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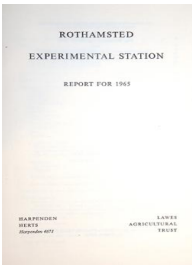
Rothamsted Experimental Station Report for 1965

Full Table of Content



Insecticides and Fungicides

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

Mrs. Maria Gwiazda of the Institute of Organic Industry, Warsaw, came to work for a year. At the request of the Indian Government, C. Potter spent three months in India advising an official committee reporting on the safe use of pesticides. R. M. Sawicki spent three months with Professor R. Milani at the University of Pavia, working on genetical techniques for the study of insecticide resistance. The visit was financed by the World Health Organisation. A. H. McIntosh was a delegate to an International Standards Organisation meeting in Paris. M. Elliott, D. C. Griffiths, K. A. Lord, A. H. McIntosh and P. H. Needham read papers at the British Insecticide and Fungicide Conference.

Much of the more directly practical work of the department this year was concerned with methods for increasing the effectiveness of insecticides and fungicides by appropriate formulation. Additives can considerably affect both toxicity and persistence and the results offer promise that improved formulations will give better control of pests and disease with smaller amounts of toxic chemical. Collaboration with the National Agricultural Advisory Service and industry continued in the search for less persistent and safer chemicals to replace those currently used for the control of wheat-bulb fly and wireworms. Some promising chemicals were found and will be more extensively tested. Systemic insecticides are now very widely used to control insects with piercing and sucking mouth parts, but there is little information on the factors that influence their effectiveness, although they can be greatly affected by the type of soil and the weather. Our laboratory experiments on sorption of systemic insecticides by soils, and field experiments on the effect of moisture content of soils, are designed to provide some quantitative information on the factors influencing the uptake, persistence and toxicity of these chemicals.

Because they are very toxic to insects but not to man, the department has long worked on pyrethrum and synthetic molecules allied to the pyrethrins. Recently, some very active compounds were synthesised. In addition to its scientific interest, this work is valuable in offering the possibility of insecticides safe to mammals that provide a satisfactory alternative to the organochlorine, organophosphorus or carbamate insecticides, where these were either hazardous or where pests had become resistant to them.

To develop better insecticides and to cope with the increasing problem of resistance to them, a detailed knowledge is needed of how they act and the reasons for their ineffectiveness. Our laboratory studies with the organophosphorus insecticides show that the simple answers generally given are inadequate, and that much still remains to be discovered about the critical sites of action and the physiological and biochemical mechanisms involved.

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Insecticides

Poisoning by organophosphorus compounds and the causes of resistance

Decomposition, distribution and sorption of diazinon in susceptible and resistant houseflies. Slow penetration of diazinon into adults is the most obvious, but not sole, cause of resistance of the SKA strain of the housefly (*Musca domestica*), and other possible causes of resistance were sought (Farnham, *et al.*, (1965), *J. Insect Physiol.* **11**, 1435). Dissected or macerated insects were mainly used so that differences in penetration of insecticide into the insect should not obscure other causes, such as differences in metabolism.

Metabolism in both susceptible and resistant flies was studied by incubating C^{14}-labelled diazinon with macerated insect material. The amount decomposed was estimated by grinding the insect tissues in hexane to remove most of the unchanged diazinon, and extracting the water-soluble decomposition products by grinding with water. The aqueous extract was acidified and extracted with ether. The ether extract contained most of the radioactivity, and was chromatographed on paper using methyl cyanide: water: ammonia 40:9:1 as solvent. Autoradiographs of the paper chromatograms showed two major decomposition products, one moved at the same speed as diethylphosphoric acid, the other faster.

Both decomposition products were always found when susceptible flies were used but often only one, corresponding to diethylphosphoric acid, with resistant flies. Most decomposition occurred in the thorax and abdomen, and little in heads. Integument removed from the thorax or abdomen decomposed diazinon.

The distribution of C^{14}-labelled diazinon in houseflies was examined by autoradiography. At two intervals after applying the insecticide especially on the pronotum, it was washed from the surface of flies with cyclohexane, and whole flies or dissected parts were then pressed in contact with thin melinex sheet placed over Ilford Industrial G. X-ray film. Darker images were formed with susceptible flies than with resistant flies because more diazinon penetrates into susceptible flies in a given time. Six hours after poisoning, radioactivity was distributed throughout the insect. No change in distribution was noticed after 24 hours. Abdomens contained the greatest concentration of poison, even though the poison was applied to the pronotum. Integument, muscles and ganglia dissected from thoraces of both susceptible and resistant flies were radioactive. After autoradiography the parts of the thorax were washed by immersing for 2 minutes in a mixture of cyclohexane: acetone = 2:1. Three successive washes in the mixture followed by an aqueous wash seemed not to modify the autoradiograms. Radioactivity remained in dissected parts of thoraces even after they were immersed in a cyclohexane–acetone mixture for 24 hours. More activity remained in the muscles and ganglia of the thoraces of susceptible than of resistant flies, but the amounts in the cuticle did not differ much. These results suggest no large differences in metabolism or distribution of diazinon between susceptible and resistant strains. They provide evidence that some radioactivity becomes firmly attached to insect tissues, although most can be extracted as unchanged diazinon by suitable extraction techniques.

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These experiments, together with anomalies in recovery of radioactivity in experiments on metabolism of diazinon in both susceptible and resistant flies, suggested that sorption occurred, which could be important in immobilising the poison and preventing it from reaching critical sites. To test for sorption, C^{14}-labelled diazinon was added to a suspension of flies macerated in 0·05*M*-Tris-(hydroxymethyl)amino-methane buffer pH 7·0 (Tris buffer). The solids were separated from the liquids by centrifugation. About one-third of the radioactivity was in the aqueous phase, both immediately after adding the insecticide and 5 hours later. Much of the radioactivity on the solids could be extracted with cyclohexane followed by a 2:1 mixture of chloroform: methanol, but the proportion extracted by each solvent depended on the time of incubation. Paper chromatography showed that most of the diazinon was recovered unchanged, even after 5 hours incubation.

Some tissues sorbed more diazinon than others. When tissues were dissected out and suspended separately in buffer solution containing 1μg/ml diazinon the integument of both thorax and abdomen sorbed more than the rest of the body; almost all the diazinon sorbed by the abdomen was on the integument and very little on its contents. There was no large or consistent difference between strains.

To test sorption and desorption in aqueous conditions, integuments of both susceptible and resistant strains were removed from the abdomen and macerated with 0·05*M*-Tris buffer pH 7·0 treated with radioactive diazinon and allowed to stand for 15 minutes. The solids were separated from the aqueous solution by centrifugation and suspended once more in Tris buffer and stood for 15 minutes before centrifuging again. The process was repeated until the solids had been washed three times. Each time the diazinon in the buffer solution was extracted into cyclohexane solution, and assayed by liquid scintillation counting. More diazinon was removed each time the integument was washed with buffer, indicating that sorption of diazinon on fly abdominal integument from aqueous Tris solution is at least partly reversible. (Lord and Gwiazda)

The effect of the cuticle on resistance of houseflies to organophosphorus compounds and DDT. Two organophosphate insecticides and their oxygen analogues were applied topically (in acetone), and injected in 70% aqueous acetone into the thoracic cavity of susceptible and diazinon-resistant (SKA) flies to find how resistance is affected when the cuticle is by-passed (Table 1).

Injection decreased the resistance factors to the four insecticides, but except to "malaoxon" did not eliminate resistance. The elimination of resistance to "malaoxon" but not malathion by injection indicates that differences in rate of penetration may account for resistance with the activated form of malathion but that with malathion itself other factors are involved. Other results are difficult to explain. Injection decreased considerably the slopes of the log dose–probit lines (ld.p lines) of the four insecticides; with "paraoxon" and "malaoxon" it also decreased considerably the LD50 values, but it increased the LD50 values of parathion against the SKA flies. This result is puzzling and could indicate that excessive amounts of parathion

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in the body inhibit its activation to "paraoxon", or that parathion is activated while penetrating the cuticle. The considerable decrease in the slope of ld.p line of injected parathion indicates that the response is not proportional to the size of the dose.

