR*) with *507* (with *kdr*) gave progeny as resistant to DDT and resmethrin at kill end-point as either parent, as did the cross *348* with *kdr-0*. The cross *507* with *kdr-0* also gave resistant progeny. These results suggest that *kdr*, *kdr-0* and *kdr-NPR* are allelic. To verify this, the cross-over rates between the brown body marker, *bwb*, and the three resistance factors were measured. The values were all approximately 50 units, but the results were inconclusive because the technique was not sufficiently sensitive to locate a gene at this distance from the fixed locus accurately.

Another marker for chromosome III, green eye, *ge*, which is nearer *kdr*, *kdr-0* and *kdr-NPR* has been obtained. Part of the population with this marker was found to resist DDT, but once the strain has been purified, the cross-over rate between *ge* and the various factors can be determined and the question of allelism unequivocally resolved. (Farnham)

Resistance of aphids* (Myzus persicae (*Sulz.*)) *to organophosphorus insecticides. The new resistant clone (DDTR) of *M. persicae* (Sulz.) started last year after the earlier clone lost resistance (*Rothamsted Report for 1971*, Part 1, 181) has now been reared for 18 months and has maintained strong resistance to organophosphorus insecticides in the absence of selection pressure (Table 3, p. 180).

The resistance of the DDTR strain to dimethoate can be decreased by using synergists; for example the synergistic factors for sesamex and tributylphosphorotrithioate are 10 and 24.

The fate of topically applied insecticide. Topical application is widely used in detecting and measuring resistance of aphids to insecticides. We found that the standard method of applying relatively large volumes (0·1–0·2 μl) of insecticides dissolved in acetone from all-glass syringes was unsatisfactory because leakage past the plunger and losses to the

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TABLE 3

Resistance factors[1] *of the resistant clone of* M. persicae *to various insecticides*

dimethoate	196
omethoate	28
ethyl parathion	462
paraoxon	27
disulfoton	89
malathion	38

[1] Resistance factor = LD50 of resistant strain/LD50 of susceptible strain

substrate caused large deviations from the intended dose. For comparative bioassays this was acceptable, but for quantitative biochemical studies on resistance, and for investigations into penetration and excretion, a precise method of application was essential.

The dosing technique was greatly improved by using a 250 μl gas-tight syringe and an 0·2 mm O.D. canula (I.D. 0·1 mm) in a standard Arnold microapplicator (*Journal of Scientific Instruments* (1965) **42**, 350) and ethyl methyl ketone as the solvent for the insecticides.

Resistance may be caused by physiological factors which decrease the amount of insecticide reaching the site of action as well as by increased detoxification. We therefore compared the rates of penetration of dimethoate and parathion and the excretion of these insecticides and their metabolites in resistant and susceptible *M. persicae*.

Groups of ten adult apterae were dosed topically with ^{14}C-labelled insecticide and kept in scintillation vials for varying times. They were then removed from the vials and the insecticide remaining on the surface of the insects was washed off with a toluene-based scintillation liquid. The difference between the amount washed off and the amount applied gave a measure of the amount penetrated. The amount of radio-activity in the storage vials gave a measure of the inseciticide or metabolites excreted.

Both insecticides penetrated more rapidly into susceptible than into resistant aphids, and parathion penetrated into both strains more rapidly than dimethoate. Approximate times for 50% penetration into the susceptible strain were 40 minutes for dimethoate and 9 minutes for parathion, compared with 56 and 16 minutes for the resistant strain. In other species dimethoate, being more polar, penetrates more rapidly than parathion.

Both strains excreted the same amount of radio-activity. Over 90% of this radio-activity was in unchanged insecticide, which could explain why aphids are more tolerant to these insecticides than houseflies (Table 4). Houseflies excrete only approximately 5% of the applied dose as unchanged insecticide.

TABLE 4

LD50s (μg/mg) *of dimethoate and parathion to resistant and susceptible* M. persicae *and* M. domestica

	M. persicae		*M. domestica*	
	susceptible	resistant	susceptible	resistant
dimethoate	0·0030	0·40	0·0005	0·04
parathion	0·0025	0·70	0·0007	0·017

Sesamex, which synergises dimethoate ten-fold and parathion slightly in resistant *M. persicae*, is known to inhibit mixed-function oxidases which are involved in insecticide degradation. We found that it also greatly inhibited the penetration and excretion of dimethoate and parathion in aphids. We do not know yet whether its primary effect is on penetration or excretion. (Devonshire and Needham)

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***The role of increased carboxyesterase activity in organophosphate-resistant* Myzus persicae (*Sulz.*).** Aphids resistant to organophosphorus insecticides have greater carboxyesterase activity than susceptible aphids (Needham & Sawicki (1971) *Nature*, **230**, 125). Starch gel electrophoresis shows that this is caused by a change in the activity of only one of the several esterases in aphids (A. P. Beranek, personal communication). We investigated the possibility that the very active carboxyesterase in the resistant strain may be a cause of resistance by hydrolysing organophosphorus esters in addition to carboxyesters. The enzyme had first to be purified by centrifugation, ammonium sulphate precipitation, gel filtration and ion-exchange chromatography because insecticide degradation cannot be attributed to a particular enzyme in a crude insect homogenate. The homogeneity of the carboxyesterase activity and its identity with the enzyme having increased activity in the resistant strain was confirmed by electrophoresis. The purified enzyme hydrolysed α-naphthyl acetate ($K_m = 1{\cdot}8 \times 10^{-4}M$), but not radio-active dimethoate ($10^{-4}M$). It may hydrolyse omethoate (the toxic phosphate analogue of dimethoate) but this could not be determined because we lack radio-active omethoate. If the enzyme proves incapable of degrading insecticides it may still contribute to resistance by combining with a portion of the insecticide present in the insect, thereby decreasing the amount available for the lethal process, the inhibition of acetylcholinesterase. (Devonshire)

Effects of environment, formulation and substrate on the persistence and distribution of pesticides

Losses of insecticides from leaf surfaces. Confirmatory work was done on the relationship between loss of DDT from cotton leaf surfaces and the density of the insecticide deposit. Different amounts of DDT were applied as a 50% wettable powder to leaves of cotton plants growing in a constant environment room at temperatures of 29°C (day) 21°C (night), 55% R.H. and ten hours of artificial daylight. Leaf samples taken weekly were ground, extracted with hexane and analysed by glc.

