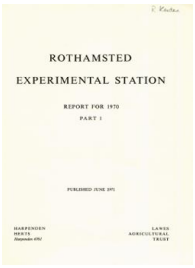
ROTHAMSTED RESEARCH

Report for 1970 - Part1

Full Table of Content



Insecticides and Fungicides Department

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INSECTICIDES AND FUNGICIDES DEPARTMENT

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Resistance of aphids to insecticides seems to be increasing in England and elsewhere. Here aphids are among the most important pests of crops because, in addition to the direct damage they cause by their feeding they also transmit many of the most important viruses.

Preliminary work on *Myzus persicae* shows that resistance to organophosphorus insecticides is correlated with an increase in aliesterase activity in all samples yet tested. This provides a convenient test for diagnosing this type of resistance. The systemic insecticide dimethoate is commonly used to control aphids and the synergist sesamex (2-(3,4-methylenedioxyphenoxy)ethyl-3,6,9-trioxaundecane) a multi-function oxidase inhibitor, almost eliminates resistance to this insecticide. Although this compound is not very suitable for use with a systemic insecticide because it does not become systemic in plants, other oxidase inhibitors may be found that also have the required properties.

Two of the pyrethroids synthesised in the department, resmethrin (NRDC 104) and bioresmethrin (NRDC 107), are now being produced commercially. Further work indicates that compounds can be synthesised that act faster and are more insecticidally active than the natural pyrethrins; other compounds can be made that are more selective in their action.

The need for better methods of applying seed dressings commercially (*Rothamsted Report for 1969*, Part 1, 225) increases with the introduction of systemic fungicides applied as seed dressings. We are collaborating with commercial firms and other organisations to meet the need and have made some progress in developing a two-stage process of applying a sticker followed by a dust formulation of the pesticide.

Insecticides

The causes of resistance

Interaction between resistance factors. The mechanisms responsible for the SKA strain of houseflies resisting organophosphorus insecticides can either interact or work independently. All three possible combinations of pairs of factors of resistance have now been inbred into different strains and tested for their combined effects on resistance. Interaction between the factor delaying penetration (*pen*) and the desethylation mechanism (*Rothamsted Report for 1969*, Part 1, 207) produced great and unpredictable increase in resistance to 14 organophosphorus insecticides. Introducing the microsomal detoxifying mechanism (*Ses*), which confers resistance against only a few of the organophosphorus insecticides, into a strain with the desethylating mechanism, greatly increased resistance, but almost exclusively against insecticides against which both factors of resistance were effective. However, it also very slightly increased resistance over that of the desethylating mechanism alone against most of the other insecticides. The greatly increased resistance to diazinon, diazoxon, malaoxon and ethyl malaoxon approximated the product of the resistances conferred by each mechanism singly, suggesting that each detoxifies these compounds independently of each other, and this is in accordance with first order kinetics. These two factors also interacted strongly to increase resistance against ethyl chlorthion and parathion, although *Ses* gives no resistance to either. The reasons for this are not

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known. The strong synergism or antagonism with sesamex and tributyl phosphorotrithionate (TBTP), which occurs in the strain(s) with the desethylating factor or *Ses* alone, disappears when these two factors are brought together, and in this, such a strain resembles the parent SKA strain.

It is not yet known whether attempts have succeeded to introduce the penetration delaying factor *pen* into a strain with the microsomal breakdown factor, *Ses*, the last remaining combination of pairs of factors of the SKA strain. In this attempt, selection by the presence or absence of visible markers, with DDT or diazinon alone was abandoned in favour of treating the test-cross progeny 466.500 × F1 (348 × 466.500) with DDT, to select for homozygosity of the microsomal detoxifying factor alone (*Ses*) derived from strain 466.500, followed in the two succeeding generations by selection with tributyltin acetate to obtain homozygosity for delayed penetration, *pen*, derived from strain 348. Delayed penetration increased resistance to DDT 10-fold but, unexpectedly, to organophosphorus insecticides little, and the reasons are being sought. Despite the apparent lack of interaction between *pen* and *Ses*, the three factors of resistance must interact, because when strains with two of the factors of resistance are crossed with strains with the remaining factor, F1s are as resistant as F1 SKA × susceptible parent. (Sawicki)

***Metabolism of organophosphorus insecticides by susceptible and resistant strains of housefly* (Musca domestica *L.*).** The metabolism of diazinon, diazoxon and parathion was studied *in vivo* to compare with the results obtained last year *in vitro*. All the metabolites identified *in vitro* were also detected *in vivo* in the appropriate strains. Diethylphosphorothioic acid and diethylphosphoric acid were the principal metabolites of these insecticides and strains with gene *a* produced more than other strains of these metabolites. Strains with gene *a* metabolised about 10, 4 and 3% respectively, of diazinon, diazoxon and parathion doses (1 μg/fly) in one hour. Diazinon, which has the higher LD50, was metabolised more than parathion by all strains.

The *in vivo* tests with resistant strains of housefly, showed more of the toxic phosphate analogues of diazinon and parathion than occur in susceptible strains. These results confirm that resistance to diazinon and parathion in strains with gene *a* depends both on increased degradation of these insecticides, which leaves less to be converted to diazinon and paraoxon, and on the increased detoxification of these oxons to their desethyl derivatives and to diethylphosphoric acid. Resistance to diazinon in the strain with the factor (*Ses*) on chromosome V is caused by detoxification of diazoxon to the unidentified metabolites. This factor does not confer resistance to parathion and paraoxon is not metabolised in this strain. (Lewis)

***Resistance to insecticides in* Myzus persicae.** Bioassays on a strain of *Myzus persicae*, derived from a glass-house population that had become resistant to organophosphorus insecticides after exposure to many insecticides (including organophosphorus insecticides and carbamates), showed it to be very resistant to parathion, dimethoate, disulfoton, oxydisulfoton, disulfoton sulphone $(C_2H_5O)_2PS.S(CH_2)_2SO_2.C_2H_5$ and DDT (Table 1), but only *ca.* ×10 more resistant to demeton-S than the susceptible strain and ×12 to the carbamate pirimicarb. The strain was continuously exposed to, and selected with, dichlorvos from a 'Vapona' plastic strip. A sub-culture of the resistant strain, started three months after getting the original strain and bred in conditions free from insecticides, retained the great resistance to dimethoate of the selected parent for about 14 generations, but lost it rapidly, for unknown reasons, during the next four generations when its resistance to dimethoate dropped to ×4.

The aliesterases of the susceptible and resistant aphids differed greatly in their ability

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TABLE 1

Resistance factors[1] *at LD50 of the strain of organophosphorus resistant* Myzus persicae *to various insecticides*

Dimethoate	212[2]
Parathion	171[2]
Disulfoton	75[2]
Disulfoton sulphone	398
Oxy-disulfoton	457
Demeton-S	*ca.* 10
Pirimicarb	12[2]
DDT	25[2]

[1] Resistance factor = $\frac{\text{LD50 of resistant strain}}{\text{LD50 of susceptible strain}}$

[2] Mean values.

to hydrolyse α-naphthyl acetate, the exact reverse of the activities reported for strains of houseflies susceptible and resistant to organophosphorus insecticides. Several organophosphorus-resistant strains of aphids were tested and all had more than usual aliesterase activity. The loss in resistance by our sub-culture of the resistant strain when not exposed to the insecticide was reflected by a corresponding loss of activity, indicating that, in aphids, resistance to organophosphorus insecticides is linked with large aliesterase activity.

