ROTHAMSTED RESEARCH

Rothamsted Report for 1966

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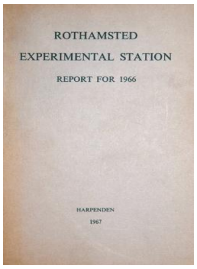

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

R. K. Callow and D. H. Calam were appointed to work on insect pheromones, and D. W. Eveling left. I. F. Henderson and E. S. El Basheir Mohammed obtained the Ph.D. degree of London University.

Physiological work on how organophosphorus insecticides act, showed that the symptoms of poisoning in the whole insect are correlated with loss of function and inhibition of cholinesterase in specific regions of the insect's nervous system. In other regions of the system death was not associated with loss of function.

Resistance to diazinon seems unlikely to be because this substance is metabolised faster, but what seems important is the increased metabolism of diazoxon, the potent anticholinesterase to which diazinon is converted in the insect.

The use of organophosphorus insecticides produces resistance to DDT in insects never exposed to this organochlorine compound. The major cause of DDT resistance in such strains differs from that in DDT selected strains. Resistance to DDT and organophosphorus insecticides by organophosphorus-selected strains has probably one common factor. This work suggests that where organophosphorus insecticides have produced resistance, organochlorine compounds may not be effective alternatives. This emphasises the need for effective insecticides of different chemical constitution and gives added importance to our work on synthetic pyrethroids. The National Research Development Corporation has protected the work done by patent applications and is making arrangements for commercial development of them in this country and abroad.

Work on insect pheromones is as yet largely confined to bees, but will be extended to those affecting the behaviour of other insects to see whether the knowledge gained can be applied in pest control.

Work in collaboration with the National Agricultural Advisory Service to find substitutes for the persistent organochlorine compounds to control wheat-bulb fly progressed well. Two compounds gave results comparable to dieldrin, and a third seems equally promising. The search for substitutes to control wireworm has been less successful, but laboratory tests have selected some compounds worth further study.

During the course of work on the chemical control of wheat-bulb fly considerable variation was found in the amount of chemical on dressed seeds. This may account for failures in control previously attributed to other causes, and we are attempting to develop techniques that will ensure reproducible, uniform seed dressings.

An objection to using insecticides to control black aphis on field beans, which we previously showed could greatly increase yields, was that under some conditions bees would be killed, and our examination of dead bees sent to the Ministry of Agriculture indicates that this is the largest single

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cause of bee poisoning. Collaborative work with the Bee Department shows that if granules of systemic organophosphorus insecticides are used, instead of sprays, bees are not harmed.

Insecticides

Poisoning by organophosphorus compounds and the causes of resistance

Sorption of insecticides by insect tissues. To find the proportion of the total amount of poison applied to insects that fails to reach its site of action, its sorption by insect tissues was studied.

(*a*) ***Conditions of sorption of diazinon.*** The sorption and desorption of diazinon in aqueous conditions by macerated eviscerated abdomens of female flies was examined using ^{14}C labelled diazinon. Ten eviscerated abdomens, dry weight about 10 mg, absorb about half the diazinon from 1 ml aqueous solution, indicating a distribution coefficient of about 100 : 1 in favour of eviscerated abdomens. The distribution is independent of the concentration of diazinon in the aqueous medium. The amount sorbed after 24 hours was the same as after 15 minutes, indicating a rapid equilibration. Desorption was equally rapid. The distribution coefficient was similar for both sorption and desorption, indicating that the process is completely reversible.

The amount of diazinon sorbed was not affected by washing the insect cuticle with cyclohexane, suggesting that cuticle waxes are not important in the sorption process.

(*b*) ***Sorption of diazoxon.*** The uptake of diazoxon by fly tissues was examined because diazinon is strongly sorbed and because it is the anti-cholinesterase compound formed from diazinon, so that its sorption would be expected to affect toxicity more than sorption of diazinon. The procedures used differed from those with diazinon, because radioactive diazoxon was not available and new apparatus made it possible to assay diazoxon by gas chromatography.

Whole flies were ground with 0·05*M*-Tris buffer pH 7·0. To test for sorption the solids were separated by centrifugation and resuspended in buffer containing diazoxon. The solids were then separated from the aqueous solution by centrifugation. To test for desorption, these solids were suspended once more in Tris buffer and again separated by centrifugation, when the amount of insecticide extracted was assayed.

The buffer solutions separated from the flies' tissues were shaken with an equal volume of methyl isobutyl ketone, which quantitatively extracted the diazoxon, which was then assayed by gas chromatography using a 5-ft $\times$ $\frac{1}{8}$-in. column of 5% SE30 on chromosorb W at 200° C and nitrogen as carrier gas and a phosphorus detector. The fly tissues sorb diazoxon, although less than diazinon. The insoluble materials from 20 flies sorb about one-third of the diazoxon from 1 ml of solution in Tris buffer pH 7·0 containing amounts of diazoxon ranging from 10 to 1,000 µg. With smaller amounts of diazoxon a larger proportion seems to be sorbed by tissues, so greater sorption may be expected at the concentrations at which

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diazoxon is effective as an inhibitor of cholinesterase ($10^{-8}M = 0{\cdot}003$ μg/ml and less). Diazoxon, like diazinon, is rapidly sorbed by fly tissues, and the amount sorbed did not change significantly with time from 2 to 45 minutes. Longer times were not examined because diazoxon decomposes slowly in aqueous solution. Diazoxon, like diazinon, seems to be desorbed from tissues when they are resuspended in buffer which does not contain insecticide.

When insects are poisoned by diazinon some diazoxon is formed, so there is need to know how sorption of one is affected by the presence of the other, especially diazoxon by diazinon. In the concentration ranges 10–100 μg/ml diazoxon and 3–30 μg/ml diazinon, which are greater than those in poisoned insects, no interaction between the sorption of the two insecticides were observed, and at smaller concentrations, the presence of one compound is even less likely to affect the sorption of the other. (Lord)

Decomposition of diazoxon. It seems unlikely that decomposition of diazinon is an important cause of resistance in our SKA strain of housefly (*Musca domestica*), because only a small proportion is metabolised in either the SKA or susceptible strain. Diazoxon, the oxygen analogue of diazinon, is formed in small quantities in tissues, and the rate it is formed and destroyed seems more likely to affect resistance, because, unlike diazinon, it is a potent cholinesterase inhibitor. The rate insecticides penetrate into different strains of houseflies is likely to differ and to make decomposition studies of diazoxon difficult to interpret. To avoid these uncertainties, decomposition was studied in suspensions of macerated flies, and the amount of diazoxon remaining unchanged was determined by the cholinesterase inhibition method described by van Asperen, Mazijk and Oppenoorth (*Entomologia exp. appl.* (1965), **8**, 163). Flies of the susceptible strain did not decompose diazoxon, but the SKA strain decomposed about 0·035 μg diazoxon per fly per hour at 25° C. Decomposition was not affected by the concentration of diazoxon over a more than ten-fold range. Ability to decompose diazoxon seems to be an important cause of resistance, because the amount decomposed in an hour is similar to the dose that kills either resistant or susceptible houseflies when iniected. (Lord and Gwiazda)

Dominant factors responsible for resistance to diazinon in the SKA strain of houseflies. The two dominant factors of resistance to diazinon in the SKA strain were separated and each inbred independently into different strains of houseflies, using techniques described previously (*Rothamsted Report* for 1965, p. 156). These incompletely dominant factors (Table 1) are Dz_4 on the IV linkage factor, and gene *a* (low-aliesterase) on the V linkage group. A third factor, a recessive that confers only slight resistance (R.F. *c.* 4) was provisionally allocated to the II linkage group.

The separated dominant factors give a similar and rather slight resistance to diazinon (Table 1) and very little resistance to diazoxon (*c.* × 3–5), but differ in their response to diazinon in the presence of sesamex. Sesamex almost eliminates resistance to diazinon and diazoxon in strains with Dz_4 only, but increases resistance to diazinon by a factor of ×2, and has no

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TABLE 1

Results of bio-assays with diazinon on parents and progenies of flies of the ocra; ar SRS × SKA cross (dose in μg/fly)

Strain	Females: Regression equation*	Females: LD50 ± S.E.	Females: R.F.	Males: Regression equation*	Males: LD50 ± S.E.	Males: R.F
SKA	−23·78 + 5·73	14·13 ± 0·74	336	−25·28 + 6·76	5·50 ± 0·31	201
Susceptible recessive mutant strain (SRS)	−7·97 + 4·92	0·042 ± 0·00082	—	−8·21 + 5·74	0·027 ± 0·00054	—
F1 (SKA × *ocra*[1]; *ar*[2] SRS)	−15·63 + 4·90	1·58 ± 0·31	38	−13·91 + 4·95	0·65 ± 0·15	24
ocra progeny of ♂ F1 (SKA × *ocra*; *ar* SRS) × ♀ *ocra*; *ar* SRS	−9·27 + 3·42	0·51 ± 0·041	12	−11·26 + 4·45	0·34 ± 0·026	13
ar progeny of ♂ F1 (SKA × *ocra*; *ar* SRS) × ♀ *ocra*; *ar* SRS	−12·65 + 4·96	0·36 ± 0·024	9	−10·91 + 4·47	0·28 ± 0·021	10
ocra; a†	−13·02 + 4·70	0·59 ± 0·0099	14	−13·75 + 5·48	0·32 ± 0·0063	12
ar; Dz_4†	−10·78 + 3·92	0·53 ± 0·013	13	−11·62 + 4·54	0·36 ± 0·0082	13

* In Normal Equivalent Deviates.
† Weighted mean result of several tests.
[1] *ocra*—recessive mutant on IV linkage group.
[2] *ar*—recessive mutant on V linkage group.

effect on diazoxon in strains with gene *a* only. *N*-propyl paraoxon and S-S-S-tri-butyl-phosphorotrithioate, both aliesterase inhibitors, synergised diazinon against susceptible, Dz_4 and gene *a* strains almost equally, showing that these synergists are not specific inhibitors for diazinon "phosphatase".

