ROTHAMSTED RESEARCH

Report for 1971

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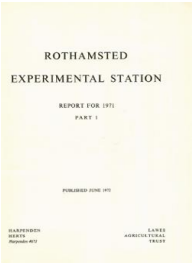

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

Although work in the department on substances influencing insect behaviour has not had any direct application, possibilities are indicated by the effects of extracts from oat and wheat plants on Wheat Bulb fly larvae. Extracts of oats not only decrease the arrestant response of the larvae to wheat-plant extracts in the laboratory, but when applied to soil they also decrease secondary attack.

Our success in synthesising active pyrethroids has stimulated work in other countries on the synthesis of this type of compound. However, much less interest has been shown in their mode of action and the mechanisms whereby insects become resistant to them. A knowledge of these subjects should not only help in developing yet more active compounds but also compounds more likely to retain their usefulness. The evidence that the knockdown and the lethal effects of pyrethroids are probably progressive stages at one site in the central nervous system rather than separate actions, one peripheral and one central, should simplify the search for more effective compounds.

Physiologically selective insecticides with adequate persistence do not exist to control most insect pests and may not be commercially attractive to produce. However, formulation as microcapsules can make the existing insecticides selective for insects with biting mouth-parts, and enclosing short-lived ones in microcapsules that slowly leak can greatly prolong the action of contact insecticides at sites where they are needed. Such formulations require less poison to be used to produce a given biological effect on the target organism and allow readily degradable poisons to be used where they are ineffective with conventional formulations. Such formulations also decrease the risk of harmful side-effects.

Insecticides

The causes of resistance

Resistance of* Myzus persicae *Sulz. and* Musca domestica *L. to dimethoate. Many populations of the potato-peach aphid (*Myzus persicae* Sulz.) in glasshouses, and houseflies (*Musca domestica* L.) that are resistant to malathion or parathion now resist dimethoate. Some of the resistance mechanisms to dimethoate in both species may be alike because sesamex synergises dimethoate against resistant aphids and houseflies. We studied resistance to dimethoate in both species, but more with houseflies because we know more about them.

Genetics of resistance of houseflies to dimethoate. The factors of resistance to dimethoate were located and isolated in a homozygous state from two dimethoate-resistant strains of houseflies (239 f b and 49 r_2b), both kindly presented by Dr. Keiding, Springforbi, Denmark. Strain 49 r_2b (R.F. *c.* $\times$ 75) was more than twice as resistant as strain 239 f b (R.F. *c.* $\times$ 30) against dimethoate, but both strains were equally resistant to diazinon (R.F. *c.* $\times$ 40) and parathion (R.F. *c* $\times$ 20). Both strains had individuals very resistant to malathion.

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Weak to very weak factors of resistance were located in both strains on chromosomes I (R.F. *c.* × 1·5) and V (R.F. *c.* × 3). The resistance factors on II autosome derived from strain 239 fb conferred weak resistance to dimethoate (R.F. *c* × 3) and malathion (R.F. *c.* × 3), but the same autosome from strain 49 conferred slightly greater resistance to dimethoate (*c* × 6) and near immunity to malathion. Sesamex synergised dimethoate a little against the strains with autosome II derived from strain 49 (R.F. 1·5–2), but slightly more (*c* × 3) against a similar strain derived from strain 239. These results suggest that there are at least two factors of resistance on autosome II in strain 49, one similar to that of strain 239, about 40 cross-over units from the marker *ar*. This factor gives little resistance to malathion and is incompletely affected by sesamex. The other factor, which confers near immunity to malathion, enhances resistance to dimethoate and is unlikely to be much affected by sesamex. It is probably located close to *ar* and is not the carboxyesterase mechanism commonly present in malathion-resistant houseflies, because TBTP (S,S,S-tributyl-phosphoro-trithioate) does not synergise malathion against the strain with autosome II derived from strain 49. Resistance to parathion on this autosome is not linked with the gene for a light ali-esterase activity, because strains 239 and 49 have normal ali-esterase activity, and are resistant to parathion ethyl phosphonate.

The weak resistance to dimethoate on autosome III is probably caused by factor *Pen* (delayed penetration), but there may be another weak mechanism of resistance.

The resistance to dimethoate on autosome V is completely inhibited by sesamex. This autosome carries no detectable tolerance to parathion.

The results suggest that strains 239 and 49 r_2b are moderately resistant to dimethoate because they have several factors of resistance that interact. Individually each factor is weak. This might explain why dimethoate resistance took so long to become established and why it is strong only where this insecticide is used continuously. Dimethoate replaced malathion or parathion because the resistance mechanisms to these insecticides (carboxyesterase, or phosphatase) are ineffective against dimethoate. These mechanisms, not selected by dimethoate, disappeared and were replaced by new and different mechanisms linked with resistance to dimethoate, which confer resistance to dimethoate, parathion, malathion and other organophosphorus insecticides. Thus, selection with dimethoate has not only caused resistance to dimethoate but has also retained resistance to parathion and malathion but altered the nature of the mechanisms of resistance to these insecticides. (Sawicki)

Resistance of* Musca domestica *L. to pyrethroids. The genetical study of resistance to pyrethroids in strain NPR selected with natural pyrethrins continued to try to isolate the factors on chromosome II, to identify the factors on chromosome III and to compare the resistance systems in NPR with those of the resmethrin-selected strain 104.

Chromosome II from NPR was isolated in strain 381 by substitution into the susceptible quadruple marker strain during earlier genetical work (*Rothamsted Report for* 1970, Part 1, 165). 381 bred true for lack of the aristapaedia marker and therefore theoretically was homozygous for chromosome II from NPR, but it was heterogeneous for resistance to synergised pyrethrolone esters. All further attempts to isolate this incompletely dominant factor in the homozygous state failed. Single pair matings had poor fertility and selection of 381 for homozygous resistant flies with a mixture of natural pyrethrins and sesamex or with 'Zectran' followed by mass culture failed, as survivors did not breed for more than two generations.

To identify the two factors isolated on chromosome III, three standard strains, *organotin-stw*, *kdr-O* and *bwb kdr*, which had known resistance mechanisms on chromosome III, were acquired. 314, the strain with the penetration factor alone from NPR on chromosome III was crossed with *organotin-stw*. The progeny were homozygous for resistance

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to tributyltin acetate indicating that the genes controlling the resistance factors in these two strains were the same.

The other pyrethroid resistance factor on this chromosome, isolated in strain 348, was also investigated. It is closely linked, if not identical, with a resistance factor for DDT. Strain *kdr-O* proved unsatisfactory for identifying the factor because it was not homozygous for its resistance factor. Strain *bwb kdr* was also unsuitable because, although the knockdown resistance factor under examination that affects both DDT and pyrethroids was homozygous, this strain had the mechanism DDT-dehydrochlorinase which could interfere with the analysis.

Bioassay with DDT, natural pyrethrins and resmethrin of the progeny of the cross of strain 348 to a susceptible strain showed that the resistance factor was almost completely recessive. The unknown system in strain 348 will be identified when the two standard strains are homozygous only for their named resistance factor.

The genetical analysis of strain 104, selected with resmethrin to locate the resistance factors, showed that the only detectable resistance system to natural pyrethrins or resmethrin was on chromosome III, in contrast to NPR which also had a minor factor on chromosome II and a major one affecting pyrethrolone esters on chromosome V. As in NPR, resistance to synergised pyrethrins in strain 104 was on chromosomes II and III. Thus, the cross-tolerance spectrum of strain 104 to pyrethroids alone depended on one chromosome and to synergised pyrethrins on two chromosomes. The additional resistance to pyrethrolone esters in NPR mainly comes from the extra mechanism on chromosome V. (Farnham)

Loss of resistance in* Myzus persicae *to organophosphorus insecticides. Last year we reported a sudden loss of resistance to dimethoate in a culture of a strain (GR) of *M. persicae* not kept under selection pressure. This happened again in a different strain (DDTR) of *M. persicae*, which, as we show later, seems to have a resistance mechanism different from the GR strain. Resistance was lost in several sub-cultures of a clone at the same time. These were being maintained in different places and all except one, which was in a glasshouse, were in controlled environment chambers. The parent stock from which the clone was derived, maintained its strong resistance to dimethoate although the culture was not treated with dimethoate.

Aphids were reared without selection for approximately 17 generations with no loss of resistance. Resistance was then lost during the next three generations. This loss occured at the same time as the aphids lost ali-esterase activity.