The aphids and houseflies also differ in the way aliesterase activity is distributed between sub-cellular fractions and the extent to which the fractions are inhibited by organophosphorus insecticides. Susceptible and resistant strains of aphids differ in the aliesterase activity of the supernatant fluid after extracts are centrifuged at 100 000 *g*, whereas houseflies differ mainly in the activity of the 100 000 *g* microsomal pellets. It seems that the aliesterase activity can be used to determine resistance to organophosphorus insecticides in both houseflies and aphids. The method is very convenient and sensitive and could be adapted for use in the field.

To determine further the nature of the resistance mechanisms to organophosphorus insecticides in aphids, sesamex (2-(3,4-methylenedioxyphenoxy)ethyl-3,6,9-trioxaundecane) a multifunction oxidase inhibitor, was incorporated in the solutions of insecticides applied to the aphids. Sesamex decreased the resistance to dimethoate from × 200 to × 3, but increased resistance to disulfoton from × 75 to almost complete immunity. This striking contrast may be because whereas sesamex blocks the mechanism that detoxifies dimethoate, it prevents disulfoton being converted to demeton-S to which aphids are only slightly resistant (Table 1). This indication that multi-function oxidases probably play an important role in the resistance of aphids to organophosphorus insecticides is being investigated. The suppression of resistance to dimethoate suggests that synergists with translaminar or systemic properties could find practical application in overcoming resistance to some of the more widely used systemic insecticides. (Needham and Sawicki)

The factors of resistance to insecticides in the pyrethrin-selected NPR strain of houseflies. The NPR strain selected against natural pyrethrins is very resistant to natural and synthetic pyrethroids (resistance factors ranging from 80 × to immunity) and also resists DDT, dieldrin, 'Zectran' and tributyltin acetate. Factors conferring resistance to each of these insecticides were located and/or isolated using the genetical marker technique described previously (*Rothamsted Report for 1965*, 156). Each of the five autosomes of strain NPR was inbred singly into a quadruple marker susceptible strain and the contribution of each examined separately. The results were:

Chromosome I. No measureable resistance to any of the insecticides tested.

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Chromosome II. The isolated factor on this chromosome gives slight resistance to all pyrethroids (approx. ×1·5–2). A second factor on this autosome confers resistance to 'Zectran'; it is unaffected by pretreatment with the synergist sesamex, and is independent of the pyrethroid resistance mechanism. The parent NPR strain is not homogeneous for either of these factors.

Chromosome III. Of three factors on this chromosome, one gives resistance to tributyltin acetate, delays the onset of poisoning symptoms with all compounds tested, and slows the penetration of DDT, dieldrin and pyrethrin I. It confers small resistance (approx. ×1·5–2) when measured at kill end point. This factor seems similar to *pen* isolated from the SKA strain and to *organotin-R* in strain *organotin-stw*. The second factor is a knock-down resistance mechanism that affects DDT and pyrethroids only. As the first factor, it delays the time taken to reach kill end point, but confers approximately ×5 resistance to these compounds. It has no effect on dieldrin, 'Zectran' or tributyltin acetate. The third factor, which gives resistance to 'Zectran', can be blocked by pretreatment with 2 μg per fly of sesamex. It is not homogeneous in strain NPR.

Chromosome IV. Dieldrin resistance was on this chromosome. The factor confers immunity to the effect of dieldrin for 24 hours after treatment, but from then kill increases for a further 72 hours finally giving approximately ×700 resistance. The factor was present in the heterozygous state in about 20% of the parent NPR strain and only 3% were homozygous.

Chromosome V. The factor on this chromosome gives approximately ×4 resistance to natural pyrethrins but none to the esters of 5-benzyl-3-furylmethyl alcohol.

Pretreatment with 2 μg/fly sesamex increased the susceptibility of all strains to the natural pyrethrins but to different extents. The strain in which synergism was least, i.e. much less than in the susceptible strain, was the one with the factor on chromosome II. Synergism in the strain with the *kdr* factor was as in the susceptibles, and was very great only in the strain with the factor from chromosome V, indicating that most of the resistance readily inhibited by sesamex in the parent NPR strain is from factors on this chromosome. (Farnham)

Mode of action of pyrethroids

The toxicities of five pyrethroids to the central nervous systems of adult male *Periplaneta americana* were estimated by electrophysiological methods described in 1968 and 1969 and compared with their toxicities to living cockroaches.

The LD95 of pyrethrin I ((+)-pyrethronyl (+)-*trans*-chrysanthemate) applied topically was one-quarter that of bioallethrin ((±)-allethronyl (+)-*trans*-chrysanthemate) and one-third that of NRDC 107 (5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate); all three compounds were very toxic to cockroaches, whereas 5-benzyl-3-furylmethyl (−)-*trans*-chrysanthemate (an optical isomer of NRDC 107) was about one-thousandth as toxic as pyrethrin I and (±)-1-(5-benzyl-3-furyl)-ethyl (+)-*trans*-chrysanthemate (the α-methyl derivative of NRDC 107) was barely toxic (Table 2).

When the compounds were dissolved in saline containing 0·5% acetone and applied directly to the central nervous system, their toxicities followed the same order as to whole insects, although differences between the toxicities of individual compounds were usually greater. The activities of the (−)-*trans* isomer of NRDC 107 and of the α-methyl derivative of NRDC 107 were so small that symptoms of toxicity could hardly be detected even at the maximum concentrations in saline attainable, 10^{-4} and 10^{-5} *M*. The other

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TABLE 2

Toxicity of pyrethroids to cockroaches and to their central nervous systems

Dose per insect	LD95	Initial burst[1]	Min. effective concentration[2]	Axonic conduction[3]
Pyrethrin I	0·37 μg	10^{-7}	10^{-9}	10^{-7}
Bioallethrin	1·49 μg	10^{-6}	10^{-8}	10^{-5}
NRDC 107	1·16 μg	10^{-6}	10^{-7}	10^{-6}
(−)-*trans* isomer of 107	0·31 mg	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
α-methyl derivative of 107	>1·0 mg	$>10^{-5}$	10^{-5}	$>10^{-5}$

[1] Minimum concentration producing large initial burst of activity.
[2] Minimum concentration at least doubling activity during 3 hour treatment.
[3] Minimum concentration halving amplitude of action potential 1 hour after treatment begun.

compounds were all very active. Pyrethrin I, which increased the amount of endogenous activity in the central nervous system when as dilute as 10^{-9} *M*, was 10 times as active as NRDC 107 and 100 times as active as bioallethrin.

The toxicities of these compounds to whole cockroaches and to exposed nervous systems are so similar that their ability to kill cockroaches reflects their intrinsic activities at their sites of action, with intermediate processes such as penetration and detoxification modifying the activity very little. This conclusion was tested by measuring the extent to which the compounds were synergized by piperonyl butoxide, which is said to inhibit detoxification. Although the two least active compounds were synergized more than were pyrethrin I or NRDC 107, their toxicities remained relatively small, and none of the compounds tested was greatly synergised. Thus the ability of these pyrethroids to kill *P. americana* seems to reflect their intrinsic activities at the sites of action, which contrasts with such species as *Musca domestica* in which other processes probably greatly modify the intrinsic toxicity. (Burt and Goodchild)

Activity of compounds designed on a theory of DDT mode of action

Holan's theory that DDT and compounds with similar molecules are insecticidal because they collect at the lipid–protein nerve membrane interface and form a wedge that keeps the pore of the lipid part of the membrane permeable to sodium ions, was investigated using five new compounds produced by G. Holan (C.S.I.R.O.). The size and stereochemical configuration of the compounds were such that on the proposed theory they would be very active insecticides and greatly affect the activity of the central nervous system.