The effect of penetration on the resistance to DDT was studied in the SKA strain and in a DDT-selected strain (F58W). DDT applied topically

TABLE 1

Toxicity of topically applied and injected organophosphate insecticides to two strains of the housefly (M. domestica *L.*)

Insecticide	Susceptible LD50 ± SE µg/fly	Susceptible Slope ± SE	SKA LD50 ± SE µg/fly	SKA Slope ± SE	Resistance factor
		TOPICAL			
Parathion	0·05 ± 0·0032	5·25 ± 0·700	1·20 ± 0·055	6·83 ± 0·867	24·0
"Paraoxon"	0·048 ± 0·0029	4·19 ± 0·469	1·00 ± 0·078	3·39 ± 0·438	20·8
Malathion	0·59 ± 0·033	5·07 ± 0·609	2·30 ± 0·130	4·69 ± 0·590	3·89
"Malaoxon"	1·60 ± 0·140	3·42 ± 0·551	12·0 ± 1·21	2·72 ± 0·391	7·50
		INJECTION			
Parathion	0·05 ± 0·0035	6·74 ± 0·433	1·26 ± 0·189	1·75 ± 0·308	25·0
"Paraoxon"	0·021 ± 0·0022	2·20 ± 0·267	0·15 ± 0·018	1·94 ± 0·256	7·10
Malathion	0·62 ± 0·151	1·04 ± 0·343	1·41 ± 0·272	1·36 ± 0·282	2·27
"Malaoxon"	0·31 ± 0.068	1·18 ± 0·273	0·34 ± 0·070	1·27 ± 0·268	1·09

to the thorax was washed off the surface of the treated flies with *n*-hexane at various intervals. Finally, the washed flies were macerated and extracted with *n*-hexane. The qualitative and quantitative examination of the washed and extracted fractions by paper chromatography, autoradiography of Cl^{36} DDT, and gas–liquid chromatography, showed that the slower penetration of DDT through the cuticle was a significant cause of resistance to this insecticide in the SKA but not the F58W flies. Four hours after treatment (1 µg/fly), 65% of the topically applied DDT remained on the body surface of SKA flies, but only 35% on the surface of the F58W and the susceptible flies. (El Basheir)

Metabolism of DDT in diazinon-selected (SKA) and DDT-selected (F58W) strains of houseflies. Studies on the metabolism of DDT in the SKA strain which is very resistant to DDT, a DDT-resistant strain (F58W) and a susceptible strain showed that all three metabolise DDT to DDE, but most DDE was recovered from F58W and least from SKA flies. No metabolite other than DDE was detected. Hence, increased metabolism of the DDT into DDE is not a factor in the resistance to DDT of the SKA (organophosphorus-selected) strain, whereas it is a factor with the DDT-selected strain. (El Basheir)

Genetical separation of the factors responsible for resistance to diazinon in the SKA strain of houseflies. The study of the physiological and biochemical mechanisms responsible for the resistance of the SKA strain to diazinon is difficult, because although there are several factors, how many is not known, and the separate ones have not been isolated.

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There are two incompletely dominant factors (R4 and R5) that we showed to be on the 4th and 5th linkage groups, by crosses between SKA flies and susceptible marker strains. (Sawicki, R. M., Franco, M. G. and Milani, R. (1965), WHO, *Information Circular on Insecticide Resistance*, No. **53**, 35)

These incompletely dominant factors were separated by crosses between the SKA strain and a double marker strain *ocra*; *ar* SRS, which was bred from *ocra* SRS and *ar* SRS strains kindly provided by Professor R. Milani. *Ocra* and *ar* are recessive visible markers on the 4th and 5th linkage groups. The F_1s of the reciprocal crosses had intermediate resistance, there was only a slight overlap between the log dose/probit mortality (ld.p) lines of the hybrid and the resistant parent. The ld.p lines of each of the four phenotypes segregating in F_2 and of an F_2 population made up to correspond to the 9:3:3:1 ratio were very shallow but straight. Bioassays of the back-cross progeny of the F_1 and *ocra*; *ar* SRS showed that the incompletely dominant resistance factors segregate in opposition to the corresponding visible markers *ocra* and *ar*, thus confirming that these resistance factors are on these two linkage groups. The four phenotypes from the progeny of the back-cross gave straight and steep ld.p lines, and their resistance factors were *ocra*; *ar* 1·0, *ar* 5·0, *ocra* 7·0, *wild* 20·0. These four phenotypes would all be heterozygous for resistance, so any recessive factors would not be manifest and the resistance shown is from the two incompletely dominant factors. These factors on the *ocra* and *ar* linkage groups therefore confer a similar small degree of resistance when separate but still heterozygous. The *ocra*; *ar* phenotype was nearly as susceptible as the double marker susceptible parent, but took considerably longer to die. This fact, and because a proportion of the double markers in the F_2 were resistant to diazinon, indicated the presence of recessive factors for resistance, which was confirmed when the double markers bred by selfing the back-cross progeny *ocra*; *ar* proved to be resistant. Thus, there is one or more incompletely recessive factor(s) on linkage groups 2, 3 or 6, which when heterozygous delay but do not prevent death, but confer resistance to death when homozygous.

To obtain two strains, each homozygous for one marker and one dominant resistant factor, but on different linkage groups, the *ocra* and *ar* progenies of the back-cross were each selfed to give their F_3s. The single markers segregating in F_3 were crossed, either in single pairs without selection or mass crossed after treatment with diazinon to kill all heterozygotes, with the resistance factor and the marker segregating in opposition to resistance. Thus, flies were obtained in F_4 homozygous for either *ocra* and R_5 or *ar* and R_4. We have also started to couple the dominant resistant factors and the marker on the same linkage group. The recessive factor(s) will be removed from the marked strains, isolated and their linkage determined.

As expected, the three phenotypes segregating from the two F_3s gave compound ld.p lines. These seem to show that none of the factors found so far plays a major role in conferring resistance to diazinon, even when they are homozygous.

Further confirmation of the location of the two incompletely dominant

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factors R_4 and R_5 was obtained using ali-esterase activity as a biochemical marker. The segregation of gene *a* for low ali-esterase, which is coupled with, and an allele of, the resistance factor on the 5th linkage group, was followed by the Warburg manometric technique using methyl butyrate as a substrate. The F_1 (*ocra*; *ar* SRS; SKA) had about twice as much ali-esterase activity as the resistant parent, but one-third as much as the susceptible parent. The *wild* and *ocra* phenotypes segregating in the progeny of the back-cross had the same ali-esterase activity as the F_1, but the double marker and *ar* had normal ali-esterase. This confirms that gene *a* and the resistance factor on the 5th linkage group (R_5) segregate in opposition to *ar* and the resistance factor on the 4th linkage group (R_4) segregates in opposition to *ocra*.

Had this work been done with unmarked flies and the commonly used toxicological methods, it could have been inferred that resistance to diazinon is caused by a single incompletely dominant gene, because the F_2 gives a very shallow straight ld.p line which could indicate an indistinct 1:2:1 segregation, and the dose discriminating between the resistant and susceptible flies in the progeny of the back-cross is the same as the LD50 for the total progeny.

Three white-eyed adult houseflies were isolated from the Rothamsted Normal strain and mated in single pairs with normal flies. The F_1 generation yielded wild-eyed adults, which were selfed to give the F_2 generation of wild-eyed and white-eyed flies in a ratio of 3:1. A pure strain of white-eyed flies was reared, and tested in the F_3 generation against diazinon, DDT and dieldrin. The results showed that: (*a*) the white-eyed strain was twice as susceptible to diazinon as the Normal strain; (*b*) it was homogeneous in its response to DDT, unlike the parent Normal strain, which was heterogeneous; (*c*) both strains were similarly heterogeneous in their response to dieldrin.

The white-eye is a pure strain, and there has been no reversion to wild-eyed types after nine generations. Dr. Franco of Pavia University found that the gene for white-eye is w_3 and is on the second chromosome. (Sawicki and Farnham)

Effect of the carrier on the toxicity of diazinon to diazinon susceptible and resistant houseflies. The carrier can greatly affect the toxicity of

TABLE 2

Weighted mean LD50 values of susceptible and resistant (SKA) flies

	Topical application			Injection
Solvent	Susceptible	Resistant	Resistance factor	Susceptible
Acetone	0·077 ± 0·0014	8·1 ± 0·4	105	
70% Acetone in water	0·073 ± 0·003			0·042 ± 0·001
Ethanol	0·073 ± 0·002	6·1 ± 0·3	84	
70% Ethanol in water	0·073 ± 0·003			0·053 ± 0·002
Ethyl cellosolve	0·071 ± 0·002			0·028 ± 0·003
Risella oil	0·072 ± 0·002			0·118 ± 0·007
Olive oil	0·121 ± 0·004	35·3 ± 16	300	
Dimethyl sulfoxide				0·032 ± 0·003

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DDT to resistant houseflies, but effects with diazinon have not been tested. Table 2 shows the toxicity of diazinon in different solvents to resistant (SKA) and susceptible flies. The flies were treated topically with a 0·3 μl drop of diazinon in Risella oil and olive oil, and with a 1·0 μl drop of diazinon in the other solvents, or were injected with a 0·3 μl drop of insecticide in each of the solvents. Olive oil was the only solvent to show a major effect with topically applied diazinon; it decreased toxicity and did so much more with resistant than with susceptible flies, so that both strain resistance and resistance factor were altered.

All the carriers had effects when the insecticide was injected; diazinon in ethyl cellosolve was four times more toxic to susceptible flies than in Risella oil. Olive oil, neat acetone or ethanol could not be used because they were too toxic alone.