Contamination of cultures. We thought that the loss of resistance reported in 1970 may have happened because the cultures became contaminated with susceptible individuals. To test this, we established a culture with equal proportions of resistant (DDTR) and our standard susceptible clone of *M. persicae*. At intervals for about four generations these were tested with dimethoate, but equality was maintained between the numbers of resistant and susceptible individuals. When the other DDTR cultures lost their resistance, this equality broke down and the whole culture responded as suceptible. Thus, contamination of the cultures seems not responsible for the loss of resistance.

Cross resistance and synergism. After the clone of the DDTR strain lost its resistance, a new clone was started from the DDTR parent stock; it has now maintained its resistance for 19 generations without selection pressure. Table 1 shows the insecticides which we have shown it resists. It is very resistant to malathion whereas another resistant clone of the GR strain is susceptible. These two strains also differ in their reaction to the synergist sesamex (a mixed function oxidase inhibitor). Adding sesamex to solutions of dime-

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thoate applied to the GR strain lessened its resistance and it responded almost as the susceptible strain. Sesamex increased the resistance of the GR strain to disulfoton, but had no effect on the response of the DDTR strain to insecticides. This suggests that our strains of *M. persicae* have different resistance mechanisms. (Needham)

TABLE 1

Resistance factors to several organophosphate insecticides applied topically to Myzus persicae *(DDTR strain)*

dimethoate	680
ethyl parathion	660
methyl parathion	47
parathion phosphonate	100
malathion	400

Biochemical mechanisms involved in the resistance of *Myzus persicae* Sulz. and *Musca domestica* L. to organophosphorus insecticides

Metabolism of* [*ethoxy-*^{14}C] *parathion by houseflies. A further detoxication mechanism was detected in strains with chromosome II derived from the diazinon-resistant SKA strain and the dimethoate-resistant strains 239 f b and 49 r_2b. This glutathione-dependent mechanism in the sub-cellular non microsomal fraction forms diethyl phosphorothioic acid (DEPTA) from parathion. It is probably an aryl-glutathione transferase. (Fig. 1).

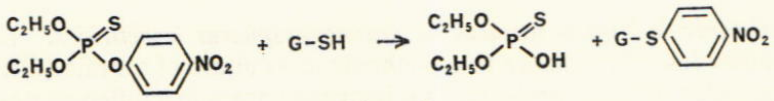

Figure 1

Metabolism of* [*methoxy-*^{14}C] *dimethoate. [Methoxy-^{14}C] dimethoate was synthesised and its metabolism studied in *Musca domestica* and *Myzus persicae*, and in fractionated extracts of *M. domestica*. Tests with extracts of *M. persicae* failed because both microsomal and supernatant fractions of macerated aphids contained substances that inhibited enzyme activity. Metabolism of dimethoate by extracts of flies was inhibited by adding extracts of aphids. The microsomal fraction of houseflies metabolised dimethoate mainly to its phosphate analogue, but only in the presence of NADPH. The 'soluble' fraction transferred one of the O-methyl groups from dimethoate to glutathione, to produce O-desmethyl dimethoate and methyl glutathione (Fig .2). This transfer is analogous to the desethylation of parathion and diazinon reported by Lewis.

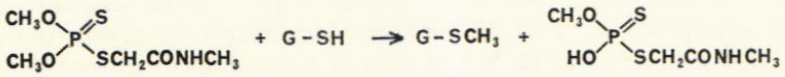

Figure 2

In vivo, the susceptible strains of both houseflies and aphids converted more dimethoate to dimethoxon than the resistant strains. This production of relatively large amounts of dimethoxon contrasts with the metabolism of parathion and diazinon of which only very small quantities of the phosphate analogues are produced. It suggests that dimethoxon has less neurotoxicity than paraoxon or diazoxon and that to kill flies dosed with dimethoate it is produced in large quantity and breaks down slowly. The major metabolic fate of parathion and diazinon in houseflies is their conversion to less toxic polar compounds.

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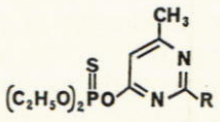
In addition to activating less of the dimethoate, resistant houseflies excrete more dimethoate metabolites than susceptible houseflies. The metabolites detected in extracts and excreta of both susceptible and resistant flies include dimethoxon, dimethyl phosphorodithioic acid, O-desmethyl dimethoate and methyl glutathione. Increased excretion of dimethoate metabolites therefore seems to be a resistance mechanism to this insecticide in some strains of housefly. The mechanism is analogous to that delaying the penetration of topically applied insecticides through the cuticle. Both rely on the controlled passage of material through membranes. (Devonshire)

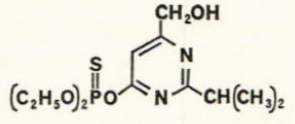
Housefly esterases. Some organophosphorus compounds (e.g. TOCP or mipafox) inhibit an esterase in the nervous system of hens and this causes delayed neurotoxicity (Johnson, *Biochemical Journal* (1969), **114**, 711). Some work was done to see whether houseflies have a similar mechanism. The activity of the mipafox-sensitive esterase in the brain of the hen is assayed with phenyl valerate after inhibiting all the paraoxon-sensitive esterases with $5 \times 10^{-5}M$ paraoxon. In the housefly, the mipafox-sensitive esterase cannot be assayed with phenyl valerate because paraoxon fails to inhibit all the paraoxon-sensitive esterases, even at a concentration of $2 \times 10^{-3}M$ paraoxon, although $5 \times 10^{-5}M$ paraoxon inhibits all esterase activity against α-naphthyl acetate. Aldridge (*Biochemical Journal* (1953), **53**, 117) classifies esterases according to their susceptibility to inhibition by paraoxon. Type A esterases are not susceptible, type B esterases are. Judged on this classification, phenyl valerate esterases are Type A in houseflies but Type B in hens. (Devonshire, in collaboration with Dr. M. K. Johnson, MRC Toxicology Unit, Carshalton)

Metabolites of diazinon and pyrimithate in mammals and in insects. Decomposition of diazinon (1) and pyrimithate (5) by UV irradiation and in mammals, especially sheep, was studied at the Central Veterinary Laboratory, complementing our work with houseflies (*Rothamsted Report for 1970*, Part 1, 164). Several metabolites (0·1–2 mg of each) that retained potential anticholinesterase activity were isolated and purified chromatographically. Their structures were established from their nmr spectra (obtained by spectrum accumulation with the computer accessory) and their mass spectra. (Table 2)

UV irradiation of diazinon (1) gives the known hydroxydiazinon (2) and the acetyl derivative (3) by oxidation of the isopropyl side-chain.



R
1. -CH(CH$_3$)$_2$
2. -COH(CH$_3$)$_2$
3. -CO.CH$_3$
4. -C(CH$_3$)=CH$_2$
5. -N(CH$_3$)$_2$
6. -N(CH$_3$).CHO
7. -NH.CH$_3$



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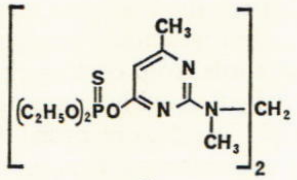

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Figure 3

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TABLE 2

Spectral data for products formed from diazinon and pyrimithate

Compound		Nmr peaks from sidechain* (τ value, multiplicity, area)	Calculated molecular weight = value found in mass spectrum
diazinon	(1)	8·67 (d, 6H); 6·87 (m, 1H)	304
hydroxydiazinon	(2)	8·44 (s, 6H)	320
acetyl analogue of diazinon	(3)	7·3 (s, 3H)	304
dehydrodiazinon	(4)	7·78 (m, 3H); 4·47 (m, 1H) 3·56 (m, 1H)	302
pyrimithate	(5)	6·85 (s, 6H)	305
N-formyl analogue of pyrimithate	(6)	0·19 (s, 1H); 6·63 (s, 3H)	319
N-demethyl pyrimithate	(7)	7·00 (d, 3H)	291
isomer of hydroxydiazinon	(8)	8·65 (d, 6H); 6·8 (m, 1H) 5·27 (s, 2H)	320
dimer	(9)	4·25 (s, 2H); 6·89 (s, 2 × 3H)	594

* Peaks from the rest of the molecule near 5·6 (dq, 4H) and 8·6 (t, 6H) (diethoxy group) and at 3·0–4·0 (s, 1H) (ring proton) are observed in all cases. The ring CH_3 at 7·3–7·7 is also present, except in compound 8.

