ROTHAMSTED RESEARCH

Rothamsted Report for 1963

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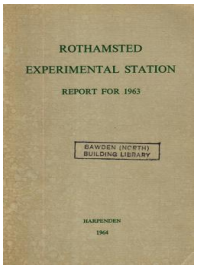

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

Frances Molloy left and I. J. Graham Bryce, J. H. Stevenson, A. W. Farnham and G. C. Scott were appointed. Mr. El Basheir from the University of Khartoum joined the department for three years. F. T. Phillips attended the "Symposium on the Use and Application of Radio-isotopes and Radiation in the Control of Plant and Animal Insect Pests" in Athens. R. Sawicki and P. H. Needham contributed to the Vth International Pesticides Congress in London, and C. Potter and A. H. McIntosh to the British Insecticides and Fungicides Conference at Brighton. At the request of the British Council and in collaboration with the department of Zoology and Applied Entomology of the Imperial College of Science and Technology, a "Symposium on Chemical Control of Insect Pests" was held for research workers from abroad.

Insecticides

The action of organophosphorus insecticides and the causes of resistance. The investigations reported last year on the causes of resistance of houseflies to diazoxon showed the need to know the relative rates the poison penetrates and the concentration it reaches in the haemolymph of susceptible and resistant individuals. To avoid the difficulties found to be associated with enzymic assay, other techniques were investigated.

Assay by gas chromatography. Using an electron capture detector, 10^{-8} g diazinon or 10^{-7} g diazoxon could be assayed. The sensitivity almost suffices to measure the diazoxon to be expected in a single poisoned insect. Techniques for applying a whole sample from an extract are not yet developed, so that at present only part of this sensitivity may be used. Tests were therefore limited to attempting to measure the amount of diazinon on the surface of flies and the total amounts of diazinon and diazoxon within flies at intervals after poisoning. In existing columns resolution is not adequate to assay diazoxon in the presence of large amounts of diazinon, but diazoxon was not detected in extracts of poisoned flies. The amount of diazinon which could be washed with cyclohexane from the surface of poisoned flies, whether susceptible and resistant to the poison, decreased rapidly, indicating rapid penetration. After 1 hour only about half of the diazinon could be recovered from flies poisoned with 1 μg of diazinon. A larger proportion was recovered when flies were treated with 10 μg of diazinon. The scatter of results was too large to show any differences between the rate of penetration into flies susceptible or resistant to diazinon.

Use of radioactive tracers. Before any precise measurements could be made it was necessary to study the physio-chemical behaviour of diazinon

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and develop a technique. In preliminary tests most of the radio-activity was lost from filter-papers treated with 1 μg of ^{14}C labelled diazinon, exposed to air for 1 week at room temperature (*ca.* 15–20° C). The effects on loss of diazinon of adding small quantities (1–100 μg) of three non-volatile liquids were examined, using radio-autography. Liquid paraffin slowed the rate of loss. "Triton X-100" had little effect on the rate of loss but prevented the movement of diazinon on filter-paper when washed with cyclohexane. Glycerol accelerated the loss from paper, probably by catalysing decomposition of the diazinon.

Covering the diazinon-treated paper with perspex, cellophane melinex or aluminium foil prevented noticeable loss for 2 weeks. Polythene sheet retarded loss, but itself became radio-active, indicating that diazinon had been transferred into the polythene.

With the technique developed from this preliminary work, the rate 1 μg diazinon penetrated into susceptible houseflies was confirmed using ^{14}C labelled diazinon, kindly donated by Messrs. Geigy, New York. The use of radioactive material made it possible to use single insects instead of batches of 10 required with gas chromatography. It is proposed to make comparable measurements of rate of penetration with resistant flies and then to study the internal distribution of the poison and its metabolites. (Lord and Solly)

General studies on mode of action and resistance to insecticides

Effect of allatectomy on the response of SKA flies to insecticides. We reported last year that the resistance of houseflies to diazinon increased considerably during the first 2 days after emergence. The levelling of resistance after the 2nd day after emergence coincides with mating. To test whether sexual maturation is responsible for the increase in resistance to diazinon with age, females of the SKA strains were allatectomised by a simple and rapid technique devised for this purpose. Removal of the corpora allata prevents the development of ovaries in Diptera. Allatectomised females (less than 1 day old) were treated with diazinon, dieldrin and pyrethrum to see whether the corpus allatum complex has any effect on the resistance to these compounds. The percentage of allatectomised flies and unoperated controls killed by diazinon and dieldrin was the same, the allatectomised flies were slightly less susceptible to pyrethrum extract than the controls, but the difference was too small to be significant. The maturation of the ovaries does not therefore affect the increase in resistance to diazinon with age. Also the corpus allatum complex plays no part in the detoxication of insecticides by houseflies. (Sawicki)

Cross-resistance of the SKA strain of housefly. Cross resistance is one of the major problems in the control of resistant strains of insects by insecticides. This is especially important among strains resistant to organophosphorus insecticides, in which the patterns of cross-resistance vary considerably between different strains.