Results confirmed previous work which showed that losses of DDT from the wettable powder formulation were related to the deposit density. For example, after one week amounts lost from deposits of 100 μg and 25 μg DDT per leaf were 30% and 8% increasing to 70% and 30% after six weeks.

Losses of insecticides from ant baits. Baits to control leaf cutting ants in the tropics must be durable and retain their effectiveness for as long as possible under extreme climatic conditions. We investigated the persistence in such baits of various insecticides with different physical properties.

Baits consisting of 0·4% insecticide dissolved in soyabean oil sorbed on dried citrus pulp were weathered for two weeks on bare earth plots in the sun and in the shade during a short visit to Trinidad. The baits contained technical aldrin, technical aldrin plus a waterproofing silicone, pure aldrin, pure dieldrin, or mirex. Baits in the shade became mouldy within a week, but the waterproofed bait was far less susceptible to fungal attack. Losses, especially of aldrin, were rapid during the first two days, but baits subsequently lost insecticide slowly. Waterproofed bait lost aldrin as easily as non-waterproofed, suggesting that the high ambient temperature (averaging about 32°C (sun) and 26°C (shade) in daytime and 22°C at night) and not the rainfall (9 cm falling during the first week and 3 cm during the second) was the main cause of the rapid loss of insecticide. Aldrin baits retained between half and two-thirds of the original amount of insecticide in the form of aldrin plus dieldrin after eight days exposure, decreasing to between a quarter and a half after 14 days. Measurable amounts of dieldrin were formed from aldrin in the baits (one-fifth of the toxicant after 14 days consisted of dieldrin). The dieldrin and mirex

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baits retained between half and two-thirds of the original amounts of insecticide even after 14 days. Because dieldrin and mirex are appreciably less volatile than aldrin, the greater amounts of these retained compared with aldrin again point to the influence of volatilisation on relative persistence.

Microcapsules. Further development work on the microencapsulation technique was done with the objective of producing formulations with a range of well-defined and predictable release characteristics. Amongst insecticides encapsulated were dieldrin, aldrin and synthetic pyrethrin. Various concentrations of the insecticides in toluene, often incorporating a suitable marker dye were encapsulated in hardened gelatin/gum acacia walls by the coacervation technique (NCR process). With the larger capsules (>500 μm diam.), it was difficult to obtain a satisfactory wall thickness. Also the present method for extracting and drying the microcapsules in the slurry gave inconsistent results. It is hoped that more recent work on the viscosity of the coacervate and the use of a fluidised bed dryer will help to overcome these problems. (Phillips and Etheridge)

Factors influencing the performance of granular systemic insecticides applied to field beans. The influence of irrigation, temperature, humidity and placement on the effectiveness of pumice and fuller's earth formulations of disulfoton and phorate applied to field beans in constant environment rooms was studied previously by determining toxicity to aphids (*Aphis fabae*) caged on the foliage at intervals up to 40 days after treatment. Frequent rainfall and higher temperature increased activity and, in the presence of irrigation, pumice formulations were more effective than fuller's earth (*Rothamsted Reports for 1968–1971*, Part 1; *Pesticide Science* (1972) **3**, 781–797). To help explain these effects we have now measured adsorption of disulfoton and phorate by soil, and release from the two granular carriers by evaporation and by leaching in separate experiments.

Adsorption isotherms were determined by the method described previously for disulfoton (Graham-Bryce (1967) *Journal of the Science of Food and Agriculture*, **18**, 72–77). Both phorate and disulfoton were moderately adsorbed by the soil from Woburn Farm used in the growth-room experiments. Generally phorate is slightly less strongly adsorbed than disulfoton, but isotherms were identical with this soil, and linear over the concentration range studied.

Rates of evaporation from pumice and fuller's earth granules containing 7·5% disulfoton or 10% phorate were determined in the controlled environment rooms at temperatures of 15, 20 and 24°C day (10, 15 and 19°C night) under the standard conditions applying during the tests of performance, except that no artificial rain was applied. The granules were exposed in petri dishes placed on the trolleys used for the pot experiments and at intervals over a period of one month the toxicant content was determined by extracting with hexane and analysing by glc.

At 20°C day (15°C night) over 97% of the toxicant was lost from pumice after one month compared with only 10–20% from fuller's earth. This difference would be expected from the more open structure of pumice. There was little consistent difference between losses of phorate and disulfoton from the same carrier. At first sight this is surprising because phorate is more volatile but this is offset partly by the smaller disulfoton concentration.

As expected evaporation was generally faster at higher (day, 24°; night, 19°C) than at lower temperatures (day, 15°; night 10°C), but the patterns of release were similar to those at the intermediate temperature.