Metabolism of diazinon in sheep gives, in addition to hydroxydiazinon, dehydrodiazinon (4) and an isomer of hydroxydiazinon (8).

Previous work showed unknown metabolites in the housefly some of which could be identical with these compounds.

UV irradiation of pyrimithate (5) gives, by successive oxidations of one of the N-methyl groups, the dimer (9), the N-formyl derivative (6) and N-demethylpyrimithate (7). (Janes, with Mr. A. F. Machin, Central Veterinary Laboratory, MAFF, Weybridge)

Mode of action of pyrethroids

Action of synergised pyrethroids on the central nervous system of *Periplaneta americana* L. The synergists piperonyl butoxide and sesamex increase the toxicity of pyrethroids by slowing their rate of conversion to less toxic compounds in the insect, allowing more toxicant to reach its site of action. However, whether these compounds also synergise pyrethroids at their sites of action within the nervous system is unknown. Although adding these synergists to pyrethrum applied to cockroach central nervous systems shortened the time taken by pyrethrum alone to block conduction in giant fibres (*Rothamsted Report for 1967*, 167–168), the shortening—up to 75% with a 300 : 1 ratio of synergist to pyrethrum—was small, so in recent experiments the method of applying the synergist was altered to conform more closely with *in vivo* conditions of poisoning. One mg doses of piperonyl butoxide were applied to the metathoracic sterna of adult male *P. americana* (American cockroaches) one or two hours before the abdomens were amputated and the abdominal nerve cords exposed by dissection. When these cords were irrigated continuously with $3{\cdot}2 \times 10^{-6}M$ pyrethrin I in saline for up to 60 minutes, the strength of stimulus required to give maximum response in the giant fibres, and the amplitude of the action potentials produced, resembled those of cords from untreated cockroaches. When cords from cockroaches previously treated with piperonyl butoxide were tested after irrigation with saline alone, the amplitude of the giant fibre action potentials was slightly less than recorded with untreated cockroaches.

The amount of spontaneous nervous activity shown by sixth abdominal ganglia irrigated with 10^{-8} and $10^{-7}M$ pyrethrin I, one to two hours after the insects were treated

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topically with 1 mg piperonyl butoxide, was compared with the activity shown by irrigated ganglia of untreated insects. Activity in ganglia from both treated and untreated insects increased fairly quickly soon after irrigation began, but 20 minutes later, when ganglia from untreated insects were still very active, ganglia from treated insects were less active, and for the next 20 minutes remained less active than ganglia from untreated insects irrigated only with saline. Ganglia from cockroaches treated with piperonyl butoxide but irrigated only with saline were as active as ganglia of untreated controls.

Thus we gained no evidence that piperonyl butoxide modifies the action of pyrethrin I on the central nervous systems of cockroaches, except for some depression of spontaneous activity. As previously assumed, the synergistic action of piperonyl butoxide and similar compounds probably operates mainly by decreasing the detoxication of pyrethroids outside the central nervous system.

Increased diuresis during poisoning by pyrethroids. Casida and Maddrell (*Pesticide Biochemistry and Physiology* (1971), **1**, 71–83) showed that many insecticides, including pyrethroids, increase diuresis in *Rhodnius prolixus* larvae. This led to a decrease in haemolymph volume, and the loss of water from the tissues and disturbance of the cation balance was thought to contribute to the death of the insects. The diuresis was considered to be caused by a diuretic factor released in abnormal amounts by the insecticide acting on the nervous system. As insects of most species die in a dehydrated condition when killed by most neurotoxic insecticides, excessive diuresis could be the common cause of death.

Some experiments were made to try to decide whether insects killed by pyrethroids died because their haemolymph was depleted, or whether this was a symptom of irreversible damage to the nervous system already inflicted by the insecticide. Adult male *Periplaneta americana* weighed at intervals from 30 minutes to seven hours after they were treated topically with just-lethal doses of pyrethrin I first started to lose weight faster than untreated cockroaches one hour after treatment. By then the insects were already prostrate which suggests that serious dehydration began only when the insects were already fatally poisoned.

If the loss of haemolymph volume causes death, the transfusion of fluid into the haemocoel to replace the fraction lost could be expected at least to alleviate temporarily the symptoms of poisoning and perhaps to allow recovery. However, injecting from 20 to 50 μl of insect saline, with or without sucrose, into their haemocoels four times between one and seven hours after they had received topically just-lethal amounts of pyrethrin I, did not affect their symptoms and none of the injected insects recovered. The insects rapidly excreted the extra fluid and all died dehydrated. When haemolymph extracted from untreated cockroaches was injected instead of saline the result was similar.

Keeping cockroaches previously treated with just-lethal amounts of pyrethrin I in an atmosphere of carbon dioxide for up to seven hours after treatment completely prevented water-loss, but failed to affect the course of poisoning in any other way. Presumably inactivation of the nervous system by carbon dioxide blocks the mechanism responsible for diuresis, perhaps by preventing the release of the diuretic hormone, but has no influence on the primary intoxication of the nervous system by pyrethrin I. Depleting the haemolymph through intoxication by pyrethrin I seems not to influence the ultimate outcome of the poisoning process in cockroaches, though it may hasten death.

Knock-down by pyrethroids. The ability to knock down insects rapidly is a desirable property of insecticides intended for such purposes as the control of domestic pests or

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virus vectors. Many pyrethroids knock down insects rapidly, but some, though extremely lethal to insects, do not. Unfortunately both the mechanism responsible for knock-down and the molecular structures that favour it are imperfectly understood.

Discussion about the nature of knock-down has often been about whether knock-down and kill are separate phenomena, or whether both are stages of the same mechanism expressed at the same site. Existing evidence suggests that the fatal lesion caused by pyrethroids lies within the central nervous system, but it has been stated that some pyrethroids knock down species such as houseflies so rapidly that the action must be exerted peripherally, because there is too little time for penetration to the central nervous system.

Some experiments were made to try to define the site of knock-down action as a preliminary to more critical work using electrophysiological techniques. The speeds of action on houseflies (*Musca domestica* L.) of pyrethrin I applied topically and injected were compared. Techniques that allowed the insects to be treated unanaesthetised were used, so that knock-down could be observed immediately the dose was given. The insecticide was applied either topically dissolved in 0·2 μl of solvent or injected emulsified without solvent in 0·2 μl of saline. A range of doses was given and the proportion of flies knocked down was recorded at intervals from one minute to 48 hours after treatment. An ED50 was later calculated for each time interval and log ED50 was then plotted against log time.

Applied topically, the site where the insecticide was applied and the solvent containing it affected the rate of knock-down considerably. Pyrethrin I applied in acetone to the ventral aspect of the thorax knocked down flies much sooner than when applied to the dorsal aspect, but when pyrethrin I was applied at either site in dodecane flies were knocked down about as rapidly as when it was applied ventrally in acetone. All treatments applied topically gave curves relating log ED50 to log time of similar form. The ED50s decreased rapidly for 30 to 120 minutes after treatment but then increased until 24 hours after treatment, as a recovery phase set in. From 24 hours onwards ED50s for all topical treatments were similar.

Pyrethrin I took effect much sooner when injected than when applied topically. To knock down 50% of flies within two minutes, needed only one-tenth as much injected pyrethrin I as when it was applied topically in dodecane and one twenty-thousandth as much as when it was applied topically in acetone to the dorsal thorax. Injected pyrethrin I had most effect immediately and the ED50s increased steadily for the next 10 hours. From this time onwards ED50s for all treatments, topical and injected, were similar.

The synthetic pyrethroid 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate (NRDC 107, bioresmethrin) was tested topically only in acetone applied dorsally. Up to two minutes after treatment, the ED50s obtained with this method and by injection resembled the values for corresponding treatments with pyrethrin I. Later, because fewer flies recovered from NRDC 107, the ED50s decreased much more than with pyrethrin I, and at the end of the test were much smaller. As with pyrethrin I, ED50s for topically applied and injected NRDC 107 coincided towards the end of the test.

