ROTHAMSTED RESEARCH

Report for 1968 - Part 1

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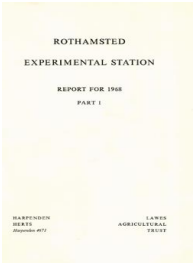

Insecticides and Fungicides Department

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INSECTICIDES AND FUNGICIDES DEPARTMENT

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The genetical work on resistance of insects to insecticides showed two points of considerable practical significance. First, a population with only two or three resistance factors, each of which alone confers only slight resistance, can develop great resistance when the factors are combined. Secondly, the likelihood of a population becoming homozygous and thus very stable for resistance is decreased by the sterility occasioned when two or more factors are present in homozygous form.

The discovery of very active compounds allied to the pyrethrins has aroused general interest in this type of compound. Although studies on the relation between toxicity to the insects and chemical structure have helped in the rational development of this group of compounds—as illustrated in the structure toxicity studies discussed below—the special features responsible for the activity could probably be further analysed if the critical sites for action in the insects were known. The activity of the compounds could then be tested directly on these sites, and toxicity separated from such complicating factors as penetration, sorption and detoxication. Previous work provides strong evidence that the critical sites lie within the nervous system, but there is little indication exactly where. Preliminary work with pyrethrin I tested on the various elements of the nervous system suggests that the critical sites are in the ganglia.

The ability to penetrate and the ability to resist detoxication, both important factors influencing toxicity, were also studied with pyrethrin I and some allied synthetic compounds.

The main reasons for the small amount of insecticides on cereal seeds commercially dressed with dry powders were identified, and attempts are being made to improve the existing methods or develop new ones.

Further materials were found that might replace organochlorine compounds for the control of Wheat Bulb fly. Two of these compounds may also be effective against wireworms.

Di-substituted organo-tin compounds, which are poor general fungicides, effectively controlled potato blight. In laboratory tests they were about $\frac{1}{15}$th as effective as fentin acetate, but they are less damaging to the plants, and are less dangerous; some are widely used as stabilisers for plastics, so are readily obtainable.

Insecticides

The causes of resistance

***Metabolism of organophosphorus insecticides by strains of housefly* (Musca domestica).** Diazinon is metabolised in both susceptible (2345 SRS, *ocra* SRS) and resistant (29R5, 393R5, 466x500R3, 348R2, Hiroyoshi nomenclature) strains of housefly (*M. domestica*) to probably three

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metabolites. Two, 0,0-diethyl phosphorothionate and 0,0-diethyl phosphate, are detectable within minutes of dosing. These occur in all strains, but in different quantities in different strains.

Starch-gel electrophoresis showed differences in the total activities of non-specific phosphatases between strains. (Lewis)

The relation between homozygosity of resistance factors and sterility. As previously described, attempts to study interaction by breeding strains homozygous for pairs of resistance factors proved difficult because of sterility, but some information was obtained.

To determine how much interaction there is between the factors for resistance that occur in the SKA strain, and to estimate the contribution of each factor to total resistance, we tried to breed strains with pairs of resistance factors (R2;R3, R2;R5 and R3;R5) from parents with single factors. This proved very difficult because the breeding method, based on the use of visible mutants that made selection with diazinon unnecessary, involved two consecutive series of single pairs, which led to a drastic loss of fertility. Further, far fewer double homozygotes were obtained than predicted from the Hardy–Weinberg equation and nearly all of these were sterile; also, in the few remaining progenies the marker and one of the resistance factors had crossed-over giving strains heterozygous for one of the two factors of resistance. Thus instead of 40 strains homozygous for two factors of resistance expected and reared from over 800 single pair progenies, we obtained only one. These difficulties show that the inbreeding of more than one factor of resistance involves, not only the successful recombination of these factors, but also the selection of fit individuals, which seem to be rare among homozygotes for resistance. Fitness is much more common in individuals homozygous for a single factor of resistance, or homozygous for one factor but heterozygous for the other. This could explain why strong resistance to organophosphorus insecticides in the field is rare and has established itself only where the insecticide was sprayed at short intervals and exerted a continuous selective pressure on both the adult and larval stages. (Sawicki and Farnham)

Organophosphorus insecticides and factors of resistance to diazinon. An experiment was made to locate and determine the action of the separate factors of resistance of the SKA strain against organophosphorus insecticides. The methyl and ethyl homologues of the phosphorothionates and the corresponding phosphates of parathion, 'Chlorthion' and malathion, were tested, together with diazinon and diazoxon, against five strains, each with a different autosome derived from the SKA strain and the two parents, a susceptible multimarker strain *bwb;ocra;ar;ac* SRS, and SKA. In four of these, the IV autosome, unmarked in the susceptible parent could be derived from the susceptible or the resistant parent, or both.

Only the strain with the SKA's V linkage group carrying the gene for low aliesterase was resistant to all fourteen organophosphorus insecticides but resistance was only from half (methyl parathion) to one-twentieth (diazinon) of the resistance of SKA. Flies with the factor on SKA's third

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linkage group were slightly less resistant to diazinon, diazoxon and malaoxon than those with gene *a* (R5) but were fully susceptible to the other organophosphorus insecticides. The penetration factor (SKA's II linkage group), delayed knock-down but had little or no effect on kill. The SKA's VI autosome gave slight resistance to chlorthion and chloroxon (*c.* × 2), but the SKA's IV autosome had no measurable factors of resistance against the organophosphorus insecticides tested.

The results of tests on the strains with the isolated factors after pretreatment with TBTP (*S*,*S*,*S*-tributyl-phosphorotrithioate) or sesamex, suggest that there are at least three different ways in which the organophosphorus insecticides tested are attacked both by the susceptible and resistant flies. The thionate are hydrolysed to give diethoxy thiophosphoric acid and the phosphates to give the corresponding phosphoric acid (see Lewis); both reactions are likely to be controlled by the aliesterase factor (R5). The phosphate is also attacked by another mechanism on the III linkage group to give unknown metabolite(s). Pretreatment with TBTP, which blocks the hydrolysis of the thionates and phosphates, synergises organophosphorus insecticides most in flies with the factor for low aliesterase. The action of sesamex is more complex because it acts as an antagonist with the thionates by inhibiting the formation of the phosphates, so that the thionates are attacked by the hydrolysing mechanism, and as a synergist with the phosphates by inhibiting their conversion into non-toxic metabolites. The response to the additives seems to depend on the mechanism predominant in breaking down a given compound. Thus hydrolysis seems to be more important than the sesamex-inhibited mechanism in breaking down ethyl chlorthion, because in flies with low aliesterase, TBTP gives a synergistic factor of 16, and sesamex almost complete immunity but a small synergism (×3) with ethyl chloroxon; the opposite occurs with ethyl malaoxon, which, in flies with the sesamex inhibited factor, is almost not synergised by TBTP, whereas sesamex gives a synergistic factor of 52. The response of the other organophosphorus insecticides is intermediate between these two compounds. Susceptible flies respond to the additives in the same way as the resistant ones, but the effects are less obvious, indicating that the differences between susceptible flies are only quantitative. However, even susceptible flies can break down phosphates to a considerable degree, because synergism was unexpectedly large when these insects were tested with phosphates after pretreatment with sesamex.

Interaction between the factors of resistance. Singly none of the three factors responsible for the strong resistance of the SKA strain to diazinon (×300>) give such resistance; two of the factors, R3 (inhibited by sesamex) and the gene for low aliesterase (R5) give *c.* ×10–15 resistance, whereas R2 the penetration factor only delays knock-down and has little effect at death. Therefore strong resistance probably occurs through the interaction between the factors when they are together, as in the SKA strain. Adding R3 to gene *a* (R5) increases resistance considerably, not only for compounds against which both factors are effective, e.g. diazinon, where there is a 6-fold increase in resistance compared with the resistance of gene *a*, but also for compounds against which R3 is ineffective, e.g. ethyl-

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'Chlorthion', where the resistance of gene *a* is increased four times. However, R3 does not always interact with gene *a*, and flies with both factors are only as resistant as flies with gene *a* against methyl parathion. The flies with both factors of resistance resemble the SKA flies in being much less affected by the additive than flies with the isolated factors. The reason for this is unknown.

Tests with strains heterozygous for R2;R3 and R2; gene *a*, show that penetration factor R2 sometimes increases resistance very much. R2, which alone gives little resistance as measured by deaths, increases the resistance of R3 to DDT from ×10–15 to complete immunity, and also increases the resistance of gene *a* 5–10-fold against diazinon. (Sawicki and Farnham)

Glandular secretions of the cotton stainer *Dysdercus intermedius* Dist. Cotton stainers secrete compounds that cause populations of the pest to aggregate and to disperse and might be used to control them. The nymphs have three dorsal abdominal glands, and analyses of the secretion from the posterior gland were given in last year's Report (p. 170). The secretion from the two anterior glands is less complex and consists mainly of *n*-tetradecane. Its analysis is made difficult by the small amount produced. Extracts of dissected glands are unsuitable for analysis because they contain so much interfering material from glandular tissue.

Adult cotton stainers have a pair of ventral thoracic glands. The appearance of the glands and smell of their secretion suggest that they have a defensive function. The secretion, which was collected by puncturing the dissected gland reservoir, is less complex than the posterior gland secretion of the nymphs; its main component is hex-2-en-1-al. (Calam and Scott)

Sex- and species-specific compounds from male bumblebees (*Bombus* spp.). Male bumblebees mark 'territories' in the field with scent secretion from cephalic glands. These scents are species-specific because males of one species do not visit marks made by males of another. The territories are thought to play an important role in the mating behaviour of bumblebees. Extracts of heads of male and female bumblebees of five species were analysed, mainly by gas chromatography and mass spectrometry, to identify the volatiles most likely to be the territory marking scents. Extracts from males of all the species examined contain substantial amounts of volatile compounds that are species-specific and either do not occur, or occur only in traces, in extracts from females of the same species. They were characterised, in part by preparing suitable derivatives.