Insecticidal activity was tested by applying the compounds topically in acetone solution to the cockroach *P. americana* (adults) and to some genetically defined housefly strains containing resistance factors, provided by Sawicki, Farnham, Plapp (Texas) and Franco (Italy).

Effect on nervous activity was examined by the method of continuous application developed by Burt, to determine the minimum concentrations of DDT and of the new compounds required to increase the spontaneous activity of the sixth abdominal ganglion of cockroaches above the normal level. The evidence so far obtained supports Holan's theory. (Shipp)

Behaviour controlling substances

The nature and mode of action of extracts of wheat and oats that influence the behaviour of Wheat Bulb fly larvae

Wheat. Larvae given a choice between a block of gel containing exudate from wheat seedlings or a block of plain gel moved to both gels in equal numbers. However, larvae

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tended to remain in contact with the wheat gel and after about one hour significantly more were in the wheat than the plain gel. Thus larvae were not attracted towards a gel of wheat, but were arrested when their random movements brought them into contact with it.

The arrestant test, which lasts 15 minutes (*Rothamsted Report for 1966*, 183), although less sensitive than the gel test, continued to be the more suitable and reliable method for assaying biological activity of extracts of wheat. Mild oxidations and reductions of crude extracts destroyed biological activity, but prolonged acid and alkaline hydrolysis did not. Further purification was achieved by partition between diethyl ether and water and by treatment with basic lead acetate. The partitioning removed some of the less polar, organic materials, and the lead acetate precipitated much biologically inactive material, leaving sugars and glycosides as the main classes of compounds. NMR examination of these purified extracts showed a relative increase in the absorptions characteristic of the *CH* OH of carbohydrates and loss of almost all absorption in the aromatic proton region. Chromatography of the most purified extracts showed that most of the components were rendered visible by spray reagents specific for sugars.

Oats. Extracts of oats, which is not a host plant of Wheat Bulb fly, not only failed to arrest larvae but decreased the arrestant activity of wheat extracts. This 'oats factor' has similar physical properties to the 'arrestant factor' in wheat: it was not destroyed when heated in the ion source of a mass spectrometer to 310°C at a pressure of 1×10^{-6} mm. The oats factor was soluble in water and methanol but could be removed from solution in water by continuous ether extraction.

Other compounds that decreased the arrestancy of wheat included two proprietary insect repellents: MGK 11 (2,3:4,5-bis(2-butylene)tetrahydro-2-furaldehyde) and deet (*N*,*N*-diethyl-*m*-toluamide). (Scott, Griffiths and Greenway)

Behavioural chemicals affecting the life cycle of codling moth (*Carpocapsa pomonella*). Other workers have established that females of the codling moth secrete sex pheromone from the abdomen. We examined extracts of female abdominal tips by gas chromatography and mass spectrometry and found a component by g.c. that seems to be the pheromone because it has the same retention time as that reported by other workers; as yet, there is too little to attempt an m.s. examination. The main components in these extracts are n-paraffins, ethyl esters of fatty acids and free fatty acids—interestingly, we noted a sex difference because male moths produce methyl esters of these same acids. Condensation of volatiles emitted by virgin female moths, followed by gas chromatography, showed several minor components and one large one, tentatively identified as ethyl oleate.

Whether there is a chemical basis for gravid female moths selecting their oviposition site is also being studied. (Greenway, in collaboration with Dr. J. E. Cranham of East Malling Research Station, Kent)

Chemicals influencing feeding and movement of leaf cutting ants *Acromyrmex octospinosus* (Reich) and *Atta cephalotes* (L). Leaf cutting, fungus-growing ants of the genera *Atta* and *Acromyrmex* are serious pests in the tropics, as they defoliate various crops while gathering material on which they culture their fungus.

Phytochemical arrestants. Some materials, notably fresh grapefruit albedo and dried citrus pulp, are especially favoured by the ants as substrates for their fungus gardens. Fractionation of the material showed that both contain an arrestant among their non-

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lipid components. A preliminary scheme for separating the non-lipids was devised and their arrestant activity is being assayed by Dr. J. M. Cherrett of University College of North Wales, Bangor.

Trail pheromone. Worker ants closely follow trails marked with ethanol extracts of abdomens of virgin queens of *A. octospinosus*. Gas chromatography and mass spectrometry show the extracts contain much ethyl palmitate and stearate, but these are not the active component, which seems to occur in very small amounts.

Pharyngeal glands. Soon after these ants eat food impregnated with dyed oil their pharyngeal gland becomes visibly stained, and when such ants are returned to their colony the dye soon appears in the pharyngeal glands of other workers. Thus the contents of the gland seem implicated in food sharing within the colony, and so also would spread any insecticide in the food. The gland produces a complex mixture of fatty acids, together with a major, but as yet unidentified, component that is not a fatty acid. (Mudd, in collaboration with Drs J. M. Cherrett and D. J. Peregrine, U.C.N.W., Bangor)

Chemicals influencing parasitization and crowding of the larvae of the Mediterranean flour moth (*Ephestia kühniella*). *E. kühniella* is a major pest of stored cereals in many countries. The mandibular glands of its larvae contain a pheromone that stimulates its parasite *Nemeritis canescens* to lay eggs and one that causes crowding of the larvae. Six groups of components were separated from the contents of the gland, the oviposition stimulant was isolated and some progress made in identifying it. (Mudd, with Dr. S. A. Corbet, Westfield College, London University)

Gas chromatography–mass spectrometry in the study of constituents of insects

Techniques of examining very small amounts of lipids from insects have been so elaborated that they can usefully be applied to the neutral and acid fractions from glands, portions of the body, or the surface of individual insects. Honeybees have provided the material for the bulk of this work which, in collaboration with the Bee Department, was directed mainly to seeking a single pheromone that could account for a queen honeybee attracting workers. Such a compound has not been found and more attention needs to be paid to the conception that a worker recognises a queen in a definite physical context by a pattern of odours rather than by an olfactory stimulus from a single compound. Extracts of whole bodies, heads, glandular material and body and cell washing were examined and much incidental information gleaned that can be classified in chemical terms. Some 40 compounds have been encountered as normal constituents of honeybee lipids, in addition to several that are evidently contaminants derived from propolis, pollen, pasticisers, pump oil, fingerprints and cosmetics.

Higher hydrocarbons occur especially in the cuticular lipids. Small amounts of tridecane, $C_{13}H_{28}$ and pentadecane, $C_{15}H_{32}$, were detected and then there was a gap in the series until the group of straight-chain hydrocarbons with odd numbers of carbon atoms from $C_{19}H_{40}$ to $C_{31}H_{64}$, of which $C_{21}H_{44}$, $C_{23}H_{48}$ and $C_{25}H_{52}$ were the most prominent members. In large samples of material, the hydrocarbons with an even number of carbon atoms and some ethylenic hydrocarbons were detectable, but not always exactly identified. Branched-chain hydrocarbons were either lacking, or minor constituents. Drones were rich in cuticular hydrocarbons; queens had rather less; workers had very little, and $C_{23}H_{48}$ and $C_{25}H_{52}$ were the only ones unambiguously identified, though $C_{21}H_{44}$ and $C_{27}H_{56}$ were probably present.

This type of hydrocarbon mixture occurs on the cuticle of other insects. Thus, washings

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of the surface of two strains of housefly differing in resistance to insecticides, contained as prominent constituents $C_{23}H_{48}$, $C_{25}H_{52}$ and $C_{27}H_{56}$, of which the first was the more abundant, plus some $C_{21}H_{44}$, $C_{29}H_{60}$ and C_{21} and C_{23} ethylenic compounds. Neither the type nor amount of hydrocarbons differed between the two strains.