Clearly, injecting pyrethroids greatly speeds knock-down, indicating that injection gets them sooner to the site of knock-down action than topical application. In these experiments, injecting placed the insecticide much closer to the central nervous system, which suggests a site of action within the central nervous system rather than in peripheral regions. Ventral application to the thorax also gave greater knock-down potency than dorsal application, probably because the large thoraco-abdominal ganglionic mass is ventrally placed within the thorax. As there is evidence that the lethal lesion also lies within the central nervous system, perhaps knock-down and killing action are stages of the same process occuring at the same site, and differences in speed of knock-down by

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different compounds are associated only with differences between their rates of penetration and detoxication. Support for this idea came from comparing speeds of knock-down by pyrethrin I of the standard strain of susceptible houseflies and of a strain resistant to penetration of topically applied insecticides (strain 314 with the penetration factor isolated from strain NPR). The strong resistance of strain 314 to knock down by topically applied pyrethrin I was abolished by injecting the insecticide. (Burt and Goodchild)

The natural pyrethrins and related synthetic compounds

In the most active synthetic compounds related to the natural pyrethrins, the acidic component, containing a gem-dimethylcyclopropane unit, is held by a spacing group in the appropriate relationship to an alcoholic side chain containing a centre of unsaturation (*Rothamsted Reports for 1966–70*). The cyclopentenone ring of the natural pyrethrins

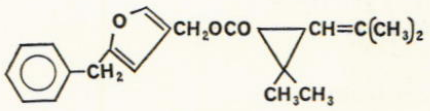

Figure 4

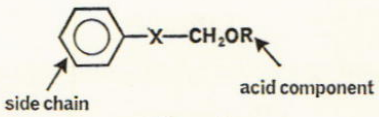

Figure 5

can be replaced, as in potent synthetic analogues such as bioresmethrin (Fig. 4), by a 3-furylmethyl group. We have now studied the effect on insecticidal activity of the nature of this spacing group (–X– in Fig. 5). Esters were synthesised all with phenyl groups separated by a range of linkages from the acid component, which was always (+)-*trans*-chrysanthemic acid. Table 3 shows these compounds and their toxicities to houseflies and mustard beetles.

None of the open chain compounds approached the toxicity to the insects of 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate (bioresmethrin). The most active of them contained an acetylenic link, which was most effective when adjacent to the CH_2OH group of the parent alcohol (9); increasing the chain length by introducing a CH_2 group on either side of the triple bond, (10) or (11), diminished activity. However, the compound (13), related to (10) but with –O– instead of $–CH_2–$ next to the phenyl group was slightly more toxic to houseflies than (9) and was easier to synthesise. The *cis*-ethylenic compound (12), produced by semihydrogenation of the acetylene (9), was much less active. Although some of these acetylenic compounds were more active than the natural pyrethrins against houseflies, they were less effective than bioresmethrin, and had little action against mustard beetles, to which the most toxic compound was pyrethrin I. Substitution of the phenyl ring in (13) (compounds not shown in Table) by chlorine, methyl or methoxy at any position diminished activity.

The relatively low toxicity of these compounds compared with that of pyrethrin I and bioresmethrin indicated that a ring as a spacing group was necessary for greatest toxicity and therefore *o*, *m*, and *p*-benzyl esters were examined. Unlike the allyl benzyl chrysanthemates synthesized earlier (*Rothamsted Report for 1965*, 163), of which the *p*-compound was most active, the benzyl unit was most effective in the *m*-position. In

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TABLE 3

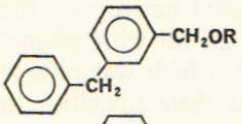
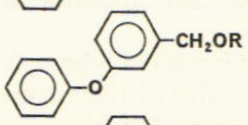
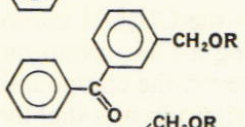
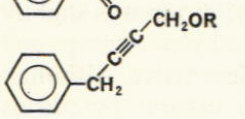
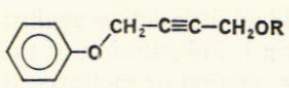
Relative toxicities of chrysanthemates related to bioresmethrin

Compound (R = (+)-*trans*-chrysanthemoyl)	Relative toxicity[a]	
	Houseflies	Mustard beetles
1 Bioresmethrin (Figure 3)	1000	1000
2 $PhCH_2CH_2CH_2CH_2OR$	10	1
3 $PhCH_2CH_2CH_2CH_2CH_2OR$	1	—
4 $PhCH_2CH(CH_3)CH(CH_3)CH_2OR$	1	<0·3
5 $PhOCH_2CH_2OR$	<2	
6 $PhOCH_2CH_2CH_2OR$	1	0·3
7 $PhCH_2OCH_2CH_2OR$	1	<0·3
8 $PhCH_2.CH_2CH{=}CH.CH_2OR$	1	1
9 $PhCH_2C{\equiv}C.CH_2OR$	50	8
10 $PhCH_2CH_2C{\equiv}C.CH_2OR$	<2	0·5
11 $PhCH_2.C{\equiv}C.CH_2CH_2OR$	<2	2
12 $PhCH_2.CH{\overset{c}{=}}CH.CH_2OR$	5	0·3
13 $PhOCH_2C{\equiv}C.CH_2OR$	80	7
14 $PhOCH_2CH{\overset{c}{=}}CH\,CH_2OR$	3	
15 4-Benzylbenzyl-OR	40	20
16 3-Benzylbenzyl-OR	150	520
17 2-Benzylbenzyl-OR	<2	
18 3-Benzoylbenzyl-OR	11	90
19 3-Phenoxybenzyl-OR	110	230

[a] by topical application

TABLE 4

Toxicities and synergism of synthetic chrysanthemates

Compound	LD_{50} (μg/insect) to houseflies		Synergistic factor	LD_{50} (μg/insect) to mustard beetles
	Unsynergised	Synergised		Unsynergised
Bioresmethrin (see Fig 4)	0·0054	0·00057	9	0·0040
	0·028	0·0013	23	0·0052
	0·022	0·0025	9	0·012
	~0·04	0·0045	~90	0·030
	~0·15			0·40
	0·057	0·004	8	1·0

this *m*-compound, the angle between the bonds of the *m*-positions (120°) approaches that (145°) between the corresponding bonds in 5-benzyl-3-furylmethyl alcohol. Thus, in the two compounds the phenyl groups are in similar spatial dispositions with respect to the essential structure in the acid. Further, it is known from physical evidence that in diphenylmethanes, of which the 3-benzylbenzyl chrysanthemates are examples, steric hindrance between the rings disposes them at an angle with respect to each other.

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Although molecular models show that there is much less restriction of rotation in 5-benzyl-3-furylmethyl compounds, the similarity in action of the two series suggests that they probably act in a conformation in which the side chain phenyl ring is skew to the plane of the furan nucleus.

The phenoxy and benzoyl compounds related to 3-benzylbenzyl chrysanthemate were also examined and their insecticidal activities are shown in Tables 3 and 4. The phenoxy compound was somewhat, and the benzoyl compound considerably, less toxic than the benzyl derivative, but when used together with large doses of synergist to suppress detoxification, all three esters were considerably toxic to houseflies. The two rings are at an angle to each other in all three compounds, which indicates that the compounds most toxic to insects are those in which a centre of unsaturation is held by a planar ring, or, with some loss of toxicity, by an appropriate acyclic structure, at a definite distance from the carboxyl group in the acid and hence the gem-dimethyl group there. (Chemical work: Elliott, Janes, Payne and Petersen. Biological work: Farnham and Needham)

Behaviour controlling substances

Chemicals influencing feeding and movement of leaf cutting ants *Acromyrmex octospinosus* (Reich) and *Atta cephalotes* (L.)