The major component of extracts from male *B. agrorum* is hexadec-7-en-1-ol, from male *B. lapidarius* is hexadec-9-en-1-ol together with some hexadecan-1-ol, and from male *B. lucorum* is ethyl tetradec-9-enoate. In all these, the double bond is probably *cis*, for physicochemical and biogenetic reasons. The corresponding compound in extracts from male *B. pratorum* is one isomer of farnesol or a closely related terpene. An extract from young male *B. derhamellus* contained *n*-tricosene and *n*-pentacosene as major components.

All extracts from males contained more *n*-tricosane than extracts from females. This hydrocarbon might act as a fixative for the more volatile

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compounds. Some differences were observed between the composition of extracts from queen and worker bumblebees.

Extracts, prepared for comparison, from parasitic bumblebees (*Psithyrus vestalis*), solitary bees (*Osmia rufa*) and wasps (*Vespula germanica*) contained no major components. (Calam)

Worker honeybees. The composition of secretion from the Nassanoff gland of worker honeybees was re-examined by a more sensitive method and the results published. Attempts continue to identify the pheromone by which workers recognise a hive entrance. (Calam and Butler, Bee Department)

Pyrethrins and related compounds

Effect of pyrethrins on the nervous system of *Periplaneta americana* L. (the American cockroach). Work was mainly directed to locating the fatal lesion in cockroaches poisoned by pyrethrin I (py I). Allethrin at concentrations greater than 10^{-6}M is already known to affect the conduction of nerve impulses along giant fibre axons of the cockroach abdominal nerve cord and to block conduction at a concentration of 3×10^{-6}M. We find that similar concentrations of py I have similar effects on giant fibre axons and also block conduction through the cercal nerve-giant fibre pathways in the sixth abdominal ganglion.

Condition of nervous system in pyrethrin-treated cockroaches. Adult male cockroaches were treated topically on the metathoracic sterna with LD90s (0·5 μg) of py I. From 2 minutes to 25 hours after treatment, amputated abdomens from treated individuals were dissected to display the abdominal cord, and the condition of the cercal nerve-giant fibre pathway was assessed by stimulating a cercal nerve electrically and observing the resultant response in the giant fibres of the abdominal cord. From 10 minutes to 25 hours after treatment most preparations showed some abnormal symptoms, such as 'bursts' and trains of discharges, but until 25 hours, when some were blocked, all preparations gave at least a partial response to the stimuli, and the response of some seemed normal. However, all insects were affected by the py I after 1 hour and prostrate after 5 hours.

To try to decide what degree of poisoning their nervous systems had sustained before the insects were dissected, some preparations were irrigated continuously with a 2×10^{-6}M solution of py I in saline from the time the insects were dissected until the nerve pathway was blocked. The time taken was compared with that required for a similar concentration to block conduction in a preparation from an untreated cockroach (Table 1). Blocking-time for individuals dissected at the same time after dosing varied considerably; the values in Table 1 are the means of four replicates. In some preparations from insects dissected 25 hours after dosing the nerve pathways were already blocked, and blocking-times for the remainder varied greatly, so no value is given for this time interval.

The blocking-time was always as long in individuals previously treated

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TABLE 1

Effect of irrigating with a 2 × 10^{-6}M solution of pyrethrin I, sixth abdominal ganglia dissected from P. americana *already treated topically with 0·5 μg pyrethrin I*

Time after treatment when dissected	Condition of insects when dissected	Irrigation time required to block response (minutes)
2 minutes	CO_2 affected	12
10 minutes	Slightly affected	33
1 hour	Slightly/Prostrate	29
5 hours	Prostrate	14
25 hours	Prostrate	—*
Untreated	Normal	14

* See text.

with py I as in untreated individuals (14 minutes), and in those treated 10 minutes and 1 hour before dissection, the blocking-time actually increased.

Effect of pyrethrin I on the response to a mechanical stimulus. Because testing the condition of a complex nerve pathway such as the cercal nerve-giant fibre preparation with electrical stimuli is perhaps a physiologically abnormal process, a test providing a mechanical stimulus was devised. A train of 20 puffs of air, each lasting 100 msec, was discharged at a rate of 2·6 per second at the cerci on an amputated cockroach abdomen. The puffs were produced by a rotating eccentric which alternately compressed and released a rubber bulb, and were conveyed to the preparation through a tube ending a short distance from the tip of the abdomen. The response to each puff, recorded from the cercal nerve where it entered the sixth abdominal ganglion and from the abdominal nerve cord, was amplified and displayed on the screen of a dual-beam oscilloscope. The response to each sequence of 20 puffs was filmed and the nerve potentials per puff were later counted by examining the film.

As expected, the number of nerve potentials per puff in the cercal nerve changed little throughout the train of 20, but the number in the giant fibres decreased sharply during the first 5–10 puffs through synaptic adaptation, finally settling at 25–50% of the number produced by the first puff.

When tests of this kind were made on preparations continuously irrigated for 100 minutes with py I at 10^{-6}M in saline, effects on the cercal nerve response were small, and neither the rate nor the degree of adaptation in the response from the abdominal cord changed much. However, the number of potentials per puff from the cord increased until after 30 minutes slightly more were recorded than from untreated preparations. Activity then decreased until after 100 minutes the response to the initial puff was one-half and the adapted response one-fifth that obtained from untreated cords. With more concentrated py I (3·2 × 10^{-6}), preparations passed through a similar sequence but completed it sooner (40–50 minutes); sometimes the nerve pathway blocked at this concentration. The amount of spontaneous activity in the ganglion followed a similar sequence, increasing and decreasing with approximately the same timing.

Effect of pyrethrin I on spontaneous activity in the sixth abdominal ganglion. Nerve ganglia show a characteristic spontaneous activity even when all

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afferent inputs are eliminated. Its maintenance within certain limits is probably essential to normal function. Very small concentrations of py I were shown to affect the spontaneous activity.

Nerve potentials were recorded from sixth abdominal ganglia exposed in amputated cockroach abdomens, and amplified and integrated with a rate-meter, the output from which was continuously recorded with a pen-recorder. This gave continuous records of the amount of activity in the ganglia. Amounts of activity in untreated ganglia were compared with those of ganglia continuously irrigated with py I dissolved in saline. Interpreting the results is difficult, because activity was intermittent and differed greatly between identically-treated preparations. To allow comparisons, the mean activity throughout successive 20 minute periods was estimated from the recorder charts by eye and the logs of these values plotted against time. When the results of at least 6 replicates per treatment were averaged, significant differences appeared.

Spontaneous activity in ganglia exposed to 10^{-5}M py I increased a thousandfold in a few minutes, but after 20 minutes it was less than the amount before treatment, and was still declining 2 hours after treatment began. Similar initial effects were produced by py I at 10^{-6}M, but after 20 minutes activity settled at about three times that before treatment, and then declined only slowly. With py I at 2×10^{-7}M the initial burst was longer delayed, but after 20 minutes activity was similar to that produced by a 10^{-6}M solution. Concentrations of 5×10^{-8}, 10^{-9} and 10^{-10}M produced no initial burst of activity, but a steady increase to 2–3 times as much as before treatment. This amount of activity was often maintained for 40 minutes, when there was a slow decline which rarely reached the level before treatment even 3 hours after treatment began.

A concentration of py I as small as 10^{10}M therefore seems to have some effect on the level of spontaneous activity in the sixth abdominal ganglion, though it seems entirely without effect on axonic conduction. (Burt and Goodchild)

The penetration of pyrethrin I into adult *P. americana*. To get information about the concentration of py I near the nervous systems of cockroaches poisoned with it, adult males were treated topically on the metathoracic sterna with LD90s (0·5 μg) of py I, and the penetration and distribution of the insecticide was followed by chemical assay. Py I remaining on the cuticle of the insects was washed off with solvent. Py I that penetrated the cuticle was obtained by grinding the whole insect, extracting with solvent and purifying the extract. Samples of haemolymph were extracted with solvent. The py I was estimated by gas–liquid chromatography using an electron-capture detector. The proportions of the dose that penetrated and was found within were measured on single insects and varied considerably; values in Table 2 are the means of at least three replicates. For each haemolymph assay the samples from three insects were pooled before analysis, but the sample was still too small for its py I content to be detected. The insects became desiccated after 2 hours, which made the sample smaller and the assay even less sensitive. Only limiting values can therefore be given.

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TABLE 2

The penetration and distribution of LD90s of pyrethrin I applied topically to P. americana

— = no data (see text)

Time after treatment	% penetrating cuticle	% found within insect	Conc. found in haemolymph (M)
0	0	0	0
5 minutes	3	2	$<0{\cdot}16 \times 10^{-7}$
15 minutes	18	4	$<0{\cdot}13 \times 10^{-7}$
1 hour	23	9	$<0{\cdot}14 \times 10^{-7}$
2 hours	30	8	$<0{\cdot}22 \times 10^{-7}$
4 hours	39	11	$<1{\cdot}56 \times 10^{-7}$
10 hours	67	11	—
16 hours	72	13	—
24 hours	75	11	—

Pyrethrin I is absorbed and eliminated more slowly than diazoxon (*Rothamsted Report for 1967*, p. 166) and the maximum amount found within the insect is a smaller proportion (10%) of the applied dose than with diazoxon (40%). About three-quarters of the maximum concentration of py I ultimately found inside is present when the insects become prostrate. If the total quantity of py I then found in the insects is assumed to be evenly distributed throughout their bodies, the concentration of py I within the tissues would be about $1{\cdot}2 \times 10^{-7}$M. The concentration in the haemolymph at this time, however, is less than one-tenth of this. The discrepancy may be caused by sorption; this is currently being investigated.