The cross-over rate of the visible recessive mutant *ocra* (IV linkage group) and Dz_4 is between 30 and 40%.

Genetics of resistance to DDT in SKA flies. Most organophosphorus-resistant strains, including SKA, are very resistant to chlorinated insecticides, especially DDT. The reason for SKA flies retaining DDT resistance while exposed to diazinon only for 9 years was determined.

There are three factors for resistance to DDT in SKA flies—two dominants, viz. DDT-ase (V linkage group), a factor on the IV linkage group, synergised by sesamex and identical to the factor described by Oppenoorth (1965), and a recessive, probably on the II linkage group. The location of the factors for resistance to both insecticides on the same linkage groups, and the likelihood that the factor on the IV and II linkage are common to both DDT and diazinon explains why selection with diazinon has maintained the resistance of SKA flies to DDT. DDT-ase is present in only about 15% of SKA flies. Most of the resistance to DDT is caused by the factor on the IV linkage group. (Sawicki and Farnham)

Inhibition of cholinesterase and loss of function in the nervous system of the American cockroach *Periplaneta americana* poisoned by diazoxon. The condition of the central nervous system in cockroaches poisoned with diazoxon was examined further. The LD90 to adult male *P. americana* was determined by topical application. Six μg diazoxon were applied in acetone, either to the abdomen or metathorax of the insects, and they were examined 0·5–24 hours later for cholinesterase inhibition and abnormal function in the sixth abdominal and metathoracic ganglia. Function in the nerve cords was examined electrophysiologically and related to the stage

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of poisoning shown by the insect. Cholinesterase inhibition in the same cords was later examined histochemically as described in *Rothamsted Report* for 1965, p. 160. In the sixth abdominal ganglion, nerve conduction from afferent components of the cercal nerve to the ascending giant fibres was examined, and in the metathoracic ganglion, conduction in the ascending giant fibre pathways. The effects of diazoxon on nerve conduction in these pathways resemble its effects on conduction in the cercal nerve-giant fibre pathways in the sixth abdominal ganglion, where it is assumed to affect the synapses. However, like Roeder and later workers, we have so far failed to demonstrate synapses histologically in ascending giant fibres traversing metathoracic ganglia.

Cockroaches first showed symptoms of poisoning about 1 hour after dosing on the thorax, and after 2 hours when dosed on the abdomen. All became badly affected 1–2 hours after first showing symptoms, and prostrate in 2–4 hours. None recovered after reaching this stage. Abnormal function occurred sooner in both the sixth abdominal and metathoracic ganglia when the diazoxon was applied near to them, though symptoms always showed first in the metathoracic ganglion, presumably because, as shown by *in vitro* experiments, this is about three times more susceptible to diazoxon than the sixth abdominal ganglion.

Metathoracic ganglia in severely affected insects always showed symptoms of poisoning and did not tend to recover normal function, even 24 hours after treatment. Symptoms in sixth abdominal ganglia were most severe about 7·5 hours after treatment at either site, and then moderated, even in prostrate insects. After 24 hours function was nearly or quite normal. Functionally, the condition of the whole insect corresponded more closely to that of the metathoracic ganglion than to the condition of the sixth abdominal ganglion, irrespective of where diazoxon was applied.

Cholinesterase activity in the nerve cords of treated insects decreased steadily to the level in ganglia treated directly with diazoxon until conduction was just blocked, but rarely became less, even in moribund insects. The recovery of function in sixth abdominal ganglia was not accompanied by a corresponding recovery of cholinesterase activity. The nerve cord in a recovered insect functioned almost normally, though its cholinesterase activity was less than normal.

Thus poisoning of cockroaches by diazoxon seems closely associated with cholinesterase inhibition in specific areas, whether or not the inhibition causes death, and the association of functional impairment and cholinesterase inhibition in metathoracic ganglia with death suggests a causal relationship.

By comparing the time taken to block conduction in the metathoracic or sixth abdominal ganglia of insects treated with diazoxon *in vivo* with the times taken by known concentrations of diazoxon to block conduction *in vitro* the probable concentration of diazoxon in contact with the nerve cord in an insect treated with the LD90 (6 μg) of this compound was calculated to be between 10^{-5} and $10^{-6}M$. (Burt and Gregory)

Penetration of acetyl choline into nerve cord of *Periplaneta americana*. Apparent discrepancies between the reported rates acetyl-choline penetrates

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into the central nervous systems of the cockroach, *P. americana*, and the housefly, *Musca domestica*, suggested that the nerve tissues of the two species might differ in their permeability to acetylcholine. Such a difference would be very significant in the study of nerve physiology and might affect the action of drugs on insect nervous systems. To find the reason for the discrepancies, the hydrolysis of acetylcholine and acetylthiocholine by cholinesterase in cockroach ventral nerve cords was examined biochemically and histochemically, and used to indicate the penetration of the substrates into the nervous system.

Biochemical measurements of the rate acetylcholine penetrated agreed well with recent estimates by workers using a radioactive tracer method, but indicated a barrier to free penetration that is removed by treating nerve cords with acetone; speedier entry after the treatment is shown by an increase of five to ten times in the rate acetylcholine is hydrolysed within the nerve cord. Treatment with acetone also removed a barrier to the penetration of acetylthiocholine. Histochemical tests showed that the free entry of acetylthiocholine into ganglia of nerve cords is probably prevented by the glial cell layer. They also indicated that the faster hydrolysis of acetylcholine after the connective tissue sheath of the nerve cord was removed probably reflected mechanical damage making small nerves and the superficial cells of the connectives between ganglia permeable to acetylcholine. After treating ganglia with acetone, acetylthiocholine reaches the central neuropile but penetrates it for only a short distance, probably because it is decomposed by the abundant cholinesterase in this region. (Lord, Gregory and Burt)

Action of synergists on the nervous system of *Periplaneta americana*. The unexpected action of SKF-525-A (*p*-diethylaminoethyl diphenyl propyl acetate) on the nervous systems of cockroaches (*Rothamsted Report* for 1965, p. 161) suggested that synergists, most of which, like SKF-525-A, are thought to act only on systems that metabolise toxicants within insects, might also affect insect nerve tissues directly, or might exert some of their synergistic effects at the site of action of the toxicant, which for most insecticides is probably the nervous system. Piperonyl butoxide and WARF (N,N-di-*n*-butyl-*p*-chlorobenzenesulphonamide), Sesamex (2-(3,4-methylenedioxyphenoxy)-3,6,0-trioxaundecane), which synergises DDT when used against resistant strains, were tested for action on the cockroach nervous system. Piperonyl butoxide acts like SKF-525-A; it gradually blocks conduction (though this effect can be temporarily relieved by increasing the stimulus voltage) and slowly suppresses spontaneous activity, but it is less active than SKF-525-A against axonic conduction. It causes "after-discharge" at an early stage of poisoning, and its effects are less easily reversed by irrigation with saline. Treatment with a $10^{-4}M$ solution in saline for 350–500 minutes sometimes failed to block conduction in cercal nerve and in giant fibres of the abdominal cord, but in sixth abdominal ganglia $10^{-5}M$ produced symptoms and $10^{-4}M$ blocked conduction within 100–125 minutes. Metathoracic ganglia were not much affected by $10^{-4}M$.

Sesamex seems only about one-tenth as active as piperonyl butoxide, but acts similarly. Even at $10^{-3}M$ it sometimes failed to block conduction

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in axons, but in sixth abdominal ganglia $10^{-4}M$ caused after-discharge and bursts of impulses leading soon to decreased excitability and to conduction-block after very long exposures (400 minutes). At $10^{-3}M$ it blocked conduction in two sixth abdominal ganglia in 34 and 50 minutes. However, its effects were quickly reversed by saline irrigation.

WARF had no effect on cercal nerve, giant fibres or sixth abdominal ganglia at $10^{-4}M$, the largest concentration that could be dissolved in saline.

Of these three compounds, clearly WARF is unlikely to have any direct action on nerve conduction *in vivo*. Sesamex seems unlikely to reach a concentration of $10^{-4}M$ near the nervous system of an insect after topical application at conventional rates, but piperonyl butoxide, which affects ganglia at 10^{-5}–$10^{-4}M$, might attain such concentrations in the haemolymph. (Burt)

The action of pyrethrum. Before studying the combined effect of pyrethrum and synergists on cockroach nerve, the action of pyrethrum alone was examined. As reported by previous workers, pyrethrum affects both axonic and trans-synaptic conduction; cercal nerve seems the most susceptible nerve tissue so far tested. It acts very quickly, first causing spontaneous repetition of impulses, soon succeeded by partial or complete conduction-block. Symptoms are sometimes reversed by saline irrigation. It is effective at very low concentrations. Thus, cercal nerve treated with $10^{-7}M$ shows symptoms in 2–7 minutes, and the height of the action potential is halved in 3–10 minutes; with $10^{-6}M$ symptoms appear in 1 minute and conduction is blocked in 1–4 minutes. (Burt)

Perfusion of cerci of *Periplaneta americana*. A convenient preparation for studying the effect of physiologically active compounds on the cercal nerves of the American cockroach was developed. The cercal nerves are said to be solely sensory, a useful feature for some physiological studies, and their enclosure by the cercus can be used to localise the site at which a compound is applied. The amputated abdomen of an adult male *P. americana* is pinned to a cork block ventral side up, with one cercus supported on a pad of plasticine. Enough cuticle is removed from the body cavity to expose the cercal nerves and the sixth abdominal ganglion, which is crushed. Two stimulating electrodes of electrolytically pointed tungsten wire are thrust into the distal third of the cercus, and action potentials are recorded from a pair of platinum–iridium alloy electrodes, one placed on the sixth abdominal ganglion and one, with a hooked tip, under the cercal nerve close to the ganglion. With another pointed tungsten wire, held in the hand, a third hole is made in the cercus proximal to the stimulating electrodes and a little smaller than the perfusing cannula, to make a tight joint and prevent perfusate escaping around the edges of the hole. The perfusing cannula is made from the finest cannula available commercially, 0·2 mm external diameter and 1·5 cm long; each cannula is electrolytically sharpened until its diameter is about two-thirds the original, and it is mounted on an Agla micrometer syringe driven by a motor unit (*Rothamsted Report* for 1965, pp. 159–160) revolving at 10 revs/hour and delivering 1·7 µl of fluid per minute. Tests with dyed perfusates showed that most of