Drop-size tests. To test further factors affecting the response of insects in bio-assays, the effects of drop size on the apparent toxicity of topically applied diazinon to susceptible and resistant flies were examined. Acetone and Risella oil were used as examples of a volatile and non-volatile solvent respectively, with drop sizes ranging from 0·1 to 0·8 μl/fly. Tests at the various drop sizes followed the general design of probit assay. Table 3

TABLE 3

Weighted mean LD50 values of susceptible and resistant (SKA) flies by topical application with varying drop sizes of diazinon in acetone and Risella oil (μg/fly)

Drop size (μl)	Acetone			Risella oil		
	Susceptible	Resistant	Resistance factor	Susceptible	Resistant	Resistance factor
0·1	0·047 ± 0·002	13·2 ± 0.7	281	0·038 ± 0·001	8·4 ± 0·3	220
0·2	0·055 ± 0·002	15·7 ± 0·9	285	0·057 ± 0·002	10·3 ± 0·4	181
0·4	0·056 ± 0·002	19·3 ± 1·1	344	0·065 ± 0·002	14·5 ± 0·5	223
0·8	0·063 ± 0·003	14·6 ± 1·0	231			

shows that, with acetone as solvent, drop size had little effect, particularly at the larger drop sizes, but with Risella oil, increasing drop size diminished the apparent toxicity. The resistance factor at any one drop size for each sovent remained constant. (Farnham)

Inhibition of cholinesterase and loss of function in the nervous system of the American cockroach* Periplaneta americana *poisoned by diazoxon. The main object of this work was to determine the amount and distribution of inhibition of cholinesterase in the nervous system of cockroaches poisoned by diazoxon and to relate this to loss of function. Electrophysiological measurements on function were made in preparations of the 6th abdominal ganglion and the cercal nerves. It was first confirmed that the synapses between the cercal nerves and the giant fibres in the 6th abdominal ganglion were blocked to conduction after continuous irrigation with diazoxon.

The steady but very small output of fluid required to irrigate small regions of nerve continuously was provided by a device consisting of a

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Sangamo Weston S7 motor unit driving the micrometer screw of an Agla micrometer syringe at 30 revs/h. The needle then delivers 5 μl/min for 1½ hours at a single filling. Other rates can be obtained by changing the motor unit. The assembly is mounted on a stand with a fine adjustment for moving the needle over the preparation.

The inhibition of cholinesterase was studied in whole ganglia and connectives using the thiocholine method of Gomori (1952). To assess the degree of inhibition of cholinesterase, photographs of stained ganglia, produced under controlled conditions, were compared with a set of five standards ranging from normal to completely inhibited.

When ganglia were irrigated continuously with diazoxon at increasing concentrations cholinesterase inhibition increased steadily, usually reaching about the same intensity at block at all concentrations, though individual preparations varied. Large concentrations tended to produce more inhibition at block. Although inhibition was complete in the peripheral region at block, considerable activity remained in the neuropile. Irrigation with inhibitor after block produced little change in the appearance of the ganglion up to 150% of block time. Irrigation with saline after block failed to reverse either the block or the cholinesterase inhibition.

The association between functional impairment and cholinesterase inhibition of the ganglion as a whole is less close than might be expected, possibly because impairment is controlled by enzyme inhibition in very localised regions of the ganglion.

The condition of the nervous systems of cockroaches treated on the thorax with lethal doses of diazoxon was examined at intervals. In most insects the function of the 6th abdominal ganglion was only slightly impaired, even when the insect showed advanced symptoms of poisoning or was moribund. The degree of inhibition was appropriate to the impairment, but individual insects differed considerably. Cholinesterase was more inhibited in the thoracic ganglia than in the 6th abdominal ganglion, particularly in the peripheral region, but even moribund insects retained considerable activity in the neuropile. Inhibition was greater in the abdominal ganglia close to the thorax than in the more distal abdominal ganglia.

Current results suggest that part at least of the nervous system of cockroaches killed by diazoxon remains functional and, if loss of function by the nervous system because of cholinesterase inhibition causes death, the loss is restricted to special areas. (Burt and Gregory)

Inhibition of cholinesterase of the cockroach nerve cord by diazinon. Although diazinon is the form used as an insecticide, it is thought not to inhibit cholinesterase directly, but to need conversion in the insect to diazoxon.

When pure diazinon was applied continuously at $4{\cdot}0 \times 10^{-4}$ *M* concentrations to the 6th abdominal ganglia of cockroaches, it blocked conduction in about the same time and inhibited cholinesterase to the same extent as diazoxon at about $\frac{1}{40}$ this concentration. This considerable activity could reflect conversion of diazinon to diazoxon by tissues near the nerve cord, and this possibility was tested by applying diazinon and diazoxon continuously and intermittently to ganglia *in situ* and to ganglia

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separated from nearby tissues by polythene aprons. Continuous irrigation with diazoxon took half as long as intermittent application to block conduction in the ganglia, but with diazinon continuous irrigation took twice as long as intermittent application. Application over an apron halved the block time with diazoxon, but doubled it with diazinon. This suggests that some conversion of diazinon to diazoxon takes place, but leaves some activity unaccounted for.

The possibility of direct inhibition by diazinon was further explored by applying it together with SKF 525–A (*p*-diethylaminoethyl diphenyl propyl acetate) a compound that prevents oxidation of parathion to paraoxon and might be expected to have a similar effect with diazinon. However, when more concentrated than 4×10^{-5} *M*, the SKF 525-A alone affects the cockroach 6th abdominal ganglion. The stimulus intensity to the cercal nerves has to be increased to maintain conduction through the ganglion, which finally blocks completely, and all spontaneous activity in the ganglion stops. At smaller concentrations these effects can be reversed by irrigation with saline. SKF 525-A does not inhibit cholinesterase. When applied with diazinon or diazoxon, SKF 525-A prevents the hyperactivity in the ganglia caused by these compounds and abolishes it when applied after it is present, but cholinesterase in these preparations is inhibited as completely as if SKF 525-A had not been applied. Because SKF 525-A affects both diazinon and diazoxon similarly and does not prevent either from inhibiting cholinesterase, diazinon itself can probably inhibit cholinesterase in the nervous system of the American cockroach without being previously converted to diazoxon.

Iodoacetic acid and chloropicrin, which also inhibit oxidising enzymes, were very toxic to the nervous system and could not be used to test whether diazinon is converted to diazoxon. (Burt and Gregory)

Resistance in aphids. The susceptibility to organophosphate insecticides of *Myzus persicae* associated with sugar beet was again tested. Samples of aphids were collected in May from mangold clamps and in July and August from sugar beet. The collections were made in both sprayed and unsprayed areas. Replicated tests were made by two techniques; in one the aphids were exposed to residual deposits of dimethoate on chinese cabbage leaves to see the combined effects of stomach poison and contact and the other was topical application of the poison to determine the effects of contact alone. The large differences in susceptibility between samples found in 1964 did not recur, and all samples collected in 1965 were uniformly susceptible to dimethoate, with no indication of any resistant strain. (Needham)

Persistence and Toxicity of Cholorohydrocarbon Insecticides

(a) Effect of environmental conditions on the persistence of dieldrin crystals. Air movement greatly affected the rate dieldrin crystals volatilised from glass surfaces. Films of needle crystals aggregated in plate-like growths, giving a "fish-scales" appearance, were prepared on glass by coating the glass with a "Cellosolve" solution of dieldrin tagged with Cl^{36} and allowing the solvent to evaporate. The individual needle crystals

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were generally within the range 0·01–0·1 mm long, with diameters about 0·002–0·005 mm.

Plates with initial deposits of 5 μg/cm² were placed alternately in still air at a constant temperature of 21° C (and approx. 75% R.H.) and in a wind tunnel with a laminar air flow of 3 m.p.h. at a constant temperature of 23° C (and approx. 35% R.H.). This air flow caused no mechanical loss of crystals. Changes in the amount of deposit were measured by counting the emission from the radioactive tracer with a thin end-window Geiger counter placed 3 mm from the film surface. Initial losses were of the order of 0·1 μg/cm²/day in still air and 1·7 μg/cm²/day in the wind tunnel, the airflow of 3 m.p.h., thus increasing loss by a factor of 17. Air flow had less effect as the deposit density decreased. With deposits of about 0·5 μg/cm² the loss in still air was 0·06 μg/cm²/day and in the wind tunnel 0·45 μg/cm²/day, a difference factor of 7½. With deposits as small as 0·2 μg/cm², the difference factor was about 2.

Extremely small but persistent residues remained on the plates for several weeks, and temperatures of 45° C with a wind speed of 2·5 m.p.h. for 10 hours produced no further loss. When these deposits were washed off with hexane and analysed by gas–liquid chromatography they gave a figure of approximately 0·005 μg/cm² of dieldrin, together with very small amounts of unidentified material.

(*b*) ***Effect of formulation on toxicity.*** It was confirmed that the contact toxicity to houseflies of DDT wettable powder formulations, both with and without "Lovo 192" (a mixture of amine stearates), increases during the first 3 weeks of ageing in the dark at 20° C in still air, when applied to either glass surfaces or excised cotton leaves. After 3 weeks the contact toxicity gradually decreases. Contact toxicity of a given DDT deposit depends on the concentration of "Lovo 192" present, but the rates the DDT or toxicity are lost from both glass and cotton leaf surfaces kept in the dark at 20° C for up to 10 weeks do not, as shown by gas–liquid chromatography and by bioassay.