If, like diazoxon, py I enters the nervous system from the haemolymph, the available concentration would not affect conduction in giant fibres and probably not in peripheral nerve either. However, a smaller concentration than that detectable by assay could affect spontaneous activity in the sixth abdominal ganglion (see last section) and presumably in other ganglia too. (Burt, Forrest and Jeffs)

Penetration and metabolism of synthetic pyrethroids. The penetration of the methylbenzyl (±)-*cis-trans*-chrysanthemates (this report) and 4-allylbenzyl (±)-*cis-trans*-chrysanthemate and its 2,6-dimethyl analogue (*Rothamsted Report for 1965*, p. 163) into *Phaedon cochleariae* adults was studied to determine whether the relative rates of penetration affected the toxicity of the compounds to this species. An equal dose (4 μg/insect) was topically applied in acetone solution to the ventral abdomen of the beetles and the unabsorbed portion was removed from the cuticle after different times by successive rinses with methanol (external rinse). The treated beetles were then ground with a little anhydrous Na_2SO_4 and extracted with further aliquots of methanol (internal extract).

An initial rapid period of penetration in which *ca.* 40% of the applied dose of all compounds disappeared from the insect surface within seconds was followed by a curvilinear decrease in the proportion recovered from the insect surface. This decrease approached first order behaviour after three hours' exposure to the compound. The compound not recovered in the immediate surface wash was not all recovered in extracts of ground insects. The proportion recovered in this internal extract increased within

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the first few hours of exposure to a maximum value, which was usually maintained for at least 24 hours of exposure. The following simple model is proposed to explain the form of these curves. The compound diffuses from the outer layers of the cuticle until it is distributed throughout the insect body. Initially diffusion is rapid, but slows as equilibrium is approached. However, equilibrium is not reached because a proportion of the compound is steadily destroyed in the insect by detoxication, producing a steady state in which compound passes into the beetle from the cuticle surface to replace the portion detoxified. The loss from the surface during this period corresponds to the first-order phase of penetration.

TABLE 3

Percentage of applied benzyl (±) cis-trans-*chrysanthemate recovered in mustard beetle extracts*

	External rinse Time of exposure, in hours									Internal extract Time of exposure, in hours								
	0	1·5	3	6	12	18	24	48	72	0	1·5	3	6	12	18	24	48	72
Air	53	21	15	6	2	<1	<1	<1	0	0	9	25	29	39	22	14	7	3
Nitrogen	53	30	30	18	5	0	0	0	0	0	5	15	32	27	33	30	34	53
Dead	68	28	30	14	6	4	4	8	7	<4	<4	15	23	28	34	37	36	40

The results of several experiments supported this hypothesis. Table 3, for example, illustrates the slower penetration of benzyl (±)-*cis-trans*-chrysanthemate into dead and live insects, kept in air and oxygen-free nitrogen respectively, than into live insects kept in air. Both treatments inhibited detoxication; whereas the proportion of the applied compound in extracts of living insects kept in air decreased after 12 hours, when none remained on the insect surface, it did not with the other dead insects. A similar experiment with 2,3-dimethylbenzyl (±)-*cis-trans*-chrysanthemate confirmed that less compound was lost from the cuticle surface of dead insects. The curvature of the plot of log per cent applied dose recovered in the surface wash against the time of exposure in hours was the same with live and dead beetles, so the fact that more penetrates live insects is probably attributable to detoxication and not any other changes that occurred at death. Also, after benzyl chrysanthemate was no longer detectable in the external wash, the rate it decreased in extracts matched the rate constant of loss from the surface during the first-order phase of penetration, indicating that the rate of penetration after the first 3 hours was limited by the rate of detoxication.

The relative rates the methylbenzyl chrysanthemates penetrate during this first-order phase should indicate their relative rates of detoxication, which may be useful in interpreting structure-toxicity results. The corresponding slopes of the penetration curves at the steady-state phase (typically, 3–12 hours after application) were therefore calculated by the method of least squares, for all the compounds studied, and plotted against the mean log relative toxicity. The regression was significant ($P = 0{\cdot}1$) and the correlation coefficient was $+0{\cdot}65$. The trend of increased toxicity with slower rate constants for penetration would be expected if penetration rates are positively correlated with detoxication rates. The variation of relative toxicity about the curve was too large to be attributed to experi-

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mental error and probably depends on differences in the affinity of the compounds for the site of action. Compounds that fell near the upper confidence limits had a more satisfactory structure, as determined by other structure–toxicity studies, for toxicity at the site of action than those near the lower limits. Hence, the structure of these compounds seems to affect toxicity by determining their affinity for the site of action, in addition to their ability to reach the site of action in active form. (Ford)

Insecticidal activity of the methylbenzyl chrysanthemates. The insecticidal action of all nineteen methylbenzyl (±)-*cis-trans*-chrysanthemates previously synthesised (*Rothamsted Report for 1966*, p. 171), was tested against adult *Phaedon cochleariae* Fab. (mustard beetles) and *Musca domestica* L. (houseflies), by the method of topical application in measured drops of acetone, and their toxicities compared.

The orders of toxicities of these esters (least toxic first, positions of methyl substituents on benzene ring given) against mustard beetles are: 3,5 < 3,4,5 < 2,3,5 < 3 < 4 < 2 < 2,5 < 3,4 < 2,3,5,6 < 2,3,4,5 < 2,4,5 < 2,4 < 2,6 < 2,3,4 < 2,4,6 < 2,3,4,5,6 < 2,3 < 2,3,4,6 < 2,3,6; against houseflies: 3 < 2 < 3,5 < 2,3,5,6 < 2,3,5 < 4 < 2,5 < 3,4,5 < 2,3 < 2,3,4,5 < 3,4 < 2,3,4,5,6 < 2,4,5 < 2,4 < 2,3,4 < 2,6 < 2,3,6 < 2,3,4,6 < 2,4,6.

The order of relative toxicities was remarkably similar with both species; this may indicate that a similar system at the site of action was attacked in both insects. The most toxic compounds probably had the best combination of the following properties: (*a*) appropriate structure to be effective at the site of action; (*b*) physical properties giving favourable penetration and distribution characteristics within the insect; (*c*) greatest resistance to detoxification.

With *P. cochleariae*, polymethylated benzyl chrysanthemates took a longer time to reach poisoning end-point than did mono- and di-methylated compounds; the LD50 of pentamethylbenzyl chrysanthemate was still decreasing after 144 hours. This may have been because compounds of larger molecular weight were distributed throughout the insect and detoxified more slowly than the less substituted benzyl esters.

As a result of this series of tests the following conclusions were reached: (i) the best dimethyl compounds for houseflies and mustard beetles respectively were 2,6- and 2,3-dimethylbenzyl chrysanthemates. 2,3-Dimethylbenzyl chrysanthemate was about twice as toxic as allethrin to mustard beetles; this is the first time that the toxicity of these dimethyl compounds has been recognised. Previously, only 2,4-, 3,4- and 2,5-dimethylbenzyl chrysanthemates had been tested (Barthel, W. F., *World Review of Pest Control* (1964) **3,** 97). The compounds with one more methyl group than each of these two dimethyl esters were the most toxic of all the methylbenzyl chrysanthemates: 2,3,6-trimethylbenzyl chrysanthemate for mustard beetles and 2,4,6-trimethylbenzyl chrysanthemate for houseflies. Substitution in both *o*-positions is therefore important for toxicity and then, depending on species a 3-, or 4-methyl group also present gives the most effective compound in this series. 2,3,4,6-Tetramethylbenzyl chrysanthemate, which combines the substitution pattern common

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to both best compounds, was next most effective ester against both species. For mustard beetles the LD50 was 0·14 μg/insect, and for houseflies 0·5 μg/insect. (ii) For both houseflies and mustard beetles, compounds with only one *m*-position substituted were much more toxic than related compounds with two *m*-methyl groups. 3,5-Dimethylbenzyl chrysanthemate was even less toxic to mustard beetles than the unsubstituted compound benzyl chrysanthemate. 2,3,5,6- and 2,3,4,5-Tetramethylbenzyl chrysanthemates were both less toxic than 2,3,6- and 2,3,4-trimethylbenzyl chrysanthemates to both species examined. (iii) For houseflies 2,6-dimethyl chrysanthemate was more than ten times more toxic than 2-methylbenzyl chrysanthemate and more than half as toxic again as 2,4-dimethylbenzyl chrysanthemate. 3-Methylbenzyl chrysanthemate was the least effective monomethyl ester for this species. To mustard beetles 2,3-dimethylbenzyl chrysanthemate was more active than either of the 2- or 3-monomethyl esters and the 4-methyl compound was slightly more toxic than 3-methylbenzyl chrysanthemate.

From comparison of molecular models, it was concluded that the most effective methyl benzyl chrysanthemates had a substitution pattern of methyl groups giving them a molecular shape similar to that of toxic cyclopentenolone chrysanthemates such as allethrin. The unsubstituted *m*-position fell at a location approximately the same distance from the ester link as did the carbonyl group in allethrin and the oxygen atom of the furan ring in 5-benzyl-3-furylmethyl chrysanthemate.