The study of resistance factors of the SKA strain was delayed by the discovery that the normal Rothamsted strain contained a proportion of flies highly resistant to dieldrin. Susceptible flies sent by the Cooper Technical Bureau, from which our normal strain was obtained previously, were

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used instead of our laboratory susceptible strain to get the comparisons shown in Table 1. Preliminary experiments show that the SKA strain differs in its pattern of resistance from both its resistant parents (Keiding 203a and Sacca a) and from the Rutgers strains in the U.S.A.

TABLE 1

LD_{50}'s and resistant factor of the SKA strain with several insecticides

Compound	LD_{50} (μg per fly) susceptible strain	LD_{50} (μg per fly) SKA	Resistance factor
Diazinon	0·050	11·0	220
"Chlorthion"	0·21	14·0	67
"Dicapthon"	0·029	0·40	14
Ethyl "Chlorthion"	0·085	>20·0	>230
Fenchlorphos ("Ronnel")	0·060	2·40	40
Ethyl "Ronnel"	—	4·60	—
Carbophenothion	—	4·80	—
Malathion	0·23	—	—
Trichlorphon	0·23	2·10	9
Dichlorvos	0·017	0·15	8·8
Dimethoate	0·016	—	—
"ET-15"	0·022	—	—
"Isolan"	1·50	8·80	5·9
DDT	0·09	>100	>1100
BHC	0·028	2·20	79
Dieldrin	0·050	0·80	16

The SKA strain, like other strains resistant to organophosphorus insecticides, is very resistant to organic chlorine insecticides, although it has not been selected for resistance against this type of compound. DDT in acetone, even at very large concentrations, kills less than 40% of the flies. Two selections with dieldrin increased the percentage of flies unaffected by this insecticide from 10 to over 90%. (Basheir and Sawicki)

Effect of synergists on resistance. In tests to determine the effect of synergists on the resistance of the SKA strain to organophosphorus insecticides the most interesting result so far obtained is that tributyl phosphorotrithioate, a phosphatase inhibitor, is a very good synergist for "Chlorthion" but does not synergise "Dicapthon". "Chlorthion" and "Dicapthon" are both phosphorus phenyl derivatives containing a chlorine atom and differ only in the position of the chlorine on the phenyl group. (Basheir and Sawicki)

Dipping method for selecting resistant houseflies. The measured-drop technique, used previously to select SKA flies for diazinon resistance, was lengthy, and some of the flies could miss treatment altogether through the fault of the operator. For this reason a dipping technique was developed which speeded up and simplified selection.

One-day-old SKA flies are dipped for 3 minutes in a 70% solution of acetone in water containing diazinon in a Büchner funnel with a sintered-glass bottom. The solution is filtered off and the flies, dried for 3 minutes by the suction of air through the funnel, are transferred into plastic containers (20 flies per dish), checked for kill 24 hours after treatment, transferred into a cage and allowed to mate. The eggs are collected from the mated survivors.

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Preliminary experiments showed that healthy houseflies are unharmed when dipped in 70% acetone in water for 4 minutes. Tests with dyes showed a standard deviation of ±20% in the amount of dye retained by individual flies. The standard deviation in the percentage of insects killed in individual dishes with the two selection techniques for the same total kill was almost the same with dipping (±13%) as with topical application (±12%). The dipping method of selection is therefore nearly as accurate as topical application by measured drop. It is very much faster, simpler and more thorough, and is better than the deposit method of selection, because it is independent of the behaviour of the flies, and is now used for selecting the SKA strain with diazinon. (Sawicki and Farnham)

Housefly rearing. Difficulties in the supply of saw-dust, fungus trouble and the failure of fly rearing from unknown causes prompted this work.