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the fluid injected into segments 8–10 passes through the cercus proximally, and starts emerging into the body cavity within 2–5 minutes. The cercal nerve can therefore either be perfused continuously, or the treatment restricted to a short length of cercal nerve by making a single injection lasting about a minute. With practice the preparation can be set up quickly, so it is suitable for toxicological studies needing many tests. (Burt)

Distribution of cholinesterase activity in the central nervous system of the cockroach *Periplaneta americana*. Earlier work with whole ganglia (Burt and Gregory, *Rothamsted Report* for 1965) indicated that impairment of nervous function by the organophosphates diazoxon and diazinon might be related to localised inhibition of cholinesterase in the central nervous system of *P. americana*. Before local inhibition could be studied in detail, more precise information was needed on how cholinesterase activity is distributed in the central nervous system. This is being studied using serial frozen sections cut with a refrigerated microtome and stained for cholinesterase activity by the Gomori thiocholine method. To handle the many sections needed, grooved "Perspex" staining racks were devised that hold 17 of the cover-glasses to which the serial sections are attached. Early results indicate that most cholinesterase activity in the ganglia of the ventral nerve cord is in the central neuropile, where it is unevenly distributed. Outside the neuropile cholinesterase is much less, except for some neuron cell bodies very rich in it. The number and position of these differs in the various ganglia. (Gregory)

Insect pheromones. Some work on the isolation and identification of the chemicals responsible for aggregation of *Dysdercus intermedius* is described in the report of the Entomology Department. (Calam)

Persistence of chlorohydrocarbon insecticides

Effect of environment on the persistence of dieldrin crystals. The effect of air movement and temperature on similar dieldrin crystals formed from "Cellosolve" solution on glass plates was further studied at a wind speed of 2 mph and a temperature of 43–45° C, which is often encountered in the tropics. These crystals in deposits of 6 μg/cm² volatilised at a linear rate of 0·1 μg/cm²/day in still air at 21° C (65% R.H.) and of 24 μg/cm²/day at 43–45° C. The dieldrin, dissolved in a suitable solvent, was sprayed on to the glass surface and crystallised *in situ*. The solvent damaged the surface of cotton leaves (though "Cellosolve" was far less injurious than dioxan or benzene), and to avoid this, aqueous suspensions of dieldrin crystals were prepared and sprayed on the leaf surfaces. However, crystals broke on passing through the spray nozzle, and that of size range 75–150 μ became of the order of 10 μ. Glass plates were sprayed to give initial deposit densities of these approximately 10-μ crystals of 10 and 5 μg/cm². At 20° C (65% R.H.) they volatilised, as expected, at similar rates to those crystallised from solvents *in situ*. At 10 μg/cm² deposit density the rate was about 0·14 μg/cm²/day, and it decreased to about 0·08 μg/cm²/day at 5 μg/cm² deposit density.

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Effect of formulation on persistence. A new type of machine was designed for the "rainwashing" test so that not only the residue left on the surface after "rainwashing" but also the washings themselves from a DDT deposit on either a glass or cotton leaf surface could be collected for analysis. This not only serves as a check on residue analysis figures but is also designed for future work in measuring the rates at which deposits are washed off.

A fan-shaped jet of water from the mains supply coming at maximum pressure through a coarse T-jet (size 650067) delivers water to the surface, which is held 3 in. away. The jet, powered by an electric motor, sweeps across the surface, traversing a 3-in.-long path twice per second for 30 seconds, to give the equivalent of about 0·8 in. of "heavy rain". The whole is enclosed within a cylinder (1 ft long × 6 in. diam) which is sealed by a removable plate at the top. The open-ended bottom of the cylinder sits inside a funnel which delivers the washings to glass-stoppered test-tubes. The DDT is extracted from these washings by repeated shaking with small portions of hexane, and the DDT measured by gas–liquid chromatography.

Cotton leaves were sprayed with standard DDT wettable powder and with DDT wettable powders containing both 5 and 10% "Lovo 192" (a proprietary mixture of amine stearates) to give deposit densities about 7 μg DDT/cm². One set of leaves (excised) was cut from the plant and kept in the dark at 20° C and 65% R.H., so that they were moribund within a week. Measurements with fully functional leaves were obtained from another set rooted with hormone solution (indole acetic acid) and kept in nutrient solution in constant environment cabinets at 20° C and approximately 50% R.H. The cabinets proved unreliable and the leaves developed brown necrotic areas, so this series of tests was unsatisfactory and will be repeated. However, the results resembled those with excised leaves as given in Table 2.

TABLE 2

% DDT washed off excised cotton leaves by "rainwashing" of deposits (c. 7 μg DDT/cm²) formulated in different ways and aged for different periods

Weeks	Std.–W.P.	5% "LOVO"–W.P.	10% "LOVO"–W.P.
0	92	32	16
2	94	32	11
4	90	28	14
7	82	32	16
13	92	32	10

The amount of each formulation washed off dead leaves does not vary with the age of deposit, and adding these amounts of "LOVO 192" to wettable powders increases the resistance to "rainwashing" from cotton-leaf surfaces (three-fold and seven-fold respectively).

Tests were done to find how much of the DDT in these wettable powder formulations penetrated into cotton-leaves with time, and thus became unavailable as a contact insecticide. Both excised (dead) and rooted (living) leaves bearing these DDT wettable powder deposits were subjected to either: (*a*) "rainwashing", washing with hexane and finally extraction of residue from leaves with hexane; or (*b*) the same procedure but without

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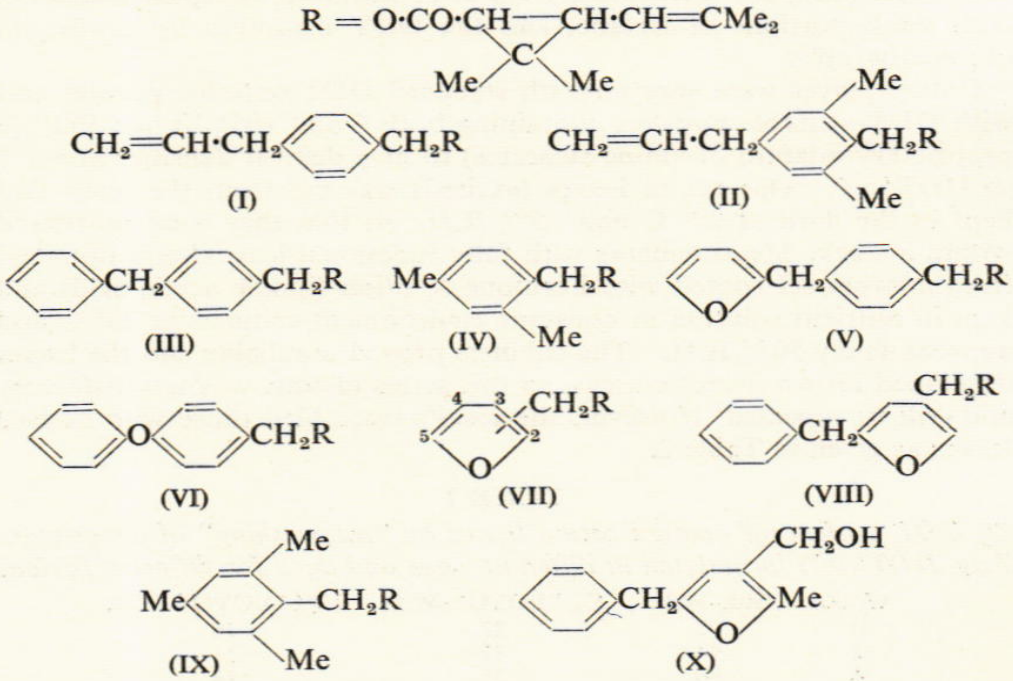
the first stage of "rainwashing". The residues were analysed for DDT by gas–liquid chromatography. The amount of DDT in the leaf increased in both living and excised leaves with time, and after 3 months at 20° C leaves sprayed with the standard wettable powder contained five times as much DDT as did recently sprayed leaves. More DDT was absorbed by leaves carrying deposits containing "LOVO" than from deposits of wettable powder alone, with 10% "LOVO" up to five times as much. (Phillips and Gillham)

Pyrethrins and related compounds

4-Allylbenzyl chrysanthemates (I) were earlier (*Annual Report* for 1965) found very toxic to houseflies (*Musca domestica* L.) but not to mustard beetles (*Phaedon cochleariae* Fab.). 4-Allyl-2,6-dimethylbenzyl chrysanthemates (II) were toxic to both species. These compounds were as active or more active against insects than the natural pyrethrins and allethrin.



Further variations of the basic structure (see *Rothamsted Report* for 1965) deduced to be necessary for great activity were prepared. 4-Benzylbenzyl chrysanthemate (III) was compared with 4-allylbenzyl chrysanthemate (I). It was as toxic as 2,4-dimethylbenzyl chrysanthemate (IV) to houseflies and more than twice as toxic to mustard beetles. Unlike alkenylbenzyl chrysanthemates (e.g. I), with benzylbenzyl chrysanthemates any alkyl groups on either aromatic ring diminished toxicity to both houseflies and mustard beetles. 4-Furfurylbenzyl chrysanthemate (V) was four times as toxic as the benzylbenzyl compound (III) to houseflies but only half as toxic to mustard beetles.