5-Benzyl-3-furylmethyl chrysanthemate and related 5-alkenyl esters

5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate (Ia) resembles pyrethrin I (IIb) and synthetic cyclopentenolone chrysanthemates such as allethrin (IIc) in the relative stereochemical positions of the —CH_2 group in the side chain of the alcohol and of the acidic part of the molecules. In the related benzyl chrysanthemates III both allyl (R = c), and benzyl (R = Ph) side chains had been shown to enhance insecticidal activity, so the 5-allylfurfuryl (IV) and 5-allyl-3-furylmethyl chrysanthemates (Ic) were synthesised for comparison with 5-benzyl-3-furylmethyl chrysanthemate, which is a very potent insecticide. 5-Allylfurfuryl chrysanthemate was obtained from the alcohol synthesised by Bohlmann's method (*Chem. Ber.* (1965) **98,** 2596).

5-Allyl-3-furylmethyl alcohol could not be synthesised by either of the routes to 5-benzyl-3-furylmethyl alcohol (*Rothamsted Report for 1966*, p. 173), so in collaboration with Dr. H. M. R. Hoffman (Chemistry Department, University College, London) we established a general method for introducing allyl and substituted allyl groups into the 5-position of 3-furoic esters. When allyl iodide was reacted with silver trichloracetate under very mild polar conditions (liquid SO_2 at −50°), the allyl cation produced persisted long enough to be trapped by the substituted furan, present in excess. Reaction mixtures were analysed by combined gas–liquid chromatography–mass spectrometry. Methyl 3-furoate (R^2 = H) gave mixtures of 5- and 2-substituted products. The chemical shifts and coupling constants from their nuclear magnetic resonance spectra, and their mass spectra, proved that the major products were, respectively

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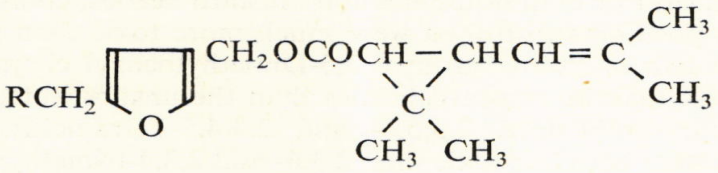
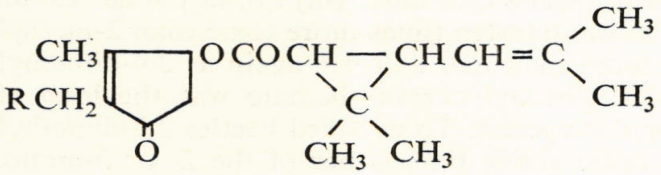
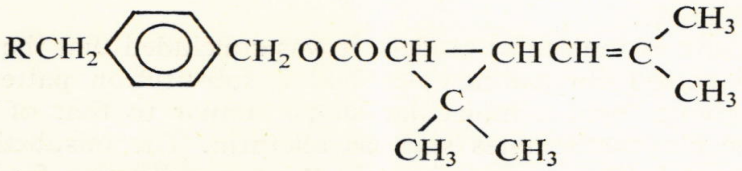
5-allyl- and 5-(2′-methallyl)-3-furoates when allyl ($R^1 = H$) or 2-methallyl ($R^1 = Me$) iodides were used. In these esters the coupling constant be-



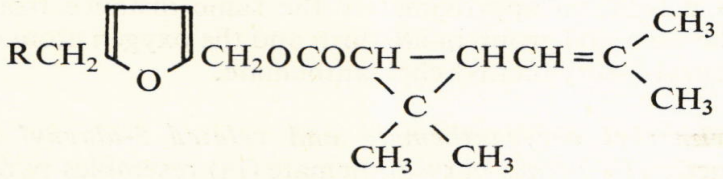
I



II



III



IV

a R = Ph

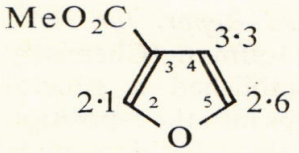
b R = CH = CH CH = CH_2

c R = CH = CH_2

tween the 2 protons was typical of 2,4 coupling and the positions of the peaks showed the absence of a 5-proton (Table 4).

TABLE 4

Chemical shifts τ and coupling constants for furans

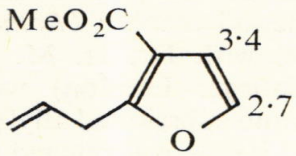


1

$J_{2,4} = 0{\cdot}7$ Hz

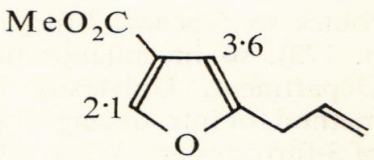
$J_{2,5} = 1{\cdot}6$ Hz

$J_{4,5} = 1{\cdot}9$ Hz



4a

$J_{4,5} = 2$ Hz



4b

$J_{2,4} \simeq 0{\cdot}8$ Hz

Methyl 2-methyl-3-furoate gave only 5-substituted products ($R^1 = H$,

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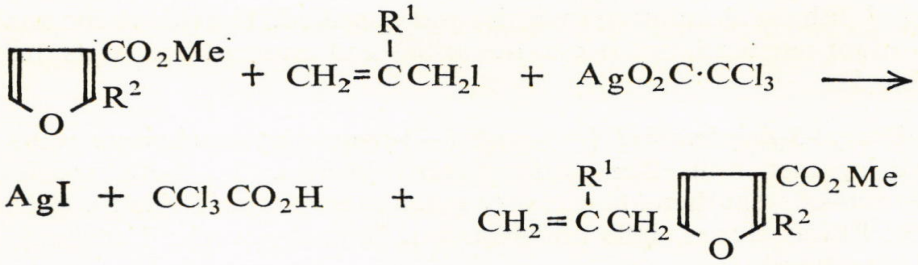


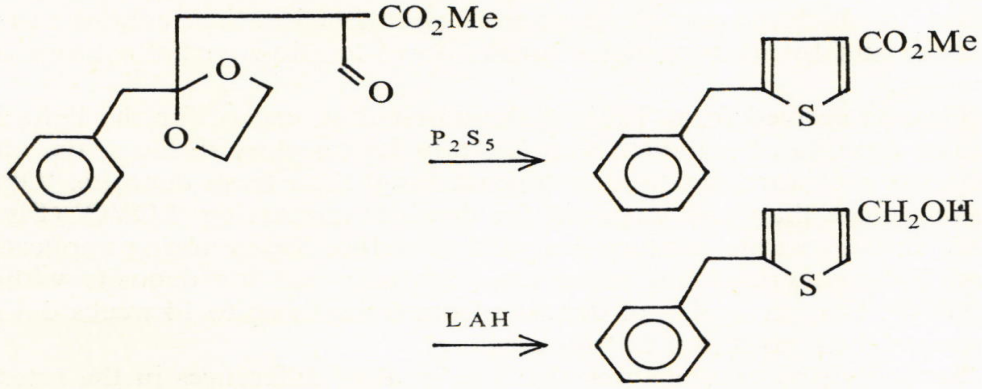
or Me, R^2 = Me). The four esters were separated by fractional distillation, reduced to the alcohols and esterified. Table 5 gives bioassays with these and related compounds.

TABLE 5

Contact toxicity to adult Phaedon cochleariae *Fab. and adult* Musca domestica *L. of synthetic pyrethroids*

	Phaedon cochleariae, Fab. LD50, in % w/v	*Musca domestica*, L. LD50, in μg/insect
5-Benzyl-3-furylmethyl (±)-*cis-trans*-chrysanthemate	0·00023	0·016
5-Propargyl-2-furfuryl (±)-*cis-trans*-chrysanthemate	0·016	0·23
5-Allyl-2-furfuryl (±)-*cis-trans*-chrysanthemate	0·120	0·49
5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate	0·00055	0·0028
5-Allyl-3-furylmethyl (+)-*trans*-chrysanthemate	0·126	0·11
5-Benzyl-3-thenyl (+)-*trans*-chrysanthemate		0·054
5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate		0·006
5-Allyl-2-methyl-3-furylmethyl (+)-*trans*-chrysanthemate		0·066
5-Methallyl-2-methyl-3-furylmethyl (+)-*trans*-chrysanthemate		0·085
5-Methallyl-3-furylmethyl (+)-*trans*-chrysanthemate		0·085
5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate	0·00077	0·0042
5-Benzyl-3-furylmethyl (−)-*trans*-chrysanthemate	0·15	0·42

***5-Benzyl-3-thienylmethyl* (+)-*trans-chrysanthemate*.** The thiophene analogue of 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate was synthesised to compare its insecticidal activity with the parent compound. Ethyl α-formyl δ-phenyl laevulate ethylene ketal, regenerated from the sodium salt with aqueous acetic acid, was heated with phosphorus pentasulphide to give ethyl 5-benzyl 3-thenoate. The ester was reduced to the



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alcohol (lithum aluminium hydride) and esterified. The thiophene analogue was about one tenth as toxic to houseflies and mustard beetles as the furan (Table 5).