The larval medium previously used (YMA) consisted of dried yeast, milk powder in water with agar. The larvae pupated in the saw-dust layer on the top of the medium. Several substitutes for saw-dust, e.g., expanded polystyrene, vermiculite and bran, were unsuitable, either because they did not absorb moisture (polystyrene) or because they caused the medium to ferment (bran) or made separation of pupae difficult (vermiculite). Fungus trouble was lessened and delayed, but not eliminated, by adding 0·1% sorbic acid to the larval medium instead of *p*-hydroxy-benzoic acid. Paper pulp was substituted for agar in the medium as a bulking agent. Filter-paper clippings were the most suitable from among several grades of paper tested. The filter-paper clippings are macerated in water, dried milk and yeast powder are added and the medium is thoroughly mixed. It is then poured into 2-lb jam jars which are inoculated with eggs. The larvae pupate a day sooner than previously.

So far, the new larval medium seems better than YMA because the pupae, which assemble at the top of the medium, are very easily separated (pupae can be sorted three times faster), the yields and average weights are as good as before and there has been no failure in the rearing. More than 500,000 flies have been reared on the new medium, fungus has not caused losses and the medium is made more quickly. The various factors affecting yield, i.e., ratio of paper : water : food : eggs are being examined. (Sawicki and Farnham)

Persistence and toxicity of residual chlorohydrocarbon insecticide films

Persistence. With the development of the spray chamber described in our Report for 1962, work was started on the volatilisation of residual insecticides from leaf surfaces. Attempts to keep detached cotton leaves alive for periods of about 2 months in this work were partially successful. Preliminary results show that with cotton leaves there is apparently no difference in the rates of volatilisation of dieldrin from the upper or lower leaf surfaces.

The linear rate of loss of dieldrin from glass surfaces down to deposits of about 0·2 μg/cm² does not occur with leaf surfaces, where preliminary experiments at 20° C in still air showed a levelling-off at around 1 μg/cm² deposit density.

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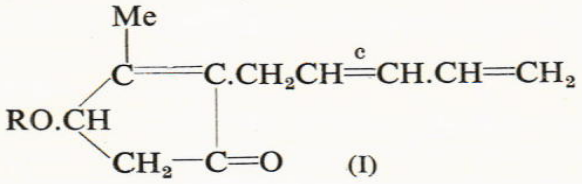
Effect of formulation on toxicity. The toxicity of several commercial DDT formulations, used as dry residual films on glass surfaces and some leaf surfaces, were tested against 4-day-old female houseflies (temp. 20° C, R.H. 60% approx., exposure time 5 minutes, toxicity measured as % kill after 24 hours). Such tests will be extended to films exposed to different conditions. A standard wettable powder and one formulated with "Lovo 192" proved to be equally toxic on glass surfaces, and were between 3 and 10 times more toxic than the other DDT formulations tested. These were emulsions of DDT stock emulsion concentrates containing either benzene or methyl naphthalene as the oil phase, and emulsions of DDT emulsifiable oil containing benzene as the oil phase, which all have similar toxicity. However, with methyl naphthalene as the oil phase in the DDT emulsifiable oil this toxicity was doubled, although the same formulation, but without DDT, was not toxic, showing that the methyl naphthalene itself was not the cause of this enhanced toxicity. Examining the deposits under the microscope showed oily droplets dispersed among the dry DDT crystal deposits, Under the conditions of the experiments these droplets sometimes took several days to evaporate completely from the glass surfaces. Oily droplets also occurred sometimes where benzene was the oil phase, but much less than DDT emulsifiable oil containing methyl naphthalene. The oily droplets may explain the enhanced toxicity and may act by increasing the pick up and penetration of DDT into the insect.

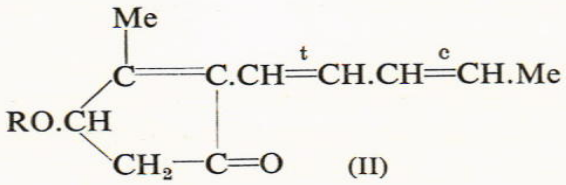
Preliminary experiments, using both upper and lower surfaces of cotton leaves, suggest the same differences in toxicity between the formulations when applied to these surfaces, although each of the formulations appear to be roughly $\frac{1}{3}$ of the toxicity of the corresponding formulation on a glass surface. DDT crystals on leaf surfaces are difficult to see under the microscope, but their presence was demonstrated by making gelatin impressions of the leaf surfaces and examining these under the microscope. (Phillips and Gillham)