The significance of the occurrence of the aromatic compounds, *p*-hydroxy- and *p*-methoxy-benzoates in bees, was for some time a subject of discussion between us and French workers. Our respective results are now in general agreement. These compounds are not derived from added preservative in the sugar given as food, and the suggestion that they have an antimicrobial function is supported by the detection of *p*-methoxy-benzoate in the washings of cells containing freshly laid eggs. (Callow, with Mrs. Koster, Bee Department)

The usual series of tissue fatty acids—lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids—occur in extracts of many portions of the bodies of honeybees. In the mandibular glands of workers, however, the preponderant constituents are the dibasic acids—all members of the series from octane-1,8-dioic to dodecane-1,12-dioic acid—and only traces of the monobasic acids are present. Of these, 9-oxodec-2-enoic acid and 9-hydroxydec-2-enoic acid produced in the queen have specific pheromonal activity, and 10-hydroxydec-2-enoic acid in the mandibular gland secretion of the worker is a major constituent of 'royal jelly'. The methyl esters of these compounds are readily detected by GC–MS methods in extracts and they are accompanied by decanoic, decane-dioic, dec-2-enedioic, 3-hydroxydecanoic, 9-hydroxydecanoic, 10-hydroxydecanoic and 3,10-dihydroxydecanoic acids. A future step is clearly to investigate what might be called the 'C_{10} economy' of the honeybee—obviously something that has a very special significance in this species. The ability of GC–MS techniques to analyse materials on a sub-microgram scale has already shown that individual bees differ greatly in the amounts of these compounds they contain, and the way is now open to study how the amount varies with race, age, physiological state and season of the year. (Callow)

The natural pyrethrins and related synthetic compounds

5-Benzyl-3-furylmethyl chrysanthemates (NRDC 104 or 107) (*Rothamsted Reports for 1966–69*) are more active against some insect species than the mixture of esters in the natural pyrethrins but act slower and are less well synergised against houseflies by methylenedioxyphenyl compounds. New compounds described below have now been developed that, although less toxic than the 5-benzyl-3-furylmethyl esters, are more active than the natural pyrethrins against houseflies, have considerable knockdown activity and are also nearly as well synergised as the natural esters.

New compounds. The six insecticidal esters in the natural pyrethrins and many related synthetic compounds are all 2-alkenyl-3-methylcyclopentenonyl chrysanthemates (I) or pyrethrates. The alkenyl group ($R = CH\overset{c}{=}CH.CH=CH_2$) in the most important natural alcoholic constituent, pyrethrolone, can be replaced in synthetic alcohols by allyl ($R = CH=CH_2$) or benzyl ($R = C_6H_5$) groups without great loss of insecticidal activity. However, it was not known whether the methyl group on the cyclopentenolone ring in such compounds was essential for toxicity, so this was investigated in esters with benzyl substituents. These were more easily synthesised than compounds with the natural *cis*-pentadienyl side chain. Thus, benzylrethrin (I) ($R = C_6H_5$), the analogue of pyrethrin I with benzyl instead of the *cis*-pentadienyl side chain, was less toxic than 2-benzyl-cyclopentenonyl (+)-*trans*-chrysanthemate (IIa) the corresponding compound without a methyl group.

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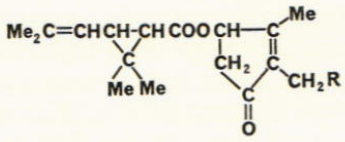
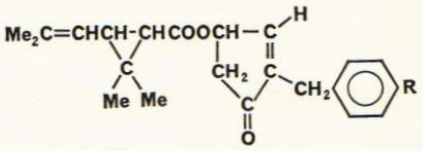


(I)



(II) a; R = H
b; R = Me
c; R = Cl

TABLE 3

Relative toxicities of synthetic pyrethroids to adult houseflies and mustard beetles

	Musca domestica L.	*Phaedon cochleariae* Fab.
5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate (NRDC 107)	1000[a]	1000[b]
5-Benzyl-3-furylmethyl (±)-*cis-trans*-chrysanthemate (NRDC 104)*	420	370
(±)-2-Benzyl-cyclopent-2-enon-4-yl (+)-*trans*-chrysanthemate ('Benzyl northrin')	88	170
(±)-2-Benzyl-3-methyl-cyclopent-2-enon-4-yl (±)-*cis-trans*-chrysanthemate*	4·2	20
(±)-2-Benzyl-cyclopent-2-enon-4-yl 2,2,3,3-tetramethylcyclopropane carboxylate	180	17
(±)-2-(*p*-methylbenzyl)-cyclopent-2-enon-4-yl (+)-*trans*-chrysanthemate	71	54
(±)-2-(*p*-chlorobenzyl)-cyclopent-2-enon-4-yl (+)-*trans*-chrysanthemate	33	18
(+)-Pyrethronyl (+)-*trans*-chrysanthemate (Pyrethrin I)	13	1600
(±)-Allethronyl (±)-*cis-trans*-chrysanthemate (Allethrin)	16	78

* Contains *ca.* 35% of (+)-*trans* isomer.
[a] LD50 0·005 μg/insect.
[b] LD50 0·004 μg/insect.

Table 3 shows the insecticidal activity of this compound ('Benzyl northrin') and two related compounds (IIb and IIc) with substituted benzyl side chains. The *p*-substituents in the benzene ring diminished toxicity as did *p*-aryl substituents in the 5-benzyl-3-furylmethyl chrysanthemates. In this series there was also a remarkable example of species specificity. Whereas 2-benzyl-cyclopentenonyl tetramethylcyclopropane carboxylate was more than twice as toxic as the corresponding (+)-*trans*-chrysanthemate to a normal

TABLE 4

Weighted mean LD50s (μg/♀ fly) and synergistic factors for houseflies pretreated with 2 μg of sesamex

	LD50 Compound alone	LD50 Compound + synergist	Approximate synergistic factor
5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate	0·0054	0·00057	9·4
5-Benzyl-3-furylmethyl (±)-*cis-trans*-chrysanthemate	0·010	0·00079	13
(±)-2-Benzylcyclopent-2-enon-4-yl (+)-*trans*-chrysanthemate	0·055	0·00061	90
Pyrethrin I	0·33	0·0011	300

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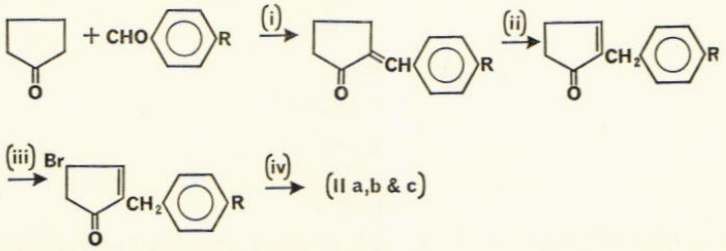
strain of houseflies, it was a tenth as toxic as the (+)-*trans*-chrysanthemate to mustard beetles. Benzyl northrin also 'knocked down' flies faster than the benzylfurylmethyl chrysanthemates and was comparable in this respect with allethrin (I, R = $CH{=}CH_2$), in addition to being more toxic than allethrin to houseflies and mustard beetles. It was less toxic to mammals than the natural pyrethrins or allethrin.

Table 4 shows that the new benzyl esters attain a toxicity to houseflies exceeding that of pyrethrin I when the detoxification mechanisms are inhibited by sesamex (2 μg/fly). The potency is then comparable with that of the benzylfurylmethyl chrysanthemates.