The presence of "Lovo 192" diminished the loss of DDT from glass surfaces when "rainwashed". The procedure for "rainwashing" was to expose the surfaces to a spray of tap water on a specially designed machine for 5 minutes to simulate the effects of 0·3 in. heavy rain. The "Lovo" protected both fresh and aged deposits, and Table 4 shows that after

TABLE 4

Retention of DDT deposits on a glass surface

State of deposit	Standard W.P.	5% "Lovo 192" W.P.
Fresh, unwashed	>80% kill 4·23 ± 0·35 μg DDT/cm²	>80% kill 3·77 ± 0·1 μg DDT/cm²
Fresh, "rainwashed"	Negligible kill 0·13 ± 0·055 μg DDT/cm²	Negligible kill 1·43 ± 0·14 μg DDT/cm²
10 weeks old, unwashed	~80% kill 2·53 ± 0·68 μg DDT/cm²	~80% kill 2·5 ± 0·23 μg DDT/cm²
10 weeks old, "rainwashed"	Negligible kill 0·23 ± 0·063 μg DDT/cm²	~20% kill 1·8 ± 0·33 μg DDT/cm²

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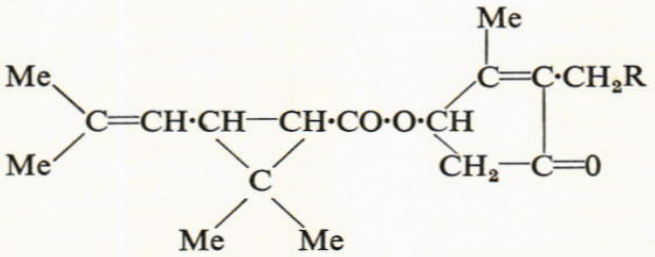
"rainwashing" approximately 10 times more DDT was retained by the 5% "Lovo" wettable powder than by the standard W.P.

With deposits on leaves, the results were more complicated, probably because of the leaf waxes. Standard wettable powder deposits (6–7 μg DDT/cm²) of all ages up to 10 weeks killed 50–90% of houseflies before "rainwashing" and none after, although chemical analysis showed that appreciable amounts (0·5–3·0 μg DDT/cm²) of DDT were retained by the washed leaf. Similar deposits (4–6 μg DDT/cm²) containing 5% or 10% "Lovo 192", some aged for 1 week and some for 10 weeks, killed 70–90% houseflies before "rainwashing" and only 0–10% after, although chemical analysis showed that most of the DDT (3–5 μg DDT/cm²) was retained by the washed leaf. Apparently the leaf substrate makes the DDT less effective as a contact poison than when on glass. (Phillips and Gillham)

Pyrethrins and Related Compounds

From knowledge gained from our work (see recent Annual Reports) on the relationship between structure and insecticidal activity, it has been possible to predict and synthesize new esters of chrysanthemic acid that are very active insecticides. These are 4-allylbenzyl (±)-*cis–trans*-chrysanthemate and 4-allyl-2,6-dimethyl benzyl (±)-*cis–trans*-chrysanthemate.

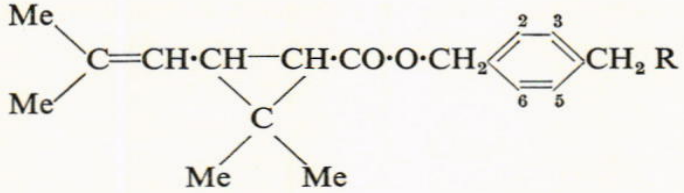
General Formulae of Pyrethroids.



Side chain
(R = alkenyl)

Chrysanthemic acid Cyclopentenolone ring

General Formulae of Benzyl Esters.



Side chain
R = alkenyl in
(most toxic esters)

Chrysanthemic acid Benzyl unit

The insecticidal activity of 2,4-dimethyl and 3,4-dimethyl benzyl chrysanthemates (W. F. Barthel, *Advances in Pest Control Research* (1961) **4**, 33, London: Interscience Publishers; *World Review of Pest Control* (1964) **3**, 97) suggested that the function of the cyclopentenolone ring in the natural esters could be fulfilled by a benzyl unit.

Detailed investigation of the variation of toxicity with structure in benzyl chrysanthemates then led to the following conclusions: (*a*) The

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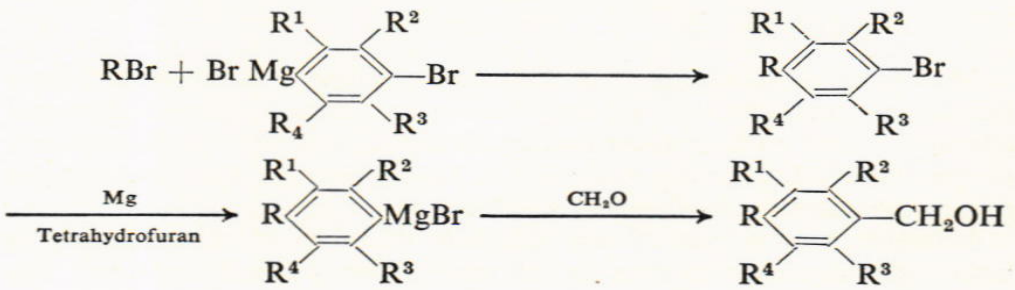
presence of an unsaturated side chain (e.g. allyl) of the type in the most toxic pyrethroids gave increased toxicity. (*b*) This unsaturated side chain was most effective in the 4 position, less so in the 3 position and gave no activity in the 2 position of the benzene ring. (*c*) The function of the double bond in the side chain was probably to activate the α-methylene group there, as with cyclopentenolone esters. Thus, without a double bond (saturated group), or with a vinyl or propenyl side chain, the esters were much less toxic. (*d*) *Phenyl* chrysanthemates (e.g. 4-allyl-2,6-dimethylphenyl chrysanthemates) of structure corresponding to active *benzyl* chrysanthemates were non-toxic, showing that the benzylic CH_2 is essential for activity, probably because such molecules more nearly approach the shape of the pyrethrins. (*e*) Methyl groups at 2 and/or 6 positions on the benzene ring increased the activity, especially towards mustard beetles. (*f*) In contrast, ethyl groups at the 2 and 6 positions removed toxicity, presumably because steric hindrance precluded a pyrethrin-like conformation. (*g*) Methoxyl groups on any position of the ring made otherwise active compounds less toxic. (*h*) 2-, 3- or 4-allyloxybenzyl chrysanthemates had even less insecticidal activity than the methoxyl compounds.

From this investigation therefore it was concluded that those benzyl alcohols whose structure relates them most closely to pyrethrolone, cinerolone, etc., gave the most toxic chrysanthemates. Two of the most active compounds synthesised (see chemical work, below) were 4-allylbenzyl (±)-*cis–trans*-chrysanthemate (ABC) and 4-allyl-2,6-dimethylbenzyl (±)-*cis–trans*-chrysanthemate (DMABC). ABC showed great specificity when tested by topical applications in acetone on adult houseflies (*Musca domestica* L.) and adult mustard beetles (*Phaedon cochleariae* Fab.), whereas DMABC did not.

ABC was twice as toxic to houseflies as were allethrin, pure pyrethrin I or the mixture of esters in natural pyrethrum. DMABC was slightly more toxic than ABC. With mustard beetles, however, allethrin was six times more toxic than ABC but only half as toxic as DMABC. The esters from (+)-*trans*-chrysanthemic acid — 4-allyl-2,6-dimethylbenzyl (+)-*trans*-chrysanthemate and 4-allyl benzyl (+)-*trans*-chrysanthemate are more toxic to houseflies than any other pyrethrin-like ester, natural or synthetic, previously recorded. (Elliott, Janes, Jeffs, Needham, Pearson and Sawicki)

Chemical work. The alkenyl benzyl alcohols were prepared by reaction of alkenyl halides with mono Grignard reagents from symmetrical *p*-aryldibromides, followed by interaction of formaldehyde with the Grignard derivative of the resulting 4-alkenyl aryl bromides:



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The symmetrical compounds *p*-dibromobenzene, 2,5-dimethyl-*p*-dibromobenzene and *p*-dibromotetramethyl benzene (dibromodurene) were used in this way. 4-Alkenyl bromobenzenes reacted easily with magnesium in tetrahydrofuran (reaction in ether was unsatisfactory, even with ethylene dibromide as entrainer). 4-Allyl, 4-(2′-methallyl), 4-allyl-2,5-dimethyl, 4-*trans*-sorbyl, 4-*trans*-crotyl and 4-allyl-tetramethylbenzyl alcohols were made by this route.