The pyrethrins and related compounds

Thermal isomerisation of pyrethrolone and its derivatives. Pyrethrolone (I; R=H) and compounds derived from it undergo thermal isomerisation at 200° C to compounds (II), in which the double bonds in the side chain are conjugated with the ring



(I)



(II)

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The stereochemistry of the new side chain has now been deduced to be *trans*-1,*cis*-3 by a study of the infra-red spectra of thermally isomerised compounds and of other compounds with spectroscopic systems related to them of known stereochemistry. The absorption bands of the isomerised compounds in the CH and CH_2 out-of-plane deformation regions correspond in form and intensity with those of *cis–trans*-dienols (peaks at 999–1,003, 982–986 and 947–952 cm^{-1}), whereas the *trans–trans* and *cis–cis*-dienols do not display these triplets of peaks. The methoxy alcohol made by reducing pyrethrolone methyl ether (I; R=Me) with sodium borohydride is also isomerised by heat, so this reaction does not depend on the presence of the keto group. (Elliott)

Intensity of the ultra-violet absorption of* cis-*penta 1-3-dienes. The intensity of the ultra-violet absorption of the *cis*-penta-2,4-dienyl system in pyrethrolone has been in doubt. Earlier it was supposed to be considerably less (ε *ca.* 14,000) than that of *trans*-pyrethrolone (ε 23–25,000) (Crombie, Harper & Newman, *J. chem. Soc.* (1956), 3963). Because pyrethrolone forms a crystalline monohydrate, it is now more accessible in the pure state. By comparing the ultra-violet spectra of the pure pyrethrolone and of its derivatives with those of related compounds with no *cis*-diene side chain, the contribution made to the absorption of pyrethrolone by the *cis*-diene was found to be *ca.* 22,000, considerably more than earlier supposed. This value was confirmed by direct measurement on the compound in which the α-unsaturated cyclopentenone contribution to the absorption of pyrethrolone was suppressed by reduction of the keto group with sodium borohydride. (Elliott)

NMR spectra of the pyrethrins and related compounds. In continuing to study the pyrethrins and related compounds by all techniques that may throw light on the reason for their high biological activity the nuclear magnetic resonance spectra of the pyrethrins and related compounds is being examined in collaboration with Mr. A. Bramwell and Dr. L. Crombie, King's College, University of London. NMR spectra give information, not only of proton–proton interactions useful in determining structure but also on the detailed stereochemistry and even the preferred conformation of molecules. This information is of especial value for biologically active compounds such as the pyrethrins, whose toxicity depends to a large extent on the shape of the molecules.

The NMR spectra of the naturally occurring constituents of the pyrethrins (pyrethrins I and II and cinerins I and II, prepared by reconstitution from the naturally derived acids and alcohols) were recorded in deuterochloroform, using tetramethylsilane as internal standard. The positions of all the protons were assigned, although signals from interaction within the *cis*-diene side chain are complex and are not yet completely worked out. It is of interest that the two methine hydrogens of the cyclopropane ring in the esters of chrysanthemic acid, which give signals at 7·95 and 8·60 τ are shifted in the esters of chrysanthemum dicarboxylic acid (pyrethrin II and cinerin II) to 7·78 and 8·28 τ respectively. This study is continuing and will be extended to include compounds related to the pyrethrins. NMR

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evidence of the structure and purity of pyrethrins I and II reconstituted especially for a study of the relative toxicity of these two esters to various strains of housefly has been very valuable, because there is conflict between values found in this laboratory (Sawicki) and in the U.S.A. (Elliott and Janes)

The constituents of pyrethrum flowers. Some experiments were done to find whether any biological activity in the fresh flowers is lost when flowers are dried for extraction. Techniques were therefore developed for preparing powders of fresh and dried flowers and assaying their activity using larvae of *Aëdes aegypti*.

The flowers were ground in a ball mill and diluted with chromatographic cellulose, an inert powder. Thorough mixing was achieved by further grinding in the ball mill, and 1 part flowers was diluted in 5,000 parts cellulose in two or three stages. Weighed amounts of this mixture (usually between 10 and 100 mg) were then placed in specimen tubes to form replicated serial doses and 15 or 20 three-day-old *A. aegypti* larvae in 10 ml water were added to each tube. After exposure, response was assessed using the Burchfield/Harris photomigration technique. After drying at 80° C, the relative potency of dried to fresh flowers was 1·00 : 1·06, and after drying at 120° C 1·00 : 1·66, confirming results of chemical and biological tests reported last year showing no significant loss after drying at 80° C but some at higher drying temperatures. (Stevenson)

Jasmolin II. The Tropical Products Institute has isolated and identified a fifth toxic constituent of pyrethrum flowers which has been called jasmolin II (*Chem. & Ind.* (1964), 371). It is the *cis*-pent-2-enylrethronyl ester of pyrethric acid; the only difference from pyrethrin II is that the terminal double bond of the alcoholic side chain is saturated. This constituent formed approximately 3% of the total pyrethrins in the samples examined.