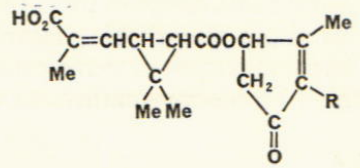
Synthesis. The three esters were made by the same route:



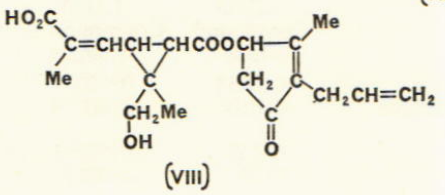
Reagents (i) NaOH, 20°
(ii) dry HCl/$MeOCH_2OH$, 130°
(iii) N-bromosuccinimide, 80°
(iv) Ag (+)-*trans*-chrysanthemate, 80°

Benzylidene cyclopentanones, obtained by condensing the corresponding benzaldehyde with cyclopentanone, gave 2-benzylcyclopentenones (cf. Phillips & Mentha, *J. Amer. Chem. Soc.* (1956), **78**, 140; Conia & Amice, *Bull. Soc. chim. France* (1968), 3327). *N*-bromosuccinimide introduced bromine at position 4 (nmr evidence) and the 4-bromo-compounds with silver chrysanthemate gave mixtures from which the pure esters (IIa, b and c) were isolated. (Chemical work: Elliott, Janes and Payne. Biological work: Farnham and Needham)

Mammalian metabolites of natural and synthetic pyrethroids. In collaboration with the Toxicology Group, Division of Entomology, University of California at Berkeley, the products formed when the two important constituents of pyrethrum extract, pyrethrins I and II, and the related synthetic compound, allethrin, are metabolised by rats, were



R	
(III)	$-CH_2CH{=}^{c}CHCH(OH)CH_2OH$
(IV)	$-CH_2CH(OH)CH{=}^{t}CHCH_2OH$
(V)	$-CH_2CH{=}^{c}CHCH(O\text{conj})-CH_2OH$
(VI)	$-CH_2CH(OH)CH_2OH$
(VII)	$-CH(OH)CH{=}CH_2$



(VIII)

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examined. The ease with which oxidative products are formed from these esters probably explains their very small toxicity to mammals.

The structures of three metabolites of pyrethrin I and II and three metabolites of allethrin were deduced from nmr and mass spectral evidence after the products had been isolated and separated with the help of tritiated esters. The acid side chain of all the metabolites isolated contained a carboxyl group formed by oxidation of the *trans*-methyl group in esters of chrysanthemic acid (pyrethrin I and allethrin) and by hydrolysis of the methoxycarbonyl group in pyrethric acid esters (pyrethrin II). All the metabolites of pyrethrins I and II (III, IV and V) and two of those of allethrin (VI, VII) were also modified on the alcoholic side chain; the conjugating acid in metabolite (V) was not identified, but nmr evidence suggests it is aromatic. The third metabolite from allethrin (VIII) was doubly modified on the acid, but the alcohol was unchanged. (Elliott and Janes, with Professor J. E. Casida and Miss E. C. Kimmel, Division of Entomology, University of California.)

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

Persistence on cotton leaves. ^{36}Cl-labelled dieldrin crystals (*ca.* 80 μm long) in aqueous suspension were sprayed on glass and living excised cotton leaf surfaces to give deposit densities 1–2 μg/cm^2, and kept in a CT room at 20°C. Losses followed the usual exponential curves and after 3 weeks the deposits remained almost constant. GLC analyses confirmed radiometric measurements (direct G–M mounting of surfaces) and showed that whereas after 3 weeks on glass surfaces dieldrin was barely detectable (less than 0·01 μg/cm^2), the leaves still contained about 0·1 μg/cm^2; after 8 weeks, when the leaves were dead, deposits were 0·08–0·06 μg/cm^2. (Phillips and Sethi)

Microcapsules. The National Cash Register Co. (NCR) have stopped the supply of various formulations of insecticides in microcapsules for experimental purposes, so we have produced batches for testing, using their coacervation technique. These microcapsules are of the simplest type, with gelatin/gum acacia walls and oily internal phases such as toluene and triolein. Spherical microcapsules ranging in size from 2 mm to a few μm in diameter were prepared, but there are still problems in preparing microcapsules with thicker walls and in separating and drying the individual microcapsules from the slurries. (Phillips and Gillham)

Stickers and microcapsules. The ability of the stickers Acronal 4D and 7D to retain large (750 μm–1 mm diameter) NCR microcapsules of gelatin/gum acacia (with a toluene internal phase) on surfaces was further studied. Formulations were aged on the surfaces and 'rainwashed' for increasing or decreasing periods over several weeks (groups A and B respectively, Table 5).

The smaller percentages of microcapsules retained on cotton leaves than reported last year indicates the deterioration in the stickers after about 6 months' storage. Probably because of this, the differences previously found between Acronal 4D and Acronal 7D are not easily discernible, but the new results again show differences between the effect of 'rainwashing' on 'fresh' (2 days old) and 'aged' (2 weeks old) deposits. The 'rainwashing' is stringent, because every 30-second period represents 2·5 cm of rain. More microcapsules were retained during the first 3 weeks in 'aged' than in 'fresh' deposits with increasing 'rainwashing' periods (group A), but no differences were found with decreasing 'rainwashing' periods (group B). This implies that a 2 weeks' ageing period increases the retention of deposits provided the rainfall is at first light to moderate, but

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TABLE 5

Percentages of microcapsules retained on living excised cotton leaf surfaces after different 'rainwashing' periods over several weeks

	Weeks						No. of replicates
	0	1	2	3	4	5	
(A) 'Rainwashing' period (secs)	30	60	150	300	150	150	
'Fresh' 4D	41	24	10	7	6	4	4
7D	40	32	19	12	9	4	4
Mean of 4D and 7D	**41**	**28**	**15**	**10**			
'Aged' 4D	53	37	26	8	4	—	10
7D	62	50	40	17	5	—	10
Mean of 4D and 7D	**58**	**44**	**33**	**12**			
(B) 'Rainwashing' period (secs)	300	150	60	30	150	150	
'Fresh' 4D	38	22	18	17	15	3	7
7D	27	14	12	11	8	2	10
Mean of 4D and 7D	**33**	**18**	**15**	**14**			
'Aged' 4D	21	12	8	6	—	—	10
7D	33	29	23	10	—	—	12
Mean of 4D and 7D	**27**	**20**	**15**	**8**			

not when it is very heavy. Table 5 shows that after 3 weeks, when all deposits had received the same total amount of 'rain', similar amounts of microcapsules were retained. (Phillips and Gillham)

Estimation of insecticide residues in plant material. Impurities (coloured or otherwise), which cause 'quenching' effects by lowering the counting efficiency and at the same time give poor reproducibility in the scintillation counting of radioactive insecticide samples of leaf extracts, have to be eliminated if no External Standard Ratio method is available to estimate the degree of 'quenching'.

Of various cold solvent systems used in a glass grinding apparatus to extract dieldrin residues from cotton leaves, chloroform–methanol (1 : 1) gave the best recovery of insecticide, followed closely by hexane–isopropanol (2 : 1), then acetone, and finally hexane.

Three methods tested for their ability to decolorise extracts of cotton leaves in order to eliminate 'colour quenching' were: (1) shaking with an absorbent material; (2) passing through an adsorbing column (chromatographic leaching); (3) shaking with an oxidising or bleaching agent.

(1) Polyethylene powder (BDH Ltd.), which has been used by other workers to absorb carotene and most of the chlorophyll from acetone–water (70 : 30) leaf extracts did not sorb all the chlorophyll and sorbed some of the dieldrin. The sorbed dieldrin could be recovered by eluting with various solvents but they also eluted chlorophyll. Charcoal removed all colour from acetone and hexane–isopropanol leaf extracts, but also sorbed some dieldrin.

