ROTHAMSTED RESEARCH

Report for 1973 - Part1

Insecticides and Fungicides Department

I. J. Graham-bryce

I. J. Graham-bryce (1974) *Insecticides and Fungicides Department ;* Report For 1973 - Part1, pp 167 - 191 **- DOI: https://doi.org/10.23637/ERADOC-1-130**

INSECTICIDES AND FUNGICIDES DEPARTMENT

I. J. GRAHAM-BRYCE

Although potent organic insecticides have been available for many years, basic knowledge of how they act is still very limited. To use existing toxicants to best advantage and to develop new ones more rationally, better understanding of the factors influencing activity is essential. Much of the fundamental work done by the department is devoted to these problems; this includes long-term investigations into the relationships between molecular structure and insecticidal activity. In recent years these investigations have been concentrated on the pyrethroid insecticides which are particularly safe to mammals and have very favourable properties in the environment. One outcome has been the discovery of several very active new synthetic pyrethroids, some of which are produced commercially. Further structural modifications have now led to additional extremely effective compounds. The pyrethroids available at present decompose rapidly in air and sunlight and are relatively expensive so that their use has been limited mainly to the control of pests indoors and in glasshouses. A major objective of recent work, therefore, has been to find photostable pyrethroids that retain the other favourable characteristics of the group. This has now been achieved with one of the latest compounds, temporarily coded NRDC 143, which appears particularly promising for development as a practical insecticide. It results from a systematic programme of synthesis in which the centres known to be susceptible to photo-oxidation in previous pyrethroids were replaced, while the features essential for activity were retained. The greater stability should extend the scope of the pyrethroid group, especially for the control of agricultural and horticultural pests, particularly as the new compounds should be simpler to produce and therefore cheaper. The performance and behaviour of NRDC 143 are being examined under practical conditions.

The discovery of more potent insecticides should make it possible to control pests with less chemical and thus provide one method of decreasing the risk of harmful effects in the environment. Many of our other studies on the physicochemical properties of pesticides and on their behaviour in the environment should also suggest ways of increasing the efficiency and selectivity of pesticide applications by better timing, distribution or formulation. Selectivity between insect species is particularly desirable in integrated control programmes where the objective is to obtain as much benefit as possible from natural enemies of pests and thereby decrease amounts of pesticide used. Further work has therefore been started to evaluate the effects of pesticides on predators and parasites and to determine the least damaging toxicants and methods of application.

In the past, work on insecticides in the department has greatly exceeded that on fungicides. Recently, however, there have been rapid and very significant advances in the chemical control of diseases. In particular, several very effective new systemic fungicides have been introduced. It is therefore particularly timely to increase our work on fungicides. The new compounds are already used extensively in agriculture and horticulture, but, as with insecticides, the factors that determine their biological activity are still poorly understood. An additional worker has therefore been appointed to the department to study biochemical and physiological aspects of the mode of action of fungicides. Although the new materials have been extremely successful, a major disappointment has been the rapid appearance of tolerant strains of fungi. In these studies,

https://doi.org/10.23637/ERADOC-1-130

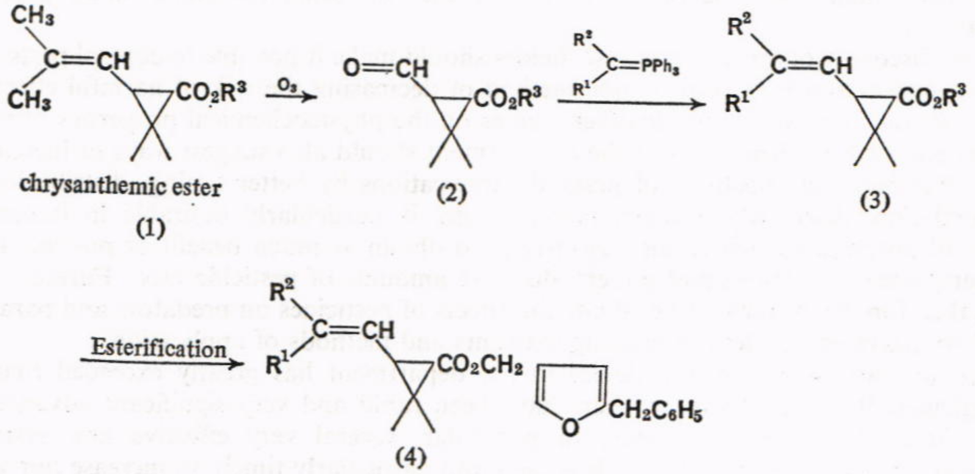
therefore, priority will be given to work on the mechanisms of tolerance. Other new lines of work on fungicidal seed treatments and on the behaviour of systemic fungicides in soil are described in this year's report.

During the year the Chemical Liaison Unit moved into a new temporary building. Although some facilities are still lacking, the new accommodation has made it possible to assemble all the members of the Unit and their equipment under one roof. This should greatly improve efficiency of working and the service available to departments interested in the fate of pesticides.

Insecticides

Relationships between molecular structure and insecticidal activity of pyrethroids. Further work was done to define the features required for activity and to find new active compounds, particularly ones that would be photostable. For some years, most attention has been given to the alcoholic component of the pyrethroid esters; this year in contrast the influence of the acid was examined. Both acidic and alcoholic components of previous pyrethroids contain centres susceptible to photo-oxidation but earlier work showed that these are not features essential for insecticidal activity so that it should be possible to discover combinations of new acids and alcohols that would be photostable but retain the activity of the more labile compounds.

Modified substituents at position 3 on the cyclopropane ring. The isobutenyl side chain in the natural compounds pyrethrin I, cinerin I and jasmolin I, and in the related synthetic compound bioresmethrin (4; $R^1 = R^2 = Me$) is known to be important for high insecticidal activity. For example, the analogue of bioresmethrin lacking this side chain is much less potent. In the ethanochrysanthemate [4; R^1 and $R^2 = (CH_2)_4$] the modified side chain gives greater activity than in the parent compound, so much work last year was aimed at finding more effective side chains, seeking especially those which might confer photostability.



Analogues of bioresmethrin with the substituent at position 3 replaced by other groups were prepared (Fig. 1). Varying R^1 and R^2 or using other intermediates similar to those shown gave 69 esters related to (4). The most effective side chains were A, $CH_3.CH_2.CH\overset{c}{=}CH$; B, $CH_2{=}CH.CH\overset{c}{=}CH$; C, $Cl_2C{=}CH$; D, $Br_2C{=}CH$ (entries 3–6 in Table 1); E, $CH_3ON{=}CH$.

https://doi.org/10.23637/ERADOC-1-130

Esters of various alcohols with the most effective new acids. The most effective new acids (A–D above) were combined with various alcohols likely to give esters with useful insecticidal properties. The isobutenyl side chain of the parent compounds is known to be attacked during photo-oxidation. Esters in which this is replaced by a more stable group, for example those of acid C above, should therefore be attacked less easily. However, although the 5-benzyl-3-furylmethyl ester (4; $R^1 = R^2 = Cl$) had outstanding insecticidal activity, it was still labile in air and light despite the more stable acid. In contrast, 3-benzylbenzyl and 3-phenoxybenzyl alcohols formed more stable esters, those with the dichlorovinyl acid having very considerable insecticidal activity, although their chrysanthemates were relatively ineffective. The most active and potentially practicable ester was 3-phenoxybenzyl (±)-*cis, trans* (20:80)2,2-dimethyl-3-(2,2-dichlorvinyl)-cyclopropane carboxylate which showed the following combination of favourable properties:

(1) Greater toxicity than resmethrin (NRDC 104) to insects (Table 1).
(2) Relative ease of synthesis from accessible starting materials.
(3) 10–100 times greater stability in light (depending on conditions) than resmethrin, bioresmethrin or other highly potent previous pyrethroids. Thus deposits of bioresmethrin (0·2 mg/cm^2) retained activity for only 4–6 hours when exposed to daylight on glass plates, whereas NRDC 143 was still active after three weeks.
(4) Low toxicity to mammals in all tests so far (Table 1).

TABLE 1

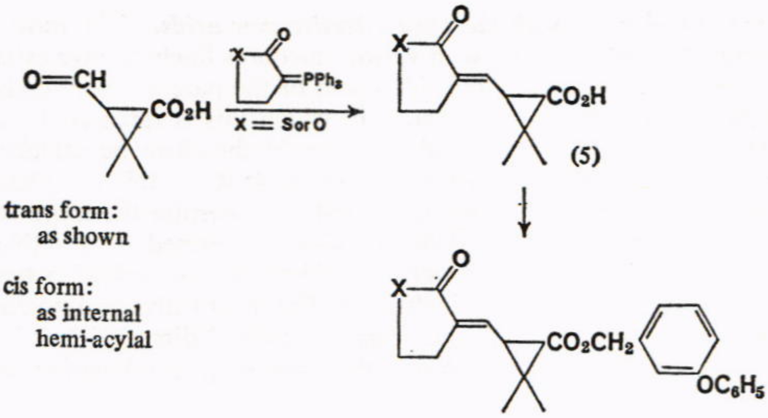
Toxicities of synthetic pyrethroids

	Relative toxicities by topical application to		Toxicity to rats[a] (mg/kg) administered in glycerol formal	
	Houseflies (*Musca domestica* (L.))	Mustard beetles (*Phaedon cochleariae fab.*)	Oral	Intravenous
Bioresmethrin (4; $R^1 = R^2 = Me$)	1000[b]	1000[c]	8000	340
Resmethrin ((±)*cis-trans* form of bioresmethrin)	420	370	>3000	160
(4; $R^1 = C_2H_5, R^2 = H$)	1600	1600	800–1000	120
(4; $R^1 = CH{=}CH_2, R^2 = H$)	2000	3900	—	—
(4; $R^1 = R^2 = Cl$)	2500	2700	>400	~30
(4; $R^1 = R^2 = Br$)	1100	1700	—	—
(3; R^3 = 3 phenoxybenzyl $R^1 = R^2 = Cl$; (±)-*cis, trans* form) NRDC 143	590	1200	>3200	>420
(6; X = 0, *cis* form)	31	36	—	—
(6; X = 0, *trans* form)	3·8	22	—	—
(6; X = S, *cis* form)	500	70	—	—
(6; X = S, *trans* form)	5·4	20	—	—
(7; $R^1 = H, R^2 = Me$)	220	15	—	—
(7; $R^1 = R^2 = Me$)	Non-toxic	Non-toxic	—	—
(7; $R^1 = H, R^2 = Cl$)	—	20	—	—