Because unsymmetrical aromatic dibromides were not expected to form one Grignard derivative exclusively, other alkenyl benzyl alcohols were obtained by an alternative route. Bromobenzyl alcohols were made by reduction of benzoic acids or esters or by chloromethylation reactions. The alcoholic function was protected as the tetrahydropyranyl ether, and the Grignard reagent from the bromo tetrahydropyranyl ether was treated with the appropriate alkenylhalide.

Bromides used in this method were as follows:

(*a*) 4-Bromo-2-methylbenzyl alcohol was made by reduction of 4-bromo-2-methylbenzoic acid, obtained by oxidation of 4-bromo-*o*-xylene.

(*b*) Chloromethylation of 3,5-dimethylbromobenzene gave 2-bromo-4,6-dimethylbenzyl alcohol after hydrolysis.

(*c*) 2- and 3-Bromobenzyl alcohols were made from the corresponding (known) bromoacids.

(*d*) 4-Bromo-3-methoxybenzyl alcohol was made by methylation of 4-bromo-3-hydroxy-benzoic acid, then reduction of the resulting methoxyl benzoic ester.

(*e*) 4-Bromo-2-methoxy-8-benzyl alcohols were made from 4-amino salicylic acid by diazotization, coupling with cuprous bromide and methylation with dimethyl sulphate.

By this route via the corresponding bromides, the following benzyl alcohols were made: 4-*trans*-crotyl, 4-(2′-methallyl), 4-allyl-2-methyl, 2-allyl, 3-allyl, 2-allyl-4, 6-dimethyl, 2-(2′-methallyl)-4, 6-dimethyl, 4-allyl-2,6-dimethyl and 2,6-dimethyl-4-(2′-methallyl) benzyl alcohols.

Esters were made in the usual way from benzyl alcohols and chrysanthemyl chlorides, or more conveniently, when benzyl halides were available, by reaction with the triethylamine salt of chrysanthemic acid.

4-Allyl-2,6-dimethylbenzyl alcohol was best synthesised by the reactions shown on the next page.

N-allyl-2,6-xylidine did not rearrange on heating in good yield to 4-allyl-2,6-xylidine, unlike *N*-crotylaniline, which Hickinbottom (*J. Chem. Soc.* (1934) 1981) found gave 4-crotylaniline in the presence of aniline hydrochloride or hydrobromide. Rearrangement took place smoothly to the required 4-allyl-2,6-xylidine in the presence of zinc chloride. By a Sandmeyer reaction 4-allyl-2,6-xylidine gave 4-allyl-2,6-dimethylbromobenzene. A side product of the reaction, which also confirmed the structure of the main product was 4-allyl-2,6-xylenol, identical with the product from rearrangement of *O*-allyl-2,6-xylenol.

4-Propenylbenzyl alcohol was made by reaction of propionaldehyde with 4-bromophenylmagnesium bromide. The alcohol obtained was

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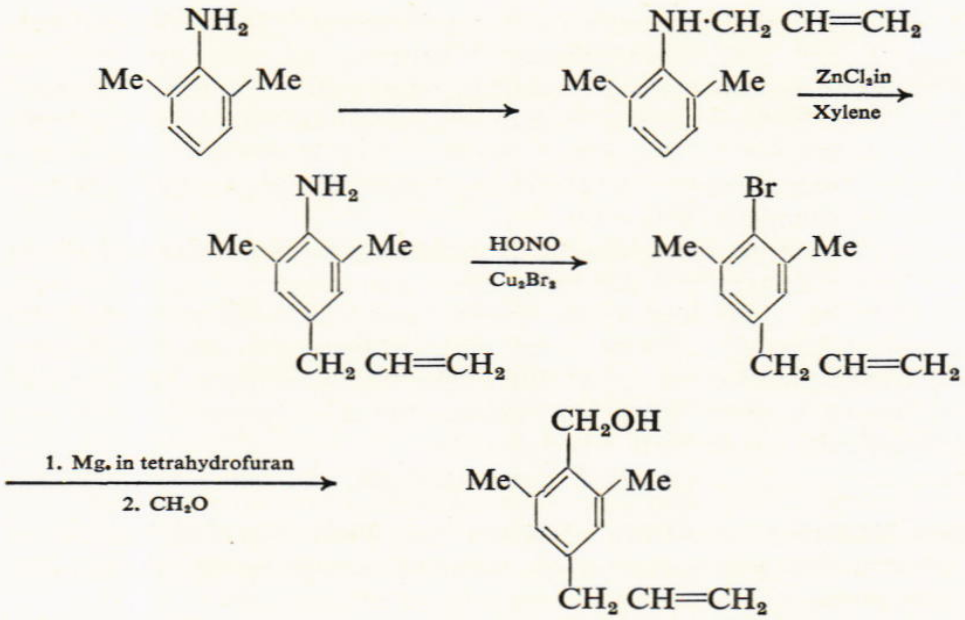

dehydrated to 4-bromopropenylbenzene, which was converted to 4-propenylbenzyl alcohol via the Grignard reagent and formaldehyde.

4-Vinylbenzyl alcohol was made by a modification of the method of Abramo & Chapin (*J. org. Chem.* (1961) **26**, 2671).

2,4,6-Trimethyl and 2,4,6-triethylbenzyl alcohols were made by chloromethylation of the corresponding hydrocarbons.

All important esters gave satisfactory analyses, and structures were confirmed by ultra-violet, infra-red and nuclear magnetic resonance spectra. (Elliott, Janes, Jeffs and Pearson)

Apparatus

An instrument was developed to make glass microelectrodes for physiological studies such as intra-cellular investigations and the measurement of membrane potentials. The electrode glass is formed in two stages, the first stage by gravity, the second by compressed air. The use of a pneumatic system with air cushioning results in a compact yet versatile instrument.

Work on the development of an apparatus for the sorting and counting of trapped insects started. Several possible techniques were examined and the movement of insects was studied in wet and dry conditions. A primary sorting technique was developed, although the degree of separation and replication has not yet been established. (Arnold)

Toxicity of Insecticides to Bees

Poisoning of bees in the field. Sixty-six samples of honey bees (*Apis mellifera*) were received from the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food, 36 more than in 1964.

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Of the 42 containing insecticide, 30 had organophosphates, and information supplied with the samples showed that 15 of these were affected by spraying bean crops, seven of which were sprayed from the air. Two further poisonings were ascribed to aerial spraying, one on runner beans and one on beet where clover was being worked by bees near by. Although individual organophosphates cannot be identified with certainty, supplementary evidence supplied with the samples indicated that dimethoate had been used on at least five occasions and demeton methyl and phosphamidon each on at least one occasion.

The spraying of mustard while in flower was responsible for poisoning two of the six samples found to contain dieldrin, and one was of bees foraging near a sheep dip. One sample containing dieldrin was received from India. Four samples contained BHC; supers for the hives of one had been stored above a room in which logs had previously been treated with BHC.

One sample of bees and one of foundation contained both BHC and dieldrin. The bees were from hives that had been stored on the carpet in a spare room of a house previously treated to control moths; the foundation was stored in a room treated with wood preservative.

One of the samples attributed to organophosphate poisoning also contained a sub-lethal amount of BHC, as did a second sample in which no further evidence of insecticides was found. As in previous years, most cases of poisoning attributed to organophosphate insecticides occurred in Eastern England; of the 30, 26 were in the National Agricultural Advisory Service's Eastern Region. (Needham and Stevenson)

Toxicity to bees of nectar from plants treated with systemic insecticides. To investigate whether nectar taken from plants treated with systemic insecticides is likely to be toxic to bees or other pollinating insects, a biological assay method was developed using *Drosophila melanogaster*. Nectar (usually 50–200 μl) was pipetted on to an 8-mm × 3-mm piece of cotton-wool roll and placed in a 75-mm × 30-mm-diameter glass vial. Twelve adult male *D. melanogaster* were confined in the vial by using gauze on which was placed a cotton-wool roll soaked in sucrose solution to provide moisture for the flies. The vials were stored at 27° C for 24 or 48 hours when the flies killed were counted. The LD50 value for dimethoate under these conditions was about 0·3 μg/vial. Dimethoate was watered on to *Fuchsia* (glasshouse var.) *Tropaeolium* sp. and *Borago officinalis* plants grown in vermiculite in 5-in. pots in the glasshouse. The dose was 0·025 g in 100 ml water/plant. The nectar was toxic to *D. melanogaster* until about 14 days after application. Nectar from plants treated with dimethoate at 0·025 g/plant was also toxic to honeybees when fed to them at a rate of 20 μl/bee. The doses used were larger than are applied to crops, and smaller ones are now being tested; also the toxicity of other insecticides. (May and Stevenson)

Systemic Insecticides

Sorption of organophosphorus insecticides by soil. Sorption by soil of systemic organophosphorus compounds is being studied because it is