4-Phenoxybenzyl chrysanthemate (VI) was much less toxic than 4-benzylbenzyl chrysanthemate (III). This indicated that the methylene

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group between the rings was essential for toxicity and that the relative stereochemistry of the two benzene rings, similar in (III) and (VI), was not by itself sufficient to confer toxicity.

Because the furan ring approaches the size and shape of the cyclopentenolone ring in the natural pyrethrins much more closely than does the benzene ring, furfuryl and furylmethyl (VII), instead of benzyl esters, were next examined.

It was found that:

(*a*) Whether the chrysanthemoyloxymethyl group was at position 2 or 3 on the furan ring, polymethyl esters were more toxic than di- and mono-methyl compounds to both houseflies and mustard beetles.

(*b*) 5-Methyl-3-furylmethylchrysanthemate was more toxic than the 5-methyl-2-furfuryl ester.

(*c*) 4- or 5-Benzyl esters were more toxic than 4- or 5-methyl esters, respectively.

(*d*) *p*-Methyl groups on benzyl side chains diminished toxicity.

(*e*) Benzylfurfuryl and 3-furylmethyl chrysanthemates were more toxic than the corresponding esters with methyl substituents as well. A similar conclusion (above) was reached with benzylbenzyl chrysanthemates.

These conclusions indicated that 5-benzyl-3-furylmethyl chrysanthemates (VIII) should be effective compounds, and the (+)-*trans*-chrysanthemate proved to be the most toxic compound in this series. It was 55 times as toxic as the mixture of esters of the natural pyrethrins to houseflies and nine times as toxic as the natural esters to mustard beetles.

The table compares the toxicity to houseflies and mustard beetles of 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate and related compounds.

TABLE 3

Comparative toxicity of the new synthetic esters and other insecticides to adult houseflies and mustard beetles

Compound	*Musca domestica* L.[a] Weighted mean LD50 (μg/♀ fly)	Weighted mean relative potency[b]	Synergistic factor	*Phaedon cochleariae* Fab. Relative potency
5-Benzyl-3-furylmethyl (+)-*trans*-chrysanthemate (A)	0·0063 ± 0·0002	250 ± 10		260
5-Benzyl-3-furylmethyl (±)-*trans*-chrysanthemate	0·012 ± 0·0005	130 ± 8		160
5-Benzyl-3-furylmethyl (±)-*cis–trans*-chrysanthemate (B)	0·016 ± 0·0003	100		100 [c]
5-Benzyl-2-methyl-3-furylmethyl (±)-*cis–trans*-chrysanthemate (C)	0·029 ± 0·0008	46 ± 2		
5-Benzyl-3-furylmethyl (+)-*trans*-pyrethrate (D)	0·055 ± 0·003	25 ± 2		130
5-Benzylfurfuryl (±)*cis–trans*-chrysanthemate (E)	0·15 ± 0·003	11 ± 0·5		4·6
Natural pyrethrins	0·35 ± 0·01	4·6 ± 0·2		27
(A) + piperonyl butoxide (1 : 1)	0·0059 ± 0·0002	230 ± 10	0·98 ± 0·05	
(B) + piperonyl butoxide (1 : 1)	0·011 ± 0·0005	140 ± 8	1·4 ± 0·08	
(C) + piperonyl butoxide (1 : 1)	0·024 ± 0·001	67 ± 4	1·6 ± 0·1	
(D) + piperonyl butoxide (1 : 1)	0·040 ± 0·002	34 ± 2	1·4 ± 0·09	
(E) + piperonyl butoxide (1 : 1)	0·084 ± 0·003	19 ± 1	2·1 ± 0·1	
Allethrin				2·4
Parathion	0·017 ± 0·0006	85 ± 5		19
Diazinon	0·036 ± 0·002	42 ± 3		3·5
Dimethoate				1·7
Demeton-methyl				1·3

[a] Susceptible strain obtained through the courtesy of Mr. J. C. Wickham, The Cooper Technical Bureau.
[b] Derived from LD50 values for the compounds and (II) (taken as standard and given potency 100) in simultaneous comparisons. Drops (1 μl) of the compounds dissolved in acetone were applied topically to 3–4-day-old adult females (*Musca*) or adult males and females (*Phaedon*).
[c] LD50, 0·0007% w/v.

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It is significant that the toxicity of these compounds, containing only carbon, hydrogen and oxygen, is greater than that of some other insecticides regarded as very toxic to insects, such as parathion, aldrin, dieldrin and carbamates, some of which are also very toxic to mammals.

Although less toxic than the (+)-*trans*-chrysanthemate, 5-benzyl-3-furylmethyl (+)-*trans*-pyrethrate had a somewhat better knockdown power when estimated 10–15 minutes after treatment and killed more houseflies after 24 hours than the same dose of pyrethrum, when tested by the topical application technique.

The new compounds are protected by patents assigned to the National Research Development Corporation.

Piperonyl butoxide barely synergised the most active esters at a toxicant : synergist ratio of 1 : 1, but even when synergised to the greatest extent, the natural pyrethrins were still less toxic to houseflies than 5-benzyl-3-furylmethyl (±)-*cis-trans*-chrysanthemate.

In addition to the methyl-furfuryl and -furylmethyl chrysanthemates, methylbenzyl chrysanthemates were studied. Previously only 2,4- and 3,4-dimethylbenzyl chrysanthemates had been examined for insecticidal activity. 2,4,6-trimethylbenzyl-(±)-*cis-trans*-chrysanthemate (IX) was more toxic to houseflies and mustard beetles than 2,4-dimethylbenzyl chrysanthemate (IV) and was a crystalline solid. Therefore all the 19 monomethyl and polymethylbenzyl chrysanthemates, many of them new compounds, were synthesised. Bioassays are not yet complete, but the most accessible compound with the greatest activity seems to be 2,4,6-trimethylbenzyl chrysanthemate. The (±)-*cis–trans*-chrysanthemate was about 60% as toxic as allethrin to houseflies and mustard beetles, as well as being crystalline and much cheaper to make.

Chemical work. Benzylbenzyl alcohols, for esterification with chrysanthemic acid, were made by a route related to that used to prepare alkenyl benzyl alcohols (*Rothamsted Report* for 1965). Although allyl bromide couples with aryl magnesium halides, benzyl halides do not react under these conditions. However, phenyl magnesium bromide couples smoothly with benzyl chloride in *benzene*, and for the present work this reaction was extended. 4-Bromobenzyl bromide coupled with phenyl magnesium bromide in benzene (but not in ether), and benzyl chloride was reactive enough to react with 4-bromophenylmagnesium bromide. However, furfuryl chloride coupled satisfactorily with 4-bromophenyl magnesium bromide in ether. 4-Benzylbromobenzenes gave the corresponding benzyl alcohols when their Grignard derivatives were treated with formaldehyde, and this reaction was used to make a range of derivatives of 4-benzylbenzyl chrysanthemates from various benzyl chlorides and bromides, some available commercially.

4-Benzyl-2,6-dimethylbenzyl alcohol was synthesised by a route related to that used to make 4-allyl-2,6-dimethylbenzyl alcohol (*Rothamsted Report* for 1965). The rearrangement of *N*-allyl-2,6-xylidine to 4-allyl-2,6-xylidine with zinc chloride in xylene is analogous to a Claisen rearrangement of *O*-allyl-2,6-xylenol in which nitrogen has replaced oxygen. *O*-Benzyl phenols do not usually undergo the Claisen rearrangement and,

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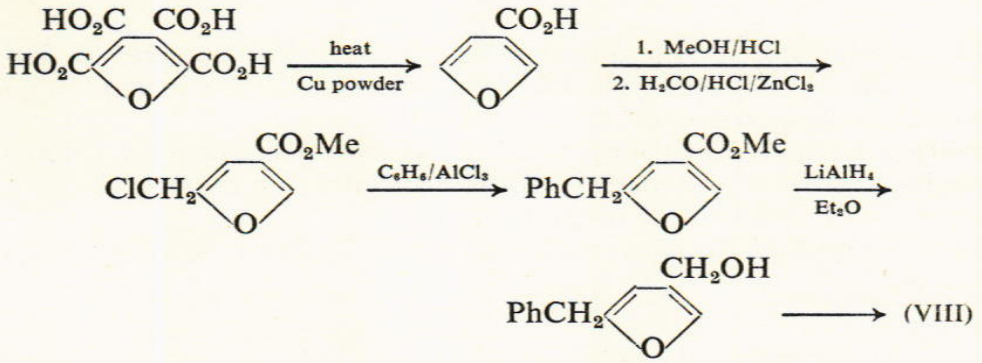
analogously, *N*-benzyl-2,6-xylidine gave no 4-benzyl compound on heating in the presence or in the absence of zinc chloride. However, when *N*-benzyl-2,6-xylidine hydrochloride was heated alone, 4-benzyl-2,6-xylidine was obtained in 65% yield, although a comparable experiment with *N*-allyl-2,6-xylidine hydrochloride gave no 4-allyl compound.

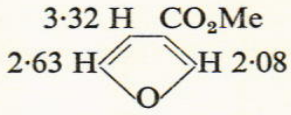
Unlike these alkenyl and benzylbenzyl chrysanthemates, there was no general route to the methyl and benzylfurfuryl- and -furylmethyl chrysanthemates. The alcohols were obtained by reduction of methyl esters of the corresponding acids; description of the preparation of known or easily accessible acids is not given.

Two syntheses of 5-benzyl-3-furylmethyl alcohol were developed. The first showed that the chrysanthemate had promising toxicity, and so the second route was devised to make larger quantities more readily.

Scheme I



Furan tetracarboxylic acid (scheme I) was not decarboxylated smoothly when heated in high-boiling coal-tar bases nor in quinoline, but gave the monocarboxylic acid smoothly by direct pyrolysis with a trace of copper powder. Methyl 3-furan carboxylate gave only the required 5-chloromethyl derivative. This was established from the nmr spectrum in which the protons had τ values allocated as shown. When chloromethylated only the proton signal at 2·63τ disappeared and all other values remained as before.