Release of insecticide to water leaching over the granules was investigated by exposing them to artificial rain in the growth rooms under the same conditions as during the tests of performance. Weighed portions of granules were spread evenly on fine nylon sieves

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and the leachate collected into graduated flasks through funnels attached to each sieve, extracted with hexane and analysed by glc. Patterns of leaching were similar for disulfoton on fuller's earth and phorate on both fuller's earth and pumice. Amounts released increased directly with the amount of water applied, so that the concentrations in the leachate remained approximately constant at values approaching the aqueous solubility which therefore appeared to limit the rate of release. Slightly more phorate was released from pumice (4·7 mg in 300 ml) than from fuller's earth (3·2 mg) which is consistent with the more open structure of the pumice, but the differences were small. However very much more disulfoton (84·7 mg) was leached initially from pumice although the concentration in the leachate decreased considerably with further leaching and the amounts released appeared to reach a limiting value. The initial concentration was much greater than the solubility, presumably due to effects of additives in the formulation. Results were similar for toxicant concentrations of both 10 and 7·5% and when the experiments were repeated at 15° and 24°C.

These results show that the toxicants are more readily available from pumice than from fuller's earth and also at higher than at lower temperatures and they also emphasise the importance of leaching by rain or dew, thus largely explaining the previously observed differences in effectiveness against aphids. However, the differences in patterns of release are large in relation to their effects on performance suggesting that effectiveness is determined largely by certain basic properties of the environment and the chemical, such as adsorption by soil which would regulate the supply of chemical to the roots. We conclude that while granules may be useful for providing a particular initial distribution of a toxicant, for example by penetrating the leaf canopy to reach the soil, and may have other advantages in persistence, safety and handling over sprays, the possibilities of controlling performance by controlling release rates under conditions such as applied during these experiments appear to be limited. (Graham-Bryce, Stevenson and Etheridge)

Control of bean aphids. Comparisons of methods and times of applying insecticides to control bean aphids are described in the report of the Entomology Department (p. 203). (Stevenson, with Bardner and Fletcher, Entomology Department)

Control of soil inhabiting pests

***Susceptibility of plants to attack by wireworms* (Agriotes *spp.*).** Several types of plant are reputed to resist or escape attack by wireworms but quantitative data are lacking. To obtain such data, we exposed test plants and wheat, which is known to be susceptible, to attack by large individuals of *Agriotes* spp. in box tests and compared the numbers of emerged plants, unattacked plants and the fresh weights of emerged plants with those of similar species growing in the absence of wireworms. The one variety of onion and the kidney-vetch tried in our tests showed no resistance and were as readily attacked and damaged as wheat. However, the two varieties of peas and two varieties of beans showed some tolerance to wireworm attack; they were attacked at least as frequently as wheat but despite considerable damage continued to grow and eventually gave plants not much lighter than unattacked plants. In contrast, the other group of plants (mustard, cabbage, *Tagetes*, clover and flax) that survived relatively better than wheat in wireworm infested soil, were small and easily damaged but many were apparently missed by wireworms or, if found, not noticeably bitten. Shallow sowing contributed to the relative immunity of these plants but does not completely explain it. (Griffiths)

Saddle gall midge. The Agricultural Advisory and Development Service asked us to cooperate with their investigations into saddle gall midge (*Haplodiplosis equestris* (Wagn.))

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which may become increasingly important as a pest of cereals, by examining the possibilities of control with soil insecticides. Overwintering larvae, collected from the field, were tested in the laboratory in containers of various soils, at different moisture contents, containing aldrin at 10 μg/g. In none of the tests was there appreciable larval mortality, even after exposure to treated soil for several weeks, although vestigial winged *Drosophila melanogaster* Meig. released on the surfaces of these soils died within a few hours. The saddle gall midge larvae may have survived because they are too inactive to pick up a toxic dose and although prospects of killing saddle gall midge larvae in soil therefore seem poor, more tests will be done next year using different test methods and more volatile insecticides. (Griffiths and Scott)

Egg laying by wheat bulb fly. For the past eight years, female wheat bulb flies have been collected regularly from wheat fields in July and August for egg laying. Up to 1972, the mean number of eggs/fly was 24, ranging from 20 to 33. In 1972, however, there was a marked decrease, with a mean of 9 eggs/fly. This decrease can be largely attributed to the extensive incidence of the parasitic fungus, *Entomophthora muscae* which killed the flies before they laid their eggs. The fungus was particularly evident at a site in Bedfordshire. (Scott)

Residual effects of soil treatments. Work on residual effects of aldrin and 'D-D' applied to soil is described in the report of the Entomology Department (p. 210). (Lord, with Henderson, Entomology Department)

Seed dressings

Adhesives for powder dressings and their effects on movement of insecticides from seeds. The search for suitable adhesives to improve the retention of dry powder dressings on cereal seeds continued. Liquid paraffin damaged seeds (*Rothamsted Report for 1970*, Part 1, 178) and we have now found that 'Polyvis' emulsions with 'Mergamma D' seed dressings also damage seeds in field trials, although previous pot tests did not reveal any serious effects on germination.

The 'Polyvis'/'Mergamma D' treatment, although improving retention, did not protect wheat against wheat bulb fly as successfully as 'Mergamma D' alone. This is probably because strong retention decreases movement of the insecticide from the seed and its availability to the pest. Experiments were therefore started to determine the effects of adhesives on the movement of γ-BHC from treated seeds into the plant and surrounding soil. Seeds dressed with γ-BHC powder alone and following pretreatment with 3% 'Polyvis' emulsion or 6% gum arabic solution were planted singly in tubes containing soil or sand. Plants were harvested at weekly intervals, or when about 15 cm high, extracted and analysed by glc, together with the soil or sand in which they were grown. In all treatments, there was little difference between the small amounts of γ-BHC found in different parts of the plant and most remained on the seed. About half as much insecticide (1–2 μg) was recovered from the sand in which 'Polyvis' treated seed was grown as in the sand which had contained seed treated with insecticide alone (2–5 μg). (Jeffs and Ladyman)

Vegetable oils as adhesives. Vegetable oils have shown promise as adhesives for powder dressings in recent tests. Seeds treated with 'Mergamma D' after previous spraying with 6% aqueous emulsions of olive oil or soyabean oil at rates of 0·6, 1·2 and 2·0 ml/100 g seed retained an average of 1099 ppm γ-BHC. In our standard retention test, 80% of the dressing was retained using 0·6% of either emulsion and 98–100% using the 1·2

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or 2·0% rates. Tests in pots containing loam soil in the glasshouse showed that neither emulsion by itself or with insecticide affected germination. These treatments will be tested in the field next year.