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likely to have an important influence on the movement and availability of the chemical to the plant. Following last year's preliminary experiments (*Rothamsted Report* for 1964, p. 167), the sorption of disulfoton (diethyl S-[2-(ethylthio)ethyl]phosphorothiolothionate) from aqueous solutions by soil was studied in detail with sieved air-dry samples from two contrasting soils; (i) the FYM plot from Broadbalk field; (ii) a plot from the market-garden experiment at Woburn which has not been receiving organic manures. Portions of these soils, weighing from 2 to 20 g, were shaken at 20° C with $M/100$-$CaCl_2$ solutions containing various initial concentrations of disulfoton. After equilibration the suspensions were centrifuged and the insecticide extracted from portions of the supernatant solutions by shaking with hexane in a separating funnel. After draining, the centrifuged soils were mixed with anhydrous sodium sulphate, powdered and shaken with a mixture of 3 parts acetone and 2 parts hexane for 3 hours. The extracting mixture was decanted from the soils and the acetone removed by shaking with 2% sodium sulphate solution to leave a solution of the extracted disulfoton in hexane. The disulfoton extracted was estimated by gas–liquid chromatography using electron capture detection. Results were expressed in the form of adsorption isotherms by plotting the amount of insecticide sorbed by the soil against the solution concentration with which it was in equilibrium. Less insecticide was usually extracted from the soil than was lost from the solution during equilibration, and when the fall in solution concentration was taken as a measure of the insecticide sorbed isotherms were not reproducible, but depended on such conditions as soil/solution ratio and the nature of the vessel used for the equilibration. When the quantity of insecticide in the soil extracts was taken as the amount sorbed, however, isotherms were reproducible and independent of these conditions. The difference between the quantity of insecticide lost from solution and that extracted from the soil could be because extraction of the soil was inefficient or because dissolved insecticide was removed by processes other than sorption on soil. Prolonged extraction with acetone using a soxhlet failed to release significantly more disulfoton; also, in a few favourable tests (using hard-glass tubes and making contact between soil and solution brief) the disulfoton extracted from the soil equalled that lost from solution. Inefficient extraction of the soil, therefore, is not regarded as an important source of error. In addition to sorption by soil, dissolved insecticide could be lost by evaporation, by chemical or microbial decomposition or by adsorption on glass. Evidence was obtained that adsorption by glass sometimes contributes to the removal of dissolved disulfoton, and decomposition is being investigated. Because of the difficulty of interpreting figures for loss from solution, isotherms were all plotted using the quantities in soil extracts.

For the two standard soils, linear isotherms were obtained after 24 hours shaking for solution concentrations up to 10 ppm. The slopes of these isotherms expressed as ppm insecticide in soil/ppm insecticide in solution were 22 and 15 for the Broadbalk and Woburn soils respectively. The rate of equilibration between soil and solution was studied by measuring the distribution of insecticide after periods of 15 minutes, 3 hours, 15

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hours and 24 hours from the initial mixing. For the Broadbalk soil the quantity of disulfoton sorbed increased from 15 minutes to 3 hours, when equilibration seemed complete and the isotherm should no further change. Results for the Woburn soil were similar, except that the quantity sorbed increased slightly between 3 hours and 15 hours. On the basis of these results a 15-hour period of shaking was generally adopted. The extent to which sorption is reversible could have an important influence on the effectiveness of insecticides applied to soil. Reversibility was studied by equilibrating soils with saturated aqueous solutions of disulfoton and then shaking them immediately with less concentrated solutions for 15 hours. The desorption curves obtained were identical with the adsorption isotherms, suggesting that the process is readily reversible. Similar results were obtained when the soils were dried thoroughly in air for 48 hours between adsorption and desorption.

The applicability of these results to natural soils was tested by determining isotherms for moist samples of the two standard soils taken directly from the field. Comparison with the isotherms for the laboratory samples showed that air-drying and sieving had not affected the sorption properties of the soils.

In addition to the two soils studied in detail, adsorption isotherms for 14 other soils ranging in clay content from 2 to 87% and in organic carbon content from 0·7 to 30% were determined under standard conditions. Isotherms were always linear, with slopes ranging from 7.5 to 190. The slopes of these isotherms were linearly related to the organic carbon contents of the soils. (Graham-Bryce)

Uptake of systemic insecticides by potatoes in the field. The effect of soil moisture on the movement and subsequent uptake by potatoes of four organophosphorus insecticides was studied in the field, using irrigation. Menazon, phorate, dimethoate and disulfoton were applied as granules at the equivalent of 2 lb a.i./acre below the seed tubers at the time of planting (26 April 1965). The toxicity of the plants to aphids was assessed by counting natural aphid populations on the plant at regular intervals and by confining aphids on leaf surfaces both in the field and in the laboratory. Leaf samples were also taken throughout the season to assay insecticide chemically.

Several different irrigation treatments were originally planned, but because of the very wet season the moisture deficit (calculated from daily rainfall figures by means of H. L. Penman's evaporation equation) did not exceed 1½-in. from field capacity until the beginning of July, and only during early July did it exceed 2 in. Irrigation was therefore largely unnecessary, so that the differences between treatments were very small, and the four insecticides could be compared only at the same soil moisture. Phorate and disulfoton were the most effective, and persisted well throughout the period of aphid attack, i.e., from the beginning of June to mid-August. The natural aphid infestation on these plots was only 21 and 16 aphids/100 leaves respectively at the time of maximum infestation (July 19) when the untreated plants had 973 aphids/100 leaves. Dimethoate and menazon were less toxic and less persistent; aphid populations at maximum

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infestation were 120 and 198 aphids/100 leaves respectively. (Etheridge and Graham-Bryce)

Insect rearing. The following animals were reared during the year:

PLANT FEEDERS

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

OTHERS

Orthoptera	*Acheta domesticus* (L.)
	Blaberus discoidalis (L.)
	Blatella germanica (L.)
	Blatta orientalis L.
	Periplaneta americana (L.)
Lepidoptera	*Plodia interpunctella* Hübn.
Coleoptera	*Oryzaephilus mercator* (Fauv.)
	Tenebrio molitor L.
	Tribolium castaneum (Herbst.)
	Tribolium confusum J. du V.
	Trogoderma granarium Everts
Diptera	*Aedes aegypti* (L.)
	Drosophila melanogaster (Meig.) (3 strains including a wingless mutant)
	Musca domestica L.

Strains. Normal susceptible
SKA (diazinon resistant)
F 58 W (DDT resistant)
ar; SRS
ocra; SRS
ocra; *ar* SRS
bwb; *ac*; *ocra*; *ar*; SRS
ocra R_5; SKA
ar R_4; SKA
ocra; *ar*; SKA

Effects of chemicals on survival and behaviour of wireworms. Of the compounds tested against wireworms in the laboratory this year, N2790 (*O*-ethyl *S*-phenylethyl phosphonodithioate) proved effective when applied as granules and was comparable with γ-BHC. N2790 is structurally similar to Bayer 38156, previously found to be effective in laboratory and field tests (*Rothamsted Report* for 1963, p. 141).

Papers disks soaked in nutrient solution were again used to study the effect of insecticides on feeding behaviour of wireworms. Wireworms whose feeding ability had been affected by contact for 4 days with soil treated

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with 4 ppm insecticide were kept for up to 64 days in untreated soil and tested at intervals. The effects of exposure to thionazin, Bayer 38156 and aldrin persisted for the whole period of test, and wireworms died after they were removed to untreated soil. Exposure to γ-BHC killed fewer wireworms, and some recovered their biting ability about 3 weeks after treatment. Paper disks treated with 70 μg insecticide were bitten less often than untreated disks, but effects on biting did not persist when untreated disks replaced treated ones, except with aldrin; wireworms kept in tubes for 1 day with disks treated with 70 μg aldrin failed to recover their biting ability after 64 days.

The experiment on chemical control of wireworms on New Zealand field ended with yields of treated and untreated plots not differing significantly. Effects of the treatments on the soil fauna are described in the report of the Entomology Department.

The persistence of thionazin and Bayer 38156 in the plots was measured using a Collembola bioassay based on the method of Way & Scopes (*Ann. appl. Biol.* (1965) **55**, 340–341). Sensitivity for thionazin was 0·05 ppm and for Bayer 38156 was 0·15 ppm. On 13 March 1964 field plots were sprayed with insecticides at 1·5 lb active ingredient/acre. Bayer 38156 was detected in all samples taken 1 month after spraying but not in those taken after 6 months. Thionazin at 1·5 lb/acre left detectable residues 1 month after spraying in one plot that was acid (pH 5·8), but not in the other three more alkaline plots. More frequent samples taken from thionazin-treated sub-plots in 1965 showed a similar pattern of persistence, and laboratory tests, using an acid soil mixed with various amounts of powdered calcium carbonate, confirmed that thionazin persisted longer in the more acid soils. (Griffiths)

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

Egg counts in autumn 1965 were again high for the 4th successive year, and there have been reports of damage during the past 2 years in areas not previously affected by this pest.