This synthesis was cumbersome, because three moles of carbon dioxide were shed from furan tetracarboxylic acid, and so much starting material gave only a small weight of product. Therefore the following alternative synthesis was developed (scheme II). The keto group in δ-phenyllaevulic ester was protected as the ethylene ketal, and the product was condensed with ethyl formate in the presence of sodium hydride. The sodium salt of the product cyclised directly with concentrated aqueous hydrochloric acid. The alcohol obtained with lithium aluminium hydride was best converted to the chrysanthemate by ester interchange with ethyl chrysanthemate in the presence of a small quantity of sodium ethoxide.

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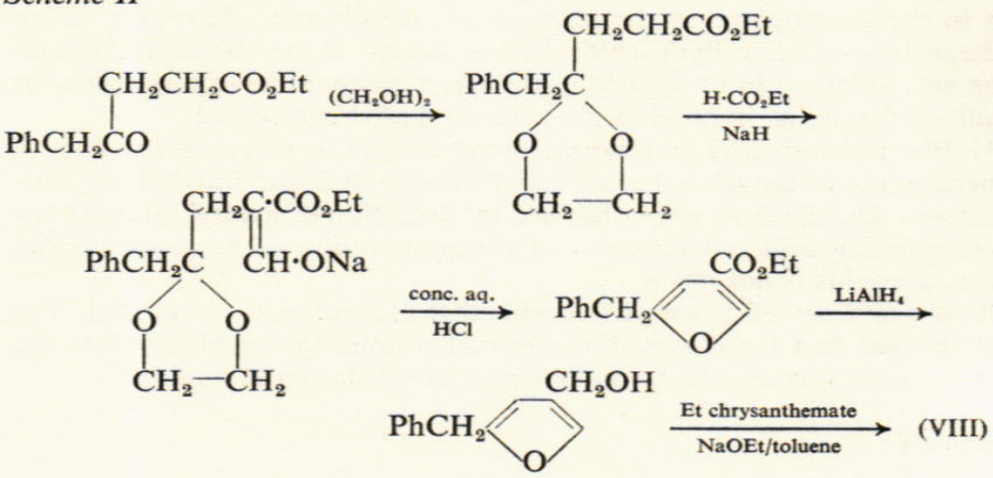
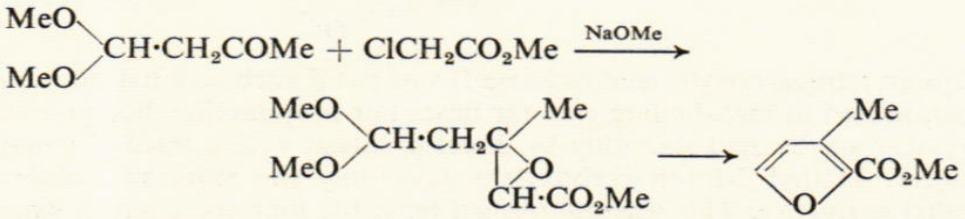
Scheme II



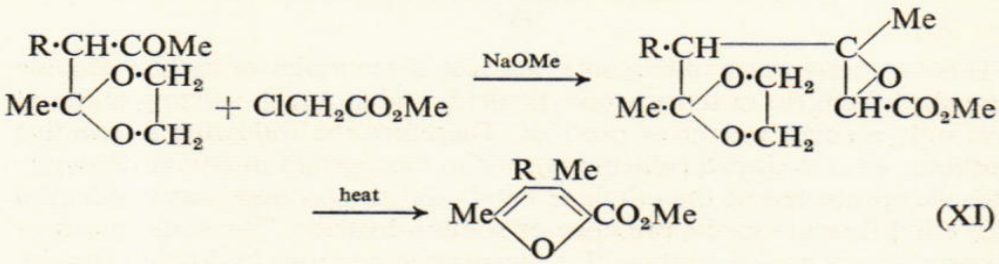
For a related synthesis, 5-methyl-3-furoic acid was obtained in improved yield by condensing ethyl formate with the ethylene ketal of ethyl laevulate, instead of the enol ether, used earlier.

5-Benzyl-2-methylfurylmethyl alcohol (X) was synthesised by chloromethylation of methyl 2-methyl-3-furoate and reacting the product with benzene in a Friedel–Crafts reaction.

Methyl 3-methyl-2-furoate was synthesised by the following known reactions:



This synthesis was extended for the present work. Acetyl acetone, used instead of 3-oxobutyraldehyde, did not itself condense with methyl chloracetate under the conditions normally used for the Darzen's reaction. However, the monethylene ketal of acetyl acetone (XI, R=H) reacted as shown, but the synthesis failed with the monoethylene ketal



of allylacetylacetone (XI, R=$CH_2CH{=}CH_2$).

Methylbenzyl chrysanthemates were made by esterification of the alcohols with (+)-*trans*- (naturally derived) or (—)-*trans* (by optical

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resolution) or (±)-*cis–trans*- (synthetic) chrysanthemic acid chlorides in the presence of pyridine.

2,3,4-, 2,4,5-, 2,4,6-Tri- and 2,3,4,5- and 2,3,5,6-tetra, and 2,3,4,5,6-penta-methylbenzyl chlorides were made by chloromethylating the hydrocarbons, and these gave chrysanthemates directly by reaction with the triethylamine salt of chrysanthemic acid.

2,3-, 2,5- and 2,6-Dimethylanilines gave the corresponding bromo compounds by the Sandmeyer reaction and then the benzyl alcohols by reacting the Grignard derivative with formaldehyde.

To make 2,3,6-trimethylbenzyl alcohol, 3-bromopseudocumene-5-sulphonic acid was made from pseudocumene (1,2,4-trimethylbenzene), and when hydrolysed this gave 2,3,6-trimethylbromobenzene and thence the benzyl alcohol.

3,4,5-Trimethylbenzoic acid was prepared by oxidising 3,4,5-trimethylacetophenone, obtained by isomerising acetomesitylene with aluminium trichloride. The acid was reduced to the benzyl alcohol with lithium aluminium hydride.

2,3,5-Trimethylbenzyl alcohol was also obtained by reducing the benzoic acid, which was the only product (from a Tiffeneau rearrangement) isolated when the Grignard reagent from 2,4-dimethylbenzyl chloride was treated with phosgene and then hydrolysed.

Pentamethylbenzyl chloride was obtained by chloromethylation (above). The alcohol was also synthesised by chlorinating hexamethylbenzene with sulphuryl chloride and selecting the required mono substitution product by fractionating the mixture of acetates obtained from the chlorides.

Methylbenzyl alcohols not specifically mentioned were made by conventional reactions. (Elliott, Farnham, Janes, Jeffs, Needham and Pearson)

Toxicity of insecticides to bees

Poisoning of honeybees in the field. Thirty-one samples of honeybees (*Apis mellifera*), alleged to be poisoned, were received from the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food, 35 fewer than last year. Of the 20 containing insecticide, 12 had organophosphates. Information supplied with these samples showed that six of them were affected by spraying bean-crops, five of which were sprayed from aircraft. Information on three of the other samples indicates that one was caused by sugar-beet spraying, one by strawberry spraying and the third by the aerial spraying of peas.

Paint containing dieldrin applied to the hive was responsible for one of the three cases of dieldrin poisoning. How the other two happened is unknown, but a second sample of bees from another hive in one of the affected apiaries contained both dieldrin and BHC.

The reason for the single poisoning with BHC is unknown.

One of the two samples containing carbaryl was of bees that had been foraging on waste from a jam factory where wasp baits containing carbaryl were used.

The bioassay on one sample indicated the presence of an insecticide, but the poison could not be identified.

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Applying organophosphate sprays to beans continues to be the main cause of bee poisoning. Most of this could be avoided by using a granular formation of insecticide (see Bee Department report, p. 215). (Needham and Stevenson)

Toxicity of insecticides to bumblebees. Acute toxicities of honeybees are determined in the laboratory (*Rothamsted Report* for 1964, p. 166), and it is desirable to compare these figures with toxicities to other bees and also to other beneficial insects. Colonies of two species of bumblebees (*Bombus locorum* and *Bombus agrorum*) were collected and biological assay tests of acute contact toxicity of four insecticides made using the method described for honeybees. Because of the small number of insects available, regression lines and LD50 values could not be calculated. However, Table 4 shows

TABLE 4

Contact toxicity of four insecticides to bumblebees

(Dose range (μg per bee) within which LD50 lies, compared with LD50 value for honeybees.)

	Demeton-methyl	Dimethoate	Disulfoton	Phorate
B. lucorum				
Queens	6–24	5–20	Over 40	6–23
Workers/males	1–2	2–5	2–10	1–2
B. agrorum				
Queens	10–24	1–5	5–10	1–5
Workers/males	1–3	0·5–2	1–4	1–2
A. mellifera				
Workers	0·5	0·1	5·0	0·3

the limits of the LD50 values and the LD50 value of the same insecticide for honeybees. A series of concentrations of each insecticide was applied to groups of three to six bumblebees on several occasions and those killed were counted 24 hours afterwards. (Stevenson and Racey, Bee Department)

Evaluation of fluorescent tracers to study distribution of spray deposit on honeybees. A dieldrin spray containing a fluorescent tracer ("Saturn Yellow") was applied to flowering crops, being worked by foraging honeybees. Honeybees were collected as follows: (1) post spray, flying bees swept at hive entrance; (2) post spray, dead and dying bees picked up in front of hive; (3) dead bees in front of hive 28 hours after spray; (4) young bees taken from hive apron 7 days after spray; (5) dead bees taken from hive entrance 7 days after spray. Bees from each group were divided into one of five classes according to the quantity of tracer they had picked up, and the dieldrin content of each was determined using gas–liquid chromatography (*J. Sci. Fd Agric.* (1966), **17**, 133) to see if the distribution of the tracer was correlated with that of the insecticide.