Treatment of seeds with liquid dressings in the laboratory. The apparatus used to apply adhesive emulsions to seeds was modified for use with commercial liquid formulations of pesticides. The apparatus treats 600 g of seed per batch, sufficient for 5–6 rows of plants at 10 g seed per row. When the dressing is applied at the normal commercial rate the standard deviation for the loading on individual seeds is 22%, compared with 83% when the dressing is added from a micrometre syringe on to 50 g batches in a revolving dish or 147% for seeds treated commercially with liquid formulations. The apparatus was used to apply liquid formulations to seeds at various rates for single row trials. (Jeffs)

Side effects of pesticides

Poisoning of honeybees in the field. Eighty-two samples of honeybees thought to be poisoned were received from beekeepers via the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food. Forty-five samples gave evidence of poisoning, but of these four came from two incidents, in each of which two apiaries were affected, bringing the number of poisoning incidents to 43, compared with 40 in 1971.

Bees from 32 incidents reacted positively to our test for organophosphate poisoning, a further sample also contained BHC and three gave inconclusive results. This test measures residual cholinesterase after poisoning, and does not specifically identify the insecticide residues. Evidence supplied with some of these positive samples suggested that 13 incidents were caused by spraying beans (nine from the air), seven by spraying peas (five from the air), one by spraying peas or beans from the air, and three more by aerial application to rape or mustard. In three further incidents, spraying of strawberries, raspberries and 'fruit' was reported. In two incidents, treatment of beekeeping equipment with preparations containing insecticide was suggested, one of these being the sample which also contained BHC.

Aldrin was found in one sample associated with pea spraying, and dieldrin in three where spraying of oil seed rape was reported, two cases being from different apiaries affected by the same application and the third involving application from the air. In addition to the case reported above, BHC poisoned four other samples, one of which was probably malicious while two were almost certainly from the same (unknown) cause. We identified carbaryl in one sample associated with reports of its use as a fruit blossom thinner.

The most striking aspect of these figures is the large number of incidents (19) reported to involve spraying from the air; we know of none in 1971 and only three in 1970, although more such cases were reported in earlier years.

Of the 25 samples received at Rothamsted by the end of June, we were unable to detect poisoning in 19. The spring of 1972 was unusually cold and this large number of negative samples may be explained by deaths due to starvation of overwintering bees being mistaken for poisoning. However there was a similar, if less marked tendency in previous years; in 1971 the corresponding numbers were 37 and 16, in 1970, 38 and 10 and in 1969, 15 and 9.

Acute toxicity of pesticides to honeybees in the laboratory. The methods already reported (*Annals of Applied Biology*, (1968) **61**, 467) were used to determine the acute oral and contact toxicity to worker honeybees of four further pesticides. Dimethoate

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was included as a standard. Table 5 gives the results as median lethal doses (LD50) with 95% fiducial limits. Pirimicarb is of particular interest because its success as an aphicide may lead to its use where bees are at risk.

TABLE 5

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

	Oral			Contact		
	LD50 (μg/bee)	Fiducial limits		LD50 (μg/bee)	Fiducial limits	
dimethoate	0·11	0·091	0·13	0·10	0·091	0·11
dialifor	29·2	23·5	36·8	28·6	21	37
				9·5	5·7	14
fenazaflor	2·9	1·7	3·7	12·2	4·4	18
pirimicarb	2·4	1·3	3·5	3·4	1·1	5·9
NRDC 119*	0·067	0·029	0·082	0·029	0·025	0·032

* 5-Benzyl-3-furylmethyl (+)-*cis*-chrysanthemate

(Stevenson)

Uptake of pesticides from aqueous solutions by slugs and worms. As part of a long-term study on the effects of pesticides on soil invertebrates, the factors determining the uptake of chemicals and their toxicity in soil are being investigated. In a wet soil, a chemical distributes between soil, water and organisms and in preliminary tests to characterise this distribution, the uptake of pesticides from aqueous solutions by slugs and worms was investigated by measuring the chemical remaining in solution at intervals.

Uptake of both dieldrin and diazinon by two varieties of slugs (*Arion hortensis* and *Agriolimax reticulatus*) and two varieties of worms (*Allolobophora longa* and *Lumbricus terrestis*) was similar. More than half the insecticide was removed from 50 ml solution by 2–5 g of invertebrate within six hours. More detailed studies with *L. terrestris* showed that the rate of uptake of both diazinon and dieldrin was proportional to the insecticide concentration over at least a 100-fold concentration range, suggesting that uptake was by a physical mechanism rather than by active transport.

Ligaturing worms to prevent ingestion of fluid had no effect on uptake of either diazinon or dieldrin. This indicates that uptake is through the cuticle and is probably limited by slow diffusion processes in the worm because stirring the aqueous solution did not significantly change the rate of uptake.

Uptake of endrin was similar to that of dieldrin, 95% being removed from solution after 24 hours but uptake of phorate and chlorfenvinphos was slower and half the chemical remained in the solution after this time. (Lord with Edwards, Entomology Department)

Behaviour-controlling substances

We continued to investigate a wide range of different species where there are prospects of improving pest-control or management of beneficial insects by exploiting responses to behaviour-controlling chemicals.