Commercial γ-BHC seed dressings for wheat-bulb fly control. Because the amounts of insecticide on experimentally dressed wheat seeds were found to vary and because control of wheat-bulb fly attack by commercial seed dressings is liable to be erratic and patchy, 10 samples of wheat seed, commercially dressed with γ-BHC against wheat-bulb fly attack, were examined. The amounts of γ-BHC on the seeds estimated by gas–liquid chromatography differed widely. In all but one sample the average amount was less than 40 μg γ-BHC/seed, the amount expected from the recommended dressing of 2 oz of 40% γ-BHC dressing/bushel of seed. Little γ-BHC and no other chlorinated insecticide was found on seeds from three samples. In the other seven the average amount of γ-BHC found per seed ranged between 24 and 44 μg. The variation seems large enough to account for erratic control, and more tests are being made in collaboration with the N.A.A.S., the Ministry of Agriculture and seed merchants. (Scott, Lord and Griffiths)

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Behaviour of γ-BHC seed dressings in soil. Little is known of the behaviour in soil of insecticides used as seed dressings, and a test was made using γ-BHC as a model compound. Wheat seeds dressed to control wheat-bulb fly attack (2 oz/bushel of 40% BHC dressing) were sown by hand in November 1964 2 in. deep at ½-in. intervals in 3 lines spaced 8 in. apart.

Soil samples 6 in. deep and ½ in. diameter were taken from the plot at various times after planting and examined for γ-BHC content. The first samples taken on 29 January 1965 showed little spread of insecticide from the line of the seeds, for there was only little in cores taken 1 in. from the line of seeds and none at 2 or more inches away. Later samples, taken up to and including 24 March, showed no regular pattern of distribution of γ-BHC. Some taken from the line of plants did not contain γ-BHC in detectable amounts, whereas others taken midway between the plant rows did. The reasons for this very uneven distribution are not known. (Lord, Jeffs and Griffiths)

Seed-dressing formulations. Insecticidal seed dressings must not damage the germinating plants and must persist throughout the period of wheat-bulb fly attack. Bardner (*J. Sci. Fd Agric.* (1960) **12**, 736–744) showed that seed dressings formulated with polyvinyl acetate and activated carbon as carriers had more persistent insecticidal action and were less damaging to young plants than seed dressings based on siliceous earth. He suggested that polyvinyl acetate and activated carbon absorbed insecticides and released them slowly over a period.

An experiment was done to compare control of wheat-bulb fly by seed dressings using insecticides formulated with: (*a*) polyvinyl acetate, (*b*) polypropylene; (*c*) wax; and (*d*) a standard dressing based on siliceous earth. The insecticides used were diazinon, parathion, dimethoate and V–C 1–13 (*O*-2,4-dichlorophenyl *O*,*O*-diethyl phosphorothioate). All the special formulations allowed more insecticide to be placed on the seeds without affecting germination than did the standard dressing. Seeds treated with the most concentrated dressings that were not toxic to the plants were sown in boxes out of doors, and wheat-bulb fly eggs were added to the boxes in February. Counting damaged shoots showed: (1) that the siliceous earth formulations of diazinon gave such good control that it could not be improved by special formulations; (2) that control with siliceous earth formulations of dimethoate was poor and was improved only little by the special formulations; (3) that control with siliceous earth formulations of parathion and V-C 1-13 was moderate and was improved by all special formulations. The results suggest that special formulations are of value with compounds that are moderately effective, but where the amount of insecticide in standard seed-dressings cannot be increased without phytotoxicity. (Lord, Scott and Griffiths)

Single-row trials of insecticide. Twelve organophosphate insecticides V-C 3-670 (V-C Chemical Company) V-C 3-759, V-C 3-764, V-C 3-786, V-C 9-85; Cyanamid 43064 (American Cyanamid Company), Cyanamid 47031; SD 3562 (Shellstar Ltd); GS 13005 (Fisons Pest Control); ethion, vamidothion and disulfoton, were tested against wheat-bulb

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fly in a co-operative field trial with the entomologists of N.A.A.S. (Eastern Region). The treatments, applied as seed-dressings formulated with siliceous earth, consisted of four replicates of single 10-ft rows, sown on 27 November 1964 in a peaty soil containing over 1 million eggs/acre. Plant examinations in March showed that ethion was the most effective compound; it acted by preventing larvae from entering the plants. Seeds treated with ethion at 1% active ingredient to weight of seed gave only 5% attacked plants on 22 March compared with 54% attacked plants from seeds treated with fungicide alone. Plants from seeds treated with ethion at 0·15 and 0·04% active ingredient were examined 1 week later, when 16% and 29% were damaged and the controls still had 54% damaged plants. (Griffiths and Scott)

Timing of sprays for control of wheat-bulb fly. A small-plot field experiment was done on the timing of sprays in relation to stage of plant growth and of wheat-bulb fly larval development. The field had 4,850,000 eggs/acre, and plots 7 ft × 10 ft were sown with fungicide-dressed seed on three dates, 15 October (early-sown), 6 November (mid-sown) and 26 November (late-sown). Some plots from each sowing were left untreated, others were sprayed on 2 February, 8 March or 6 April with dimethoate at approximately 9·6 fl. oz a.i. in 100 gal water/acre. Dissections of sample plants taken from plots before and after spraying indicated that the 8 March spray killed more larvae than did the early or late sprays.

At harvest yields were variable, but differences between different sowing dates were large: mean yields from untreated early-sown, mid-sown and late-sown plots were 28·2, 15·7 and 0·7 cwt/acre respectively. Spraying had little effect on yields of early sown plots; it trebled the yield of late sown plots, but the crop was still a failure. An economic increase in yields was obtained on mid-sown plots sprayed on 8 March, when the plants had begun to tiller but had 86% damaged shoots; the plants on these plots recovered remarkably to yield 31·9 cwt/acre. (Griffiths and Scott)

Host-plant exudates attractive to larvae. Attempts were made to improve methods of testing extracts of wheat plants for attractiveness to wheat-bulb fly larvae. In standard tests, larvae are released between two blocks of gel (one plain, one containing wheat extract). Temperature is not critical, because the larvae react equally well at 15°, 20° and 25° C. A disadvantage of the method is that some larvae continue to wander round the dish throughout the test, and several attempts to overcome this failed. However, when larvae were placed in actual contact with blocks cut from a gel in which wheat seedlings had been growing, 29 out of 30 larvae were still in the gel after 2 hours, whereas only 5 out of 30 remained in contact with a block of plain gel. (Scott and Lord)

Molluscicides

Work continued on the mode of action and relative toxicities of different chemicals to slugs. Metaldehyde had a fumigant effect on slugs, which was almost certainly because of its breakdown to acetaldehyde. The fumigant

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activity of acetaldehyde to *Agriolimax reticulatus* at 10° C, (expressed as a median lethal concentration over a 1-hour exposure period) was 7·69 ± 0·21 mg/l, and to *Arion hortensis* 8·91 ± 0·18 mg/l, which, although smaller, was less susceptible to acetaldehyde in vapour form.

Several substances were tested as contact poison by immersing *A. reticulatus* in aqueous solutions. Their relative toxicities at 10° C (expressed as mean median lethal concentrations after a 1-hour exposure) were: Ioxynil 8·3 ± 0·3 ppm, sodium pentachlorophenate 22·0 ± 0·7 ppm, copper sulphate 71·9 ± 1·9 ppm and acetaldehyde 4,822·0 ± 815·9 ppm. At 20° C the toxicity of copper sulphate was unchanged, that of sodium pentachlorophenate was approximately doubled and that of Ioxynil trebled.

The contact activity of copper sulphate and metaldehyde were compared when applied as dry powders at 10° C. The mean median lethal concentration figures after a 1-hour exposure were: copper sulphate 2,027·0 ± 197·2 ppm, metaldehyde 43,370·0 ± 7,406·3 ppm.

When tested as stomach poisons by the technique developed last year the following mean median lethal dose figures were obtained for toxicity to *A. reticulatus* at 10° C: copper sulphate 131·6 ± 5·6 μg/slug, metaldehyde 85·2 ± 4·0 μg/slug and sodium pentachlorophenate 22·9 ± 2·5 μg/slug. Although less toxic than copper sulphate as a contact poison, metaldehyde was more toxic as a stomach poison, but neither was as toxic as sodium pentachlorophenate. Copper sulphate was more toxic when injected into the haemocoel of *A. reticulatus* in solution than as a stomach poison; the mean median lethal dose was 18·4 ± 1·1 μg/slug. (Henderson)

Fungicides

Laboratory tests

Formulation. The bioassay method (described last year) was used to compare formulations of fungicides to control potato blight. Figures for relative effectiveness given below are mean ratios of EV50s from at least two replicate comparisons.

Adding 1% "Dri-Sil 37" (containing the water-soluble silicone, sodium methyl siliconate) to simple suspensions of copper oxychloride improves the physical properties of the spray material (*Rothamsted Reports* for 1961 and 1962). In bioassay tests the siliconate increased the effectiveness by about six times, but it is expensive, and residues are rather brittle and unlikely to persist for long in the field.