[a] We thank Mr. R. D. Verschoyle and Dr. J. M. Barnes, Medical Research Laboratories, Carshalton, for these results
[b] LD50 0·005 μg/insect
[c] LD50 0·004 μg/insect

3-Phenoxybenzyl esters of other acids. The combination of favourable properties found for NRDC 143 could not have been predicted from a consideration of the properties of the same alcoholic and acidic components in other esters. This suggested that all accessible combinations of effective acids and alcohols should be examined. Therefore

https://doi.org/10.23637/ERADOC-1-130



trans form:
as shown

cis form:
as internal
hemi-acylal

(5)

(6)

the more stable 3-phenoxybenzyl alcohol was esterified with acids (5) (British Patent 1 308 650 to Roussel-Uclaf) known to give esters with good insecticidal or knockdown action when combined with 5-benzyl-3-furylmethyl alcohol (Fig. 2). The acids were obtained as shown by Wittig reaction of the lactone or thiolactone phosphorane with *cis* or *trans* forms of formyldimethylcyclopropane carboxylic acid. The 3-phenoxybenzyl esters showed poorer knockdown and insecticidal characteristics than the corresponding 5-benzyl-3-furylmethyl esters.

Acyclic chrysanthemates. The most potent pyrethroids (pyrethrin I, bioresmethrin) have unsaturated side chains supported by a ring system: cyclopentenonyl in pyrethrin I and furylmethyl in bioresmethrin (4; $R^1 = R^2 =$ Me). There have been many attempts to replace this ring with acyclic structures having similar steric characteristics. Further compounds of this type, related to 3-phenoxybenzyl derivatives (see Fig. 3), have been synthesised from β-chlorocrotonic ester, α-methyl-β-chlorocrotonic ester, or from dichloroacrolein as follows:

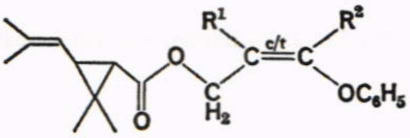
$$\underset{(R^1=H\text{ or Me})}{CH_3CO.CHR^1.CO_2Et} \xrightarrow[\text{(ii) } H^+,\text{ MeOH}]{\text{(i) } PCl_5} Cl.C(CH_3)=CR^1.CO_2Me$$

$$\xrightarrow[\text{(ii) Reduction with SDA}]{\text{(i) PhOH, } K_2CO_3\text{, acetone}} PhO.C(CH_3)=CR^1.CH_2OH \rightarrow (7;\ R^2 = \text{Me})$$

$$CCl_2=CH.CHO \xrightarrow[\text{THF}]{\text{PhOH, } K_2CO_3} \underset{\text{(mixture of isomers)}}{PhO.C(Cl)=CH.CHO}$$

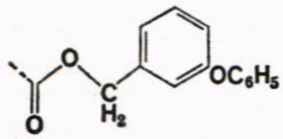
$$\xrightarrow{\text{SDA}} PhO.C(Cl)=CH.CH_2OH \rightarrow (7;\ R^1 = H,\ R^2 = Cl)$$

Measurements of insecticidal activity (Table 1) show that the ester (7; $R^1 = H$, $R^2 =$ Me) is the most active of this type yet discovered against houseflies. (Chemical work: Elliott, Janes and Pulman; Biological work: Farnham and Needham)



(7)

cf



https://doi.org/10.23637/ERADOC-1-130

Mode of action of insecticides

Neurotoxic action of cyclodienes. There is considerable interest in the mode of action of the important cyclodiene group of insecticides. Recently, Wang, Narahashi and Yamada (*Pesticide Biochemistry and Physiology* (1971), **1**, 84–91) showed that certain compounds related to dieldrin (photodieldrin, photoaldrin, *trans*-6,7-dihydroxydihydroaldrin (aldrin-*trans*-diol) and a *trans*-diol-like metabolite of dieldrin) when applied to cockroach nervous systems produced abnormal symptoms more quickly than dieldrin itself. At Dr. G. T. Brook's suggestion we tested several other related compounds which he considered might provide more information about the nature of the poisoning process.

Symptoms of dieldrin poisoning were characterised by applying just lethal doses (7·5 μg) of dieldrin to adult male *Periplaneta americana* in 1 μl acetone and examining the condition of their nervous systems by electrophysiological tests up to seven days afterwards. As early as one day after treatment after-discharge sometimes appeared in crural nerves when the abdominal nerve cord was stimulated; after four days this nerve pathway was usually blocked. After-discharge also appeared in giant fibres of the abdominal nerve cord after stimulation of cercal nerves, but block did not occur. Impulse conduction in giant fibres traversing metathoracic ganglia was slightly affected but giant fibres in the abdominal cord conducted normally up to seven days after treatment. The condition of the insects correlated well with that of their nervous systems; two days after treatment all were normal, but at four days seven out of nine were prostrate.

Compounds to be tested electrophysiologically were applied directly to metathoracic ganglia at 100 μl/hour as $10^{-5}M$ emulsions in saline prepared by adding solutions of the compounds in acetone to cockroach saline and shaking vigorously. Spontaneous nervous activity was recorded continuously, and at frequent intervals the abdominal nerve cord was stimulated electrically and the response from a crural nerve observed.

The results of the electrophysiological tests were inconsistent and difficult to interpret, but the following conclusions could be drawn from the limited number of tests done. *Cis*-6,7-dihydroxydihydroaldrin (aldrin-*cis*-diol) and heptachlor epoxide caused most after-discharge in the crural nerve following stimulation of the abdominal nerve cord, heptachlor diol B*, *bis*-dechlorodieldrin†, and HEOM‡ considerably less, and heptachlor diol A*, aldrin-*trans*-diol, dieldrin and HEOM *trans*-diol** least of all.

Measurements of spontaneous activity gave somewhat more consistent results. Exceptionally severe bursts of activity occurred in ganglia treated with dieldrin, aldrin-*trans*-diol, *bis*-dechlorodieldrin, heptachlor epoxide and heptachlor diol A. Aldrin-*cis*-diol and HEOM *trans*-diol caused some abnormal activity, but activity following treatment with heptachlor diol B and aldrin-*cis*-diol could not be distinguished from that of ganglia treated with saline alone. Dieldrin and heptachlor epoxide were slower to act than aldrin-*trans*-diol or heptachlor diol A. *Bis*-dechlorodieldrin was intermediate in speed of action. Heptachlor epoxide, *bis*-dechlorodieldrin and aldrin-*cis*-diol were the most active compounds when all the tests were taken into account. (Burt and Goodchild, with Dr. G. T. Brooks, ARC Unit of Invertebrate Chemistry and Physiology.

Toxicity of cyclic polyethers. Many insecticides interfere with the conduction of nervous impulses which involves the controlled movement of potassium ions through the axon membrane. Some recently described cyclic polyethers (Pederson, *Journal of American Chemical Society* (1967), **89**, 7017) being studied by the Molecular Structures

* Isomers of *trans*-1,2-dihydroxy-3-chlorodihydrochlordene
** trans-6,7-dihydroxy-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene.
†1,4,10,10-tetrachloro-6,7-*exo*-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene
‡ 1,2,3,4,9,9,-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene
trans-6,7-dihydroxy-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene

https://doi.org/10.23637/ERADOC-1-130

Department, which form non-polar complexes selectively with potassium, were therefore tested for effects on nerve impulse conduction.

When applied continuously in cockroach saline at $10^{-5}M$ for 2 h to the abdominal nerve cords of adult male *Periplaneta americana*, dibenzo-30-crown-10 (2,3:17,18-dibenzo-1,4,7,10,13,16,19,22,25,28-decaoxacyclotriaconta-2,17-diene) did not change significantly either the amplitude of action potentials of the giant fibres or the magnitude of the stimulus required to trigger them; spontaneous nerve activity in terminal abdominal ganglia was also unaffected.

As there is evidence that pyrethrin I acts by affecting the properties of axon membranes, pyrethrin I ($10^{-6}M$) and dibenzo-30-crown-10 ($10^{-5}M$) were applied together in saline to cockroach abdominal nerve cords to test for synergism. However, the effect of the mixture was the same as that of pyrethrin I applied alone at $10^{-6}M$.

Very large doses (500 μg/insect) of two more cyclic polyethers injected into cockroaches in 5 μl acetone prostrated the insects for 4 h after treatment. One-third of those treated with benzo-15-crown-5 were still prostrate 24 h after treatment, but those treated with 4-acetylbenzo-15-crown-5 recovered. Doses of 100 μg/insect of either compound had no permanent effect on the insects.