***5-Benzyl-3-furylmethyl* (+)- *and* (—)-*trans*-chrysanthemate** were compared against houseflies and mustard beetles. To both species, the (+)-*trans*-chrysanthemate was very much more toxic (Table 5). (Chemical work: Elliott, Janes, Jeffs and Pearson. Bioassay work: Farnham, Ford and Needham)

Effects of environment and formulation on the persistence of insecticides

Volatilisation. With the help of a computer, equations were fitted to the daily measurements of the rates pure crystals of the insecticides dieldrin and aldrin were volatilised from glass and cotton-leaf surfaces under constant conditions of 20° C and still air. These are of the form:

Model 1 (simple exponential), $y = Ae^{-kt}$
Model 2 (double exponential), $y = Ae^{-kt} + Be^{-k't}$
Model 3 (compound exponential), $y = (A + Bt)e^{-kt}$

where y is the amount of insecticide remaining on the surface at time t, and A, B, k, k' are constants.

Under these conditions the losses of dieldrin from cotton-leaf surfaces usually follow a Model 1 type of equation, but on glass surfaces Model 2 or Model 3 types usually provide the best fit.

On glass surfaces, two components show in the process of volatilisation. One is fast and dominates at the beginning, but decreases sooner than a slower component that is more effective towards the end at low deposit densities.

Persistence to 'rainwashing'. The rates at which wettable powder formulations of DDT are removed in accelerated 'rainwashing' tests were measured, using heavy and light deposits of both fresh and aged residues of two types of formulation on two types of surface.

A Model 2 type of exponential equation could always be fitted, indicating that there are two separate factors working simultaneously. These are probably the impaction effect, in which the more friable particles are removed piecemeal by purely mechanical action, and the longer term effect, in which the particles have to be wetted before their cohesion to the surface and to other particles breaks down to allow partial solution and erosion.

Graphs derived from Table 6 show first that, except for the light and heavy deposits of standard wettable powder on glass surfaces, the older deposits are more resistant to 'rainwashing' than fresh deposits. 'Older' means more than two days old for deposits containing 'LOVO' (Fisons Ltd. amine stearate mixture designed to reduce losses during application and by weathering) but more than 3 weeks old for deposits without 'LOVO'. Further ageing of deposits from 6 weeks up to 14 weeks did not increase their resistance to 'rainwashing'.

Secondly, the graphs show that the greatest differences in the rates of

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removal by this accelerated 'rainwashing' method occur during the first 20 seconds, when the more friable parts of the deposits are removed. After this, the rates, which now show the removal of the more tenacious parts of

TABLE 6

Percentages of DDT remaining on surfaces at intervals during continual 'rainwashing' of deposits aged for different times

Light deposits contain 2–5 μg DDT/cm². Heavy deposits contain 20–30 μg /DDTcm²
— = no readings

(i) Glass

	(a) Standard W.P.				(b) 5% 'LOVO' W.P.			
	Light deposit		Heavy deposit		Light deposit		Heavy deposit	
Time (seconds)	2–14 days old	6 wks old	2–14 days old	6 wks old	2–14 days old	6 wks old	2–14 days old	6 wks old
0	100	100	100	100	100	100	100	100
2	23	22	20	18	94	90	97	99
5	14	14	11	8	89	87	95	98
10	11	11	8	6	85	84	93	96
20	9	8	5	4	80	77	90	95
40	7	7	4	3	74	72	88	94
80	5	4	3	2	66	67	83	92
160	2	0	1	0	56	61	78	91

(ii) Leaf

(*a*) Standard W.P.

	Light deposit						Heavy deposit					
Time (seconds)	1–2 days old	3–7 days old	6 wks old	9 wks old	11 wks old	14 wks old	1–2 days old	3–7 days old	6 wks old	9 wks old	11 wks old	14 wks old
0	100	100	100	100	100	100	100	100	100	100	100	100
2	16	47	59	—	—	—	13	42	51	—	—	—
5	9	34	50	—	—	—	6	30	41	—	—	—
10	6	27	44	—	—	—	4	24	35	—	—	—
20	5	22	41	—	—	—	3	21	31	—	—	—
40	4	19	38	44	49	47	2	18	29	26	34	32
80	3	15	36	40	44	43	2	15	26	22	30	28
160	2	11	34	36	39	39	1	13	24	20	26	25

(*b*) 5% 'LOVO' W.P.

	Light deposit						Heavy deposit					
0	100	100	100	100	100	100	100	100	100	100	100	100
2	71	88	86	—	—	—	93	94	95	—	—	—
5	54	82	81	—	—	—	89	92	92	—	—	—
10	43	77	78	—	—	—	87	89	91	—	—	—
20	36	73	75	—	—	—	84	88	90	—	—	—
40	30	68	72	76	64	69	81	83	86	87	81	88
80	24	62	68	72	60	66	76	78	83	82	78	84
160	18	65	65	68	55	63	69	70	80	79	74	81

the deposits, are roughly similar for all the examples, although, of course, the amounts remaining in the 'LOVO' deposits are much greater.

Thirdly, they show that for deposits containing 'LOVO', whether on glass or on leaf surfaces, the heavier the deposit the greater the percentage retained at corresponding times of 'rainwashing'. No correlation in this

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respect is found for deposits without 'LOVO'. Thus, a heavy residue containing 'LOVO' and covering a surface fairly completely seems to withstand the impaction effect of 'rainwashing' better than a residue sparsely distributed over the surface.

Penetration of DDT from these formulations into the excised (moribund) leaves after ageing for 9, 11 and 14 weeks was found by analysis of the leaves after 'rainwashing'. The wax layer was stripped off by dipping in hexane and agitating for a few seconds; this procedure was repeated with fresh hexane, and the combined hexane washings gave the amount of DDT tenaciously held as a surface deposit. Maceration of the stripped leaf with hexane in a ground-glass tissue grinder then extracted the amount of DDT that had penetrated.

TABLE 7

Percentages of DDT after ageing for 9–14 weeks at 20° C, (i) removed by 'rainwashing' for 160 seconds, (ii) held by surface wax, (iii) found inside leaf

(These percentages do not account for any small amounts of DDT which may have volatilised during the ageing period)

	Leaf					
	Light deposit			Heavy deposit		
	9 wks old	11 wks old	14 wks old	9 wks old	11 wks old	14 wks old
Standard W.P.						
(i) Rainwashings	64	61	61	80	74	75
(ii) Surface wax	7	6	3	10	8	14
(iii) Inside leaf	29	33	36	10	18	11
5% 'Lovo' W.P.						
(i) Rainwashings	32	45	37	21	26	19
(ii) Surface wax	34	29	26	66	62	66
(iii) Inside leaf	34	26	37	13	12	15

The following conclusions can be drawn from Table 7:

(1) The amounts of DDT removed by dipping the leaves in hexane show that an average of six times as much DDT remains on the surface and in the surface wax after 'rainwashing' aged deposits containing 'LOVO' compared with those without 'LOVO'.

(2) The amounts of DDT recovered by macerating the wax-stripped leaves with hexane show that similar amounts of DDT penetrate into the leaf whether the deposit contains 'LOVO' or not.

(3) Similar amounts penetrate whether the deposit is aged for 9, 11 or 14 weeks, so that after 9 weeks (at least) penetration into these leaves must be negligible.

(4) The amounts of DDT that penetrate into the leaf are not directly proportional to the amounts applied to the surfaces. Thus, the initial amounts applied in heavy deposits (20–30 μg DDT/cm²) were over six times that in light deposits (2–5 μg DDT/cm²), but the proportion of DDT that penetrated from the heavy deposits (average, 13%) after

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9–14 weeks represents only about twice as much DDT as the 33% (average) that penetrated from the light deposits.

Microencapsulation. Many different types of microcapsules were tested for stomach-poison action and for any contact toxicity effects that might result from ageing or wetting and high humidity. Capsular walls included cross-linked gelatin, gelatin/silicate, and polyvinyl alcohol/bakelite materials (sometimes double-walled), with internal phases of DDT dissolved in paraffin plus Aroclor, or in sperm wax. Some of the earlier types tested leaked their contents when aged for periods at high humidity, but types being currently tested have been free from such defects after several weeks under these conditions. Suitable stickers for use with these microcapsules in aqueous suspension are currently being tested in the laboratory. These include solutions of celluloses and alginates, but the most promising stickers found to date are various polyacrylates and polyvinyl isobutyl ethers. (Phillips and Gillham)

Toxicity of pesticides to honeybees

Poisoning of honeybees in the field. Thirty-nine samples of honeybees (*Apis mellifera*), alleged to be poisoned, were received from the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food, 15 fewer than in 1967. Of the 25 that contained insecticide, 14 reacted positively to our test for organophosphate insecticide poisoning, and three gave inconclusive results. The test for organophosphate insecticide measures residual cholinesterase after poisoning and not the insecticide residues themselves. Information supplied with these samples showed that four incidents were caused by spraying field beans in flower, one by spray drift from rape on to field beans in full flower, and another by spray drift from wheat on to flowering red clover. One, which involved the loss of bees from 69 colonies, was caused by spraying from the air 80 acres of rape in flower. Two were associated with the spraying of peas, and one spraying of strawberries in flower.

One sample gave evidence of poisoning by both organophosphate and BHC. BHC was found in three samples of which one was reported to be malicious damage. Gas chromatography showed traces of BHC in two further samples, but there was too little to react positively in our bioassay. One of these involved bees dying in a chimney where BHC smoke had been used 12 months before to remove a swarm. Two samples were poisoned with carbaryl used to thin apple blossom. The smaller number of samples of organophosphate poisoning than in 1967 presumably reflects the reduced need to spray against bean aphis in 1968. (Needham and Stevenson)

Aerial application of granules. Phorate granules were applied, by aeroplane, to three crops of beans while in flower, two by Mr. B. A. Cooper (National Agricultural Advisory Service, East Midland Region) and one in co-operation with the N.A.A.S. Eastern Region. Bees introduced to the crops were flying when the insecticide was applied. The limited observa-

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tions made supported the view that this method of applying insecticide does not seriously damage honeybee colonies. (Stevenson)

Poisoning on oilseed rape. In 1967 malathion and azinphos-methyl emulsifiable concentrates sprayed on oilseed rape in full flower, killed many foraging bees, whereas endosulfan wettable powder did not. A further trial was made in 1968 to see whether the endosulfan wettable powder was less toxic to honeybees because of its formulation or because it is less toxic to them than the other insecticides.