Jasmolin II is less toxic to the insects yet tested than a similar concentration of pyrethrins produced by dilution of a 25% pyrethrum extract. The extract was 16–17 times as toxic as jasmolin II to *Aëdes aegypti* larvae (using the Burchfield/Harris photomigration test) and to *Phaedon cochleariae* adults (topical application), less than 17 times to *Tenebrio molitor* adults and at least 100 times to *Tribolium castaneum* adults.

The toxicity of jasmolin II applied topically to 3–4-day-old female houseflies was compared with that of a commercial 25% pyrethrum extract and reconstituted cinerin II. The three materials in acetone were tested with and without piperonyl butoxide (1 part insecticide : 8 parts synergist). Pure jasmolin II was nearly as toxic as pure cinerin II (relative toxicity—extract 1·0, jasmolin II 0·44; cinerin II 0·52), but much less when synergised (relative toxicity—synergised extract 1·0; synergised jasmolin II 0·32; synergised cinerin II 0·60). The synergistic factor of the synergised compounds were: pyrethrum extract 9·6; jasmolin II 7·0; cinerin II 12·0. Jasmolin II had some knock-down activity. (Stevenson, Sawicki and Farnham)

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Relative toxicity of pyrethrin I and II against houseflies. The considerable differences in the relative toxicity of pure pyrethrins I and II against houseflies obtained in the U.S.A. (pyrethrin II about 0·3 times as toxic as pyrethrin I) and at Rothamsted (pyrethrin II, 1·3–1·5 times more toxic than pyrethrin I) were thought to depend on differences in bio-assay technique. However, Chan & Kearns (*J. econ. Ent.* (1962), **55**, 919) used a measured-drop technique of topical application similar to ours, but obtained results similar to those of previous American authors, i.e., pyrethrin I was more toxic than pyrethrin II.

The discrepancies could be either because the flies on the two sides of the Atlantic differ in their response to the individual constituents of pyrethrum or that pyrethrin II, an unstable compound, was impure when bioassayed, or that different methods of anaesthesia before treatment affect the response of the insects to the pyrethrins.

To test whether different strains of houseflies show different relative toxicities, two American strains (Orlando Regular and Cradson P), a diazinon-resistant strain SKA (a cross between a Danish strain 203a and an Italian strain, Saccà a) and the Rothamsted normal strain were treated with freshly reconstituted pyrethrin I and pyrethrin II. (Previous results showed that reconstituted pyrethrins are identical with the naturally derived materials chemically and biologically.) Table 2 shows that pyrethrin II was more toxic than pyrethrin I to the five strains of flies, confirming our earlier results.

TABLE 2

Toxicity of pyrethrin II relative to pyrethrin I against four strains of houseflies 24 hours after treatment

(*Toxicity of pyrethrin I = 1·00*)

Strain	Relative toxicity of pyrethrin II
Cradson P	1·46
Orlando Regular	1·33
SKA	1·42
Rothamsted normals	1·21

The pure constituents had only a very poor knock down against the houseflies of the two strains resistant to organophosphorus insecticides (SKA and Cradson P).

Tests are now in progress to see if immobilisation by cold or CO_2 alters the relative toxicity of the constituents. (Sawicki and Elliott)

Analytical chemistry. The collaborative work with C. Edwards to investigate the long-term effects of DDT and aldrin on soil was continued (see report of Entomology Department), and various soils were examined for insecticides. (Jeffs)

The ability of "Trapex", a proprietary nematicide containing methyl isothiocyanate, to pass through various types of "protective" gloves was tested for the Nematology Department. The vapours passing through the gloves were estimated, using the gas chromotograph, and the proportion of active to inactive ingredients was increased by passage through some types. (Lord and Solly)

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To assist studies on the movement of systemic insecticides in soil, the solubility of phorate and disulfoton was determined with the gas chromatograph; it was found to be 22 ppm for phorate and 21 ppm for disulfoton at 21° C. (Lord and Solly)

Bee poisoning in the field. Thirty-five samples of bees were received from Mr. P. S. Milne, Chief Bee Advisory Officer of the Ministry of Agriculture, Fisheries and Food. Twenty yielded evidence of poisoning by cholinesterase-inhibiting substances, probably organophosphorus insecticides. It has not been found possible to identify with certainty the organophosphorus insecticides which the poisoned bees contained, but information obtained by the advisory officers supplies some useful evidence. Out of the 20 samples shown to be poisoned by organophosphates, the evidence indicated that 10 were poisoned by dimethoate, 4 with demeton-methyl, 2 with phosphamidon; there was no evidence about the other 4.