These compounds therefore have very little action on cockroaches and the tests described revealed no effect on their nervous systems. It is not known whether this is because the chemical failed to reach the critical sites or because insufficient potassium ions were complexed to modify normal behaviour. Further compounds of this type will therefore be investigated if they appear more likely to produce measurable effects. (Burt and Goodchild)

Neuroanatomy of insect central nervous system. To obtain most benefit from electrophysiological and histochemical investigations, a thorough knowledge of the organization of the insect nervous system is necessary. We therefore began detailed studies on the histology of the central nervous system, particularly the neuroanatomy of the mesothoracic ganglion of the cockroach *Periplaneta americana* (L.) (*Rothamsted Report for 1970*, Part 1, 176). The first phase of this study, the description of the tracts of fibres that form the roots of the peripheral nerves, has now been completed. Details are given in the Abstracts of Papers (9.17). The observations gave particularly interesting information about the probable functions of the fibres, which can be presumed motor when they arise from cell bodies within the ganglion and sensory when they arise elsewhere. On this basis, in the 30 nerve roots identified on each side of the ganglion, about 150 fibres appeared to be motor and over 2000 sensory. Both types included large and small fibres so that fibre diameter is not consistently related to function, as had sometimes previously been suggested. Motoneuron cell bodies lie mainly ventrally or ventrolaterally and their fibres run more or less dorsally to give branches into dorsal or lateral neuropile before leaving the ganglion in the nerve trunks. Many small sensory fibres run from the nerves into a ventral region of very fine neuropile, the ventral association centre; mainly coarser sensory fibres branch into more dorsal areas of neuropile. (Gregory)

The causes of resistance. Resistance to insecticides is becoming progressively more serious throughout the world. So far the only solution has been to change to a different insecticide when resistance develops, but this may not be possible indefinitely, particularly as there is increasing evidence that populations which have already become resistant to a range of insecticides develop resistance more rapidly to new chemicals. To suggest ways of overcoming resistance or of delaying its occurrence, a thorough knowledge of resistance mechanisms, and the ways in which they interact, is indispensable. Our previous work with houseflies has characterised several important resistance mechanisms but

https://doi.org/10.23637/ERADOC-1-130

recent studies suggest that other mechanisms remain unidentified so that further work is needed. We are also studying resistance in *Myzus persicae* (Sulz.), which involves additional problems because aphids can reproduce parthenogenetically and are controlled mostly by systemic insecticides ingested when they feed on plant sap.

Resistance of houseflies to organophosphorus insecticides. Genetical work has revealed further complexities in the mechanisms of resistance to organophosphorus insecticides. The differences in the genetic constitution of houseflies selected repeatedly with dimethoate (strain $49r_2b$) or tetrachlorvinphos (strain $39m_2b$) are relatively small, but they influence cross resistance to other insecticides considerably. Strain $49r_2b$ resists most organophosphorus insecticides strongly but strain $39m_2b$ shows strong resistance against only a few, such as malathion, malaoxon and tetrachlorvinphos itself.

Both strains have gene *D* (mechanism *a*, *Rothamsted Report for 1972*, Part 1, 178–179) on chromosome 2. However, this gene, which confers strong resistance to dimethoate and omethoate and slight to moderate resistance to many other organophosphates is relatively infrequent in flies from strain $39m_2b$, which explains why this strain resists dimethoate only slightly unlike strain $49r_2b$.

In both strains, chromosome 2 also carries a gene or genes called *M* (mechanism *b*, *Rothamsted Report for 1972*, Part 1, 178–179), which is very close to the visible marker *ar* and confers strong resistance to malathion. In strain $49r_2b$ this gene, or one very close to it, confers moderate resistance to tetrachlorvinphos but in $39m_2b$ a similar gene gives very strong resistance to this insecticide. It is not clear yet whether these genes in the different strains are allelic or whether there is an additional resistance gene in strain $39m_2b$ responsible for the different resistance to tetrachlorvinphos.

Both strains have the gene *Pen* on chromosome 3, which delays the entry of insecticides through the cuticle and the gene *Pb* which confers resistance to methylene dioxyphenyl synergists and synergised pyrethrins, but not pyrethrins alone. This gene has been allocated provisionally to chromosome 2.

The greatest differences in cross resistance between the two strains are associated with the genes on chromosome 5. The dimethoate selected strain $49r_2b$ has a gene or genes conferring slight resistance (resistance factor <3) to trichlorphon, fenthion, malathion and dimethoate, but no resistance to tetrachlorvinphos or DDT. This mechanism can be suppressed by sesamex. The tetrachlorvinphos selected strain $39m_2b$ has a gene(s) that confers resistance (resistance factor about 10) to tetrachlorvinphos and DDT, but no resistance to any of the organophosphorus insecticides tested. It can also be suppressed by sesamex. A further resistance gene *ses*, which confers resistance to diazinon and DDT in the diazinon selected SKA strain (*Rothamsted Report for 1968*, Part 1, 172–173) gives no resistance to tetrachlorvinphos. We have yet to discover if these mechanisms are allelic or independent and how previous selection influences their appearance. If, as seems likely, they involve microsomal detoxifying systems, further studies may help to clarify the role of mixed function oxidases in resistance. (Sawicki)

The major mechanism of resistance to organophosphorus compounds associated with gene *D* on chromosome 2 of the dimethoate selected strain $49r_2b$ was also investigated by biochemical methods following isolation in a substrain. The uptake and metabolism of dimethoate and the susceptibility of acetylcholinesterase to inhibition in this substrain and in a susceptible strain were compared in an attempt to identify the resistance mechanism.

Topically applied ^{14}C-labelled dimethoate penetrated rapidly (50% in 8–10 min) and at a similar rate into both susceptible and resistant houseflies. Even when fortified with the cofactors NADPH and glutathione, homogenates of both strains metabolised only a small proportion of the insecticide and differences between resistant and susceptible

https://doi.org/10.23637/ERADOC-1-130

insects were again slight. Metabolism *in vivo* was also similar in the two strains when doses of dimethoate too small to kill the susceptible insects (5ng/fly) were applied, but with larger doses (50 ng/fly) the resistant strain metabolised more than the susceptible strain (which was not killed during the 2-h period of the experiment) although the products were the same. However, neither the differences in metabolism nor the relative rates of penetration could account for the observed resistance.

Much greater differences were found in the rates at which acetylcholinesterase (EC 3.1.1.7) was inhibited in the two strains by oxygen analogues of several organophosphorus insecticides, inhibition occurring faster in the susceptible insects.

The bimolecular velocity constants for inhibition differed between the two strains by a factor of ten for omethoate, five for paraoxon, four for methyl paraoxon, seven for tetrachlorvinphos and 23 for malaoxon. This decreased susceptibility probably confers resistance not only directly but also by increasing the time during which detoxification of the insecticide can occur. This suggestion is consistent with the differences in metabolism observed when large doses of dimethoate were applied. (Devonshire)

Resistance of houseflies to pyrethroids. Last year we described the pyrethroid resistance factors *kdr*, *kdr-O* and *kdr-NPR* which are recessive genes giving similar cross resistance (*Rothamsted Report for 1972*, Part 1, 179). We have now confirmed that they are alleles and established that they are located on chromosome 3 at approximately 40 map units from the marker *ge*, green eye.

Pyrethroid insecticides are being used to control houseflies on farms in Denmark. Recently resistance has been detected in some populations, particularly those which had already developed such strong resistance to organophosphorus insecticides such as dimethoate that these compounds could no longer be used for control. Flies from farm 290, where resistance to pyrethroids had followed resistance to dimethoate, were therefore compared with the laboratory-selected strains NPR and 104 to determine if the patterns of resistance were similar. Selection with bioresmethrin showed that resistance in strain 290 was of a new type. Flies from this strain developed at least 1000-fold resistance to bioresmethrin, which was much greater than either strain 104 which was originally selected with resmethrin (resistance factor to bioresmethrin = 80) or strain NPR which was originally selected with natural pyrethrins (resistance factor to bioresmethrin = 200). A genetical analysis of the bioresmethrin-selected substrain of strain 290 is under way. (Farnham)

Resistance of aphids* (Myzus persicae (*Sulz.*)) *to organophosphorus insecticides. Although earlier clones lost resistance unexpectedly (*Rothamsted Report for 1971*, Part 1,

TABLE 2

Resistance factors[1] *of the resistant clone of* M. persicae (*DDTR*) *to various insecticides, measured following topical application*

dimethoate	187
dimethoxon	28
parathion	215
paraoxon	14
malathion	50
disulfoton	95
dieldrin	1
'Isolan'	3
dicrotophos	11
dimetilan	2
bioresmethrin	19

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

https://doi.org/10.23637/ERADOC-1-130

181), the present clone of *M. persicae* (Sulz.), strain DDTR has now been reared without selection for two and a half years with no apparent loss of resistance (Table 2). We continued to investigate the causes of resistance in this strain.

Acetylcholinesterase activity in homogenates of resistant and susceptible aphids was measured by a radiometric technique in which the hydrolysis product, [^{14}C] acetate, was separated from unchanged [^{14}C] acetylcholine by ion-exchange chromatography and estimated by liquid-scintillation counting. There was no significant difference between resistant and susceptible strains in either the total acetylcholinesterase activity or its susceptibility to inhibition by omethoate.

Investigation of the fate of topically-applied dimethoate and parathion revealed that only approximately 25% penetrates into both resistant and susceptible strains, and the remainder is lost by evaporation from the cuticle. These large losses had previously been attributed to excretion of unchanged dimethoate (*Rothamsted Report for 1972*, Part 1, 180). The loss by evaporation, which is likely to vary with the dose and properties of the insecticide applied, makes the interpretation of bioassays involving topical application of insecticides to aphids uncertain. Such losses may seriously affect the apparent resistance levels and cross resistance patterns measured by this technique. A further objection to topical application is that in practice aphids are controlled mostly by systemic insecticides. Resistance to insecticides reaching their sites of action by this route may involve mechanisms different from those causing resistance to insecticides applied to the cuticle.