Emulsifiable concentrate and wettable powder formulations of both endosulfan and azinphos-methyl were each applied to seven acre (2·8 hectare) plots at the corners of a 100 acre (40 hectare) field of oilseed rape in full flower (Table 8). Five colonies were placed at each corner early on the day of spraying and the bees were foraging the areas when they were sprayed, which was between 11·00 and 15·45 hours and in good weather. Table 8 shows numbers of dead bees collected in traps placed in front of each hive for 43 days after the spraying. The numbers killed by azinphos-methyl during the 24 hours after spraying do not include some that died on the ground around the hives.

TABLE 8

Dead honeybees collected from hives at the experimental insecticide spraying of oilseed rape (average numbers of dead bees per hive per day)

(Insecticide applied on 3rd July 1968)

Days after treatment	Azinphos-methyl				Endosulfan			
	Wettable powder		Emulsifiable concentrate		Wettable powder		Emulsifiable concentrate	
	Workers	Drones	Workers	Drones	Workers	Drones	Workers	Drones
0	3·0	1·0	6·0	0·8	39·2	0·2	5·2	0
1	2437·0	7·0	997·0	2·2	119·8	0·4	58·4	4·2
2–5	108·2	2·5	25·3	1·3	14·7	0·4	9·6	1·0
6–7	23·4	8·2	35·7	8·9	36·6	7·0	30·7	5·0
8–9	26·1	8·3	41·2	10·3	64·5	8·8	55·8	4·5
10–12	21·6	7·4	37·1	5·8	22·0	3·3	18·0	5·3
13–15	6·6	1·9	5·9	2·3	8·2	1·7	10·3	7·6
16–20	11·9	8·7	12·5	3·2	12·6	3·6	5·4	5·1
21–29	6·6	2·7	9·2	4·4	15·2	11·0	3·7	2·3
30–43	11·5	6·0	11·1	5·6	24·7	8·2	23·2	6·8

1·5 lb azinphos-methyl wettable powder (6 oz a.i.) per acre (420 g a.i. per hectare)
27 fl oz azinphos-methyl emulsifiable concentrate (6 oz a.i.) per acre
1·3 lb endosulfan wettable powder (7·3 oz a.i.) per acre (510 g a.i. per hectare)
21 fl oz endosulfan emulsifiable concentrate (7·3 oz a.i.) per acre
all in 30 gals water per acre (300 litre per hectare).

Both formulations of azinphos-methyl killed many more bees than did endosulfan. The condition of the colonies exposed to endosulfan deteriorated slightly during the trial, and as in 1967, the numbers of dead bees collected increased slightly towards the end of the observation period.

The suction sampler described elsewhere (p. 189) was used to collect samples of the insect population. There were few adult pollen beetles

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(*Meligethes*) present during the trial; the number of larvae was less after the sprayings than before, whereas they increased slightly in the unsprayed centre of the field. The catches of predatory and parasitic insects did not provide evidence on the relative toxicity of the different insecticides to these beneficial insects. (Needham and Stevenson)

Apparatus and techniques

The gas–liquid full flow control valve was further developed for multi-channel switching of a wide range of slurries and suspensions, and a provisional patent has been filed by the National Research Development Corporation (9.4).

The patents on the variable sieve and the atomiser heads were completed and an agreement has been signed for the commercial development of the sieve. Experiments were started to measure the efficiency of the variable sieve using dry materials over a wide range of sizes. Work on the automatic flow counting of insects from the insect-sorting apparatus led to the development of a high impedance transistorised portable counter unit (9.5). The counter will register with input impedances in excess of 5 megohms and probe potentials of 2 volts at 2 microamps. With this order of impedance, lesions in leaves can be counted; the leaf forms one electrode and with a felt tip pen as the other, the lesions are marked as they are counted. A further development was the introduction of a keyboard and multi-counter unit that enables the direct or indirect channelling of a count so that different phenomena can be recorded simultaneously, and a running total when required. As it is a portable self-powered unit, it may be of use in counting insects on field crops.

A portable suction sampler was designed, powered by an internal combustion engine, and successfully used to sample the insect population on oilseed rape crops on hedges and grassland. The main aim was to develop a portable sampler that retained a high vacuum even under 'blocked load' conditions, such as may happen in removing insects from dense foliage or grassland. To achieve the vacuum required a two-stage impeller unit was made. With impellers of 11·5 in. in diameter a blocked load vacuum of 50-in. water column was obtained with rotor speeds of 4800 rpm. Prolonged testing and the high axial thrusts developed by this pressure-reduction eventually distorted the rotors, but the vacuum achieved was greater than required and the apparatus is being re-designed for use in 1969. The modifications should increase the flow rate of air at the expense of some of the vacuum head, and almost eliminate the axial thrust on the motor shaft. They should also simplify and lighten the unit.

Laboratory tests and scale work on impellers and casings to establish the sampler modifications required, suggested the development of an impeller unit for use on recording volumetric spore traps. The efficiency and comparative simplicity of this type of unit against positive displacement pumps has decreased power requirements from a minimum of 11·4 watts to 5·2 watts. When arranged so that the impeller casing can rotate with the spore trap head, all the present shaft seals can be dispensed with, and the apparatus has no rotating or micro-finished components associated

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with positive displacement pumps. As the flow is non-pulsating a critical orifice is not required, and the comparative quietness of the unit is advantageous, especially when sampling within buildings. A high speed bench unit was also made using the same rotor and critical dimensions. This gave a readily adjustable vacuum up to 20 cm water column with a flow rate of 40 litres per minute, and proved satisfactory for use with an insect aspirator when sampling insects from laboratory cultures. (Arnold)

Staining insect central nervous system. In studies of the histology of the central nervous system of the cockroach *Periplaneta americana*, as a prelude to histochemical studies of insecticide action (*Rothamsted Report or 1967*, p. 169), the best staining was given by the Power (1943) modification of the Bodian protargol method. However, results were not consistent, and to try and make them so various changes in the staining conditions were tested.

In this technique, paraffin sections of central nervous system ganglia are impregnated twice in protargol (silver-protein) solutions containing metallic copper. After each impregnation, the sections are developed in a hydroquinone-sodium sulphite solution. The yellow stain so produced is intensified with gold chloride, followed by oxalic acid to give a bright red or purple stain selective for the nerve fibres.

The most important factors affecting staining proved to be the copper content of the impregnation baths, the sulphite concentration in the developer and section thickness. The three are interrelated and a change in one can be balanced by a change in one or both of the others. To produce consistent results, increase in section thickness requires increase in copper or sulphite concentration, but with constant section thickness, copper and sulphite concentrations are inversely related. With 10μ sections best results are obtained with a copper concentration of 1·3 g in 65 ml of 2% protargol solution and a sulphite concentration of 4% (of the hydrate) in 1% hydroquinone. For 20 μ sections, when 1·3 g of copper are used, 10% sulphite is needed. Under these conditions results are consistent. (Gregory)

Systemic insecticides

Sorption by soil. Studies on the sorption of dimethoate were extended (*Rothamsted Report for 1967*, p. 177) using a much wider range of soils. Sorption was small and difficult to measure using gas–liquid chromatography, and greater accuracy was obtained by using P-32 labelled radiotracers. Amounts sorbed were determined by counting aliquots of solution in liquid G.-M. tubes before and after equilibration with soil, using a Panax GX9 autoscaler. Although sorption was small, there were considerable differences between different soils. The soils are being analysed in detail to see what properties determine sorption, but preliminary examination suggests that the organic matter content is important as with other organophosphorus insecticides (*Rothamsted Report for 1965*, p. 168). For example, amounts of dimethoate held by the soil in equilibrium with solution concentrations of 500 ppm are 0·58 mg/g for a fen peat soil and

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0·03 mg/g for soil from the unmanured plot on Broadbalk which contains very little organic matter. (Graham-Bryce and Etheridge)

Diffusion of organophosphorus insecticides in soil. The influence of moisture content on the diffusion of disulfoton and dimethoate in soil from the Broadbalk FYM plot was studied using the technique described in last year's *Report* (p. 178). Dimethoate is relatively involatile, soluble in water and only very slightly sorbed, so that when added to soil the proportion partitioning into soil air is very small. The contribution of vapour movement to the total diffusion rate should therefore be negligible and the soil solution network should be the dominant pathway for diffusion, even though diffusion in air is much faster than in solution. In support of this, the apparent diffusion coefficient of dimethoate increases rapidly with increasing moisture content, from $3{\cdot}31 \times 10^{-8}$ cm²/s at 10% volumetric moisture content to $1{\cdot}41 \times 10^{-6}$ cm²/s at 43% moisture content. The experimental values agree well with values calculated by modifying diffusion coefficients in free solution to allow for soil moisture, sorption and the tortuosity of the diffusion pathway through the pores, assuming movement only in the soil solution.