Eight samples contained enough dieldrin for this or aldrin to have caused death.

Only one sample gave evidence of poisoning by lindane, and only one conclusive evidence of poisoning by a carbamate. Two samples were suspected of containing a carbamate, but this was not confirmed.

Three samples gave no evidence of insecticide or mercury poisoning.

One sample was received from Western Australia, but no insecticide was found, and information received later indicated that the bees had not been killed by insecticides. (Needham and Solly)

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

	PLANT FEEDING
Hemiptera	*Acyrthosiphon pisum* (Harris)
	Aphis fabae (Scop.)
	Megoura viciae (Buckt.)
	Myzus persicae (Sulz.)
	Rhopalosiphum padi (L.)
Lepidoptera	*Pieris brassicae* (L.)
	Plodia interpunctella Hubn.
Coleoptera	*Phaedon cochleariae* (F.)
	STORED PRODUCTS, DOMESTIC AND MEDICAL
Orthoptera	*Acheta domesticus* (L.)
	Blaberus discoidalis (L.)
	Blatella germanica (L.)
	Blatta orientalis (L.)
	Periplaneta americana (L.)
Lepidoptera	*Anagasta kühniella* (Zell.)
Coleoptera	*Oryzaephilus mercator* (Fauv.)
	Tenebrio molitor (L.)
	Tribolium castaneum (Herbst.)
	Tribolium confusum (J. du V.)
	Trogoderma granarium (Everts)

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Diptera
- *Aëdes aegypti* (L.)
- *Drosophila melanogaster* (Meig.)
  - (3 strains including a wingless mutant)
- *Drosophila subobscura* (Coll.)
- *Musca domestica* (L.)
  - Strains. Normal susceptible
    - CSMA 1948
    - SKA (diazinon resistant)
    - Cradson P
    - Orlando regular
    - NAIDM
    - F 58 W (DDT resistant)
- *Parascaptomyza pallida* (Ztt.)

Hymenoptera
- *Apis mellifera* (L.)

Crustacea
  Cladocera
- *Daphnia magna* (Straus)

Guppies (*Lebistes reticulatus*) were also reared.

Effect of chemicals on aphid behaviour. The study of settling responses of *Myzus persicae* on plant leaves and chemically treated surfaces was continued. The behaviour of individual aphids when released on the basal leaves of potato plants after 1 hour of tethered flight was to make a few short-duration probes on the upper surface and then to go to the lower surface, where, after further exploratory probes, they settled on side veins to feed and to reproduce. When surfaces were less attractive or periods of flight shorter, the aphids settled less well (Table 3), remaining on the leaves for shorter periods and producing fewer nymphs. This is in general agreement with the results obtained by B. Johnson (*Anim. Behav.* (1958), **6**, 9–26) with *Aphis fabae* on beans.

A more convenient way than tethered flight of producing the settling response is to confine aphids away from their host plants in glass tubes, and flight-mature aphids were treated in this way when many were required. One hour in the tubes was equivalent to between 30 seconds and 5 minutes of tethered flight. The behaviour of the aphids was not affected by smearing extracts of host leaves on to the surfaces of non-host leaves, and vice versa; Heathcote & Ward (*Bull. ent. Res.* (1958), **49**, 235–237) reported that

TABLE 3

Reaction of aphids, after various periods of tethered flight, to leaf surfaces

Duration of tethered flight		Potato plant					Holly leaf	*Solanum nigrum* leaf
		30 sec	5 min	15 min	30 min	60 min	60 min	60 min
Number of aphids (out of 10) which stayed on the plant for:	Less than 1 hour	1	1	0	0	0	9	2
	1–9 hours	6	3	2	1	0	1	8
	Overnight (or longer)	3	6	8	9	10	0	0
Number of aphids (out of 10) showing larviposition		3	5	8	10	10	0	5