We have therefore investigated feeding techniques as an alternative method for measuring resistance. Aphids were fed either on seedlings to which insecticide had been administered via the roots, or on artificial diets containing insecticide enclosed in 'Parafilm' membranes. Both these techniques give similar results for resistance to dimethoate, in contrast to the topical application technique which gives resistance factors ten times greater. The use of artificial diets is preferable because the system is less complex, and because to kill resistant aphids on seedlings it is sometimes necessary to apply phytotoxic doses of dimethoate. (Devonshire and Needham)

Side effects of pesticides on beneficial insects. We continued to investigate the harmful effects of pesticides on pollinating insects and began new work on ways of minimising damage to predators and parasites of aphids.

Poisoning of honeybees in the field. One hundred and three samples of honeybees thought to be poisoned were received from beekeepers via the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food. As in previous years these were analysed for the presence of insecticides. Eighty samples gave evidence of poisoning but of these

TABLE 3

Causes of bee poisoning in samples received during 1973

	Number of incidents	
1. anticholinesterases		
Probable	43	2 also contained carbaryl 1 also contained BHC
Suspected	10	1 also contained carbaryl
2. BHC	2	1 also contained anticholinesterases
3. carbaryl		
Probable	5	1 also suspected anticholinesterases
Suspected	7	1 also probable and 1 suspected anticholinesterases

https://doi.org/10.23637/ERADOC-1-130

26 came from nine spraying incidents, bringing the number of poisoning incidents to 63, compared with 43 in 1972 and 40 in 1971. These are summarised in Table 3. The anticholinesterase test measures residual cholinesterase after poisoning and does not identify the specific insecticide residues. This year the Warburg technique used previously was replaced by a spectrophotometric assay based on the method of Ellman *et al.* (*Biochemical Pharmacology* (1961), **7**, 88). Both procedures give similar results but the spectrophotometric method is quicker. Carbaryl was also determined by a new method involving high pressure liquid chromatography. This method, which is described more fully later, proved simpler, more sensitive and more reliable than the previous method based on gas chromatography.

Evidence supplied with some of the cases where poisoning by anticholinesterases (mainly organophosphorus insecticides) seemed probable, suggested that 17 were associated with the spraying of field beans, 16 with aerial application, six with spraying of rape or kale and one with the use of dichlorvos resin strip. The sample in which only BHC was detected was reported to be associated with the spraying of peas.

In all the five cases where carbaryl poisoning appeared probable, spraying of pears was implicated. In the other seven samples where carbaryl was involved, it was uncertain whether the amount present could cause poisoning; fruit spraying was also implicated in four of these incidents. In one of the samples where insecticide poisoning was not found, the bees are known to have been killed by cyanide which could not be detected in our tests, and it was suggested that herbicides might be responsible for poisoning in a few other samples.

One sample from Rwanda also reacted positively to our test for poisoning by anti-cholinesterases.

Treatment of beans and aerial application are therefore still the most important single causes of poisoning. (Stevenson)

Effects of insecticides on other beneficial insects. To assess the effects of insecticides on beneficial insects in the field suitable methods of sampling are required. Sampling methods for studying the effects of aphicides were tested by spraying large plots (0·13 ha, three replicates) and small plots (0·04 ha, two replicates) with the selective aphicide menazon (0·3 kg/ha a.i.) or the broad spectrum insecticide dimethoate (0·35 kg/ha a.i.) and comparing insect populations with those on untreated plots. *Aphis fabae* were estimated by inspection on the crop, while carabid beetles were sampled by pitfall traps and other insects by using sweep nets and the suction sampler previously developed in the department (*Rothamsted Report for 1968*, Part 1, 189).

Both insecticides controlled aphids well; yields on the large plots were 3·71 t/ha for the dimethoate treatment, 3·79 for the menazon treatment and 2·96 for the untreated control (standard error of differences 0·179). Yields on the smaller plots showed no clear trends, probably because the damage caused by sampling had proportionally greater effects than on the larger plots.

The numbers of carabid beetles trapped fell for four days after treatment on the dimethoate plots, but were not affected by the more selective menazon. Numbers of Coccinellids were reduced by both treatments, possibly because aphid prey was removed, but samples of other individual species were too small to give conclusive results. More intensive sampling will therefore be necessary in future experiments. (Stevenson, with Edwards, Free and Williams, Entomology Department)

Effects of insecticides on pollinating insects in field beans and prediction of aphid infestations. These studies are described in the report of the Entomology Department (p. 196). (Stevenson, with Bardner and Fletcher, Entomology Department and Moffatt, Farm)

https://doi.org/10.23637/ERADOC-1-130

Formulation and application of pesticides. The efficiency and selectivity of pesticides can be influenced considerably by how they are formulated and applied. Several projects in the department are concerned with improving methods of application or developing better formulations.

Seed treatments. Further work was done to improve the adhesion of powders on seeds and to learn more about the phytotoxic effects of some insecticidal seed treatments. With the co-operation of Plant Protection Limited we were able to use prototype models of the new 'Rotostat' seed treater (Elsworth & Harris, *Proceedings of the 7th British Insecticides Fungicides Conference* (1973), **1**, 349) for applying some treatments and to compare its performance with older methods. The 'Rotostat' treats seeds by a batch process in a rotating mixing chamber and promises to improve the loading, adhesion and distribution of chemicals on seeds.

Tests for retention of powders. The retention test previously used to test the effectiveness of adhesives applied to seeds before treatment with powder insecticide (*Rothamsted Report for 1970*, Part 1, 178) does not reflect practical conditions, although it proved useful for investigating adhesion. A new test was therefore designed. Treated seed is poured through a wide-stemmed funnel into a vertical glass tube, 40 cm long, and falls to a similar funnel, closed by a slide, at the base of the tube. The slide is opened and the seeds fall a further 10 cm onto a sieve, during which loose powder separates. The test is analagous to the commercial process where seed falls from the mixing unit into a header bin and then into scales and a bag. To test the method, separate 100 g samples of wheat treated with powder were passed from 1–10 times through the apparatus, after which each sample was analysed for insecticide remaining on the seeds. Retention was expressed as the percentage of insecticide originally applied. After five passes little further powder separated from the seeds. Table 4 shows the reproducibility of the method for 100 g samples of seed passed five times through the apparatus. The method is being evaluated by the Seed Treatment Panel of the Pesticides Analytical Advisory Committee (PAAC). (Jeffs)

TABLE 4

Retention of 40% gamma-BHC powder applied to wheat at a rate of 1200 μg/g.
(*Results are means of 2 replicates for each batch of seeds tested*)

	Amount retained after 5 passes	
Test	μg/g	% of amount applied
1	513	42·5
2	424	35·3
3	471	39·2
4	461	38·4
5	409	34·0
6	428	35·6
Mean	451	37·5

(Standard deviation, 38·4; coefficient of variation 8·5%)

Adhesives for powder seed treatments. Previous work with small batches (500 g) of seeds in the laboratory showed that pretreatment with 6% soya bean oil emulsion in water greatly improved the retention of powder formulations (*Rothamsted Report for*

https://doi.org/10.23637/ERADOC-1-130

1972, Part 1, 184). The effectiveness of this treatment has now been examined on a larger scale with the help of Plant Protection Limited. Ten kilograms of wheat seed were treated with 120 ml of 6% soya bean oil emulsion followed by 20 g of 40% gamma-BHC powder in a small model of the newly developed 'Rotostat' seed treater. A further 30 kg of seed were treated in a pilot-scale Plantector seed-treater at the same rate. Retention, calculated as the percentage of the insecticide on the seeds before the retention test, was compared with that on seed treated in the corresponding machine with the powder alone. Seeds pretreated with the adhesive retained 90–95% of the insecticide, compared with only 46% for the Plantector and 73% with the Rotostat when pretreatment was omitted. The figure for powder alone with the Rotostat is probably an overestimate because the seeds had to be removed from the experimental model used by hand, during which some powder fell off, so that the initial loading appeared low.

As with the earlier laboratory tests, these results on a pilot scale are promising and we hope to extend the tests to full-size commercial machines next year. The seeds treated in the experiments this year are being tested in short row trials. (Jeffs and Walker)

Application of large rates of fungicides to barley seed. Seed treatment is likely to become an increasingly popular method of applying insecticides and fungicides, so that methods of applying much larger loadings than in the past will be required.

For field experiments on the relative performance of different systemic fungicides, we needed to treat barley seed with powder formulations containing 50% a.i. of benomyl, thiophanate methyl or 'NF 48' at a rate of 235 μg/seed. Visual examination of seed treated by hand with these amounts (14 g/kg) of powder alone showed that most of the powder failed to adhere to the seed. The seeds were therefore pretreated with 30 ml of 6% aqueous gum arabic solution per kg seed. Preliminary tests showed that 86% of the applied powder adhered to the seeds following this pretreatment and that the powder was uniformly distributed from seed to seed. By increasing the amount of powder applied to compensate for the 14% loss, loadings very near the target were achieved. Average values were 248 ± 23 μg/seed for benomyl, 205 ± 11 for thiophanate methyl and 211 ± 12 for 'NF 48'. (Jeffs)

Amounts of pesticides on commercially treated seed. Analysis of seeds from commercial batches sown at Rothamsted indicated that loadings of insecticides were well below the target. One sample in particular carried only about one-tenth of the expected dose. Consultations with the seed merchants and further investigations at their premises suggested that most of the insecticide was lost in the dust extraction system.