In contrast, disulfoton is more volatile, less soluble and more strongly sorbed by soil. The stronger sorption causes smaller diffusion coefficients than with dimethoate ($2{\cdot}83 \times 10^{-8}$ cm²/s at 41% volumetric moisture content) but vapour diffusion is much more significant so that the value does not change much as moisture content decreases ($2{\cdot}74 \times 10^{-8}$ cm²/s at 8% volumetric moisture content). The contribution of vapour diffusion apparently increases with decreasing water content in approximately the same proportion as diffusion in solution decreases. (Graham-Bryce)

Factors influencing the effectiveness of granules applied to beans. Granular formulations are being used increasingly on field beans and are less dangerous to honeybees than spray formulations. In the dry summer of 1967, however, there were reports that phorate granules were less effective than previously in controlling *Aphis fabae*, and it was suggested that wet conditions were needed to release the insecticide from the granule and allow it to be taken up by the plant. The effects of environmental factors on the performance of granular formulations were therefore examined in controlled environment rooms.

Beans were grown in tall earthenware pots containing light sandy soil from Woburn Farm. Sufficient water for normal growth was supplied from a sand/water reservoir to the base of the pots. In addition, simulated rain was applied when required from an overhead spray line fitted with hollow cone jets, which produced a fine rain at a rate of approximately 1·5 in. per hour. The usual amount given in experiments was 0·1–0·2 in. per day. Ten per cent phorate granules and 7·5% disulfoton granules were applied at 0·04 g a.i. per pot, by a 'pepper pot' method and the granules were confined to the area of the pot by fitting a flexible sleeve. Conditions in the growth rooms were: temperature, day 20° C, night 15° C; relative humidity, day 70%, night 82%; 16 hour illumination per day.

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The toxicity of the plants to aphids was assessed by caging *A. fabae* on leaf surfaces for 24-hour periods at intervals during the experiments.

In most experiments so far, disulfoton formulated on pumice was slightly more effective than phorate on fuller's earth and daily rainfall made plants more toxic than when they were watered only from below. However, the trends were often confused and several factors probably influence the results. For example, there is evidence for an initial vapour effect that enhances toxicity during the first few days after treatment. Also the proportion of granules lodging on the plant influences behaviour; granules applied to the soil at the base of the plant usually took longer to become effective than when applied to the foliage. These and other factors are being studied further. (Graham-Bryce, Stevenson and Etheridge)

Analytical work

Polarography of organophosphorus insecticides. Work elsewhere indicated that aqueous solutions of disulfoton and phorate gave reduction peaks with the cathode ray oscilloscope polarograph that could be used for analysis. We confirmed the presence of these peaks but found them erratic and therefore investigated how they were affected by different conditions. Much of this work was done using a special cell, kindly lent by Dr. B. Fleet of Imperial College, in which a calomel reference electrode is connected directly to the analytical solution. Disulfoton was studied in most detail.

The change of peak size with time in different buffer solutions over the pH range 3·6 to 12·9 suggested that the polarographically active species was not the parent insecticide, but the thiol ($HS.CH_2.CH_2.S.C_2H_5$) formed by hydrolysis. Support for this hypothesis was obtained by studying the polarography of a sample of the thiol synthesised quite independently of the insecticide by refluxing $C_2H_5.S.CH_2CH_2OH$ with HBr and thiourea, and also by separating and identifying some of the products of aqueous and alcoholic hydrolysis by partitioning with various solvents. By showing that the compound reduced seems to be the thiol and not the parent organophosphorus compound, these investigations emphasise that great care must be taken to standardise conditions when using polarography to analyse solutions of organophosphorus insecticides. (Graham-Bryce)

Control of aphids and virus diseases of lucerne and peas. Collaborative work on these problems is described in the report of the Entomology Department. (Etheridge)

Insect rearing. The following species were reared:

	PLANT FEEDERS
Homoptera	*Aphis fabae* (Scop.) *Myzus persicae* (Sulz.)
Hemiptera	*Dysdercus intermedius* Distant
Coleoptera	*Phaedon cochleariae* (F.)

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OTHERS

Orthoptera	*Blaberus discoidalis* (L.) *Periplaneta americana* (L.)
Lepidoptera	*Pieris brassicae* (L.)
Coleoptera	*Tribolium castaneum* (Herbst.) *Tribolium confusum* J. du V. *Trogoderma granarium* Everts
Diptera	*Drosophila melanogaster* (Meig.) and a wingless mutant *Musca domestica* L. Strains. *bwb*; *ocra*; *ar*; *ac* SRS SKA (diazinon-selected) R2; *ocra*; *ar*; *ac* (R2 penetration factor) *bwb*; R3; *ar*; *ac* (R3 sesamex susceptible factor) *bwb*; *ocra*; *a* DDT-ase; *ac* (low aliesterase and DDT-dehydrochlorinase) *bwb*; *ocra*; DR4; *ar*; *ac* (dieldrin resistant) *organotin*; *stw* (homozygous for R2) 213b (pyrethrum resistant) *bwb*; R3; *ac*; *ac* (R3 sesamex susceptible, low aliesterase)

Commercial seed dressings. In collaboration with workers at the Plant Pathology Laboratory of the Ministry of Agriculture, Fisheries and Food and the National Institute of Agricultural Engineering, work was done to find the causes of the small amount of insecticide retained on wheat seed treated with commercial BHC dressings. Experiments were made in several premises to examine all stages of the seed-dressing process. Most seed-dressing machinery consists of a mixer and automatic scales at the bagging-off point. In all methods studied, a header-bin was interposed between the mixer and the scales. For the safety of the workers, extraction vents are introduced in several positions in the machinery.

Sampling from the mixer-outlet, beneath the header bin and from the top, middle and bottom of the bag, showed that there was no significant loss in retained insecticide between the mixer and the bag. Measured doses of insecticide powder are automatically dispensed on to automatically weighed-out quantities of grain and the two mixed before delivery to the bin. Checks showed little variation in the dose of powder delivered, the weight of grain delivered or the mixing time.

Partially or wholly blocking the extraction system produced no immediate effect, but after a time the dressing on the surface of the seeds at the sides of the bin increased and this seed was delivered to the last few bags of the run.

Further tests showed that much of the insecticide dressing separated from the grain while leaving the mixer and entering the bin, and this seems to be the main cause of the small amount retained on the seeds. In the two bins examined, the separated powder does not reach the bag unless the bin is emptied.

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A further experiment to provide a general balance sheet under usual working conditions showed that an introduced 25·44 lb of 40% BHC powder in the powder hopper gave 18·40 lb in the bags and 6·25 lb extracted through the vents. Thus the ventilation system can provide a loss of approximately 25%, but this would be less if the powder adhered to the seed. It must be emphasised that the amount in the bags in this experiment does not give a measure of the dressing on the seeds, because of the separation that occurs.

The possible losses during transport over 90 miles to a farm and during drilling were studied. There was no apparent loss between the factory and the farm, but samples of seed taken from the coulters had 25% less than the seed put into the hoppers of the drilling machine.

About half the powder applied in the factories studied separated from the seed, and whereas some dressing adhered to the seed strongly, much did not. Some of the loose powder separates as soon as it encounters the air turbulence in the header bin; it then either lodges on the surface of seeds at the side of the tank or, in some bins, the lighter dust floats in the space above the seeds, finally to be removed *via* the extraction system. Blanking of the vents does not improve conditions because it has no effect on this separation, and if there were no extraction during the whole of a run, the last bags would probably contain too much because the dressing would accumulate in the header bin. More powder separates to the sides when the bin is large than when it is small. In two examples tested a seed loading of 250 ppm was obtained for the large bin compared with better than 300 ppm for the small bin. These results at two factories using the same type of dressing machine indicate the need for work on the design of a header bin to lessen the effect of turbulence. Also, it seems necessary to formulate the dressing so that a larger proportion adheres strongly to the seed. (Jeffs with G. Bell, G. Lloyd, R. Tuppen, M.A.F.F. and D. Comely, N.I.A.E.)

Aphids and field beans. Collaborative work is described in the report of the Entomology Department. (Stevenson)

Wheat Bulb fly (*Leptohylemyia coarctata* Fall.)

Wheat extracts and the behaviour of Wheat Bulb fly larvae. The behaviour of Wheat Bulb fly larvae in contact with extracts of wheat stems indicated a type of klinokinetic response: larvae turned back when their random movements brought them to the perimeter of the area containing wheat extract.

The biologically active material in methanol extracts of wheat stems was involatile, stable to mild heat and extreme pH; it was absorbed by a strong acid exchange resin, but not by an alkali exchange resin, suggesting a polar nature. Active methanol solutions were partially purified by passage through a column of Sephadex LH20; further fractionation was obtained by thin layer chromatography on cellulose. (Scott and Calam)

γ–BHC seed dressings. Work continued on how γ-BHC affects the growth of Cappelle wheat seeds and to what extent it protects young plants

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from attack by larvae of Wheat Bulb fly. The seeds were treated with organomercury fungicide alone (controls) or with one of five different amounts of γ-BHC, and the actual amounts of insecticide retained on the seeds were measured by GLC analysis. N.A.A.S. entomologists sowed the treated seeds in a sandy clay loam on 23 November and in a lighter soil, a sandy loam, on 15 November 1967. Both sites contained many Wheat Bulb fly eggs.