Pheromones from larvae of the Mediterranean flour moth and other stored products pests. The crowding pheromone which regulates the population density of larvae of the Mediterranean flour moth *Anagasta kuehniella* (Zeller) and the oviposition stimulant of its

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parasite *Venturia canescens* (Grav) are contained in the larval mandibular glands. The clear, oily secretion from the glands was obtained free from major contaminants by draining the glands on to glass slides and shown by chromatography and mass spectrometry to contain almost exclusively a compound (I), which had the molecular constitution $C_{22}H_{40}O_4$ and which elicited strong oviposition responses from the parasite *V. canescens*.

Larvae from other lepidopteran species related to *A. kuehniella* were examined to see if the same or similar compounds were present. Extracts of dissected mandibular glands from the last instar larvae of three lepidopteran stored products pests, the Dried currant moth *Ephestia cautella* (Wlk.), the Indian meal moth *Plodia interpunctella* (Hübn.) and the Cocoa moth *Ephestia elutella* (Hübn.) all contained as a major component, compound (I) previously identified in the mandibular glands of *A. kuehniella*. These extracts also caused *V. canescens* to oviposit.

This suggests that the pheromone-mediated population regulation mechanism demonstrated for *A. kuehniella* may also operate in other Lepidoptera and that the response is not species specific. (Mudd, with Dr. S. A. Corbet, Westfield College, London University)

Chemicals influencing behaviour of leaf cutting ants *Acromyrmex octospinosus* (Reich) and *Atta cephalotes* (L). Leaf-cutting ants are serious pests of the New World tropics and sub-tropics where they cause damage comparable with that of locusts. Poison baits offer the best prospects for economic control and our work is aimed at finding chemicals which could improve the efficiency of the baits and their acceptability to the ants.

Phytochemical arrestants. Further work to identify substances responsible for the arrestant properties of the grapefruit albedo used in the baits (see *Rothamsted Report for 1971*, Part 1, 189) showed that most, but not all the activity of a complete extract of albedo could be obtained by combining the constituent carbohydrates. The complete extract is therefore being examined more thoroughly to identify other components of the albedo which contribute to the arrestant activity.

Pharyngeal glands. The contents of these glands, which are implicated in food sharing and the transmission of insecticide within the colony, were identified previously (*Rothamsted Report for 1971*, Part 1, 189). This year we have done further work to determine the origin of the gland contents, i.e. whether they are obtained exclusively from the diet, from glandular secretions or from a mixture of both. The presence of ergosterol in the gland indicates that at least some of the contents are derived from the diet because insects cannot synthesise sterols. (Mudd, with Drs. J. M. Cherrett and D. J. Peregrine, University College of North Wales, Bangor)

Trail pheromone. Poison gland extracts which contain the trail pheromone of the ants, were examined and a fraction isolated chromatographically which had high trail-following activity. However, further characterisation was not possible because only very small amounts of material were available. (Mudd and Scott)

Chemistry of aphid cornicle secretions and body lipids. The work on cornicle secretions of aphids (*Rothamsted Report for 1971*, Part 1, 191) was completed and is summarised in the Abstracts of papers (p. 355). We have now extended this work to a study of triglycerides in the bodies of aphids and found that they contain the same fatty acid radicals as the cornicle secretions, but in different proportions. Although the triglyceride composition of body extracts (like that of the cornicle secretions) was not well correlated with

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taxonomic position, these extracts provide a second chemical characteristic that can be used to define and identify a particular species. Body extracts contain enough triglycerides for their composition to be determined in single aphids and the use of body extracts allows examination of aphids lacking cornicles and of specimens that do not give secretion because of low body turgor. All summer forms of *Myzus persicae* (Sulz.) had similar body triglycerides even when selected for resistance to organophosphorus insecticides or bred for three months on an artificial diet. The composition of body triglycerides was also independent of colour in two aphids species in which pink and green forms were compared.

When cornicle secretions were collected progressively so as to draw increasingly upon body fat reserves, their composition changed gradually towards that of the body extracts. (Griffiths and Greenway)

Codling moth. Field trials using the synthetic sex attractant (*trans*-8, *trans*-10-dodecadien-1-ol) were conducted during 1972 at East Malling. Male moths were caught by this attractant earlier in the year than by conventional light-traps so that longer advance warning of infestation can be obtained. Unlike the light-traps, the material is highly specific for codling although it did collect a few other related lepidoptera. Even small populations of moths in orchards sprayed with insecticides could be detected. (Greenway, with Dr. J. Cranham, East Malling)

Chemicals affecting behaviour of wheat bulb fly larvae. Further laboratory and glasshouse tests were done to characterise the substances in cereal extracts that affect the behaviour of wheat bulb fly larvae. Previous observations showed that wheat extracts have an 'arrestant' effect and oat extracts an 'anti-arrestant' effect (*Rothamsted Report for 1970*, Part 1, 167–168). We have now found that extracts from shoots of barley, rye and ryegrass also act as arrestants. Guttation droplets from growing wheat seedlings failed to arrest larvae.

Larvae attacking wheat in the field will rarely enter a plant already occupied by a feeding larva; if a second larva does enter, it usually dies (Gough (1946), *Bulletin of Entomological Research* **37**, 251–271). However, we found no differences between the arrestant properties of extracts from attacked and healthy wheat shoots in laboratory tests. Also, an extract of actively feeding second instar larvae dissected from attacked wheat plants did not arrest other larvae or produce an 'anti-arrestant' effect when added to wheat.

The persistence of aqueous extracts containing 'wheat arrestant' and 'oats anti-arrestant' was measured by incubating them at 20°C both with and without fresh soil and comparing their biological activities at intervals with extracts stored at 0°C. After 25 days, the extracts incubated without soil lost half their activity whilst those with soil were completely inactive.