Seed sown on Broadbalk in autumn 1972 carried about 500 ppm dieldrin compared with the expected dose of 1200 ppm; the value found in autumn 1973 was about 400 ppm. (Lord)

Mercury on single seeds. The X-ray spectrophotometric method described by Lord (*Rothamsted Report for 1969*, Part 1, 226) for determining mercury on single seeds was modified to allow for absorption of X-rays by the crushed seed and filter paper. One variety of wheat and two varieties of barley were treated with liquid mercury formulations using the new Rotostat seed treater. Fifty single seeds, taken at random from samples of each cultivar, were analysed for mercury. For Maris Dove wheat the average loading was 0·67 μg per seed (range 1·1–0·13 μg) with a standard deviation of 0·23. Julia barley had a mean loading of 0·59 (range 1·1–0·30, standard deviation 0·16) and Lofa Barley had 0·93 μg/seed (range 1·73–0·39, standard deviation 0·28). The small standard deviations show that the distribution patterns were relatively even compared with many previous samples treated with traditional machines. None of the seeds carried more than twice the

https://doi.org/10.23637/ERADOC-1-130

average load, which was from about 70 to slightly greater than 100% of the target. (Jeffs, with Brown, Pedology Department)

Phytotoxicity of liquid seed treatments. Liquid seed treatments sometimes adversely affect subsequent germination and growth. To investigate the route by which phytotoxic quantities of gamma-BHC penetrate into wheat seed, the insecticide was applied topically to the different regions of the seeds. At commercial rates of application, each individual seed would carry 12 μg/BHC if distribution between seeds was uniform. Therefore batches of ten seeds were treated with 9 or 18 μg per seed of a commercial liquid formulation of gamma-BHC containing 25% active ingredient using a micro-applicator. The measured drop was placed either on the dorsal surface of the seed, opposite the crease, or directly onto the scutellum. Treated and untreated seeds were planted against the walls of glass crystallising dishes separated from moist sand by filter paper and kept at 20°C in subdued light. After ten days the longest root on each seed was measured, and the number of shoots longer than 25 mm counted (Table 5). Neither level of gamma-BHC

TABLE 5

Effects of gamma-BHC liquid seed treatments on growth of wheat
(Values are means of 4 replicates, each with 10 seeds)

<table>
<tr><th></th><th>Untreated</th><th>9 μg BHC per seed</th><th>18 μg BHC per seed</th><th>Standard error of mean</th></tr>
<tr><td></td><td></td><td colspan="2">Mean length of root, mm</td><td></td></tr>
<tr><td>Scutellum</td><td rowspan="2">74</td><td>65</td><td>26</td><td rowspan="2">7</td></tr>
<tr><td>Dorsal surface</td><td>74</td><td>78</td></tr>
<tr><td></td><td></td><td colspan="2">Mean number of shoots longer than 25 mm</td><td></td></tr>
<tr><td>Scutellum</td><td rowspan="2">9</td><td>8</td><td>3</td><td rowspan="2">1</td></tr>
<tr><td>Dorsal surface</td><td>9</td><td>10</td></tr>
</table>

affected germination or root length when applied to the dorsal surface. When applied to the scutellum the lower level had little effect but with 18 μg/seed the roots were 60% shorter than those from untreated seed and only one-third as many shoots emerged. In addition to indicating the relative sensitivity of different sites on the seed surface, the results show that it should be possible to develop standard tests for assessing phytotoxicity of seed treatments quantitatively by applying carefully measured amounts of chemical to specific sites on the seed. (Jeffs and Griffiths)

Microencapsulation. By enclosing pesticides in small capsules it should be possible to improve selectivity, control release and persistence and mask any repellent effects of the toxicant. We therefore continued work on the development of microencapsulation techniques. Earlier difficulties described in last year's report (*Rothamsted Report for 1972*, Part 1, 182) have been partly overcome. We are now able to achieve more satisfactory wall thickness and to control the capsule size range better. Thicker-walled capsules and the use of a fluidised bed drier have also made the difficult process of extraction and drying from the wet slurry more successful. Several different insecticides have been encapsulated including DDT, BHC, dieldrin, demeton-S-methyl and disulfoton, and small glasshouse tests are now in progress comparing the effectiveness of some of these microencapsulated insecticides with more traditional formulations. (Phillips and Etheridge)

https://doi.org/10.23637/ERADOC-1-130

Control of soil inhabiting pests

Wheat bulb fly. Although much work has been done on the chemical control of wheat bulb fly larvae, no entirely effective treatments are available, particularly as dieldrin is now being withdrawn. In the past evaluation of seed treatments has been confused by the deficiencies in application methods and by phytotoxic effects referred to elsewhere in this report. In collaboration with the Agricultural Development and Advisory Service (ADAS), the effects of different loadings on emergence of winter wheat and attack by wheat bulb fly larvae were therefore investigated. Liquid formulations of carbophenothion and chlorfenvinphos and a powder formulation of dieldrin, which are all amongst the most effective treatments tested so far, were compared.

Seed treated at a range of rates was sown in replicated short row trials at four sites known to contain large populations of wheat bulb fly eggs. Exact loadings were determined by analysing samples of the treated seed. Emergence counts showed that only the largest dose of chlorfenvinphos (>100 μg/seed) affected the number of seedlings to emerge. The effects of dosage on insect damage can be summarised as follows:

(1) The sites at Semer, Suffolk (clay/calcareous clay loam) and at Rothamsted (Flinty silt loam or loam over Clay-with-flints, Batcombe series) gave similar results. Carbophenothion was relatively ineffective at all dosages, but chlorfenvinphos was increasingly effective as the dosage increased, although below about 20 μg/seed results were not significantly different from untreated controls. Dieldrin appeared to be effective at smaller rates (about 10 μg/seed) particularly with respect to the percentage of dead larvae and the percentage of plants with live larvae.

(2) Results from the other two sites were less clear cut, but were broadly in agreement with those at Semer and Rothamsted. At Redbourn, Lincs (sandy clay loam) carbophenothion was again the least effective treatment but differences between the dose/response relationships for chlorfenvinphos and dieldrin were not marked. The trial at Chatteris (peaty loam) was spoilt by extensive bird damage in December–January, but measurements showed that the length of row damaged appeared to depend on the chemical used, the order of decreasing damage being carbophenothion, chlorfenvinphos, dieldrin, untreated control. (Griffiths, Jeffs and Scott)

***Saddle gall midge* (Haplodiplosis equestris) (*Wagn.*).** We continued to investigate methods of controlling saddle gall midge which may become increasingly important as a pest of cereals. A further ten insecticides were tested for toxicity against larvae collected from the field. Each larva was treated topically with a 0·1 μl drop of a 0·01% solution (equivalent to about 6·4 μg active ingredient/g larval weight). Mortality was recorded after 17–20 days. Carbophenothion, parathion and thionazin gave about 50% mortality. Aldrin, carbofuran, dichlofenthion, dimetilan, chlorpyriphos, ethion and 'Cyanamid AC 92100' were not toxic at the dose tested. (Griffiths and Scott)

Uptake of pesticides from soils by worms. Previous studies showed that the uptake of pesticides by worms is a physical process involving penetration through the cuticle (see *Rothamsted Report for 1972*, Part 1, 186). In further work the partition of chemical between macerated worm solids and water was compared with partition between soil and water and between octanol and water. Using a standard equilibration time of 15 minutes and either two or three concentrations for each substance, it was shown that uptake of captafol, captan, folpet, diazinon, 'Nemacur-P', carbaryl, parathion, phorate, aldicarb and 'Dowco 275' from water by both worm solids and soil was correlated with partition between octanol and water, indicating that sorption by worm solids and by soil are determined by similar properties. It may therefore be possible to describe distribution between worms and moist soils using much the same equations as those

https://doi.org/10.23637/ERADOC-1-130

described by Briggs for partition between soil and water (see Report of Chemistry Department, p. 62). (Lord and Neale, with Edwards, Entomology Department)

Control of weevils and associated viruses in field beans. This collaborative work is described in the report of the Plant Pathology Department (p. 141) (Etheridge, with Cockbain, Plant Pathology Department)

Behaviour-controlling substances

We maintained a broad programme of work aimed at isolating and identifying chemicals that influence insect behaviour and at developing methods of using these substances for monitoring insect populations or for controlling them by disrupting normal behaviour.

Codling Moth. Although much work on behaviour-controlling substances has been done at various centres and many active chemicals have been identified, so far practical exploitation of these chemicals has been limited. This is largely because too little is known about the ways insects respond to chemicals and about the effects of environmental factors and of the distribution of chemical on response. Synthetic attractants have been available for codling moth for several years and our most recent work has been concentrated on studying how best to use these for monitoring or control.

In field trials at East Malling, male codling moths were caught by the synthetic attractant (*trans*-8,*trans*-10-dodecadien-1-ol) up to three weeks before light traps (*Rothamsted Report for 1972*, Part 1, 188) and a much greater proportion of the total catch was trapped in the early part of the season. This is probably because the attractant traps are most effective when the moth population is small and there is least competition from wild females. To confirm this hypothesis moth populations must be monitored independently throughout the season, but, if correct, control of codling solely by trapping out virgin males would probably be successful only where populations were relatively small. Elsewhere, the use of attractant traps to locate infestations, together with other observations (e.g. date of first egg hatch, after Batiste *et al.*, *Environmental Entomology* (1973), **2**, 387–391), would make it possible to time insecticidal sprays much more accurately according to local conditions.

Comparison of 'Sectar' and 'Wing' sticky traps (3M Co. Ltd.) with the double-cone design of Sharma, Shorey and Gaston (*Journal of Economic Entomology* (1971), **64**, 361) showed that trap design had little effect on numbers of moths caught. However, the double-cone trap has the advantage that moths are preserved alive and are therefore available for release experiments.