Last year we formulated fentin acetate by dissolving it in melted lanolin or paraffin wax and emulsifying the hot solution. Unfortunately, this increased the phytotoxicity, as well as the fungicidal efficiency. In an attempt to retain the fungicidal but decrease the phytotoxic properties, we formulated fentin acetate by adding emulsified lanolin or paraffin wax to dispersed "Brestan 60" wettable powder (kindly given by Hoechst Chemicals Ltd.). The most successful additive of this type was 1% paraffin wax, emulsified with "Brij 76" and "Brij 72" (kindly given by Honeywill & Stein Ltd.). In many bioassay tests with glasshouse- and field-grown leaflets under various conditions this formulation was about

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three times more effective than "Brestan 60" alone, and in a field trial did not damage the variety Kind Edward.

The fungicidal efficiency of copper oxychloride and other fungicides usually increases as the particles get smaller. However, a simple weighing method showed that use of very small particles of copper oxychloride may harm plants. Detached King Edward potato leaflets, sprayed with suspensions of copper oxychloride, lost water about 25% faster ($P = 0{\cdot}01$) when the particles were small (95% below 1 μ) than when they were larger (95% below 8 μ), despite a fourfold difference in concentration (0·063% compared with 0·25%).

Field trials. These were of two types: (1) conventional spraying of haulms with standard and new formulations of copper oxychloride or fentin acetate; (2) soil-ridge treatment with copper oxychloride, fentin acetate and other (mostly organo-tin) compounds to try to control tuber blight.

Haulm spraying. A 6 × 6 Latin square trial was done with the variety King Edward at Rothamsted. Haulms were sprayed twice (9 and 26 July). Treatments were: A, a commercial copper oxychloride wettable powder at 0·25% Cu; B, the copper oxychloride–wax formulation, used in 1962–64, at 0·25% Cu; C, "Brestan 60" at 0·01% fentin acetate; D, "Brestan 60" at 0·03% fentin acetate; E, as C, but with wax added (see "Formulation" above); and N, unsprayed. Haulms were burnt off when 90% of the haulm on unsprayed plots was dead. Yields of total tubers in tons/acre were: A, 14·1; B, 15·6; C, 14·5; D, 15·8; E, 15·2; and N, 13·6 (5% LSD = 1·40). Thus, the copper oxychloride–wax formulation (B) gave a significantly larger yield than the commercial wettable powder (A); 0·01% fentin acetate with wax (E) gave a significant increase over the yield from unsprayed plots (N), whereas the same material without wax (C) did not; the largest yield was from 0·03% fentin acetate (D).

Mr. S. C. Melville, N.A.A.S., Starcross, Devon, again kindly included our copper oxychloride–wax formulation in his spraying trial with the variety Majestic at Crediton, Devon. Although the wax formulation seemed to protect the haulms for longer than a commercial formulation of copper oxychloride, the yields from the two treatments were identical.

Soil ridge treatment. In a microplot trial, with the variety King Edward in flinty loam soil at Rothamsted, the following chemicals were applied to the soil ridges on 28–30 June or 27–29 July: A, fentin acetate; B, tributyltin acetate; C, triphenyltin sulphide; D, triphenyltin chloride; and E, tetrachloroisophthalonitrile. The organo–tin compounds, kindly given by Pure Chemicals, Ltd., were applied as kaolin dusts at 0·18 lb metallic Sn/acre, and compound E, kindly given by Farm Protection Ltd., as a wettable powder spray ("DAC 2787") at 9 lb/acre. Haulm treatment was uniform; burning-off was at 90% haulm destruction. The percentages of blighted tubers at harvest-time from the early and late applications were: A, 5·0, 5·1; B, 6·9, 7·6; C, 9·9, 11·3; D, 5·3, 3·4; E, 6·9, 6·3; untreated, 11·0 (5% LSD = 5·6). Thus, fentin acetate (A) and triphenyltin chloride

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(D) significantly decreased the amount of tuber blight, and equally whether applied early or late.

On a larger trial, also with the variety King Edward in flinty loam soil at Rothamsted, copper oxychloride (about 3 lb Cu/acre) or fentin acetate (about 0·35 lb/acre) was applied to the soil ridges as granules (copper oxychloride in plaster of Paris, and fentin acetate in fullers' earth) a few days after planting, or as wettable powder sprays at 50% crop emergence, or at first haulm blight; or as wettable powder sprays on the haulms at first haulm blight. There were no differences in the amounts of haulm destroyed by blight; burning-off was at 42% destruction. Table 5 shows the amounts of blighted tubers at harvest-time.

TABLE 5

Percentages of blighted tubers (w/w) at harvest

Treatment	Fungicide: Copper oxychloride	Fungicide: Fentin acetate
Granules at planting	14·5	18·9
Soil spray at emergence	16·7	19·1
Soil spray at first haulm blight	16·4	11·4
Haulm spray at first haulm blight	11·2	14·3
Untreated	21·0	
LSD, 5%	5·9	

Other trials of the same type were kindly done by Mr. F. E. Shotton, Mr. S. C. Melville and Mr. G. H. Brenchley, N.A.A.S., using copper oxychloride, fentin acetate and fentin hydroxide with the varieties King Edward and Arran Banner, at Terrington St. Clement, Norfolk; Downderry, Cornwall; and Mepal, Cambs. Soil treatments at crop emergence (Downderry) or in July (Terrington and Mepal) did not significantly decrease the amounts of tuber blight, and at Terrington fentin hydroxide did not significantly decrease the amount of slug damage to tubers.

Thus, the results from this 1-year set of trials of soil fungicides for tuber blight control are conflicting. However, the efficiency of the method is bound to depend on the formulation, and on the soil type. In most of the trials the fungicides were applied to the ridges as wettable powder sprays which were designed for use on haulms. We hope that control of tuber blight by soil fungicides can be improved by more suitable formulation. (McIntosh and Eveling)

Other actions of organo–tin compounds. Use of fentin acetate and hydroxide to control potato blight is now permitted, and there is increased interest in the biological properties, other than fungicidal, of organo–tin compounds, ten of which were assessed at Rothamsted for control of various pests.

D. C. Griffiths showed that fentin acetate did not control wireworms when sprayed on to soil as a wettable powder ("Brestan 60") at 3 lb fentin acetate/acre.

Miss A. M. Shepherd (Nematology Department) kindly tested the following organo-tin compounds as possible hatching-stimulators on eggs of the beet-cyst nematode: dibutyl tin diacetate, maleate and dilaurate (kindly given by Albright & Wilson (Mfg.) Ltd.); tributyl tin oxide,

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acetate and fluoride; triphenyl tin chloride and sulphide (kindly given by Pure Chemicals Ltd.). All increased hatch action, and dibutyl tin di-acetate and maleate more than the others.

In preliminary tests to select insecticides that control wheat-bulb fly, A. W. Farnham and Mrs. E. M. Gillham kindly tested the insecticidal action of the above tri-substituted tin compounds, plus fentin acetate, on adult houseflies by standard topical application and film methods. In the application tests (0·5–1·0 μg/fly) there was some knock-down action, the order of decreasing effectiveness for both knock-down and kill being roughly: tributyl tin oxide (1) $>$ tributyl tin acetate (2) $>$ triphenyl tin chloride (3) $\geqslant$ tributyl tin fluoride (4) $\geqslant$ fentin acetate (5) $\geqslant$ triphenyl tin sulphide (6). However, in the film tests (5 minutes exposure to 30 μg/cm^2 on glass) only compounds (1) and (2) had any action. Unfortunately, the order of decreasing toxicity to germinating wheat seed (to which these compounds, like other wheat-bulb fly insecticides, were applied as 20% dusts) was almost the same as the order of decreasing insecticidal action, viz., $4 > 1 \simeq 2 > 3 \simeq 5 > 6$. D. C. Griffiths and G. C. Scott, in co-operation with the N.A.A.S., have included tributyl tin oxide (1) and fentin acetate (5) in current single-row field trials on the control of wheat-bulb fly in winter wheat.

J. W. Stephenson (Entomology Department) examined tubers from a field trial on control of potato-tuber blight at Terrington St. Clement (see above), in which fentin hydroxide was applied directly to the soil, and found that the fentin hydroxide did not significantly decrease the amount of damage by slugs.

The Effects of Spray Particles on Leaves

Work on the effect of small particles, of little chemical activity, on the epidermal permeability of leaves ended. In addition to suspensions tested before (*Rothamsted Report* for 1963, p. 144 and 1964, p. 175), suspensions of titanium oxide ("Tiona W.D.", kindly donated by Messrs Laporte Titanium Ltd.) and an alumina-aluminium oxide ("Almicide") were compared with "Stockalite" by the ammonia vapour test already described; neither titanium oxide, of smaller particle size than the "Stockalite", nor the highly absorptive "Almicide" caused the vapour to penetrate as much as did the "Stockalite".

A "Stockalite" suspension and a toxic pesticide suspension (copper oxychloride) of similar concentration and size of particle increased water loss similarly immediately the spray deposits dried out and until at least 3 weeks after spraying. (Eveling)

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