To see if plant extracts could be used to interfere with host plant location, further box tests were done to examine the effects of oat and wheat extracts on attack by wheat bulb fly larvae (see *Rothamsted Report for 1971*, Part 1, 190). Application of oat extracts decreased the numbers of wheat shoots attacked compared with wheat extracts and controls, but because of large standard errors, the differences were mostly not statistically significant in this year's tests. Application of wheat extracts had no effect on the total number of attacked tillers. Although oats are themselves immune, they did not protect wheat plants when grown next to them.

In another series of tests, boxes were divided so that one half contained compost and the other half a 3:1 (w/w) mixture of compost and activated charcoal. Larvae from eggs placed along the line separating the two sections attacked a mean of 28 shoots/100 plants

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growing in the charcoal side compared with 90 shoots/100 plants in the compost side. When activated charcoal was applied to a small area around each seed, 30 shoots/100 plants were attacked compared with 48 shoots/100 untreated plants in the early stages of the experiment. However, this difference became less marked as the experiment progressed.

It is possible that the charcoal in these tests absorbs wheat exudate so that larvae are unable to locate the plants, but it may act as a physical barrier to the larvae. Further tests are therefore needed to find out exactly how charcoal protects wheat from attack. (Scott and Greenway)

Movement of 9-oxodecenoic acid on bees' bodies. This work is described in the report of the Entomology Department (p. 220). (Callo wand Greenway, with Butler and Simpson, Entomology Department)

Equipment and techniques

Sampling and spraying equipment. The radial outflow turbine developed for use with spore trapping equipment (*Rothamsted Report for 1968*, Part 1, 189) is now produced commercially. Continuous operation of recording spore traps in heavily polluted atmospheres has shown the considerable advantages of this type of glandless, seal-free pump compared with the positive displacement pumps previously used. The insect suction sampler powered by a petrol engine (*Rothamsted Report for 1968*, Part 1, 189) is also in commercial production and marketed as the Univac Sampler. Several refinements have been made, so that the samples collected are now easier to remove and less prone to damage.

The atomisers developed in 1967 have now been evaluated using a modified Kearns and March spray chamber. The much smaller air input from these atomisers compared with conventional sprays minimises mixing and turbulence within the chamber. Spray distribution is improved by moving one of the two horizontally opposed atomisers to a position 9 cm above the centre line of the chamber. The mass median diameter of the spray deposit during these tests was 60 μm.

Measurement of insect activity. For further studies of wheat bulb fly activity (see report of the Entomology Department, p. 207) an apparatus has been developed to record their flights. The flies are contained in a 60 cm^3 cage consisting of a light wooden frame covered with terylene mesh. The top and side panels are connected to a low impedance transducer by a continuous nylon thread. Movement of the panels caused by a fly alighting on the mesh is detected by the transducer, amplified, and used to trigger an event recorder and electro-mechanical counter. The cage is suspended by elastic bands and cords which isolate it very effectively from airborne vibrations and those transmitted through the structure of the building. Because a low impedance detector is used electrical screening is unnecessary, the equipment being unaffected by nearby electrical apparatus or the fluorescent lights required to provide periods of artificial daylight. A detector capable of indicating the frequency of wheat bulb fly feeding is now being developed. (Arnold)

Electronic counting of insect nerve fibres. In studying the neuroanatomy of the central nervous system of the cockroach *Periplaneta americana* (L.) (*Rothamsted Report for 1970*, Part 1, 176) it has proved difficult to count the many very small fibres (<2 μm diam.) in some of the peripheral nerves and their root bundles. This was overcome with a camera lucida and Arnold portable electronic counter (*Laboratory Practice* (1969) **18**, 444–445). The image of the tip of the counter probe could be seen superimposed on the microscope field in the same way as the pencil point when drawing, and the nerve fibres counted by

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touching the probe tip against the drawing-board at the position of each fibre. Using a felt-tipped pen as the probe enabled fibres to be marked off on paper to prevent counting any twice. The method showed that there are more small sensory fibres than thought previously: about 700 in the mesothoracic nerve 2, 150–200 in nerve 3 and over 600 in nerve 5. (Gregory)

Artificial feeding of aphids. The use of purer materials, and practice in techniques, now enables us to keep *Myzus persicae* (Sulz.) for several months on the artificial diet described by Mittler *et al.* (*Journal of Insect Physiology* (1970) **16**, 2315–2326) and so begin using this technique to study the effects of plant constituents on aphid behaviour. However, nine components of the diet, although perhaps necessary for proper nutrition, deterred many individuals of *M. persicae* from feeding and are better omitted when immediate acceptability is required, for example for tests on uptake of insecticides. Diets lacking these materials were also more readily accepted by *Acyrthosiphon pisum* Harris and *Aphis fabae* Scop. Neither diet was suitable for *Megoura viciae* Buckt. which can penetrate the parafilm membranes retaining the diet but which may require specific plant substances to encourage feeding. (Griffiths and Greenway)

Insect rearing. The following species were reared:

PLANT FEEDERS

Homoptera	*Aphis fabae* (Scop.)
	Myzus persicae (Sulz.)
	Strains. Susceptible
	Two organophosphate-resistant
	Megoura viciae Buckt.
Hemiptera	*Dysdercus intermedius* Distant
Coleoptera	*Phaedon cochleariae* (F.)