Phytochemical arrestants. To identify the substances(s) responsible for the arrestant properties of grapefruit albedo (*Rothamsted Report for 1970*, Part 1, 168), the non-lipid components were fractionated and tested. The results so far indicate that carbohydrates (sucrose, glucose, fructose, xylose and rhamnose) are largely responsible. (Mudd, in collaboration with Drs. J. M. Cherrett and D. J. Peregrine, UCNW, Bangor)

Trail pheromone. The trail pheromone of these ants has been reported to be produced in the poison gland. Recently, the trail pheromone of a closely related ant, *Atta texana* (Buckley), was identified as methyl 4-methylpyrrole-2-carboxylate (I). Because *Atta cephalotes* and *Acromyrmex octospinosus* will follow trails marked with extracts of the poison gland of *A. texana*, this compound (I) was synthesised and assayed for activity. It had some activity for *A. octospinosus* but very little for *A. cephalotes*, implying either that the trail pheromone of these ants differs from (I) or that more than one compound is involved. Constituents of their poison glands are being examined. (Mudd, in collaboration with Scott and T. Lewis)

Pharyngeal glands. These glands are implicated in food sharing and the transmission of insecticide within the colony (*Rothamsted Report for 1970*, Part 1, 169). Analysis shows their contents to be largely ($> 85\%$) triglycerides (mainly dioleoylpalmitin with lesser amounts of 11 others). However, the gland also contains free sterols mainly ergosterol, sterol ester, and free fatty acids, mainly palmitic with myristic, linolenic, oleic, stearic and linoleic acids. (Mudd, in collaboration with Drs. J. M. Cherrett and D. J. Peregrine, UCNW, Bangor)

Chemicals influencing parasitization and crowding of the larvae of the Mediterranean flour moth (*Anagasta kuehniella*, Zeller). In further work (*Rothamsted Report for 1970*, Part 1, 169) on the crowding pheromone of the larvae and the oviposition stimulant of its parasite *Nemeritis canescens*, the oviposition stimulant was isolated and a tentative structure proposed. (Mudd, in collaboration with Callow and Dr. S. A. Corbet, Westfield College, London University)

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Attini—trail pheromone. Two nests of *Atta cephalotes* and two of *Acromyrmex octospinosus*, originally obtained from Trinidad by Dr. J. M. Cherrett (UCNW, Bangor), have been reared at Rothamsted since October 1970. Each contains an egg-laying queen, and the colonies, together with their cultured fungus gardens, have developed 10 to 20 times.

In the laboratory, the ants' nest was connected by a string to a table containing food. The ants established a trail from the nest via the string to the food on the foraging table. When the end of the string connected to the foraging table was connected to an artificial trail, prepared from an ether extract of the contents of the abdominal 'poison sacs' (i.e. trail gland reservoirs) of foraging ants, and traced on a sheet of filter paper, the ants followed the artificial trail. Attempts to identify the trail pheromones are being made, using gas chromatography and mass spectrometry. (Scott and Mudd)

Codling moth. Samples of synthetic sex attractant (*trans*-8, *trans*-10-dodecadien-1-ol) were kindly provided by Dr. W. L. Roelofs (Cornell University) and Zoecon Corporation (Palo Alto, U.S.A.) for use in field trials. Marked male moths were released in an unsprayed orchard at East Malling and more than 50% were recaptured, but in the laboratory recovery was poor. However, we are studying further the efficiency and the uses of this technique for trapping moths. (Greenway, in collaboration with Dr. J. Cranham, East Malling)

Ladybird defensive secretion. When attacked, the seven-spot ladybird (*Coccinella septempunctata*) yields a yellow defensive fluid with a characteristic smell from leg glands. Extracts containing this material were obtained by vacuum distillation of adult ladybirds and collecting the condensate. The odorous material is in the neutral fraction and chemical tests indicate that it is an aldehyde or ketone. (Callow and Greenway with Dr. Miriam Rothschild)

Substances controlling behaviour of Wheat Bulb fly larvae. In laboratory tests, Wheat Bulb fly larvae were arrested by extracts of wheat; this activity was decreased by the addition of extracts of oats (*Rothamsted Report for 1970*, Part 1, 167–168). When larvae were placed in contact with an oat extract for five minutes, washed with distilled water, and then used in an arrestant test, their response to wheat decreased compared with that of larvae pre-treated in a similar way with water. The oat extract had affected the insects' ability to respond to wheat.

The effect of applications of wheat extract or oat extract on attack by Wheat Bulb fly larvae on wheat growing in seed boxes in the glasshouse was examined. Wheat Bulb fly eggs were added to soil in boxes midway between two rows of Cappelle wheat seedlings. Beginning one week before the introduction of the eggs, the boxes were watered daily with either water alone (controls), or water + wheat-stems extract, or water + oat-stems extract. The amount of water was varied from day to day according to the requirements of the plants, but the amount of extract was maintained at a level equivalent to 1·5 g of wheat or oat stems per box per day.

The progress of attack was followed by counting and marking damaged shoots each week. The initial damage to shoots was similar for each treatment. Two weeks later, however, when larvae were leaving their first sites of infestation and attacking further shoots, there was a significant decrease in total numbers of attacked shoots in boxes treated with oat extract compared with the other treatments. Further tests will be done to find out how oat extracts produce this effect. Work is also continuing on the isolation and identification of the 'wheat arrestant' and the 'oats factor'. (Scott and Greenway)

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Cornicle secretions of aphids. Many aphid species have cornicles (= siphunculi) from which a liquid that solidifies rapidly is secreted when the insect is attacked. A defensive function is ascribed to this material and it has been identified as a mixture of triglycerides (F. E. Strong, *Annals of the Entomological Society of America*, (1967), **60**, 668). Examination of these secretions by mass spectrometry shows that they consist of relatively few triglycerides of sorbic, hexanoic, myristic and palmitic acids.

Aphids produce the same secretion independently of the stage of their development or of their type of host plant, but different aphids produce a range of secretions that differ in the proportions of the above triglycerides. We examined secretions of 25 species of aphids and classified them into three main groupings:

(1) Aphids possessing all the above fatty acids in their cornicle secretions.
(2) Aphids with little palmitate in their cornicle secretions.
(3) Aphids with little sorbate in their cornicle secretions.

The composition of these secretions may reflect the fatty acid composition of the whole aphid, but these groupings do not agree with accepted morphological taxonomy.

Although the secretions are host-independent and different secretions are obtained from different aphid species feeding on the same plant, their compositions may be connected in some subtle way with the food of individual species. We noted that *Megoura viciae* and *Aphis fabae*, which feed mainly on stalks and main veins of plants, contain more palmitate in both cornicle secretion and the whole insect, than does *Myzus persicae*—a minor-vein feeder—and that the plant phloem where the first two species feed is relatively rich in palmitate. (Callow, Greenway, Griffiths, and in part, Miss C. S. Morris, Luton College of Technology)

Techniques

Fluorescence microscopy of insect central nervous system. The Bodian protargol silver stain, even in the improved form used to study the neuroanatomy of the central nervous system of the cockroach *Periplaneta americana* (L.) (*Rothamsted Report for 1969*, Part 1, 220), has limitations. It cannot be used to stain selected nerve fibres and tracing individual fibres among many others similarly stained through successive serial sections is slow and laborious. The fluorescent dye Procion Yellow M-4R largely overcomes these problems and the technique of its use was established. It can be made to diffuse along selected nerve fibres without staining neighbouring ones and so enable fibre pathways through the neuropile to be followed more easily. Superimposed camera lucida drawings of selected features from successive sections produce three-dimensional pictures of fibre pathways. The method has answered questions about nerve root neuroanatomy that could not be decided from silver preparations and confirmed many of the conclusions drawn from these preparations (*Rothamsted Report for 1970*, Part 1, 176). Also, and particularly important for studies of insecticide action, the dye stains even the fine terminal arborizations of the nerve fibres, where synaptic contacts with other fibres are probably most numerous. Areas of synaptic contact between functional units can thus be much more precisely identified and are being related to earlier histochemical studies of cholinesterase distribution within the CNS to identify the probable sites where cholinesterase-inhibiting insecticides act. (Gregory)

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

Persistence of dieldrin on cotton leaves. ^{36}Cl-dieldrin crystalline deposits of approximately 4 μg/cm^2 density on cotton-leaf surfaces were subjected to different environments in a

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heated wind tunnel. After 24 hours in still air at 20°C, 10% of the deposit was lost by volatilisation, but 25% was lost with a 60 m/min wind and 30% with one of 90 m/min. However, at a tropical temperature of 40°C and wind speed of 60 m/min, the loss was 95%. In each of the four conditions the rate of loss was linearly related to the amount of dieldrin on the cotton-leaf surface, which points to simple exponential laws governing the losses. (Phillips)

Movement of dieldrin within cotton plants. Although dieldrin does not distribute itself through a plant from a point where it is applied in the same way and in such quantities as the accepted systemic insecticides, measurable amounts do move.

When young cotton plants in a glasshouse were suspended with their roots in 25 ml nutrient solution containing 40 μg of a crystalline suspension of ^{14}C-dieldrin, small quantities (*c.* 0·007 μg/g) of dieldrin moved into the leaves after one week, and more (*c.* 0·4 μg/g) after three weeks. The amounts taken up apparently depended on the transpiration of the plants.