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wetting agents on Brassica leaves influences the colonisation of plants by the aphids, but this was not confirmed when potato leaves were tried. A major difficulty of the work is the variation in physiological state of greenhouse plants, grown at different times throughout the year, and it will be necessary to know considerably more about how these changes affect the behaviour of alighting aphids before the effects of chemical treatments can be assessed. (Griffiths)

Vapour-phase movement and root uptake of systemic insecticides. Further tests were made, similar to those described in the Report for 1962, in which aphids were confined on the leaves of wheat seedlings whose roots were exposed to the vapour of four systemic insecticides. These confirmed that, in these conditions, phorate and disulfoton volatilise, pass into the roots of the plants and reach the leaves in sufficient quantities to kill the aphids; menazon and dimethoate decreased the aphid populations only slightly.

Measuring the toxicity to mosquito larvae of the water that partially covered the roots during a test showed that, although some insecticide passes to the roots directly across the air space, some passes through the water.

Leaves of wheat seedlings whose roots dipped in aqueous solutions of the four insecticides used in the vapour tests were equally toxic to aphids; hence the differences in toxicity observed in the vapour tests are not caused by differences in the inherent toxicities of the insecticides, but by differences in their volatility. (Etheridge and Burt)

Control of aphids and virus diseases of field beans. A further experiment, done in collaboration with A. J. Gibbs of the Plant Pathology Department, compared three organophosphorus insecticides for their ability to control aphids and virus diseases in field beans.

The variety Herz Freya was used, as it is particularly susceptible to pea leaf-roll virus, the most damaging virus common in field beans. The seed rate was 200 lb/acre. The following treatments were applied: 70% menazon dispersible powder applied as a slurry dressing to the seed with a methyl cellulose sticker at the equivalent of 1 lb and 3 lb a.i./acre; 5% w/w disulfoton granules applied by a Gandy applicator to the foliage at the equivalent of 1 lb a.i./acre and 50% v/v demeton-methyl emulsifiable liquid sprayed at the equivalent of 12 fluid oz/acre in 80 gal. The two seed dressings and the granules were applied alone and in combination with the demeton-methyl spray, and these treatments were compared with a single demeton-methyl spray. Thus, together with the controls, there were eight treatments. Each treatment had four replicates.

The beans were sown very late, on 9 April. The disulfoton granules were applied on 11 June when the plants were about 18 in. high, and the spray applied on 27 June when they were about 3 ft high. The date of spraying was determined by the degree of infestation and the desirability of spraying before flowering. The spray was applied when aphids averaged between 1 and 2 per untreated plant. The plants were examined at regular intervals and aphid infestation and virus infections recorded. To determine how

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long the plants remained toxic to aphids, aphids were caged on some of the plants at regular intervals.

In spite of the severe winter and the late spring, the aphid migration was earlier and larger than in 1962 and, by 27 June 26% of the control bean plants carried aphids, with a geometric mean of 1·17 per plant. The infestation remained at about this level until mid-July, when there was a secondary migration into the crop and by August 98% of the plants in the control plots were infested, with a geometric mean of 395·6 aphids per plant.

The early aphid attack was prevented by both the menazon seed treatments alone, and greatly checked by the disulfoton granules alone. Tests with caged aphids showed that both the menazon seed treatments gave plants that were very toxic until late June and slightly so in early July.

The single demeton-methyl spray made plants completely toxic to aphids for only 2 weeks, but this sufficed to prevent damage to blossom and delay to some extent late multiplication from the second migration; loss of toxicity may have resulted partly from the very rapid plant growth during this period; some plants attained a height of over 7 ft.

The menazon seed treatments may have been more effective in 1963 than in 1962 because the soil was much wetter throughout the season, there is evidence from laboratory tests that moisture content of the soil and the effectiveness of menazon are correlated.

The only virus disease at all common in the crop before flowering was pea leaf roll, and by 14 July only about 1% of the untreated plants were infected and the incidence did not increase later. All the insecticidal treatments decreased the incidence, but only the menazon seed dressings did so significantly (Table 4). The incidence of virus was too small to affect yield.

TABLE 4

Mean number of plants infected with pea leaf roll virus per 10 yd of row (Sampled 14.7.63)

Treatment	Control	Menazon S.D. (1 lb/acre)	Menazon S.D. (3 lb/acre)	Disulfoton granules
Unsprayed	1·72	0·50	0·44	1·06
Demeton-methyl	0·89	0·39	0·22	1·17

S.E. Treatment means ±0·275.