In the sandy clay loam only the largest dressing of γ-BHC, 46 μg insecticide/seed, decreased the plant stand. Dressings of 46, 24 and 11 μg/seed decreased attack by Wheat Bulb fly larvae and, judged by the number of healthy shoots in late March, the optimum dose was 24 μg/seed. However, there were fewest live larvae in plants grown from seed with 46 μg γ-BHC/seed and these plants improved to give the best plant stands in May. In the sandy loam, a different method of sowing was used and the amounts of γ-BHC on seeds *as sown* slightly exceeded those at the sandy clay loam site. In the sandy loam, even the smallest amounts of γ-BHC decreased the plant stand, and although the three largest dressings, 55, 30 and 14 μg γ-BHC/seed, decreased attack by Wheat Bulb fly larvae, no treatment had as many healthy shoots as the controls at any examination. (Griffiths and Scott)

Single row trials of insecticides. In collaboration with entomologists of the N.A.A.S. (Eastern Region and East Midland Region), seed dressings of a further eight experimental insecticides were compared with heptachlor and ethion standards for control of Wheat Bulb fly. Methyl cellulose was used to stick insecticides on seeds of Cappelle winter wheat at 0·1% and 0·5% a.i. to weight of seed, and short rows of treated seeds were sown, in autumn, (15 November and 21–22 November 1967) in randomised blocks on two sites containing many Wheat Bulb fly eggs.

Examination of plants during spring showed that R 42211 [*O*,*O*-diethyl *O*-(2-diethylamino-6-methyl-pyrimidin-4-yl) phosphorothioate] was about as good as ethion in protecting plants against Wheat Bulb fly attack and did not damage the plants. B 77488 (*O*,*O*-diethyl phosphorothioate *O*-ester with phenylglyoxylonitrile oxime) and B 80833 (*O*-methyl *O*-3,4-dichlorophenyl methyl phosphonothionate) were only slightly less effective than ethion and did not damage the plants at 0·1% a.i. to weight of seed, an amount corresponding to a commercial dressing. (Griffiths and Scott)

Control of wireworms (*Agriotes* spp.)

Because seed dressings of R 42211 and B 77488 gave promising results against Wheat Bulb fly (see previous section), an emulsifiable concentrate of R 42211 and granules of B 77488 at 2 and at 8 ppm a.i. to weight of soil were tested in the laboratory against wireworms. When first tested, soils treated with both insecticides killed many wireworms, but those with B 77488 granules retained their insecticidal activity longer. (Griffiths)

Sex attractants in click beetles. Jacobson *et al.* (*Science* (1968) **159** (3811), 208–209) identified a sex attractant in adults of the American

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sugar-beet wireworm (*Limonius californicus*). The compound, valeric acid, was said to occur in large amounts in unfertilised female beetles and to decrease rapidly after mating. To study whether British click beetles have similar attractants, individuals of *Agriotes obscurus*, *A. sputator* and *A. lineatus* were collected from pitfall traps in the field from the beginning of April till the third week in June. None of the females, even those caught in early April, was attractive to males, and we could not demonstrate any response by male click beetles to solutions of fatty acids reported to attract males of American click beetles.

To obtain freshly emerged, unfertilised female beetles we reared individual large larvae through the pupal stage in the laboratory. The few females of *Agriotes* spp. obtained in this way failed to mate with males in the laboratory. However, several females and one male of *Athous bicolor* also emerged and these mated readily. A preliminary chemical examination of an ether extract from the abdomen of an unfertilised female of *Athous bicolor* showed constituents that should be amenable to analysis by GC-MS methods. (Callow and Griffiths)

Fungicides

Tests of fungicides to control blight (caused by *Phytophthora infestans*) on potato haulms and tubers, and cereal take-all (caused by *Ophiobolus graminis*) continued, and began on the control of potato common scab (caused by *Streptomyces scabies*). Most of the materials were kindly given by the makers. The following code numbers are used: Dow 'M2452' for *O,O*-diethyl phthalimidophosphonothioate and Du Pont '1991' for 1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester.

Laboratory tests

Potato blight. Organo-tin compounds were formulated as aqueous saponin suspensions, and tested for possible control of blight on potato leaflets (for methods, see *Rothamsted Report for 1967*, p. 187). Each compound was compared weight by weight with fentin acetate, formulated in the same way, on several occasions. Table 9 shows the results. The following other compounds were almost inactive: dibutyltin di(isooctyl thioglycolate), di(lauryl mercaptide), dioleate, distearate, maleate, sebacate and succinate; dioctyltin dichloride; and diphenyltin sulphide. Differences in the stability of the suspensions may account for some, but not all, of the differences in effectiveness. Only tributyltin acetate damaged the leaflets as much as fentin acetate.

Thus, many dibutyltin compounds were, in our tests, about 15–20 times less effective than fentin acetate for controlling potato blight. Tributyltin acetate, the most phytotoxic compound tested, was surprisingly ineffective considering published results on other fungi *in vitro*. The most effective compound tested, bis(triphenyltin) sulphide, is considerably less toxic to mammals than fentin acetate; the next best compound, diphenyltin dichloride, is related to possible breakdown products of fentin acetate. (McIntosh)

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TABLE 9

Relative effectiveness of organo-tin compounds for control of P. infestans

Compound	Effectiveness relative to fentin acetate (=100)
Dibutyltin adipate	2
,, diacetate	7
,, dibenzoate	5
,, dibutyrate	7
,, dichloride	8
,, di(2-ethyl hexoate)	6
,, dilaurate	7
,, di(methyl maleate)	8
,, dipropionate	5
,, disalicylate	4
Tributyltin acetate	5
Diphenyltin dichloride	18
Bis(triphenyltin)sulphide	40

Potato common scab. Pot tests, in which Majestic plants were grown in chemically-treated scab-infested soil from Woburn, gave variable results. We hope to improve and continue these tests. (McIntosh, with Lapwood, Plant Pathology Department)

Nematodes. Dibutyltin dilaurate is widely used in U.S.A. to control helminths in chickens; however, it had no effect on tomato root-knot nematodes when a suspension was applied as a soil drench at 256 ppm of a.i. (McIntosh, with Jones, Nematology Department)

Cereal take-all. Various fungicides (drazoxolon, folpet, triamiphos, quintozene, Dow 'M2452', and Du Pont '1991') were applied in three ways to winter wheat grown in pots containing soil expected to be infested with the take-all fungus, but their effect on the disease could not be measured because the untreated plants failed to control the disease. (McIntosh, with Slope, Plant Pathology Department)

Field trials

Potato-haulm blight. In a trial at Rothamsted with the variety King Edward, haulms were sprayed at 70 gal/acre on 20 July, when blight was just beginning (in general 0·1% destroyed, with some primary foci); haulms were burnt-off on 31 August. Treatments were: A, 'Brestan 60' at 0·03% fentin acetate; B, 'Brestan 60' at 0·01% fentin acetate; C, as B but with 1% wax added (see treatment E in *Rothamsted Report for 1965*, p. 175); D, dibutyltin dilaurate at 0·1% in 0·03% saponin suspension; E, bis (triphenyltin) sulphide at 0·04% in 0·03% saponin suspension; and N, unsprayed. Yields of total tubers in tons/acre were: A, 13·46; B, 12·66; C, 13·08; D, 12·50; E, 12·98; N, 12·09 (5% LSD = 1·08). Thus, with one spraying, only fentin acetate at 0·03% significantly increased yield.

Potato-tuber blight. In a microplot trial with the variety King Edward at Rothamsted, fungicides were applied to the soil or stems in an attempt to protect the tubers from infection by spores washed down from haulms

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to soil. The chemicals (with rates in lb a.i./acre) were: copper oxychloride (9), fentin acetate (0·6), tetrachlorisophthalonitrile (3), captafol (3) and tetraphenyltin (0·6) placed, as plaster of Paris granules, on the soil close to the stems in June, or (except tetraphenyltin) sprayed on the lower stems in early July. Blight had killed 50% of the haulms by 14 August. The proportion of tubers blighted at harvest ranged around 5%, but no treatment significantly affected it. (McIntosh)

Potato common scab. A trial at Woburn with the variety Maris Piper tested the control of common scab by soil-treatments before planting. Dusts were applied to the soil on 11 April; all plots were rotavated within 1 hour of application, and potatoes were planted the same day. Scab indices (the proportions of the skins disfigured by scabs) were calculated at harvest from samples of 50 tubers per plot (Table 10).

TABLE 10

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

Treatment	Nominal rate, lb a.i./acre	Total tubers, tons/acre	% Ware	Scab index
quintozene	150	14·22	91·9	2·98
captan	150	12·75	91·3	23·32
captan	50	12·04	89·6	27·00
Dow 'M2452'	150	10·36	84·4	18·99
drazoxolon	150	11·16	85·5	12·74
untreated	—	12·62	90·2	25·83
5% LSD		1·36	3·7	6·82

Some damage (dwarfing and bronzing of leaflets) from Dow 'M2452' was noticed in June, but was not noticeable in July; the other treatments caused no visible damage. Quintozene almost eliminated scab, and significantly increased yield, possibly by controlling *Rhizoctonia solani* as well; captan had no effect; Dow '2452' and drazoxolon significantly decreased the scab index but also both yield and % ware. (McIntosh, with Lapwood and Hide, Plant Pathology Department)

Staff and visiting workers

K. A. Lord returned from a year in Pakistan helping the Department of Plant Protection of the Ministry of Agriculture. D. C. Griffiths and C. Potter attended the 13th International Congress of Entomology in Moscow; C. Potter read a paper to the section of chemical control and toxicology. C. Potter visited Brazil, Argentine and Paraguay as a consultant to the Food and Agriculture Organisation of the United Nations on resistance of insects to insecticides. Visiting workers included Dr. T. D. Mukerjea of the Tea Research Association, Assam and Miss M. Mankowska of the Polish State Institute of Hygiene.

The department collaborated with the Plant Pathology Laboratory of the Ministry of Agriculture, Fisheries and Food in giving a course on insecticides and fungicides to members of the National Agricultural Advisory Service.

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