Also, when either crystalline suspensions or emulsions of ^{14}C-dieldrin were applied as 1 μl droplets to the upper leaf surfaces of cotton leaves of mature cotton plants in the glasshouse, at least one-fifth of the dieldrin had moved 8 mm laterally within a week; after several weeks measurable amounts penetrated to the lower leaf surfaces. When leaves were grown in a different environment ('Saxcil' cabinet) so as to produce thin leaves, the penetration of dieldrin to the lower surfaces was quicker, showing that movement through the leaf depended on the thickness of the cuticle. (Phillips and Kavadia)

Microcapsules. Because satisfactory encapsulated material was not obtainable commercially, the technique of microencapsulation was studied. A 0·1% DDT solution in toluene was microencapsulated in hardened gelatin/gum acacia walls by the coacervation technique (NCR process). Microcapsules of 500 μm diameter were used to test leakage in different environments and to compare with a standard wettable powder (WHO specification) for persistence on cotton plants.

The relative humidity affected leakage and an increase from 55% R.H. to 95% R.H. doubled the amount of DDT leaking through the walls, although little was lost.

In the tests comparing persistence, microcapsules suspended in an aqueous 'Acronal' sticker suspension and a 50% DDT wettable powder in aqueous suspension were applied to different leaves on cotton plants growing in three different environments. These were: variable temperature (15°–30°C) and humidity, with approximately 12 hours daylight (glasshouse); 32°C day/22°C night, 55–60% R.H. and 10 hours artificial daylight ('Saxcil' cabinet); 28°C day/20°C night, 55–60% R.H. and 10 hours artificial daylight (C.E. room). Leaves were picked at weekly intervals up to five weeks, and those bearing microcapsules were first washed with hexane and the washings analysed by gas-liquid chromatography (glc) to measure the DDT that had leaked through the capsule walls. They were then ground and extracted with hexane before analysis by glc, in the same way as were leaves bearing the wettable powder deposit. The effects of the different environments were small. Losses of DDT from the wettable powder formulation depended on the deposit density; 80% was lost from 2000 μg and 60% from 400 μg DDT deposits per leaf after one week, increasing to 90% and 70% respectively after five weeks. During the same period only 15% was lost from 25 μg of encapsulated DDT, increasing to 20% after five weeks. (Phillips and Kavadia)

Other microcapsules of hardened gelatin/gum acacia containing the dye Sudan Blue in sunflower oil were prepared for Dr. Cherrett, University College of North Wales, Bangor, for bait studies with leaf-cutting ants. (Phillips)

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Factors influencing the effectiveness of granules of systemic insecticides applied to field beans. The performance of granular formulations of disulfoton and phorate applied to field beans in controlled environment rooms and in the field was studied further by the methods reported previously (*Rothamsted Reports for 1968–70*, Part 1). In growth rooms, granules containing equal amounts of the active ingredient of each insecticide were applied to: (a) the foliage and soil; (b) the soil only; (c) the leaves only, the soil being protected by covers. Treatment (c) was less effective than the other two when toxicity was tested by caging aphids (*Aphis fabae*) to the leaves for 24 hours at intervals up to 40 days after treatment.

Attempts to demonstrate the supposed fumigant effect of these formulations immediately after application have been inconclusive. Disulfoton on pumice was applied to 16 pots of beans, closely arranged around four untreated pots and ventilation of the growth room was reduced. *Aphis fabae* caged to the untreated plants immediately after the other plants were treated, to test the fumigant effect, were unaffected. This contrasts with field results reported below.

The field trial done at Woburn in 1970 to test the effect of wetting the foliage daily on the insecticidal activity of the disulfoton and phorate formulations, was repeated at Rothamsted. There were three moisture regimes: (1) covered plots receiving neither rain nor irrigation; (2) plots receiving rain only; (3) plots receiving rain and, on dry days, enough water to wet the foliage thoroughly. Phorate and disulfoton on both pumice and fullers earth granules were applied to the foliage at 2 lb/acre a.i. (2·3 kg/ha) on 1 July. Effectiveness was assessed by caging *A. fabae* on the leaves for periods of four days at weekly intervals. The insecticides did best on the irrigated plots, where plants remained toxic to aphids until early August; plants treated with disulfoton on pumice were most toxic, but all the formulations gave satisfactory results. Treatments (1) and (2) remained effective only until mid-July. Assessing toxicity during the first 10 days after treatment was complicated by aphids dying on untreated plants, suggesting a fumigant effect, in contrast to the results in the growth room. (Etheridge, Stevenson with Dr. I. J. Graham-Bryce, Plant Protection Limited)

Artificial feeding of aphids. Artificial feeding of aphids has been used for various reasons, but mainly to study the direct effects of changes in environment on aphids, without indirect effects caused by changes in the host plant. It can also be used: (1) to study stomach toxicity of systemic insecticides; (2) to study the reaction of aphids to substances extracted from host or non-host plants; (3) to provide a uniform nutrient background for studying the loss of resistance to insecticides that sometimes occurs suddenly in laboratory-reared stocks of aphids.

Table 5 shows the results of tests using groups of 10 individuals of the polyphagous species *Myzus persicae* or the oligophagous species *Megoura viciae* confined in tubes closed by membranes of 'Parafilm M' containing different liquids, compared with the same species of aphids on growing plants or on membranes containing no liquid.

Both species of aphids survived well, produced young freely and excreted many spots of honeydew when on their host plants. *Megoura viciae* performed poorly on all the liquid diets but, because it is a large and vigorous species, only about half the aphids had died at two days, even when confined on a dry membrane. In contrast, the survival, reproduction and excretion of *Myzus persicae* was much greater on sucrose solutions or on water, than on dry membranes. However, reproduction and excretion were only a fraction of that on the host plant. Sucrose solution containing extracts of the host plant was less suitable than sucrose alone. Our poor results with *M. persicae* on the diet are unexplained, and later tests with only minor variations in chemicals and techniques,

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TABLE 5

Aphids on host plants or artificial diets

	Myzus persicae				*Megoura viciae*			
	LA	TL	LL	SP	LA	TL	LL	SP
Plant[1]	8·3	44·5	44·5	250+	10·0	58·0	54·4	250+
Sucrose	8·3	10·6	9·6	8·4	5·6	16·8	6·2	3·8
Water	7·0	8·25	3·5	9·8	5·8	14·8	3·3	3·2
Diet[2]	6·0	0·3	0·3	5·0	6·5	6·5	1·0	2·5
Sucrose + plant extract[3]	2·4	1·7	1·4	3·4	4·5	16·3	8·3	0·5
Dry membrane	0·4	0·0	0·0	2·0	4·8	14·3	2·3	0·5

Ten aphids per tube, four to six tubes per test, mean numbers of live adults (LA), total larvae (TL), live larvae (LL), and excretory spots (SP) at two days

[1] Turnip for *M. persicae* and field bean for *M. viciae*

[2] Complex diet of sucrose, amino acids, salts and vitamins (as Mittler, Tsitsipis & Kleinjan, 1970, *Journal of Insect Physiology*, **16**, 2315–2326)

[3] Extracted in 80% ethanol, evaporated, redissolved in water

gave greater survival, larviposition and excretion than those shown above. (Greenway and Griffiths)

^{14}C-sucrose solutions containing dimethoate were used to study the stomach toxicity of this insecticide to *M. persicae* and the effect of dimethoate on feeding by this species of aphid. The slope of the dosage-mortality response line was slight and the LD_{50} was large because dimethoate deterred *M. persicae* from feeding. Dimethoate at only 0·01 ppm in sucrose solution affected aphid feeding and aphids took up 70 times as much sucrose from sucrose solution alone as from solutions containing 100 ppm dimethoate.

Radiotracer studies on solutions without dimethoate revealed that different individuals differed greatly in their amounts of feeding, that all aphids that died had taken up little sucrose solution, and that aphids consumed much more sucrose solution at R.H. < 20% than at R.H. 85%. The radiotracer technique is too cumbersome for routine use in developing an adequate synthetic diet, but is a useful check at critical points in the process (Griffiths and Javadi)

Acute toxicity of pesticides to honeybees in the laboratory. The method already reported (*Annals of Applied Biology*, (1968), **61**, 467) was used to find the acute oral and contact toxicity to worker honeybees of four further pesticides. Dimethoate was included as a standard and Table 6 gives the results as weighted mean median lethal doses (LD_{50}).