OTHERS

Orthoptera	*Blaberus discoidalis* (L.)
	Periplaneta americana (L.)
Lepidoptera	*Pieris brassicae* (L.)
Diptera	*Drosophila melanogaster* (Meig.)
	Strains. Normal
	Vestigial wings
	Musca domestica (L.)
	Strains. *ac; ar; bwb; ocra* SRS—fully susceptible to DDT, dieldrin and organophosphorus insecticides
	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, DDT or dieldrin. 239 fb and 49 r_2—dimethoate selected, very resistant to many organophosphorus insecticides
	Several strains derived from 239 fb and 49 r_2, each with one or more factors of resistance to dimethoate, and other organophosphorus insecticides
	NPR—pyrethrum extract selected, very resistant to pyrethroid insecticides, 104—resmethrin selected, very resistant to resmethrin, *ac; ar; bwb; ocra*—called 608Q fully susceptible to pyrethrum knockdown, pyrethroid insecticides and to carbamates

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Some strains derived from NPR each with one or more factors of resistance to pyrethroid insecticides, DDT and dieldrin
Two strains with DDT knockdown resistance, *kdr-0*; *507*
A wild type susceptible strain
Calliphora erythrocephala (Meig.)
Hymenoptera *Acromyrmex octospinosus* (Reich)
Atta cephalotes (L.)

Fungicides

Tests of fungicides to control common scab on potato tubers were continued, and experiments on the efficiency of mercury seed dressings in controlling *Septoria nodorum* and *Fusarium nivale* on wheat were started.

Glasshouse tests. A few tests last year indicated that some simple chlorinated 1,4-benzoquinones were effective as soil-treatments to control potato common scab (caused by soil-borne *Streptomyces scabies*). We have now tested many other quinones as possible scab-control chemicals; this was done, as before, by mixing them with scab-infested soil from Great Hill Bottom, Woburn, in which Majestic plants were then grown in the glasshouse.

No anthraquinones or naphthoquinones were effective; thus, the following either failed to control scab ($P = 0{\cdot}05$), or damaged the plants, or both, at 50 ppm: 1-chloro- and 2-chloro-anthraquinone; 1,4-naphthoquinone, 2-chloro-, 2-hydroxy-, 2-methoxy-, 2-methyl- and 2,3-dichloro-1,4-naphthoquinone (dichlone).

Table 6 shows results with substituted 1,4-benzoquinones and hydroquinones, including chloranil (tetrachloro-1,4-benzoquinone) and also quintozene as a standard for comparison. Yields and amounts of scab are given as percentages of those in the corresponding 'nil'-treatments; the figures are the mean percentages from the numbers of tests shown (15 plants per treatment per test). Roman type indicates effects that were not significant (i.e. $P > 0{\cdot}05$), and bold type those that were significant at $P < 0{\cdot}01$ (yield) or $P < 0{\cdot}001$ (scab).

The quinones varied greatly in their activity against scab and their phytotoxicity. However, as would be expected, corresponding 1,4-benzoquinones and hydroquinones

TABLE 6

Effects of soil-treatments with substituted quinones (50 ppm) on relative yield and scab incidence in the glasshouse

	1,4-Benzoquinones			Hydroquinones		
Substituent	No. of tests	Yield	Scab	No. of tests	Yield	Scab
no substituent	6	**90**	**22**	6	95	**22**
carboxy-	—	—	—	2	109	93
chloro-	7	96	**15**	9	**92**	**15**
methyl-	4	**83**	**19**	2	**80**	**17**
phenyl-	2	98	**23**	—	—	—
2,5-di-tert.butyl-	2	**30**	**21**	2	**34**	**36**
2,5-dichloro-	5	106	**24**	2	108	**20**
2,6-dichloro-	4	99	**29**	—	—	—
2,5-dihydroxy-	2	109	124	—	—	—
2,6-dimethoxy-	3	**87**	88	—	—	—
tetrachloro-	2	109	**61**	—	—	—
quintozene	23	**95**	**24**	23	**95**	**24**
'nil'-treatment		100	100		100	100

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which are easily converted into one another in biological systems behaved almost identically. For example, both the 2,5-di-tert.butyl- compounds damaged the plants severely and both the 2,5-dichloro- compounds controlled scab well without decreasing yield.

All these tests were done at 50 ppm of chemical in soil. However, in one test, chloro-1,4-benzoquinone and chloro-hydroquinone controlled scab almost as well at 25 ppm as at 50 ppm, with slightly smaller decreases in yield.

In general it is clear that, allowing for compounds not tested here, there must be many 1,4-benzoquinones and hydroquinones which control scab as effectively as quintozene, without affecting yield. The most acceptable of those tested were possibly the 2,5-dichloro-compounds. However the established fungicide chloranil (tetrachloro-1,4-benzoquinone) was only slightly active, and dichlone (2,3-dichloro-1,4-naphthoquinone) which is chemically more stable than chloranil, was ineffective; by contrast, the simpler benzoquinones and hydroquinones were more effective.

Because of this, we tested some unsubstituted polyhydric phenols, and a few of their ethers, in the same way. Catechol was slightly less effective than hydroquinone, but did not decrease yield; all the others tested had slight or no effects on scab and yield, viz. catechol monomethyl ether (guaiacol), catechol dimethyl ether (veratrole), resorcinol, hydroquinone mono- and dimethyl ethers, pyrogallol and phloroglucinol.

These results confirm the view that the best scab-control chemicals in this series are likely to be simple quinones, i.e. either catechols or hydroquinones (or their corresponding benzoquinones); neither chlorination nor stability seems to be necessary.

Field trials

Control of scab by haulm-sprays. Last year we reported laboratory tests which showed the possibility of controlling scab by spraying the foliage with ethionine. This has now been confirmed in two similar small field trials (var. Maris Piper) at Woburn, one in Great Hill Bottom and the other in Schoolfield.