In the range 10–1000 μg attractant per trap, the curve relating amount of attractant to numbers of male moths caught was fairly flat, with a shallow maximum at 100 μg. However, because of evaporation of the attractant, which has a half-life of 15 days in still air conditions at 20°, it was necessary to renew the lures approximately every six weeks to maintain attractiveness in the field. (Greenway, with Dr. J. E. Cranham, East Malling Research Station)

Chemicals affecting behaviour of wheat bulb fly larvae. In laboratory tests, wheat exudates and extracts have an 'arrestant' effect on wheat bulb fly larvae, whilst extracts of oats, a non-host, have an 'anti-arrestant' effect (*Rothamsted Report for 1970*, Part 1, 167–168). In an attempt to interfere with host location by adsorbing the arrestant exuded from wheat, Scott and Greenway showed that attack could be decreased by growing wheat in a 1 : 3 (w/w) mixture of activated charcoal and compost (*Rothamsted Report for 1972*, Part 1, 188–189).

https://doi.org/10.23637/ERADOC-1-130

Further tests were done in 1973, using smaller amounts of charcoal. Wheat was sown in boxes in two rows of 20 seeds so that one row was growing in compost and the other in a 5 cm-wide band containing a mixture of compost and activated charcoal powder or granules in various proportions. Thirty wheat bulb fly eggs were added to each box in a single line mid-way between the rows and plants subsequently damaged by larvae were counted (Table 6).

TABLE 6

Effect on wheat bulb fly attack of charcoal and compost mixtures

Treatment	Mean no. of attacked plants/row from 5 replicates: Treated row	Untreated row	SEDM*
1 : 6 w/w charcoal granule + compost	10·6	11·8	2·18
1 : 6 w/w charcoal powder + compost	2·2	15·6	1·66
1 : 12 w/w charcoal powder + compost	4·0	15·4	1·25
1 : 24 w/w charcoal powder + compost	4·2	14·8	1·50

* Standard error of the difference between the means

Significantly fewer plants were damaged in the rows treated with charcoal powder at all rates tested ($P<0{\cdot}01$). It is uncertain exactly how the charcoal produces this effect; it probably absorbs the wheat exudate, but the possibility that it acts as a physical barrier cannot be excluded. The charcoal granules had no significant effect on attack. One possible explanation for this is that the granules did not form such an intimate mixture with the compost, so that insufficient exudate was adsorbed to interfere with plant location.

The insect repellents, 'MGK 11' (2,3,:4,5-*bis*(2-butylene)tetrahydro-2-furaldehyde) and deet (*N,N*-diethyl-*m*-toluamide) were previously shown to have anti-arrestant properties (*Rothamsted Report for 1970*, Part 1, 168). We have now done further work on the possibility of exploiting this for control. In a single observation box, wheat plants growing in compost were treated daily with an emulsion of both repellents in water. They were each applied at a rate equivalent to 0·2 ml/compound/day beginning one week before wheat bulb fly eggs were introduced. None of the plants was attacked compared with a mean of 16 attacked plants in untreated boxes, indicating that the treatment was either toxic or prevented larvae from finding the plants.

Oats are not normally attacked by wheat bulb fly larvae; we attempted, unsuccessfully to induce an artificial attack by treating growing oats with an extract of wheat at a rate of 0·6 g wheat shoots/oat plant/day. (Scott and Greenway)

Control of leaf-cutting ants, *Acromyrmex octospinosus* (Reich) and *Atta cephalotes* (L.). Leaf-cutting ants are among the most serious pests of growing crops in the New World tropics and sub-tropics where they cause great economic damage by defoliating a wide variety of plants while gathering material on which they culture their fungus. Conventional methods of control are ineffective because the nests have complex structures and are difficult to find. Poison baits offer much better prospects, but the traditional dried citrus pulp bait disintegrates rapidly, becomes mouldy under tropical conditions and its irregular particle size and shape makes it difficult to distribute by air. With support from the Overseas Development Administration, we are therefore attempting to devise more effective and durable synthetic baits which will be cheap, easy to prepare

https://doi.org/10.23637/ERADOC-1-130

and acceptable to the ants. This requires the discovery of ingredients which will attract the ants to the bait and induce them to carry it back to the nest and we are investigating potentially useful phytochemical arrestants and pheromones. It also requires the development of suitable matrix materials and of formulations in which all the components of the bait are compatible.

Phytochemical arrestants. Work to identify substances responsible for the arrestant properties of grapefruit albedo and citrus pulp has continued (*Rothamsted Report for 1972*, Part 1, 187). Fractionation of extracts of these materials showed that the activity is contained in the water-soluble, non-lipid part of the extract. Separation of this fraction into its main constituent groups (amino acids, bases, acids, carbohydrates, etc.) indicated that the activity of the total extract was distributed to different extents between all of the components. The most active subfraction was that containing neutral non-ionic compounds which are mostly ($>95\%$) carbohydrates, but further subfractionation produced no components, apart from the sugars, having major arrestant activity. It seems likely that the arrestant activity of the whole citrus extract represents the ants' response to the whole range of components combined in the extract, rather than a response to separate individual constituents.

Leaf-cutting ants do not use the plant material that they gather for food directly but as a substrate on which they culture the fungus that they eat and feed to their brood. It therefore seems possible that the ants select particular plant materials primarily because they support the growth of their fungus best, although other factors, such as the texture of the leaf material, may play some part in determining preference. This explanation would account for the complex foraging patterns of the ants and their variable response to fractions of plant extracts. It would also explain, among other characteristics, why the non-lipid fraction of citrus pulp, which contains all the necessary fungal nutrients, is most arrestant and why this arrestant activity diminishes as various fungal nutrients are removed from the extract during further fractionation. We have therefore started experiments to determine whether the rate of fungal growth on various plant extracts can be correlated with the ants' preferences for certain plants. (Mudd, with Dr. J. M. Cherrett and Dr. D. J. Peregrine, University College of North Wales, Bangor)

Use of scent trail pheromone as an attractant in baits. The possibility of using trail pheromones to attract ants to baits has been suggested previously. Tests became possible with the isolation, identification and subsequent synthesis of methyl-4-methylpyrrole 2-carboxylate (M4MP2C), a volatile component from the *Atta texana* trail secretion. When untreated filter paper discs and discs impregnated with M4MP2C were offered to *Acromyrmex octospinosus* and *Atta cephalotes*, there was no significant preference for pick-up of discs containing the pheromone. However, when sugar impregnated discs were used, those discs which also contained M4MP2C were preferentially picked up and carried back to the nest, as was also found by workers at UCNW, Bangor. However, when the pheromone was used in more complex baits, although the pheromone-treated baits were usually the most attractive in individual tests, the differences were not significant. (Phillips, Etheridge and Mudd)

Insecticides for use in ant baits. Toxicants for use in ant baits should have a delayed action over a wide dosage range, should be transferred readily from one ant to another, causing death of the recipient, and should not be repellent (Lofgren *et al.*, *USDA, ARS*-81 (1967), 14). Work was started to find compounds having these characteristics as alternatives for the organochlorine insecticides at present used in baits for leaf-cutting ants.

https://doi.org/10.23637/ERADOC-1-130

Test methods were similar to those used by Lofgren. The toxicants were dissolved or emulsified in the bait medium (5% w/v aqueous honey solution) and presented on small cotton wool plugs to the ants in plastic test chambers 8 cm high and 5 cm in diameter. The air in the chambers was kept humid by supplying water to their porous bases. Twenty worker ants (*Acromyrmex* sp.) from laboratory colonies were placed in each test chamber 24 h before treatment to allow them sufficient time to recover from handling.

All insecticides were tested at concentrations of 1·0, 0·1 and 0·01%. The treated bait was left in the containers for 24 h after which it was removed and replaced by untreated honey solution which was replenished as required. Mortality was recorded daily for up to 14 days after treatment.

So far 14 compounds have been tested. Of these, methomyl, dichlofenthion, mecarbam, benomyl and the experimental compound 'M & B 20266' showed a delayed toxic action. Other new insecticides are currently being evaluated and the most promising compounds are being investigated in more detail. (Phillips and Etheridge)

Bait matrices. A wide variety of materials have been prepared and tested as possible replacements for citrus pulp. These include vermiculite, expanded polystyrene, a urea-formaldehyde polymer, pumice, fuller's earth formulations, wood products and gelatin products. All the materials were unattractive to the ants without the addition of an arrestant substance, although untreated vermiculite was sometimes picked up by *Acromyrmex* sp. They were therefore treated with a variety of additives to improve pick up. These substances included solutions of sugars, honey, sugar syrups and treacles, and extracts of whole or parts of lemons, grapefruits and oranges. Vegetable oils were also added as they would be required as solvents for insecticides in the finished bait. Sucrose and soya-bean oil were directly incorporated in the urea-formaldehyde polymer 'Ufoam' (ICI Ltd.) during manufacture. After several trials we now use a standard bioassay method similar to that used at UCNW, Bangor. Candidate bait materials are presented to the ants as discrete particles on a glass plate which is divided into 2-cm squares. A different material is placed in each square, chosen randomly. Tests are concluded and the glass plate removed when only one piece of the most attractive material remains on the plate, and the numbers of the other material particles are recorded. The most promising materials tested so far are vermiculite, and to a lesser extent, 'Loam-a-lite', a fuller's earth compound. Several formulations of these materials were at least as attractive or better than the standard dried citrus pulp. Similar results have been obtained by workers at UCNW, Bangor. (Phillips and Etheridge)

Control of mould infection on baits. Considerable wastage of citrus pulp baits containing aldrin has occurred in Trinidad, both during storage and after application in the field, because of mould infection. Mould growth occurs after two to three days in warm humid conditions and spreads rapidly, and the bait becomes completely unattractive to ants. Tests carried out at Rothamsted, and field testing in Trinidad of material prepared by us, have shown that treating citrus pulp bait with as little as 0·1% w/w propionic acid will give considerable protection against infection without loss of attractiveness. Benomyl, a candidate in our insecticide screening, was also tried as a mould suppressant, but was much less effective and gave only partial protection. Tests using vermiculite-based baits indicated that mould infection would not be a serious problem and that it would probably be unnecessary to add propionic acid. (Phillips and Etheridge)

Chemistry of aphid body lipids. The composition of lipids in aphids and their cornicle secretions is being studied to give information about the factors which influence para-

https://doi.org/10.23637/ERADOC-1-130

sitism and host selection, to learn more about the possible defensive function of cornicle secretions, and as a possible basis for classifying different species.