In each group of bees the amount of dieldrin increased with the amount of tracer seen on the bees, and was often in excess of the median lethal dose. However, when bees with the same amount of tracer but from different groups were compared there was a big variation in the amount of dieldrin present for a given amount of tracer. Some contained dieldrin but showed

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no tracer. Fluorescent tracers should therefore be used to follow the fate of insecticide sprays only under carefully controlled conditions. (Needham and Stevenson, with Mr. B. A. Cooper, N.A.A.S., East Midland Region)

Apparatus

A compact and robust hand-operated microapplicator was devised and made that selects and delivers five sizes of drops ranging from 0·25 to 5 μl. To test the accuracy and consistency of the apparatus, 10 individual drops of water were weighed for each drop size. The coefficient of variation with the smallest drop of 0·25 μl was less than 2%.

The commonly used electric-barrier method of confining insects was modified, using an inexpensive mains- or battery-operated relaxation oscillator. The output of 25 V at 300 c/s is coupled to the electric barrier. When wired in conjunction with a change-over relay and battery, the barrier remains energised should the mains fail. A wide range of insects were satisfactorily contained.

The development of an apparatus for the sorting and counting of trapped insects continues and a counting system that should allow more clearly defined fractions to be collected is being developed. (Arnold)

Systemic insecticides

Work on soil factors affecting uptake of organophosphorus insecticides from soils by plants continued.

Sorption by soil. Studies on the sorption of disulfoton (diethyl S-[2-(ethylthio)ethyl]phosphorothiolothionate) by soil (see *Rothamsted Report* for 1964, p. 167) were concluded. As described in last year's report, adsorption isotherms for all soils studied fitted the straight-line relationship $x/m = bC$ well (where x/m is the insecticide sorbed per unit weight of soil and C is the concentration of the equilibrium soil solution). Correlations were all greater than 0·96, but more detailed statistical analysis showed that, with nearly half the soils, the Freundlich isotherm $x/m = KC^n$ (where K and n are constants) gave a significantly better fit. The Freundlich plots for different soils had different curvatures, as shown by values of n, which ranged from 0·8 to 1·1. For the range of solution concentrations up to saturation, however, these isotherms deviate only slightly from linearity, so that for practical purposes the linear isotherm can be used as a good approximation.

To interpret the movement in soil and availability to plants of other insecticides and to throw light on the nature of the insecticide–soil bond, adsorption of other insecticides is being studied. Measurements were made with phorate (diethyl S-(ethylthiomethyl)phosphorothiolothionate), using the same equilibration method as with disulfoton. For the 10 soils studied, isotherms have all been similar to those for disulfoton except that phorate has slightly less affinity than disulfoton for soil. At solution concentrations of 10 ppm, about 80% as much phorate is adsorbed as disulfoton. (Graham-Bryce)

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Effect of soil moisture content on uptake of organophosphorus insecticides. Previous field trials (summarised by Burt *et al.* *Ann. appl. Biol.* (1965), **56**, 411) suggested that soil moisture content influenced uptake by plants of systemic insecticides, and that the performance of different insecticides was affected differently by different moisture conditions. The effects of soil moisture content are therefore being studied in the field and in the laboratory.

In the field an irrigation experiment similar to that described last year (*Rothamsted Report* for 1965, p. 169) was planned. Menazon, phorate, dimethoate and disulfoton were applied as granules at the equivalent of 3 lb a.i./acre below potato tubers at the time of planting (10 May 1966). The toxicity of the plants to aphids was assessed by counting natural aphid populations on the plants at regular intervals and by confining aphids *Macrosiphum euphorbiae* (Thos.) on leaf surfaces both in the field and in the laboratory. It was intended to establish several different water regimes by different irrigation treatments, but, as in 1965, the season was wet and the differences between treatments were very small. The four insecticides could therefore be compared only at the same moisture content. The natural aphid infestation was much smaller than in 1965, and was thus less useful as an index of toxicity. At the time of maximum infestation (12 July) the control plots had only 157 aphids/100 leaves and the numbers on the treated plots were: dimethoate 35 aphids/100 leaves, menazon 132 aphids/100 leaves, phorate 5 aphids/100 leaves and disulfoton 7 aphids/100 leaves. The natural population counts together with caging tests confirmed previous results. Phorate and disulfoton controlled aphids very effectively throughout the growing season; dimethoate was somewhat less satisfactory and menazon much less.

The effects of soil moisture content on the uptake and toxicity of systemic insecticides were also studied by pot experiments, where conditions can be better controlled and soil moisture content made the only variable. In preliminary experiments, moisture content was controlled simply by frequent watering to maintain pots as near as possible to a constant weight calculated to give the desired average soil moisture content. When the moisture content of a sandy clay loam soil was maintained at 10 and 20% w/w, dimethoate and menazon moved through the soil rapidly. With the largest amounts of dimethoate in the wetter soil nearly all the aphids caged on broad-bean plants were killed in 24 hours. Disulfoton and phorate moved more slowly.

The water control in these experiments was not satisfactory, and a better technique was devised using osmotic control. Soil is separated from a suitable solution by a semi-permeable membrane, and water moves from solution to soil when the water potential in the soil exceeds the osmotic pressure of the solution. By maintaining the solution concentration constant, water can be added to the soil continuously and automatically at a known potential to replace water lost by evaporation from the surface or by plant transpiration. A suitable robust semi-permeable membrane is "Visking" dialysis membrane, and Union Carbide polyethylene glycol 20M (molecular weight approx. 20,000), which does not pass through the membrane, is a suitable solute for the osmotic solution. The polyethylene glycol (PEG) is inert and soluble enough to give a wide range of osmotic

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pressures. The method was tested extensively with a sandy clay loam for soil moisture contents from 5 to 17% w/w with pots containing soil only and others planted with wheat. After an initial adjustment, which took up to 1 week, the weights of pots remained constant within 2% until the end of experiments, even when the pots were in a greenhouse in June, showing that enough water was entering the pots to balance evaporation and transpiration. Experiments could not be continued for longer than 3–4 weeks after the seedlings emerged, because of microbial decomposition of the membrane in contact with the soil. At the end of experiments, examination of the soils showed that below a drier surface layer of approximately $\frac{1}{2}$ in., moisture content varied little. Coefficients of variation for water contents of 15 samples taken from different points in each pot ranged from 6 to 15%, and average moisture contents for different pots in the same solution differed by less than 2%. Apart from a small tendency for moisture contents to increase from top to bottom of the pots, moisture gradients were not apparent. For small plants that transpire little, therefore, this method can establish widely different moisture régimes, which are reproducible and defined to a large extent.

The matric potential at which water enters the soil with this method is equivalent to the osmotic pressure of the PEG solution. It is therefore important to determine this osmotic pressure. Direct measurements of osmotic pressure are difficult, so two indirect methods were used. First, freezing-point depressions were determined and the equation $OP = 12{\cdot}06\ \Delta T$ applied (where OP is the osmotic pressure in atmospheres and ΔT the freezing point depression in ° C). Freezing points were determined by following the change of temperature with time during melting of the continuously agitated frozen solutions as indicated by a thermistor coupled with a recording potentiometer in a Wheatstone bridge circuit. Apparatus for these measurements was kindly provided by Mr. R. Greenwood of The City University, London. Second, concentrations of KCl solutions isopiestic with the PEG solutions were determined, and osmotic pressures calculated from data in the International Critical Tables. A simple technique was devised to determine the required KCl concentrations. Filter-paper circles were soaked in PEG solution, blotted and placed in airtight brass cylinders, separated by a small air space from similar circles supporting KCl solutions. The cylinders were sealed and kept at 20° C (± 1) for 3 days when movement of water vapour from one circle to the other was detected by changes in weight. By using a range of KCl concentrations with vapour pressures bracketing that of the unknown PEG solution, the concentration of the isopiestic KCl solution that would cause no change in weight could be estimated accurately. The plots of PEG concentration against osmotic pressure for the two methods agreed reasonably well, and the curves obtained showed that the solutions did not obey van t'Hoff's law as indicated by Lagerwerff *et al.* (*Science* (1961), **133**, 1486). Osmotic pressures ranged from approximately 0·5 atm at 5% PEG to approximately 15 atm at 25% PEG. Very much larger osmotic pressures are given by more concentrated solutions, so that matric potentials over the whole range normally experienced by plants growing in soil can easily be produced with these solutions.

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This osmotic method of controlling the potential at which water is supplied to soil is being used to find how soil moisture conditions affect uptake of organophosphorus insecticides by wheat. (Graham-Bryce and Etheridge)

Control of virus diseases of lucerne. Collaborative work on this problem is described in the report of the Entomology Department. (Etheridge)

Analytical. Collaborative studies on the assay of residues of dimethoate continued in collaboration with the Joint Dimethoate Residues Panel of the Scientific Subcommittee on Poisonous Substances used in Agriculture and Food Storage. (Lord)

Insect rearing. The following were reared during the year:

PLANT FEEDERS

Hemiptera	*Acyrthosiphon pisum* (Harris)
	Aphis fabae Scop.
	Macrosiphum euphorbiae (Thos.)
	Megoura viciae Buckt.
	Myzus persicae (Sulz.)
	Rhopalosiphum padi (L.)
Coleoptera	*Phaedon cochleariae* (F.)

OTHERS

Orthoptera	*Blaberus discoidalis* (L.)
	Periplaneta americana (L.)
Lepidoptera	*Plodia interpunctella* (Hübn.)
	Pieris brassicae (L.)
Coleoptera	*Oryzaephilus mercator* (Fauv.)
	Tenebrio molitor L.
	Tribolium castaneum (Herbst.)
	Tribolium confusum J. du V.
	Trogoderma granarium Everts
Diptera	*Drosophila melanogaster* (Meig.) and a wingless mutant
	Musca domestica L.