(2) Chromatographic leaching was by either Alumina 'H' or Alumina 'W200 basic' columns with various eluting solvents, which separated carotenes, chlorophylls and xanthophylls but not all the dieldrin, of which various proportions remained associated with the chlorophylls.

(3) Hydrogen peroxide did not decolorise leaf extracts and benzoyl peroxide only to a small extent, but sodium hypochlorite or acidified potassium permanganate solutions did after shaking for considerable periods. These reagents, by preventing 'colour quench-

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ing' may provide satisfactory 'clean-up' methods but their use has not yet been fully tested in scintillation counting. (Phillips, Sethi and Kavadia)

Poisoning of honeybees in the field. Of 63 samples of honeybees (*Apis mellifera*), alleged to be poisoned, received from beekeepers in England via the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food (22 more than in 1969), 45 gave evidence of poisoning; of these, 29 reacted positively to our test for organophosphate poisoning (OP in Table 6) and 9 gave inconclusive results (? OP); this test measures residual cholinesterase after poisoning and not the insecticide residues themselves. Evidence supplied with some of the samples (see Table 6) showed the sprayed crops that led to the poisoning.

Dieldrin was in four samples, both DDT and BHC in one, and BHC only in one. Contaminated hive equipment may have been the cause of the organophosphate plus BHC in one sample and for the dieldrin in another; deliberate poisoning was suspected for the DDT plus BHC in one sample and for the organophosphate plus dieldrin in another. One sample contained carbaryl.

TABLE 6

Confirmed honeybee poisonings by organophosphate insecticides in 1970

		Crops sprayed (see text)				
	Total	Field beans	Peas	Potatoes	Brassica	Fruit
OP	27*	5	5	4	3	2
OP + dieldrin	2				1	
?OP	8	2		4		
?OP + BHC	1					

* Includes three aerial applications.

The few poisonings associated with spraying field beans, compared with previous years, probably reflects the small *Aphis fabae* infestation. Poisoning associated with spraying potatoes during July and August is a new development, which we hope will not recur.

The confirmed poisonings involved an estimated total of 472 colonies, compared with 220 in 1969 and 200 in 1968.

A sample of *Apis indica* received from Pakistan contained aldrin and some dieldrin; one of *A. mellifera* received from India gave negative results. (Stevenson)

Apparatus and techniques

Particle (insect) counter. An apparatus was developed by which many dead insects can be counted automatically. A positive liquid flow system is used to carry the insects through an optical sensing head. The detector and associated circuitry allows the unit to be used with any scaler that will accept randomly spaced pulses at rates of up to 800/sec and trigger on pulse amplitudes of 10 V or less. The output from the sensing head is continually monitored so that changes in the light intensity or optical density of the liquid can be detected. As the system will count all particles with a refractive index different from that of the fluid carrying them, some form of discriminator is necessary that can cancel pulses emanating from particles smaller than a pre-set size. This discrimination is obtained by increasing the light intensity to the point at which the output to the scaler moves from '1' to '0'. Increasing the light intensity further increases a meter

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deflection which can be calibrated as a particle size discriminator. Results indicate that an accuracy of $\pm 3\%$ can be expected when counting *Aphis fabae*. Most of the test counting has been done with aphids, as the wide range in size on any one plant increases the difficulty of carrying them through the detector and in accurately counting them. As expected, larger insects, all of similar size as obtained from the sorting apparatus are easier to count and the count is more accurate. The maximum concentration of particles for a given rate of flow has yet to be determined. This information is important because too great a concentration results in errors, largely from coincidence counting. A batch of several thousand insects can be counted in approximately 5 minutes, inclusive of the time spent in feeding them in and collecting them from the system.

Atomisers. An improved atomiser was devised for use with the Kearns–March insecticide testing equipment. Developed from the spray heads patented by the National Research Development Corporation in 1967 the atomiser dispenses 0·05–0·1 ml in a specified time while using less than one litre per minute of air at 70 kN/m². The atomiser normally fitted to this equipment has an air flow rate in excess of 20 litre/minute. The significantly lower volume of air entering the chamber greatly reduces the turbulence level and keeps the loss of insecticide due to leakage down to a very low level. (Arnold)

Neuroanatomy of the insect central nervous system. Neuroanatomical studies of the central nervous system of the cockroach *Periplaneta americana* (L.), begun to provide a basis for studying histochemically local cholinesterase inhibition in the CNS by organophosphates, were continued using the improved Bodian protargol silver stain (*Rothamsted Report for 1969*, Part 1, 220). The thoracic ganglia were examined first, because their condition in poisoned insects is most closely related to that of the whole insect. Four aspects of the mesothoracic ganglion were then studied intensively: (1) the roots of the peripheral nerves; (2) the groups of nerve cell bodies that surround the central neuropile; (3) the longitudinal and (4) the transverse nerve fibre tracts of the neuropile. The surrounding layers, of glial cells, perineurium and neural lamella, were omitted as they were studied by earlier workers. Work on the nerve roots is complete and on the other aspects well advanced.

Each nerve root divides within the ganglion into a number of bundles of fibres. The courses of these were traced and their likely function, motor or sensory, deduced from whether they end in cell bodies (motor) or not (sensory). The results tally well with the known functions of each peripheral nerve. The paths of the more important branch fibres were also followed and approximate areas of synaptic contact between fibres located, chiefly in dorsal and mid regions of the neuropile. In the ventral neuropile a specialised region receives many fine, almost certainly sensory, fibres from five of the peripheral nerves and also endings of association neurons. Such knowledge of how functional units are arranged within the CNS will enable the ways insecticides affect the CNS to be better understood. Localising probable areas of synaptic contact between units should allow the sites that may be expected to be important in the local action of insecticides to be predicted. (Gregory)

Systemic insecticides

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 was studied further by the methods reported in 1968 and 1969; toxicity of the plants was assessed by confining *Aphis fabae*

https://doi.org/10.23637/ERADOC-1-125

on leaf surfaces for 24 hour periods at intervals during the experiments. The number of aphids killed was increased by: (1) simulated rain given daily, rather than every three or more days; or no rain at all; (2) formulation on pumice rather than on fullers earth; (3) applying the granules to the foliage and soil, rather than to the soil only; (4) warmer ambient air.

With our standard temperature conditions (20°C day, 15°C night), relative humidities of 45% and 97% were compared and found to have no significant influence on the kill of aphids, except that kill was slightly greater in the first cage test 24 hours after treatment at 97%.

The effects of rain and formulation were also studied in the field at Woburn. Three moisture regimes were established: (1) covered plots receiving neither rain nor irrigation; (2) plots receiving rain only; (3) plots receiving rain and, on rainless days, irrigation that thoroughly wetted the foliage. 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). Because of dry weather, the crop was poor, especially under the covers and the experiment will be repeated at Rothamsted in 1971. Effectiveness was assessed by caging *A. fabae* on the leaf surfaces for periods up to five days at weekly intervals and, with no natural infestation, by estimates of an artificial *A. fabae* infestation established on the plants from laboratory cultures. Cage tests showed that the pumice formulations were on average more effective, and higher kills were obtained in the irrigated plots. As expected, kills during the first week were large, but there was a second peak of activity about 30 days after treatment, possibly from insecticide washed into the soil and entering the plants via the roots. On the irrigated plots the pumice/phorate treatment was less successful than expected during the first week, but both pumice treatments did particularly well during the second peak. The population counts agreed with these results. (Etheridge, Graham-Bryce and Stevenson)

Control of aphids and virus diseases of peas. Collaborative work on this problem is described in the report of the Entomology Department, p.187. (Etheridge)

Control of aphids on field beans in relation to toxicity to bees and the growth and yield of the crop. Collaborative work on these problems is described in the report of the Entomology Department, p. 183. (Stevenson)

Insect rearing. The following species were reared:

PLANT FEEDERS

Homoptera	*Aphis fabae* (Scop.)
	Myzus persicae (Sulz.)
	organophosphorus and DDT resistant strains
	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

https://doi.org/10.23637/ERADOC-1-125 *pp 16*

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
A number of strains derived from SKA, each with one or more factors of resistance to organophosphorus insecticides, DDT or dieldrin
NPR—pyrethrum extract selected, very resistant to pyrethroid insecticides
104–5B3FC selected, very resistant to 5B3FC
ac; ar; bwb; ocra—called 608Q, fully susceptible to pyrethroid insecticides to pyrethrum knockdown and to carbamates
A number of strains derived from NPR each with one or more factors of resistance to pyrethroid insecticides, DDT and dieldrin
A wild type susceptible strain

Hymenoptera *Acromyrmex octospinosus* (Reich)
Atta cephalotes (L.)