TABLE 6

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

	LD_{50} (μg/bee)	
	Oral	Contact
dimethoate	0·15 ±0·0056	0·11 ±0·0044
demeton-s-methyl	0·21 ±0·017	0·26 ±0·0069
fenitrothion	0·019±0·0016	0·018±0·00057
oxydemeton methyl	0·31 ±0·016	0·54 ±0·019
tolylfluanid*	43 ±2·5	>100

* proposed BSI name for *N'*-dichlorofluoromethane-sulphonyl-*NN*-dimethyl-*N*-*p*-tolysulphamide

(Stevenson)

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Poisoning of honeybees in the field. Seventy-eight samples of honeybees thought to be poisoned, and three of comb, were received from beekeepers in England via the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food. Fifty-three samples gave evidence of poisoning, but 17 of these came from four spraying incidents, in which two or more apiaries were affected, bringing the number of poisoning incidents to 40, compared with 45 in 1970.

Bees from 23 incidents reacted positively to our test for organophosphate poisoning and five gave inconclusive results; this test measures residual cholinesterase after poisoning, and not the insecticide residues themselves. Evidence supplied with some of the positive samples suggested that five incidents were caused by spraying peas, three orchards, two mustard/rape, two a mixture of peas and beans, one potatoes and one beans and runner beans. Dichlorvos resin strips, used in rooms where parts of the hive were stored, were implicated in three of the positive organophosphate tests and one of the inconclusive ones.

Of the 10 poisonings where BHC was identified, one involved spraying of rape, in one the hive had been treated with a woodworm killer, in another hive parts were stored in a house where an electrically operated thermal insecticide vapouriser was in constant operation in another room, and one was thought to be malicious. There was one dieldrin poisoning and one sample in which poisoning was confirimed but we could not identify the insecticide.

We examined by bioassay with *Drosophila melanogaster* three samples of comb thought to have caused bee deaths. One, which had been stored in a room with a dichlorvos strip, was toxic; the other two were not.

For the second year very few poisonings were reported associated with spraying field beans; again this probably reflects the small *Aphis fabae* infestation. The increased number of BHC poisonings arises from various causes and not one specific use of the insecticide. (Stevenson)

Assay of carbaryl in bees. Methods previously used to assay carbaryl in poisoned bees were tedious and often gave doubtful results. An improved technique was developed showing the presence of carbaryl in bees poisoned with 0·5 μg carbaryl per bee. Five bees (approximately 0·5 gm) are macerated with 2·5 g anhydrous sodium sulphate and 7 ml methylene dichloride and then filtered. The solids are re-extracted with 5 ml methanol, followed by more methylene dichloride; the combined extracts are taken to dryness and the residue redissolved in methylene dichloride. Carbaryl is separated from components of bees that interfere with its assay by passing this solution through a column of Florisil deactivated with water and eluting the carbaryl with methylene dichloride. An aliquot of the purified carbaryl solution is taken to dryness and the carbaryl acetylated by standing overnight in the dark at room temperature with a mixture of 0·2 ml acetic anhydride and 0·05 ml methane sulphonic acid. After decomposing the acetylating agent with ice, the N-acetyl carbaryl is extracted into methylene dichloride. Finally, the N-acetyl carbaryl is transferred into acetone solution and assayed by gas chromatography on a column of 5% SE30 silicone gum on 'Chromosorb' W using an N sensitive alkali flame detector.

Carbaryl was not found in two samples of bees alleged to have been poisoned by it. (Lord and Stevenson)

Beneficial insects on motorway verges. Some modern developments, such as the use of herbicides, destroy food of pollinating and other beneficial insects, but others, such as the large verges on motorways, could provide important new habitats for them. As a start to studying the effects of these environmental changes, a 200 m length of embank-

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ment on the M1 near Watford, Hertfordshire, was surveyed. It contained more than 50 species of flowering plants, 28 of which were being visited by insects; the species included three of Apoidea, three of Coccinellidae and 17 of Syrphidae, which may be considered beneficial as pollinators or predators. (Stevenson, with Free and Gennard, Bee Department).

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 49r_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 knock-down, pyrethroid insecticides and to carbamates
	Some strains derived from NPR each with one or more factors of resistance to pyrethroid insecticides, DDT and dieldrin
	A wild type susceptible strain
	Calliphora erythrocephala (Meig.)
Hymenoptera	*Acromyrmex octospinosus* (Reich)
	Atta cephalotes (L.)

Seed dressings

Wheat Bulb fly—single row trials, 1970–1971. The following chemicals were tested as seed dressings against Wheat Bulb fly larvae: three organophosphorus compounds 'C8874' (*O*,*O*-diethyl *O*-2,5-dichloro-4-iodophenyl thiophosphate), 'C18244' (*O*-ethyl-*O*-(2,5-dichloro-4-iodophenyl) ethyl thiophosphonate) and coumithoate ('Dition');

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three insect repellents, deet, 'MGK 11' (2,3: 4, 5-bis(2-butylene) tetrahydro-2-furaldehyde) and 'MGK 874' (2-hydroxyethyl-*n*-octyl sulfide) and the synthetic pyrethroids resmethrin and bioresmethrin. All were formulated as 20% dusts and stuck on the seeds with methyl cellulose. There were also five treatments with γ-BHC, namely 'Mergamma W' powder applied directly to the seeds, or stuck on the seeds with 'Lutanol' or 'Polyvis', or with two different amounts of liquid paraffin. Amounts of γ-BHC, estimated by glc, were similar for all treatments, ranging from 680–740 ppm. Seeds were sown in fields infested with Wheat Bulb fly eggs, a peaty loam in the Eastern Region, and a sandy clay loam in East Midland Region of Agricultural Development and Advisory Service. The efficiency of the treatments in protecting young plants from attack by Wheat Bulb fly larvae was compared with a standard seed dressing (liquid chlorfenvinphos at 3 ml/kg) and with a control (seeds treated with fungicide alone).

At the sandy clay loam site, attack by Wheat Bulb fly larvae was slight (only 15% damaged shoots in the controls) and not affected significantly by any treatment, but some of the chemicals damaged the seedlings: seeds treated with all the γ-BHC formulations, exept 'Mergamma W' powder alone, and with the insect repellent deet at 0·2% a.i. to weight of seeds, gave significantly fewer plants than did control seeds.

On the peaty loam, attack was greater (38% damaged shoots in the controls) and damage by the chemicals less. The main conclusions from this trial were: (1) deet was phytotoxic (at 0·2% a.i. to wt of seed); none of the repellents had much effect on attack by the larvae; (2) resmethrin at 0·2% was the best pyrethroid treatment, although it did not significantly decrease numbers of damaged shoots or live larvae; (3) 'Mergamma W' powder stuck to seeds with 1·2 ml liquid paraffin/100 g seed decreased the numbers of plants that emerged. Only 'Mergamma W' powder, without sticker or stuck to seeds with 0·1 ml liquid paraffin/100 g seeds, significantly increased the number of unattacked shoots per metre row; (4) organophosphorus compounds gave the best results. 'C 8874' at 0·1% and 'C 18244' at 0·1% and 0·5% a.i. to wt of seed almost halved the percentage damaged shoots and increased the number of healthy shoots/metre row. These compounds were better than chlorfenvinphos used as the standard, but it is improbable they will be developed commercially. (Griffiths, Scott and Jeffs)

Soil insecticides for control of *Sitona*. Tests of insecticides against *Sitona* larvae are described in the report of the Entomology Department (p. 201). (Griffiths)

Control of aphids and virus diseases of peas and brassica crops. Work on these subjects is described in the report of the Entomology Department (p. 203) and Plant Pathology Department (p. 133). (Etheridge)

Fungicides

Tests of fungicides to control blight on potato haulms, common scab on potato tubers, and eyespot of wheat were continued.

Naming of chemicals. The chemicals used are referred to by their common or chemical names, except the following: 'W 524' (*N*,*N*′-bis-1-formamide-2,2,2-trichloroethyl)-piperazine: Cela); 'PH 50–82' ((2,4,5-trichlorophenyl) sulphonylmethyl thiocyanate: Philips–Duphar).

Laboratory tests

Potato-haulm blight. In tests on possible control of blight by systemic action, chemicals were mixed with potting loam in which King Edward plants were then grown in the glasshouse. After five weeks, leaflets were detached and inoculated with zoospore

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suspensions of *Phytophthora infestans*. No resistance to infection was found with any of the following chemicals at 50 ppm: dichlofluanid, edifenphos, fluorene, 2-bromofluorene, DL-ethionine, DL-methionine.