Strains. Normal susceptible
SKA (diazinon resistant)
ocra SRS
bwb, *ocra* SRS } fully susceptible to
bwb, *ocra*, *ar* SRS } diazinon and DDT
29, 52 (resistant to diazinon. Homozygous for low aliesterase only, and marked with *ocra*)
8, 18 (Homozygous for Dz4 only and marked with *ar*)

Wheat-bulb fly (*Leptohylemyia coarctata* Fall.)

Commercial seed dressings for wheat-bulb fly control. The amount of γ-BHC on seeds treated with dry dressings to control wheat-bulb fly

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was again measured, and the work extended to include other insecticides and methods of treating seeds. The results of the tests, made in collaboration with the N.A.A.S., the Ministry of Agriculture and seed merchants, showed large differences between the mean amounts of insecticide on seeds treated at seven different seed-dressing plants. The standard deviations of the estimated amount of BHC left on a seed at a particular seed-dressing plant was ±30% at five plants and ±15% at two others. All the samples of concentrated seed dressings used contained the correct amount of γ-BHC, and had similar particle-size distribution. The amount of insecticide on the seed seemed not to be related to the type of machinery, the dressing, weather or seed variety. Analyses on a few samples of seed followed from the seed-dressing plant to the farmer's drill showed that the insecticide was not lost from the seed during transit. (Lord and Jeffs)

Preliminary studies on the adult behaviour of *Leptohylemyia coarctata*. Some preliminary studies were done on feeding and oviposition of adult wheat-bulb fly in June–September 1966 to see whether behavioural mechanisms could be used in combination with chemical control. Open cylinders of perspex, coated on the inside with "Stictite", were placed over commonly occurring plants in the wheat crop and in surrounding hedgerows to see whether adult wheat-bulb fly were attracted to particular food plants in the field. The largest catches were between 15 June and 6 July when five males were caught on a trap over goosegrass (*Galium aparine* Linn.) and another five on a larger trap over hogweed (*Heracleum sphondylium* Linn.). When the number of cages was increased to three for each species of plant even fewer male flies were caught, perhaps because the main flowering period of the plants was over. Female flies were not caught in traps placed over any plants during the whole period 15 June to 8 August.

Oviposition tests were made in breeding cages where adult female flies, collected from the field in July and August, were fed on a diet of honey, milk and blood. Standard cages consist of a hurricane lamp glass closed at the base with perforated zinc, which stands on a platform of copper wire on a black filter-paper in a petri-dish lid. The bases of the cages, where eggs are laid, were modified in various ways to examine the effect of different physical conditions on oviposition. In cages with the filter-paper divided into white and black halves, flies laid 175 eggs on the white portion and 1,033 eggs on the black. Flies preferred a perforated to a flat surface, and a small mesh size of 1,444 holes/in² to the standard perforated zinc with 81 holes/in². (Griffiths and Scott)

Single-row trials of insecticide. Eleven more experimental materials, azinphos-ethyl, bromophos, N 2790 (*O*-ethyl *S*-phenyl ethylphosphonodithioate), R 30472 (*O*-butyl *S*-(5-methyl-2-oxotetrahydrofuran-3-yl) methylphosphonodithioate), R 30569 (*O*-butyl *S*-(5-ethyl-2-oxotetrahydrofuran-3-yl) methylphosphonodithioate), RD 15721 (4-methylthio-3,5-xylyl-*N*-acetyl-*N*-methylcarbamate), SD 8211 (2-chloro-1-(2,5-dichlorophenyl) vinyl dimethyl phosphate), SD 8447 (2-chloro-1(2,4,5-trichlorophenyl) vinyl dimethyl phosphate), triphenyl tin acetate, tributyl tin oxide

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and carbophenothion ("Trithion") were formulated as seed dressings and compared with heptachlor, ethion and γ-BHC standards in co-operative field trials with entomologists of N.A.A.S. (Eastern Region and East Midland Region). Rows, 10 ft long, were sown with dressed seeds on two infested sites, a clay loam and a peaty loam. Plant examinations in the spring showed that carbophenothion was the most promising new material: plants grown from seed treated with carbophenothion at 0·5 and 0·1% active ingredient to weight of seed had 12% and 21% attacked shoots respectively in the peaty loam soil (compared with 80% attacked shoots in the controls) and 20% and 44% attacked shoots respectively in the clay loam (compared with 81% attacked shoots in the controls). Azinphos-ethyl, bromophos and the liquid dressing of N2790 were less effective than carbophenothion against wheat-bulb fly, and the other new materials tested were either ineffective or damaged the plants. (Griffiths and Scott)

Timing of sprays for control of wheat-bulb fly. A field experiment, similar to the small trial done last year on the timing of sprays in relation to the stage of plant growth and of wheat-bulb fly larval development, was sown on a medium boulder clay soil containing 4,600,000 eggs/acre. Large plots, 110 × 8½ ft were used to give reliable yield figures. All plots were sown with fungicide-dressed Cappelle seed, half on 22 October and half on 1 November 1965. Some plots from each sowing were left untreated, and others were sprayed with dimethoate on 23 February, 2 March, 9 March or 16 March 1966. A small portable sprayer was used because the ground was wet in early spring, and all sprays were applied at 8 fluid oz dimethoate in 60 gal water/acre (i.e. at slightly less than the recommended rate of 24 fluid oz/acre of the 40% formulation "Rogor E"). At each spraying the stage of plant growth and of larval development, in early and late sown plots, was recorded. By 23 February half the larvae in the early sown plants and one-tenth of those in the late-sown plants had already moulted once. In addition, slug damage decreased yields, particularly in the early sown plots. Table 5 shows that the time of spraying was very important. The

TABLE 5

Yields of grain in cwt/acre (85% *d.m.*)

		Sprayed			
	Unsprayed	23/2	2/3	9/3	16/3
Sown 22 October	18·9	28·2	28·8	25·5	23·2
Sown 1 November	11·6	31·0	27·5	17·9	14·9

L.s.d. at $p = 0{\cdot}05 = 4{\cdot}5$
$p = 0{\cdot}01 = 6{\cdot}1$

first two sprays, applied when plants from both sowings had produced sufficient growing buds to replace the damaged central shoots and when only a small proportion of larvae were third instar, increased yields much more than did the later sprays, especially on the late-sown plots. (Griffiths and Scott)

Recovery of wheat plants from damage by wheat-bulb fly larvae. The ability of wheat plants, at various stages of growth, to recover from damage

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caused by wheat-bulb fly larvae, or from artificial damage simulating wheat-bulb fly attack was studied.

Plants at the two-leaf stage that were cut off at ground level survived by regrowing their centre shoots. When they were cut down on several occasions they continued to regrow their centre shoots each time until, after 10–12 successive cuts, they died. In other tests the meristem of the centre shoots was completely destroyed with a needle: two-leaf plants were not tested, but many plants at the three-leaf stage died when treated in this way, although four-leaf plants survived. When live larvae were placed in contact with plants at the three-leaf stage the plants were still alive 48 days later but had smaller dry weights than unattacked plants. When live larvae were placed in contact with older plants that had three to four tillers and the larvae were removed from the plants 5 days later, so that they could only damage a single shoot, the attacked plants gave yields similar to unattacked plants when grown free from competition.

The results indicate that the main growth of young plants is of the centre shoot, and that destroying the meristem of the centre shoot kills the plant. At some point during the three-leaf stage the bud that forms the side shoot reaches a stage able to grow independently when the centre shoot is damaged. These tests gave no evidence that tillering was stimulated by wheat-bulb fly attack or artificial damage, and injections of ground-up suspensions of wheat-bulb fly larvae into the centre shoots of three-leaf plants also failed to increase the number of shoots. (Bardner, Entomology Department and Griffiths)

Larval response to plant extracts. Tests for attractiveness, used in the chemical study of the factor thought to influence the behaviour of wheat-bulb fly larvae near wheat plants, have usually been unsatisfactory, but progress was made using a different type of test, which measured arrestant rather than attractant activity. A drop of centrifuged extract of the mashed plant material or of distilled water as a control was placed at the centre of a filter-paper in a moistened petri dish. Ten larvae were then placed on the treated area and observed for 30 minutes. Larvae remained longer on extracts of young wheat stems and roots than on distilled water, or on extracts of wheat leaves or oat plants (oats is not a natural host plant of wheat-bulb fly larvae). Qualitative results indicate that the biologically active part of wheat stem extracts is organic, water-soluble, involatile and not destroyed by brief treatment at pH 2 or 10. (Scott and Janes)

Effects of chemicals on survival and behaviour of wireworms. Laboratory tests of AC 43064 (2-(diethoxyphosphinothioylimino)-1,3-dithiolane), "Aphidan" (*S*-(ethylsulfinylmethyl) *O*,*O*-diisopropyl dithiophosphate), azinphos-ethyl, Bayer 25141 (*O*,*O*-diethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate), "Bidrin" (3-(dimethoxyphosphinyloxy)-*N*,*N*-dimethyl-*cis*-crotonamide), chlorfenvinphos, "Dursban" (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), "Fitios" (*S*-(*N*-ethylcarbamoylmethyl) *O*,*O*-dimethylphosphorodithioate), mecarbam, parathion, P 1973 (*S*-(*N*-methoxycarbonyl-*N*-methylcarbamoylmethyl) dimethyl phosphorothiolothionate), P 2188 (experimental organophosphate, Murphy Chemical Company) and a carbamate (3-*sec*-butylphenyl *N*-phenoxyacetyl

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N-methyl carbamate) showed that azinphos-ethyl, "Dursban", "Fitios", mecarbam, parathion, P1973 and P2188 killed wireworms at 4 and 8 ppm active ingredient in soil. Tests 3 months later with fresh wireworms showed that most of these compounds had been inactivated in the soil, but "Dursban" and P2188 still killed many wireworms.