Examination of triglycerides from 30 species of aphids and their cornicle secretions, summarised in the abstracts of papers, showed that the main constituents were as follows:

$$\begin{array}{l} CH_2OR_1 \\ | \\ CHOR_2 \\ | \\ CH_2OR_3 \end{array} \qquad \begin{array}{l} R_1, R_3 = C_{13}H_{27}CO \text{ (myristic) and/or } C_{15}H_{31}CO \text{ (palmitic)} \\ \\ R_2 = C_5H_7CO \text{ (sorbic) or } C_5H_{11}CO \text{ (hexanoic)} \end{array}$$

Examination of further specimens from other aphid families showed the presence of similar triglycerides and supported earlier evidence (Callow, Greenway & Griffiths, *Journal of Insect Physiology* (1973), **19**, 737–748) that there is little correlation between taxonomic position and variations in the proportions of these triglycerides.

To help predict aphid infestations on crops during the summer, it is important to be able to distinguish between *Aphis evonymi* F. and *Aphis fabae* Scop. and possible hybrids of these species when they are together on spindle trees during winter. As this is difficult to do by examination, the composition of body lipids and cornicle secretions in the two species was compared. Although there were systematic differences in the composition of different seasonal forms, triglycerides found in any given form of both species were almost identical. Triglycerides in apterous and alate (summer) viviparae contained about equal amounts of myristic (C_{14}) and palmitic (C_{16}) acid moieties, while myristic predominated in fundatrices and fundatrigeniae (spring forms). Males collected in autumn resembled the spring forms. However, oviparae and eggs of these and other species differed considerably from the other seasonal forms and fatty acid analyses showed this was due mainly to the presence of hexenoic ($C_{6:1}$), sorbic ($C_{6:2}$) and myristoleic ($C_{14:1}$) acids.

The triglycerides that are typical of aphids (shown above) were consumed preferentially by Braconid parasites developing within *Myzus persicae* (Sulz.) so that when the parasitism was advanced only those triglycerides where R_1, R_2, R_3 were all long-chain fatty acid radicals remained. The adult parasites themselves contained very little triglyceride. (Greenway and Griffiths, with Mr. C. Furk and Mr. R. Prior, MAFF Plant Pathology Laboratory, Hatching Green, Harpenden)

Equipment and techniques

Cobalt staining of insect central nervous system. In further work on the neuroanatomy of the mesothoracic ganglion of the cockroach *Periplaneta americana* (L.) detailed work was begun on the composition and arrangement of the groups of cell bodies of the motor and interneurons within the ganglion. In addition to the Bodian silver and Procion Yellow fluorescence methods used previously, the cobalt chloride stain of Pitman, Tweedle and Cohen (*Science*, *Washington* (1972), **176**, 412–414), in a modification of the method used by Iles and Mulloney for Procion Yellow (*Brain Research* (1971), **30**, 397–400), was tried. A similar technique has recently been employed by Pearson and Fourtner (*Canadian Journal of Zoology* (1973), **51**, 859–866). A freshly dissected ganglion was placed in a bath of saline and one peripheral nerve or branch led through a seal of 'Apiezon N' grease into a bath of 0·5*M* cobalt chloride. The cobalt chloride diffused into the cut end of the nerve and passed along the nerve fibres to their cell bodies, which, after treatment of the ganglion with dilute ammonium sulphide solution to precipitate the cobalt, stained black. Staining time was fairly critical and ranged from 2 h at 25–

https://doi.org/10.23637/ERADOC-1-130

27°C for large nerves cut short to 8 h for long or very small nerves or branches. Ganglia were then fixed and either examined as whole mounts or embedded in paraffin wax and sectioned. This method is being used to map the motor neuron cell bodies of each peripheral nerve and has also resolved some questions left unanswered by the earlier work. (Gregory)

Liquid chromatography. A high pressure liquid chromatograph was assembled using a simple gas-pressure pump made by the workshops, and an ultra-violet absorption detector with a micro-cell. The equipment was used to explore the possibilities of assaying traces of u.v. absorbing pesticides which cannot be analysed by gas-chromatography or other simple methods. Using a column 0·5 m long, with internal diameter 2 mm, packed with an ether phase bonded onto silica beads (Permaphase ETH) and methanol–water as the mobile phase, a method for separating and measuring nanogram quantities of carbaryl was developed and used to determine carbaryl in bees. Extraction and preliminary separation of carbaryl were as described previously (*Rothamsted Report for 1971*, Part 1, 195). The liquid chromatography system was about ten times more sensitive than the previous gas chromatography system and was also more satisfactory because it did not require the formation of derivatives, it was easier to use and was less liable to interference from constituents of bees.

A similar system was used for separating and measuring nanogram quantities of benomyl and MBC in aqueous and other solutions. The method was used to determine the solubility of these fungicides in water and the effect of pH on the rate of conversion of benomyl to MBC. (Lord and Williams)

Artificial feeding of aphids. Methods of feeding aphids artificially are required for studies on systemic insecticides and on factors influencing host selection. In previous work (*Rothamsted Report for 1972*, Part 1, 190) the artificial diet described by Mittler *et al.* (*Journal of Insect Physiology* (1970), **16**, 2315–2326) was modified by increasing the concentrations of alanine, leucine, phenylalanine, serine, folic acid, calcium pantothenate, pyridoxine and sucrose, and omitting nine other components. This diet was more readily accepted by *Myzus persicae* (Sulz.) but resulted in poorer growth of aphids in prolonged tests. Therefore each of the nine omitted components was re-tested at a series of concentrations in an attempt to establish a readily accepted but nutritionally adequate diet.

These tests resulted in the inclusion of histidine, valine, nicotinic acid, glutamic acid, riboflavin, arginine and tryptophan at one-quarter, ascorbic acid at one-half, and choline chloride at the same concentration as in the Mittler diet. Larvae survived well and increased in weight rapidly on this diet during the first generation. Although adults produced more live young on this diet than on the Mittler diet during nine-day tests, they still preferred to settle on diets completely lacking these nine compounds when given a choice. (Griffiths and Greenway)

Insect rearing. The following species were reared:

PLANT FEEDERS

Homoptera	*Aphis fabae* (Scop.)		
	Brevicoryne brassicae (L.)		
	Myzus persicae (Sulz.)		
		Strains.	Susceptible
			Two organophosphate-resistant
	Megoura viciae Buckt.		

https://doi.org/10.23637/ERADOC-1-130

Hemiptera	*Dysdercus intermedius* Distant
Coleoptera	*Phaedon cochleariae* (F.)

OTHERS

Orthoptera	*Blaberus discoidalis* (L.)
	Periplaneta americana (L.)
Diptera	*Drosophila melanogaster* (Meig.)
	Strains. Normal
	Vestigial wings
	Musca domestica (L.)
	Strains. A wild-type susceptible strain
	ac; ar; bwb; ocra—called 608Q, a multi-marker susceptible strain. SKA-diazinon selected, very resistant to many organophosphorus insecticides
	Several strains derived from SKA, each with one or more factors of resistance to organophosphorus insecticides or DDT
	39m$_2$b—tetrachlorvinphos resistant strain
	49r$_2$b—dimethoate selected strain
	Several strains derived from 49r$_2$b each with one or more factors of resistance to dimethoate and other organophosphorus insecticides
	Several substrains of 49r$_2$b derived by selection with pyrethroids
	290rb—dimethoate selected strain
	Several substrains of 290rb derived by selection with pyrethroids
	NPR—pyrethrum extract selected strain
	Several strains derived from NPR each with one or more factors of resistance to pyrethroids and DDT
	104—resmethrin selected strain
Diptera	*Calliphora erythrocephala* (Meig.)
Hymenoptera	*Acromyrmex octospinosus* (Reich)
	Atta cephalotes (L.)
	Diaretiella rapae McIntosh

Fungicides

Control of common scab on potatoes. Glasshouse and field experiments on chemical control of potato common scab, caused by soil-borne *Streptomyces scabies*, were continued. Chemicals were either mixed into the soil or were sprayed in solution on the haulms, the soil being protected from spray, in glasshouse tests.

Glasshouse tests of soil-applied chemicals. In our routine tests, chemicals were added, usually at 50 ppm, to scab-infested soil from Great Hill Bottom, Woburn, in which Majestic shoots were immediately planted.

Nearly all the chemicals tested were polyhydroxybenzenes or related compounds and thus extend the series reported last year. Quintozene was included as a standard. Table 7 shows yields and amounts of scab as percentages of those in the corresponding 'nil'-treatments; the figures are the mean percentages from the numbers of tests shown (15 plants per treatment per test). Roman type indicates effects that were not significant, and bold type those significant at $P<0{\cdot}001$.