Potato common scab. Possible scab-control chemicals were tested by adding them to soil (mostly from Great Hill Bottom, Woburn) in which Majestic plants were then grown in the glasshouse. The following chemicals either failed to control scab ($P = 0{\cdot}05$), or damaged the plants, or both, at 50 ppm or at the rate shown: anhydrous ammonia (450 ppm), 2-bromofluorene, 2-bromopyridine, isobutylamine and sec.butylamine, caffeic acid, 1- and 2-chloroanthraquinone, 1-chloro-2,4-dinitrobenzene, cinnamic acid, copper oxychloride, 3,4-dichloronitrobenzene, 2,6-dichloro-4-nitrophenol, 2,6- and 3,5-dichloropyridine, 2,4-dinitrophenol, 2,4-dinitrophenylthiocyanate, dodemorph benzoate (10 ppm), griseofulvin, menaphthone, DL-methionine, 4-nitropyridine-1-oxide, 2,3,5,6-tetrachloroanisole, 1,2,4,5-tetrachlorobenzene, tetrachloro-*o*-benzoquinone, 2,3,5,6-tetrachloro-4-cyanopyridine, 2,3,5,6-tetrachlorophenol, 2,3,5,6-tetrachloropyridine, 2,4,6-trichloropyridine. Table 7 shows results with the more effective chemicals, and also with the established fungicides quintozene, chloranil and dichlone for comparison. Binapacryl, dinocap phenols (previously referred to as 'MC 2810'), DL-ethionine and fluorene decreased yield to below 86% of that in the corresponding 'nil'-treatments ($P < 0{\cdot}001$), but the others did not decrease yield. The amount of scab in the 'nil'-treatment varied from test to test (15 plants per treatment per test) so, to make comparisons simpler, the scab incidence for each chemical treatment was calculated as the percentage of that in the corresponding 'nil'-treatment. The figures in the Table are the mean percentages from the numbers of tests shown. The soil was taken from either Great Hill Bottom or Schoolfield, Woburn. The scab organism in the Schoolfield soil seemed to be generally more resistant to chemical control; in three tests even quintozene did not affect it. Despite the differences between the two strains of the organism, the simple chlorinated *p*-benzoquinones were quite good scab-control chemicals. They were very slightly less effective than quintozene, and clearly more effective than the closely related but better-known quinones chloranil and dichlone, and they did not decrease yield.

TABLE 7

Relative amounts of scab on glasshouse-grown Majestic tubers after soil-treatments (50 ppm)

Treatment	Soil from Great Hill Bottom			Soil from Schoolfield		
	No. of tests	Scab incidence	Effect not sig. (n.s.) or sig. at	No. of tests	Scab incidence	Effect not sig. (n.s.) or sig. at
nil	11	**100**	—	6	**100**	—
quintozene	11	**26**	$P<0{\cdot}001$	6	**53**	$P<0{\cdot}001$
binapacryl	2	**26**	$P<0{\cdot}001$	—	—	—
dinocap phenols	3	**32**	$P<0{\cdot}001$	1	**80**	n.s.
DL-ethionine	2	**48**	$P\simeq0{\cdot}002$	2	**121**	n.s.
fluorene	2	**47**	$P<0{\cdot}001$	1	**103**	n.s.
furcarbanil	2	**53**	$P<0{\cdot}001$	1	**122**	n.s.
2-pyridinethiol-l-oxide, Na salt	3	**46**	$P<0{\cdot}001$	—	—	—
'PH 50–82'	2	**50**	$P=0{\cdot}001$	1	**111**	n.s.
chloranil	1	**73**	n.s.	2	**84**	n.s.
dichlone	1	**106**	n.s.	—	—	—
p-benzoquinone	1	**55**	$P\simeq0{\cdot}01$	1	**88**	n.s.
chloro-*p*-benzoquinone	1	**16**	$P<0{\cdot}001$	1	**56**	$P\simeq0{\cdot}005$
2,5-dichloro-*p*-benzoquinone	1	**33**	$P<0{\cdot}001$	1	**68**	$P\simeq0{\cdot}01$
2,6-dichloro-*p*-benzoquinone	3	**32**	$P<0{\cdot}001$	2	**58**	$P<0{\cdot}001$

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TABLE 8

Relative amounts of scab on glasshouse-grown Majestic tubers after foliage sprays

Treatment	% a.i.	Soil from Great Hill Bottom: No. of tests	Soil from Great Hill Bottom: Scab incidence	Soil from Schoolfield: No. of tests	Soil from Schoolfield: Scab incidence
nil	—	4	**100**	1	**100**
'W 524'	0·025	1	**91**	—	—
DL-ethionine	0·2	2	**69**	1	**127**
DL-methionine	0·2	1	**109**	1	**118**
2-pyridinethiol-l-oxide, Na salt	0·1	2	**103**	—	—

A few systemic chemicals are said to be able to move downwards in plants; if this were so, root diseases might be controlled by spraying the foliage. To see whether this applied to control of potato scab, the foliage of potted Majestic plants growing in untreated soil in the glasshouse was sprayed at the time when tubers were forming, i.e. when they were susceptible to attack by *Streptomyces scabies*. Table 8 shows the results; the amounts of scab are expressed in the same way as in Table 7. DL-ethionine was the only chemical that significantly affected scab; and, as in the soil-treatment tests, it was effective only on plants growing in soil taken from Great Hill Bottom (scab incidence = 69 : $P \simeq 0{\cdot}03$). The corresponding figures from the standard soil-treatment with quintozene (50 ppm) in the same tests were larger than usual, viz 47 ($P < 0{\cdot}001$: Great Hill Bottom) and 64 (not significant: Schoolfield).

Field trials

Potato-haulm blight. Blight attack came late, so the effects of spraying (27 July and 17 August) were small. Of five treatments, only 'Brestan 60', at 0·03% fentin acetate, significantly increased yield.

Potato common scab. In a trial at Woburn with the variety Maris Piper, chemicals were applied as 10 or 20% dusts on 21 April. All plots were rotavated within one hour of application, and potatoes were planted the same day. Scab indices, which are estimates of the percentages of the skins disfigured by scabs, were calculated at harvest from samples of 50 ware tubers per plot (Table 9).

The trial was in Schoolfield; all previous trials (1968–70) were in Great Hill Bottom, where scab is evidently more easily controlled by chemicals (see glasshouse tests, above).

TABLE 9

Effect of soil-treatments on yield and incidence of potato common scab

Treatment	Rate: lb/acre	Rate: (kg/ha)	Total tubers: tons/acre	Total tubers: (tonnes/ha)	Scab index
quintozene	70	(78)	15·5	(38·8)	13·4
captafol	70	(78)	19·7	(49·4)	14·9
captafol	35	(39)	19·7	(49·6)	16·1
dinocap phenols	70	(78)	17·9	(44·9)	29·0
pentachloropyridine	70	(78)	8·0	(20·2)	21·9
nil	—	—	18·1	(45·4)	22·1
LSD, $P = 0{\cdot}05$			3·4	(8·6)	4·4
$P = 0{\cdot}02$			4·2	(10·4)	5·3
$P = 0{\cdot}01$			4·7	(11·7)	5·9
$P = 0{\cdot}001$			6·3	(15·9)	8·1

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Dinocap phenols and pentachloropyridine, which controlled scab quite well in glasshouse tests on plants growing in soil from Great Hill Bottom (*Rothamsted Report for 1970*, Part 1, 181), failed to do so in this trial in Schoolfield. However, captafol was almost as effective as quintozene; this confirms the 1970 result. Indeed, the combined results for the 70 lb/acre treatments in both trials show that captafol gave a slight increase in yield, whereas quintozene decreased it ($P \simeq 0{\cdot}04$) by about 13%. (McIntosh)

Cereal diseases. The systemic fungicide benomyl was used as a seed-dressing and spray in a field trial on control of eyespot on winter wheat. Details are given in the report of the Plant Pathology Department, p. 147. (McIntosh, with Prew, Plant Pathology Department)

Staff and visiting workers

Dr. K. A. Lord returned from secondment to the Government of Pakistan. Mrs. M. C. Payne and Mrs. M. Petersen left and D. Pulman was appointed. Visiting workers were G. O. Osborne from New Zealand and Iraj Javadi from Pahlavi University, Shiraz, Iran.

K. A. Lord, A. H. McIntosh and C. Potter read papers at the 6th British Insecticide and Fungicide Conference, Brighton. C. Potter and F. T. Phillips attended the Inauguration of the Nuclear Research Laboratory at the Indian Agricultural Research Institute, New Delhi, and read papers at the International Symposium on the Use of Isotopes and Radiation in Agriculture and Animal Husbandry Research, which took place after the inauguration.

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