In further tests on the effects of insecticides on the biting behaviour of wireworms, paper discs soaked in nutrient were treated with large amounts of insecticide, up to 100 μg active ingredient per disc. Discs treated with nutrient and aldrin were readily bitten, but those treated with nutrient and γ-BHC, or nutrient and thionazin, were bitten less often, either because these insecticides repel wireworms or have a quick toxic action. (Griffiths and Scott)

Fungicides

Laboratory and field testing of formulations of fungicides to control blight, *Phytophthora infestans*, on potato haulms and tubers continued; experiments with fungicides for possible control of cereal take-all, caused by *Ophiobolus graminis*, were begun, but results are not yet known. Most of the materials used were kindly given by the makers.

Laboratory tests

Formulation. The bioassay method (*Rothamsted Report* for 1964) was used to compare formulations of fungicides for control of potato haulm blight; at least two replicate comparisons were done with each pair of formulations.

Last year the effectiveness of fentin acetate was increased by adding 1% paraffin wax, emulsified with the non-ionic "Brij 76" and "Brij 72" (Honeywill & Stein Ltd.) to a dispersed wettable powder ("Brestan 60": Hoechst Chemicals Ltd.). Adding the same 1% wax emulsion increased the effectiveness of dispersed zineb wettable powder (Murphy Chemical Co. Ltd.) at 0·3% a.i. by 2·2 times ($P < 0{\cdot}01$), but did not improve dispersed tetrachloroisophthalonitrile wettable powder ("Daconil" or "DAC 2787": Farm Protection Ltd.) at 0·01% a.i.

In tests without added wax, wettable powder formulations of some organo-tin compounds were compared with fentin acetate ("Brestan 60"), at 0·009% metallic Sn (0·03% fentin acetate). Decyltriphenylphosphonium bromochlorotriphenyl stannate (as "6651": Cela Landwirtschaftliche Chemikalien G.m.b.H.) was 2·2 times more effective than "Brestan 60" ($P \simeq 0{\cdot}02$), but triphenyltin chloride (as "Hoe 2871" and "Hoe 2872": Hoechst Chemicals Ltd.) was no more effective.

Apparent systemic action. A small trial with King Edward potatoes in 1965 showed that fentin acetate and triphenyltin chloride, applied to the soil-ridges, gave some control of potato-tuber blight. In looking for other materials that might control tuber blight, a few compounds were tested for systemic fungitoxic action. Each was applied to the surface of the soil round potted glasshouse-grown King Edward plants in three successive doses at intervals of about 4 days; each dose was applied in 2·5 g kaolin

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(English Clays Lovering Pochin & Co. Ltd.). About 5 days after the third application, leaflets were taken from the plants and compared, for susceptibility to infection by *P. infestans*, with leaflets from similar untreated plants (McIntosh & Eveling, *Eur. Potato J.* (1965), **8**, 98). The materials used were: A, 1-phenylthiosemicarbazide, which is said to be systemically fungicidal to *P. infestans* in potatoes (van der Kerk, *Wld Rev. Pest Control* (1963), **2**, 29); B, decyltriphenylphosphonium bromochlorotriphenyl stannate ("A36": C. H. Boehringer Sohn); and C, fentin acetate (Hoechst Chemicals Ltd.). In Table 6, which summarises the results, "dose" is the weight of compound per plant on each of three occasions; "ratio" is the weighted mean ratio of concentrations of zoospores needed to prevent infection on 50% of the leaflets (treated : untreated plants); *t* and *P* have the usual statistical meanings.

TABLE 6

Effect of soil applications on resistance of potato leaflets to infection by P. infestans

(See text for details)

	Compound applied				
	A	A	B	B	C
Dose, g	0·05	0·01	0·5	0·1	0·24
No. of tests	3	3	5	2	3
Ratio	1·6	1·5	5·0	2·0	5·9
t	1·9	2·0	5·6	2·3	5·0
Sig. at *P*	$>0·05$	$\simeq 0·05$	$\ll 0·001$	$\simeq 0·02$	$\ll 0·001$

When applied in this way, 1-phenylthiosemicarbazide (A) caused some marginal scorching of the leaflets, and had almost no effect on their resistance to infection. However, the two organo-tin compounds, although used in rather large amounts (a total of 0·2 g metallic Sn per plant for C and the larger rate of B), caused no visible damage to the plants, and increased the resistance of leaflets to infection by two to six times.

Organo-tin compounds as larvicides. Organo-tin compounds, besides being fungicides, have some insecticidal action, particularly on larvae. Thus they might possibly be used to control wireworms; or, when applied to the soil as fungicides (see "Field Trials", below), they might affect the soil fauna. The following preliminary laboratory tests were done to see which compounds are the best larvicides.

Eggs of rust-red flour beetles (*Tribolium castaneum*) were added to whole-meal flour impregnated with various organo-tin compounds, usually from alcohol solution; adults were counted after about 8 weeks at 28° C. In some tests eggs from surviving adults were similarly kept for a further 8 weeks, in untreated flour, to see whether the chemicals had made them sterile.

Approximate LD50s (as w/w percentages of compounds in flour) were 0·0005% for fentin acetate, triphenyl tin chloride and sulphide (Pure Chemicals Ltd.) and 0·0025% for tributyl tin fluoride and oxide (Pure Chemicals Ltd.), tributyl tin sulphide (Albright & Wilson (Mfg) Ltd.) and decyltriphenylphosphonium bromochlorotriphenyl stannate. Thus, the simple triphenyl tin compounds were better larvicides than the others.

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The tributyl tin compounds slightly lengthened the life-cycle of *T. castaneum*, but had no sterilant action.

Field trials. These were of two types: (1) conventional spraying of potato haulms with standard and new formulations of fentin acetate; (2) treatment of soil-ridges in potato crops with various materials, to try to control potato-tuber blight.

Haulm spraying. Mr. S. C. Melville, N.A.A.S., Starcross, kindly included one of our wax formulations of fentin acetate in his spraying trial with the variety Arran Consul in Devon; however, the trial failed because the crop was badly affected by leaf-roll, but not by blight.

Soil-ridge treatment. In a microplot trial with the variety King Edward at Rothamsted, various fungicidal dusts and skin-forming emulsions were applied to the ridges to protect the tubers, chemically or mechanically, from infection by spores washed down from haulms to soil. All organo-tin compounds, except L, were applied at 0·18 lb metallic Sn/acre; and compounds A–G and M as kaolin dusts. Treatments were: A, 1,4-dichloro-2,5-dimethoxybenzene (Du Pont Co. (UK) Ltd.) at 40 lb a.i./acre on 24 May; B, tributyl tin fluoride on 24 May; C, as B, on 1 July; D, tributyl tin oxide on 1 July; E, tributyl tin sulphide on 1 July; G, decyltriphenylphosphonium bromochlorotriphenyl stannate on 5 July; H, "Epok V 8020" (British Resin Products Ltd.), an emulsion of polyvinyl acetate and polybutyl acrylate, with no fungicide, at 350 gal of 37% emulsion/acre on 30 June; J, "Unisol 91" (International Synthetic Rubber Co. Ltd.), an emulsion of synthetic rubber, with no fungicide, at 240 gal of 25% emulsion/acre on 6 July; K, "Intex 100" (International Synthetic Rubber Co. Ltd.), an emulsion of synthetic rubber, with no fungicide, at 210 gal of 34% emulsion/acre on 7 July; L, emulsion containing 40% bitumen and 0·06% triphenyl tin chloride (Armour Hess Chemicals Ltd.) at 470 gal/acre on 14 July; M, 1-phenylthiosemicarbazide at 30 lb/acre on 4 August; and O, untreated. Haulms were unsprayed and were about 50% destroyed by blight by 13 August. None of the treatments decreased the proportion of tubers blighted at harvest (14–27 September). In contrast, treatments H and K significantly increased the proportion; and treatments A, C, D, E and H significantly decreased the yields of total and healthy tubers.

We conclude: (1) that the skin-like residues left by H and K, which were applied to cracked soil in dry weather, prevented the cracks from closing again when the soil later became moist, and in this way allowed spores of *P. infestans* to reach tubers directly; (2) that treatments A, C, D, E and possibly H damaged the roots badly enough to decrease yields, although no damage was visible on the haulms.

In a larger trial at Rothamsted, applying fungicidal dusts (kaolin) to the soil-ridges was compared with spraying the haulms with wettable powder formulations. As before, all organo-tin compounds were applied at 0·18 lb metallic Sn/acre. Treatments were: A, fentin acetate, and B, triphenyl tin chloride, as dusts on ridges in May; C and D, as A and B, in June; E, tetrachloroisophthalonitrile (Farm Protection Ltd.) as dust on ridges at

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3 lb a.i./acre in June; G, zineb (Universal Crop Protection Ltd.) as dust on ridges at 3 lb a.i./acre in June; H, fentin acetate wettable powder ("Brestan 60") on haulms on 2 August; J, tetrachloroisophthalonitrile wettable powder ("Daconil" or "DAC 2787") on haulms at 5 lb a.i./acre on 2 August; K, zineb wettable powder (Murphy Chemical Co. Ltd.) on haulms at 3 lb a.i./acre on 2 August; and L, untreated. Haulms in treatments A–G and L were unsprayed. Differences in percentages of haulm destroyed by blight were slight: about 50% was destroyed by 13 August. None of the treatments decreased the proportion of tubers blighted at harvest (26 September), or affected yield; this conflicts with the 1965 microplot trial, in which treatments corresponding to B and C decreased the proportion of blighted tubers. Possibly the fungicides were not put close enough to the stems.

A similar experiment at Woburn failed because the crop, which was evidently deficient in magnesium, was not attacked by blight.

Miss C. Frost kindly included our fentin acetate and triphenyltin chloride dusts in her soil-ridge treatment trial with the variety Kerr's Pink at Oak Park, Carlow, Ireland. Neither compound significantly decreased the proportion of blighted tubers. (McIntosh and Eveling)

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