Most of the chemicals either failed to give satisfactory control of scab, or decreased yield, or both. Catechol did not decrease yield but was less effective than hydroquinone

https://doi.org/10.23637/ERADOC-1-130

TABLE 7

Effects of soil-treatments on relative yield and scab incidence in the glasshouse

Treatment	Ppm	No. of tests	Yield	Scab incidence
phenol	50	1	93	182
tert. butyl hydroquinone	50	2	**66**	**3**
chloroneb	50	4	**78**	92
2,5-dimethyl-1,4-benzoquinone	50	1	**31**	**17**
	12·5	1	83	89
catechol	50	9	100	**34**
guaiacol	50	2	95	83
veratrole	50	2	105	130
4-methyl catechol	50	2	93	74
4-tert. butyl catechol	50	4	**84**	**6**
4-tert. butyl catechol	50*	2	106	**9**
4-tert. butyl catechol	25	2	90	**15**
4-tert. butyl catechol	12·5	2	97	**16**
quintozene	50	13	95	**15**
nil	—	13	100	100

* Planting delayed for two weeks

(previously tested) or quintozene. The most promising chemical in the polyhydroxybenzene series was 4-tert.butyl catechol, which has also been used as a soil conditioner at much higher rates (about 250 ppm). It decreased yield to 84% of untreated controls in our routine tests when added to soil at 50 ppm immediately before the shoots were planted. The loss of yield could be avoided by delaying planting for two weeks (as indicated by the asterisk in Table 7) or, more simply, by using less chemical. Thus, at 12·5 ppm, 4-tert.butyl catechol was as effective as quintozene at 50 ppm and had no effect on yield.

Glasshouse tests of haulm-sprays. Some growth regulators, applied to the haulm, affect the growth of the whole plant and, as a result, could affect its resistance to root-disease.

The haulms of potted Majestic plants growing in untreated scab-infested soil in the glasshouse were sprayed with aqueous solutions of growth regulators. Treatments were applied once only, about two weeks after potting, just before the tubers began to form and to be susceptible to attack by *S. scabies*. The following chemicals did not affect scab incidence at the concentrations shown: 2,4-D (0·001%); 'Ethrel' (0·05%); flurecol, *n*-butyl ester (0·0005%); maleic hydrazide (0·05%); DL-α-methoxyphenyl acetic acid (0·005%); 1-naphthylacetic acid (0·01%); 2,3,5-triiodobenzoic acid (0·002%). Similarly, chlormequat chloride, given as one application of 50 ml of 0·3% solution per pot, also failed to affect scab incidence. By contrast, spraying with daminozide (formerly known as aminozide) clearly decreased the amounts of scab. Table 8 shows the combined results of two tests, in the same way as Table 7; quintozene, at 50 ppm in soil at planting,

TABLE 8

Effects of single haulm-sprays of daminozide on relative yield and scab incidence in the glasshouse

Treatment	Yield	Scab incidence
daminozide spray, 0·15%	100	75
daminozide spray, 0·3%	103	**48**
daminozide spray, 0·6%	94	**58**
daminozide spray, 1·2%	100	**30**
quintozene in soil, 50 ppm	89	**10**
nil	100	100

https://doi.org/10.23637/ERADOC-1-130

is included as standard. As care was taken to prevent the spray material from reaching the soil directly, the results show the possibility of controlling a root disease by what appears to be a downward moving systemic action.

Field trial of soil-applied chemicals. In a trial at Great Hill Bottom, Woburn, with the variety Maris Piper, chemicals were applied as 20% dusts on 10 April at 78 kg/ha (70 lb/ac, or about 35 ppm). All plots were rotavated within a few hours of application, and potatoes were planted the same day. Experimental treatments were catechol, hydroquinone and chlorohydroquinone (which do not persist in soil), with quintozene as standard. None of the experimental chemicals decreased scab, although they had all given good control in corresponding glasshouse tests (see Table 7 here and Table 6 in last year's report (*Rothamsted Report for 1972*, Part 1, 191)).

The soil used in the glasshouse tests, although taken from the same field, was often stored for some weeks before use, and was usually fairly dry at the time the chemicals were added; it was not rewetted until after the shoots had been planted. By contrast, the dusts in the field experiment were applied directly to moist soil. Residue determinations showed that these chemicals were stable in the dusts and in dry Woburn soil, but very unstable in moist soil (10% water) taken directly from the field. An effective concentration of added chemical was therefore probably achieved for considerably longer in the glasshouse, where the application rate was 50 ppm, than in the field, where the rate was 35 ppm. The applied chemicals may even have largely decomposed before rotovation. Differences in persistence may therefore explain the difference between the two sets of results. (McIntosh)

Control of seed-borne pathogens with organomercury fungicides. Although organomercury fungicides have been widely used for many years to control seed-borne diseases of cereals, little is known about how they act or even about the dose-response relationships for the main deseases they control. We began work on these problems by studying control of natural seed-borne infections of *Septoria nodorum* on wheat (cv. Capelle-Desprez) using two compounds applied in commercial formulations, phenyl mercuric acetate (PMA) and ethyl mercuric chloride (EMC). To ensure even distribution between seeds, solutions of these chemicals in ethanol were applied as microdrops, and other batches of seed were dipped into solutions of the compounds in ether. The effects of dose on disease control were studied in growth room experiments. The influence of soil moisture level and of the period of storage between treatment and planting on effectiveness was investigated for two levels of infection. Effectiveness was assessed by scoring infection on individual seedlings using a 0–3 disease severity scale from which a disease index for each treatment could be calculated. The dose required to decrease the disease index to half that on untreated seed was taken as the ED50.

Table 9 shows control on seedlings grown in soil at constant low moisture content following application as microdrops: EMC was more effective than PMA, but the effectiveness of PMA increased on storage. ED50s for treatments without storage were somewhat smaller for the less infected seed. This was consistent with a preliminary test using seed with only 20% infection and may reflect a smaller degree of infection on individual seeds as well as fewer infected seeds in the batch. For both PMA and EMC soil moisture content had little effect on the ED50, although increasing soil moisture decreased the level of disease.

Preliminary work was also done on control of *Fusarium nivale* in barley seed about 50% infected. Growth room experiments showed that control of seedling disease was incomplete following microdrop applications of 0·5 μg PMA per seed or 0·25 μg EMC per seed. (Bateman)

https://doi.org/10.23637/ERADOC-1-130

TABLE 9

Effects of storage and level of seed infection on control of Septoria nodorum *by phenyl mercuric acetate (PMA) and ethyl mercuric chloride (EMC). (ED50s as μg Hg/seed)*

Compound	Storage time	Seed 55–60% infected	Seed 85–90% infected
PMA	Nil	0·12±0·016	0·16±0·019
PMA	5 weeks	0·06±0·009	0·05±0·005
EMC	Nil	0·04±0·004	0·06±0·005
EMC	5 weeks	0·05±0·005	0·03±0·004

Factors influencing the effectiveness of systemic fungicides applied to soil. Several recently introduced systemic fungicides are converted to the same product, methyl benzimidazol-2-ylcarbamate (MBC) which is thought to be the active fungicidal agent. However, they vary in effectiveness against several diseases. Factors likely to influence the relative performance of different MBC precursors following application to the soil were therefore studied in the laboratory and related to relative effectiveness in the field.

Conversion of benomyl, thiophanate-methyl and 'NF48' to MBC in aqueous solutions and soil suspensions was followed using u.v. spectrophotometry and high pressure liquid chromatography. In aqueous solution benomyl is converted within a few hours, whereas thiophanate methyl and 'NF48' are more stable, as was found by other workers. In the presence of soil, conversion of 'NF48' is much more rapid and thiophanate-methyl is also somewhat less stable. These effects are much less with autoclaved soil, suggesting that micro-organisms contribute to conversion. Measurement of adsorption of the different parent compounds by soil is difficult because they are unstable, but measurements with autoclaved soil and with suspensions equilibrated at low temperatures which decrease microbial activity indicate that thiophanate methyl and 'NF48', like MBC, are only moderately adsorbed by soil (distribution ratios for adsorption <5).

In the field, relative effectiveness of benomyl, thiophanate methyl and 'NF48' against mildew (*Erisyphe graminis*) and smut (*Ustilago nuda*) of barley was studied following application as a seed treatment. All treatments significantly decreased smut, but benomyl and 'NF48' were more effective than thiophanate methyl. Average values from six replicate plots were 1·5 smutted heads per plot for benomyl, 0·5 for 'NF48', 22 for thiophanate methyl and 122 for untreated controls. Control of mildew followed a similar pattern but results were much less clear-cut. Effects on yield of grain were consistent with disease control, mean values being 5·57 t/ha for benomyl, 5·71 for 'NF48', 5·34 for thiophanate methyl and 4·88 for untreated controls (standard error of differences 0·15). Although the results appear to reflect the relative stabilities of the different compounds, the differences in the field seem larger than could be accounted for by stability alone and further work on the behaviour of these and other precursors is underway. (Graham-Bryce and Williams)

Control of moulding in hay with propionic acid. Studies on the use of propionic acid to prevent moulding and heating of hay are described in the report of the Plant Pathology Department (p. 122). (Lord and King, with Lacey, Plant Pathology Department)

Staff

D. W. Hollomon was appointed to work on the mode of action of fungicides. I. H. Williams from the Canada Department of Agriculture, Vancouver, spent a year in the Department as a visiting worker. Sandwich course students who worked in the Department were Mrs. Valerie Rhenius, M. C. Neale and I. P. Walker.

K. A. Lord spent four weeks in Rome acting as a consultant to the Food and Agriculture Organisation for a project to examine the fate of chemicals used to control locusts.

https://doi.org/10.23637/ERADOC-1-130

INSECTICIDES AND FUNGICIDES DEPARTMENT

I. J. Graham-Bryce and A. H. McIntosh attended the 2nd International Congress of Plant Pathology in Minneapolis, USA. Several members of the Department attended the 7th British Insecticide and Fungicide Conference at Brighton: I. J. Graham-Bryce acted as a session organiser and presented an invited paper, M. Elliott and K. A. Jeffs presented research reports and D. C. Griffiths and G. C. Scott contributed an exhibit on control of wheat bulb fly. J. H. Stevenson participated in a conference on Integrated Approaches in Plant Protection organised by the European and Mediterranean Plant Protection Organisation in Vienna.

https://doi.org/10.23637/ERADOC-1-130