Seed dressings

Distribution and retention of seed dressings. Insecticides applied as dry powders do not adhere strongly to seeds, and often separate from the seeds later. Work was started to improve the adherence and retention of powder formulations, but it is difficult to incorporate in a formulation a sticker that does not interfere with the flow of powder, so we decided to try applying an adhesive to seeds before adding the powder. Methyl cellulose, natural gum, polyvinyl ethers and polybutenes as 3% aqueous solutions or emulsions or undiluted paraffin oils were tested, applied to wheat seeds in a revolving drum. A commercial powder dressing containing gamma BHC was then added in the proportion of powder to seed to give a target loading similar to that used commercially. Preliminary results suggest that with adhesives at 3–6 ml per 500 g seed, the adhesiveness is greater with polybutenes and paraffins than with methyl cellulose and natural gums, perhaps because the last two become dry. Thus seeds treated with 6 ml of 3% gum arabic solution retained 60% of the insecticide applied to them whereas the aqueous polybutenes and paraffin oils retained 80–90%. Untreated seeds retained only 18% of insecticide. Liquid paraffin applied at the rate required damages wheat seedlings, so polybutenes are being further tested. Information on another method was sought by finding how the insecticides are distributed on wheat seeds when these are dipped in an aqueous emulsion of aldrin or ethion. (Made by mixing the required amount of a 50% solution of aldrin or ethion in xylene containing 10% of Agrilan A as surface active agent, with one litre of water). The amount of liquid retained after dipping was found by weighing the seed before and after immersion in water containing 0·01% 'Teepol', and from this the concentration of insecticide needed to deposit the desired loading on seeds was calculated (0·8 and 1·6% for aldrin, 2·4% for ethion). Wheat seed dipped in the aqueous emulsions of insecticide and dried over a stream of warm air (45°C, velocity 4 ft/sec) for 9 minutes, weighed 0·6% more than before dipping, presumably mainly from an increase in moisture content. The insecticide loading was near to the target (40 or 20 μg per seed for aldrin, and 87·5 μg for ethion) and a retention test showed that 90–100% of the insecticide adhered strongly. The range in amount of aldrin or ethion on single seeds was only three to six fold whereas it is 200–300 fold with commercial liquid treatments. Applied

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by this method aldrin had no effect on germination, but ethion was slightly more damaging to seed sown in the field than when applied as a powder dressing. Despite the good distribution and retention by this method, work has been discontinued at present because of the difficulty of drying large quantities of dipped seeds. (Jeffs)

Biological efficiency of seed dressings against Wheat Bulb fly *Leptohylemyia coarctata* (Fall.)

γ-BHC and organophosphates. Collaborative work with the National Agricultural Advisory Service (Eastern Region and East Midlands Region) on how known amounts of γ-BHC and organophosphorus seed dressings protect young plants from attack by larvae of Wheat Bulb fly, was completed and is summarised in the Abstracts of papers (p. 328).

Seed dressings of experimental compounds. Short row trials of seed dressings of synthetic pyrethroids, insect repellents, organophosphorus and carbamate insecticides against larvae of Wheat Bulb fly were spoilt by an inappropriate choice of amounts and by severe damage to the crops by birds and rodents.

Combinations of carboxin and insecticides. Because of the increasing use of systemic fungicides on cereals, tests were done to see whether enough of a powder formulation of carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) could be put on seeds in addition to insecticides; also whether the materials interact in ways that affect plant growth, control of smut by carboxin, or control of Wheat Bulb fly larvae by the insecticides. Seed was dressed carefully in the laboratory, using a methyl cellulose sticker for powder formulations to give the full theoretical amounts. A powder formulation of carboxin was tested in combination with (a) a powder formulation of γ-BHC, (b) a liquid formulation of aldrin and (c) a liquid formulation of chlorfenvinphos. Powder γ-BHC dressings alone decreased the number of plants that germinated, and γ-BHC with carboxin was even more damaging in a sandy loam soil. With the other combinations plant growth was normal and insect control unimpaired.

Seeds dressed commercially had only small amounts of carboxin (about 30–50% of the theoretical dose) but larger amounts of the liquid insecticides aldrin, carbophenothion and chlorfenvinphos. Largest loadings of insecticides, >80% of the theoretical dose were obtained by dressing the seed with liquid insecticides first and then adding the carboxin powder. Again, insect control was good but neither laboratory nor commercial dressings of the seed adequately tested systemic fungicide action because of atypically late sowing dates and small infestation of smut. (Griffiths, Scott and Jeffs)

Soil insecticides

Microbiological breakdown of parathion. The work done in collaboration with Dr. N. Walker of the Soil Microbiology Department was completed and is summarised in the Abstracts of Papers (p. 309).

Behaviour of diethyl and dimethyl organophosphorus compounds in soil. Of the many different diethyl and dimethyl organophosphates tested against soil-inhabiting insect pests, diethyl compounds have usually been more effective. To help explain whether this is because diethyl organophosphates are inherently more toxic to insects or behave differently in soil, four dimethyl/diethyl pairs were chosen and used against a convenient test insect, adult vestigial wing *Drosophila melanogaster*, on glass surfaces and in soil. Dimethyl and diethyl forms of azinphos were much less toxic to *D. melanogaster*

https://doi.org/10.23637/ERADOC-1-125

than diethyl or dimethyl forms of parathion, bromophos or carbophenothion on glass surfaces, but the two members of each pair were similarly toxic so differences in effectiveness cannot be ascribed to differences in intrinsic toxicity. When *D. melanogaster* were allowed to walk over treated soil, for 24 or 48 hours, the toxicities of each compound depended greatly on the soil type and moisture conditions, several hundred times more insecticide was needed to kill equivalent numbers of insects in moist peat than in moist sand. However, the ratio of toxicities of dimethyl and diethyl pairs was much the same in all the tests, and it seems improbable that they were adsorbed onto or volatilised from soils to greatly differing extents. Tests of persistence of toxicity in a moist clay–loam soil showed that although diethyl and dimethyl forms of carbophenothion both lost their activity in 3 weeks, the diethyl forms of azinphos, parathion and bromophos remained active longer than the corresponding dimethyl compounds. Possibly such differences in persistence account for some diethyl compounds giving better control of soil-inhabiting pests. (Griffiths and Smith)

Fungicides

Tests of fungicides to control blight (caused by *Phytophthora infestans*) on potato haulms, common scab (caused by *Streptomyces scabies*) on potato tubers, and soil-borne diseases of cereals continued.