Aqueous solutions of DL-ethionine, with and without dimethyl sulphoxide (to increase penetration into leaves) and 'Manoxol OT' (a wetter) were sprayed on the haulms at about 130 gal/acre (1460 litres/ha) on 13, 16 and 20 June, when tubers were beginning to form and were susceptible to attack by *S. scabies*. Scab indexes, which are estimates of the percentages of the skins disfigured by scabs, were calculated at harvest from 40 ware tubers per plot (three plots per treatment per trial). Table 7 summarises the results. Ethionine decreased the amount of scab, but neither of the additives altered its effectiveness. The total amount of ethionine applied, on all three occasions, was about 9 kg/ha; the resulting control compared favourably with that given in previous field trials by soil-treatment with quintozene at about 80 kg/ha.

The plots in these trials were too small (one row × 3·3 m) to give reliable estimates of yield, but there was no indication that any of the treatments affected yield.

TABLE 7

Effects of haulm-sprays on incidence of potato common scab in the field

Spray treatment	Scab indexes (means from two trials)
nil	40
0·2% DL-ethionine	28
0·2% DL-ethionine + 0·5% dimethyl sulphoxide	31
0·2% DL-ethionine + 0·02% 'Manoxol OT'	29
LSD, $P = 0·01$	7
$P = 0·001$	10

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Control of scab by soil-treatment. Residual effects from last year's trial on Schoolfield, Woburn, were measured by re-planting the site with potatoes (var. Maris Piper), without further application of chemicals to the soil. As before, yields and scab indexes were measured at harvest. Table 8 shows that there was a clear residual effect on yield by pentachloropyridine and on scab incidence by quintozene, corresponding to the direct effects in 1971. However, captafol, which affected scab almost as much as quintozene in 1971, had no residual effect this year.

TABLE 8

Residual effects of soil-treatments on yield and incidence of potato common scab in the field

Treatment in 1971	Rate, kg/ha	Total tubers, tonnes/ha		Scab index	
		1971	1972	1971	1972
quintozene	78	39	42	13	32
captafol	78	49	46	15	38
captafol	39	50	50	16	38
dinocap phenols	78	45	50	29	43
pentachloropyridine	78	20	32	22	46
nil	—	45	48	22	42
LSD, $P = 0{\cdot}05$		9	9	4	7
$P = 0{\cdot}01$		12	12	6	10
$P = 0{\cdot}001$		16	16	8	—

(McIntosh)

Treatment of potato seed tubers to control diseases. Work on the behaviour of fungicides applied to potato seed tubers is described in the report of the Plant Pathology Department (p. 150). (Lord, with Tisdale, Plant Pathology Department)

Behaviour of propionic acid in hay. Studies on the use of propionic acid to prevent moulding and heating of hay are described in the report of the Plant Pathology Department (p. 129). (Lord, with Lacey, Plant Pathology Department)

Plant growth regulators

Behaviour of chlormequat on wheat and barley. Chlormequat is a plant growth regulator used to prevent lodging of wheat by retarding stem growth, but it has comparatively little effect on barley. The reasons for this difference are not known, but Alcock and Morgan (*Proceedings of the 9th British Weed Control Conference 1968*, **1**, 238) postulated that chlormequat is translocated more freely in wheat than barley. We therefore compared uptake and movement of ^{14}C-labelled chlormequat in wheat and barley leaves.

Small drops (2 μl) of 0·25% ^{14}C-labelled chlormequat in 50% aqueous acetone were applied to leaf surfaces of intact wheat and barley plants. Aqueous solutions without wetter are usually used in practice but acetone was essential in our tests to improve wetting of the leaf surfaces so that drops could be applied reproducibly.

Measurements at the point of application with a thin-end-window Geiger counter showed that about one-third of the radio-activity was lost from the leaf surface in one hour, the material entering wheat faster than barley. Measurements above and below the point of application showed that radio-activity moved faster towards the leaf tip than towards the root. The results were confirmed by repeated scanning of leaves using a thin-layer chromatogram scanner with a windowless counter.

The distribution of radio-activity was measured quantitatively by cutting treated

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leaves into 1 cm lengths 24 hours after applying the chemical. Each section was washed with water to remove chlormequat from the surface and then ground with ethanol to extract radio-active chemicals from the leaf tissues. Radio-activity in both washings and extracts was assayed by liquid scintillation counting. At least 90% of the radio-activity was found in the surface washings from barley compared with less than half for wheat. Amounts in surface washings from sections on either side of the point of application were negligible. The corresponding extracts contained significant amounts of radio-activity, however, with more moving towards the tip than towards the root. Examination of the extracts by chromatography on silica gel loaded paper followed by autoradography showed that there had been little metabolism of the chlormequat.

Similar general trends were observed when tests were extended to 4–7 days, although as expected uptake and movement were more extensive. (Lord)

Staff

C. Potter retired in April after 34 years with the department, the last 25 as head. Fortunately, however, he will remain associated with the department. R. K. Callow also retired. G. L. Bateman was appointed.

At the invitation of the organisers, P. E. Burt read a symposium paper at the 14th International Congress of Entomology in Canberra and subsequently visited research laboratories in Australia. I. J. Graham-Bryce contributed an invited paper to an International symposium on 'Pollution, Engineering and Scientific Solutions' in Tel Aviv and visited research institutes in Israel as a guest of the Volcani Centre for Agricultural Research. He also read an invited paper at the 11th British Weed Control Conference, Brighton, and was a member of the United Kingdom delegation to the FAO Conference on 'Ecology in Relation to Plant Pest Control' in Rome. A. R. Greenway read a paper at the NATO Advanced Study Institute on the Chemistry of Insects, Varenna, Italy.

At the invitation of the Pakistan Government, K. A. Lord spent two weeks in Karachi advising the Department of Plant Protection, Ministry of Agriculture on methods of studying pesticides. F. T. Phillips spent one month in the West Indies investigating the persistence of insecticides on baits and the possibilities of using slow-release formulations against tropical pests.

Sandwich course students who worked in the Department were S. Ladyman, Barbara Morison and A. Mulkerrins.

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