Naming of chemicals. The chemicals used are referred to by their common or chemical names, except the following: 66109 (*N*-(4-chlorophenylthiomethyl) phthalimide: Bayer); A 3596 (confidential: Geigy); BAS 3180F, 3191F and 3200F (confidential: BASF); 'Dexon' (*p*-dimethylaminobenzenediazo sodium sulphonate: Bayer); GS 16306 (4-tert. butyl-7-decylamino-3,4,5,6-tetrahydro-2H-azepine-chlorohydrate: Geigy); 'Imugan' (*N*-(2,2,2-trichloro-1-(3,4-dichloroanilino)-ethyl) formamide: Bayer); M2452 (*O*,*O*-diethyl-phthalimidophosphonothioate: Dow); MC 2810 (confidential: Murphy); 'Parnon' (bis(4-chlorophenyl)-3-pyridinemethanol: Eli Lilly); W 524 (*N*,*N'*-bis-(1-formamide-2,2,2-trichloroethyl)-piperazine: Cela).

Laboratory tests

Potato haulm-blight. Tests of organo-tin compounds were concluded. The relative effectiveness of dibutyltin diacetate, compared with fentin acetate, was the same in '1-day' and '2-day' tests. This means that triphenyltin sulphide is the only compound whose relative effectiveness is known to be increased by inoculating with spores two days after spraying instead of one day (see *Rothamsted Report for 1969*, Part 1, 228).

In tests on possible control of blight by systemic action, chemicals were mixed with potting loam in which young King Edward plants were then grown in the glasshouse. After 5 weeks, leaflets were detached and inoculated with zoospore suspensions of *P. infestans*. No resistance to infection was detected with any of the following chemicals, used at 25 ppm of a.i., or at the rate shown: BAS 3180F, 3191F and 3200F, benomyl, captafol, carboxin (at 10 ppm), dimethirimol (at 7·5 ppm), dodine, GS 16306, glyodin, M2452, MC 2810, mebenil (at 10 ppm), 'Parnon' (at 7·5 ppm), pentachloropyridine, pyridinitril, thiabendazole, thiophanate, thiophanate-methyl and W 524.

Potato common scab. Possible scab-control chemicals were tested in the glasshouse in the same way as last year. Quintozene, which nearly always controlled scab very effectively ($P \leqslant 0{\cdot}005$), was again used as standard. The following materials either failed to control scab ($P = 0{\cdot}05$), or damaged the plants, or both, at 50 ppm of a.i. or at

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TABLE 7

Relative amounts of scab on glasshouse-grown Majestic tubers after soil-treatments

Treatment	No. of tests	Scab incidence
Nil	10	100
Quintozene, 50 ppm	10	40
Captafol, 35 ppm	1	38
MC 2810, 50 ppm	2	44
Pentachloropyridine, 50 ppm	2	50
Tecnazene, 50 ppm	6	33
2,3,4,5-tetrachloronitrobenzene, 50 ppm	2	56
2,3,5,6-tetrachloro-4-nitrophenol, 50 ppm	2	27

the rate shown: 66109, A 3596, BAS 3200F, chloranil, chlorthal-methyl, 'Dexon', 2,4-dichlorobenzyl alcohol, dichlorophen, dinocap, 1,4-dinitro-2,3,5,6-tetrachlorobenzene, dithianon, edifenphos, GS 16306, guanoctine, hexachlorobenzene, hexachlorophene, 8-hydroxyquinoline sulphate, 'Imugan', mancozeb, maneb (at 35 ppm), mebenil (at 10 ppm), pentachloroanisole, pentachlorobenzyl alcohol, pyridinitril, sulphamic acid, 2,3,6-TBA, 2,3,5,6-tetrachloroaniline, 2,3,5,6-tetrachloro-4-nitroaniline, 2,3,5,6-tetrachloro-4-nitroanisole, tetrachlorophthalic anhydride, tetrachlorophthalimide, tetradifon, tetrasul, thiophanate, thiophanate-methyl, triarimol (at 5 ppm), 3,4,5-trichlorobenzyl alcohol, tridemorph (at 10 ppm), and W 524. The following chemicals gave significant control of scab at 50 ppm, but could hardly compare with quintozene: ethoxyquin ($P = 0{\cdot}02$–$0{\cdot}05$) and tetrachloroanthranilic acid ($P = 0{\cdot}01$). Results with quintozene and chemicals of comparable effectiveness (Table 7) are taken from 10 separate tests, in each of which there were 15 plants per treatment. The severity of the scab attack varied from test to test, so the results are given as the mean amounts of scab, relative to the variable amount on the tubers from the untreated plants, which is taken as 100 throughout. All the chemicals listed decreased scab significantly ($P \simeq 0{\cdot}003$); tecnazene significantly decreased yield in four of the six tests, but none of the others did. The effects of tecnazene and captafol confirm those found in similar tests last year, and are themselves confirmed by field results (see below). The most effective chemical (2,3,5,6-tetrachloro-4-nitrophenol) is the only one to have given significantly better control than quintozene in the glasshouse.

Field trials

Potato-haulm blight. The 1969 trial at Rothamsted was repeated, but with no blight attack in the exceptionally dry summer, no useful information was gained.

Potato common scab. A trial at Woburn with the variety Maris Piper tested the control of common scab by soil treatments before planting. The chemicals were applied as 10 or 20% dusts on 30 April; all plots were rotavated within 1 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 8). With the dry summer, scab was much more severe than in 1968 or 1969. No treatment affected the percentage of ware. Maneb did not affect scab, but all other chemicals decreased it ($P \leqslant 0{\cdot}001$). Captafol was as effective as quintozene, and did not significantly affect yield; it seems to be an acceptable substitute for quintozene. The higher rate of tecnazene controlled scab significantly better than quintozene ($P = 0{\cdot}05$), but both rates significantly decreased yield; thus tecnazene cannot be used at

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TABLE 8

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

Treatment	Rate, lb a.i./acre	Total tubers, tons/acre	% Ware	Scab index
Quintozene	70	13·5	92	38
Captafol	70	13·7	94	37
Maneb	70	14·9	92	58
Tecnazene	70	10·3	91	28
Tecnazene	35	12·1	91	43
Nil	—	15·1	92	59
LSD, $P = 0·05$		2·2	3	9
$P = 0·02$		2·7		11
$P = 0·01$		3·0		12
$P = 0·001$		4·0		17

planting time. The effects of all treatments on both scab and yield confirm those of glasshouse tests this year and last. Residues in soil, 1 and 7 months after application, were: quintozene, 30 ± 5 and 5 ± 1·0 ppm; tecnazene (70 lb/acre), 24 ± 7 and 4 ± 1·1 ppm; tecnazene (35 lb/acre), 13 ± 4 and 1 ± 0·3 ppm. (McIntosh)

Cereal diseases. The systemic fungicides benomyl, oxycarboxin and thiabendazole were used as seed dressings in a field trial with winter wheat. Details are given in the report of the Plant Pathology Department. (McIntosh, with Prew, Plant Pathology Department)

Staff and visiting workers

I. J. Graham Bryce and J. B. Lewis left and A. L. Devonshire was appointed.

At the request of the Pakistan Government, K. A. Lord was seconded for a second time to establish a government laboratory dealing with the chemistry of pesticides.

M. Elliott returned from a year's secondment to the Toxicology Group of Professor J. E. Casida in the Division of Entomology, University of California, Berkeley, U.S.A.

Visiting workers were Dr. Erik Shipp and Mr. Dennis Horn from Australia and Dr. K. S. Kavadia and Mr. G. R. Sethi from India.

A. H. McIntosh, F. T. Phillips, R. M. Sawicki and J. H. Stevenson read papers at the VIIth International Congress of Plant Protection held in Paris from 19–22 September 1970.

https://doi.org/10.23637/ERADOC-1-125 pp 21