TABLE 5

Mean yields (at 85%) dry matter wt/acre

Treatment	Control	Menazon S.D. (1 lb/acre)	Menazon S.D. (3 lb/acre)	Disulfoton granules
Unsprayed	25·0	30·6	33·0	28·8
Demeton-methyl spray	33·7	34·7	34·5	34·3

S.E. Treatment ±1·01

Table 5 shows that the insecticide-treated plots all yielded more than the untreated plots, with the greatest increases from the high-rate menazon seed dressing alone and the demeton-methyl spray treatments, either alone or in combination with the seed treatments and granule treatments. In this

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year, when none of the treatments prevented aphids multiplying late in the season and virus disease was unimportant, the increase in yield came from the control of aphids during the period of blossom and seed set. In years with more virus disease, or when the course of aphid infestation and seasonal growth of the plant are different, the relative effects on yields of the different treatments may differ. (Etheridge and Gibbs, Plant Pathology Department)

Control of potato virus. The co-operative work with L. Broadbent and G. D. Heathcote was concluded. The last of a series of three experiments designed to show whether systemic insecticides applied at planting, either in the soil or directly to the tubers, could check the spread of leaf-roll virus or virus Y within potato crops, was done at Efford Experimental Horticulture Station in 1962. In untreated plots both leaf roll and virus Y increased five-fold, a rather small spread. Disulfoton and dimethoate prevented leaf-roll spread, and both menazon treatments decreased it significantly; menazon applied to the seed tubers was better than menazon granules applied in the soil. Only disulfoton significantly decreased the spread of virus Y (Table 6).

TABLE 6

Percentage of plants infected with virus when tubers were grown in 1963

Treatment and rate of application	Percent infected	
	Leaf roll	Virus Y
Untreated	5·7	5·3
Menazon tuber treatment, 1·3 lb/acre	1·2*	3·8
Menazon granules, 1·6 lb/acre	1·9*	5·0
Dimethoate granules, 3·0 lb/acre	0·4*	4·4
Disulfoton granules, 1·0 lb/acre	0·1*	2·0*

* Significantly different from untreated percentage at 5% level.

Shoots produced by the tubers harvested from this experiment were tested for residual effects of the treatments on aphids and on shoot growth as previously described (*Rothamsted Report* for 1959, pp. 127–128). Although the aphids on shoots of tubers from treated plots never exceeded the number on shoots from untreated plots, the differences were not significant. The insecticides did not significantly slow the growth of the shoots.

In this series of experiments the systemic insecticides dimethoate, disulfoton, menazon and phorate applied in the soil as granular formulations before potato tubers were planted, and menazon applied directly to the tubers, protected the plants from aphids for at least 8 weeks after the foliage emerged, and prevented or greatly decreased the spread of leaf-roll virus. Disulfoton and phorate were more consistently effective than menazon and dimethoate. The spread of virus Y was sometimes checked, but incidence was always at least half that in untreated plots. Provided that the insecticides were applied at the recommended rates, harmful side effects were negligible.

This method of controlling aphids and virus diseases is therefore effective and has important advantages over spraying, because although costs are similar, the crop is damaged less and insecticide application is easier. (Burt)

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Effect of chemicals on the survival and behaviour of wireworms. Studies on the mechanism of toxic action of chemicals on wireworms were continued. Bardner (*Rothamsted Report* for 1962, p. 149) showed that BHC-treated soil took a considerable time to kill wireworms, but that it soon influenced their behaviour, and that affected wireworms were often found on the surfaces of treated soils. Similar effects have been produced when the soil was treated with Bayer 38156 (*O*-Ethyl *S*-*p*-tolylethylphosphonodithioate). Giving wireworms a period of contact with BHC-treated soils decreased their burrowing ability in proportion to the length of time that they had been in contact with the insecticide and the rate at which the insecticide was applied. Using wireworms marked with paint, it was possible to record how often individuals occurred at the surface and to identify those occurring there most frequently as forming a large proportion of the ones that subsequently died in, or on the surface of, the soil. Whether wireworms whose burrowing ability has been affected in this way can recover when transferred to untreated soil has not been determined.

Because of the need to find effective substitutes for the more persistent organic chlorine compounds, the search for new compounds for control of wireworms was continued and intensified. The experimental organophosphate Bayer 38156 proved to be effective in laboratory tests, and this material, together with "Zinophos" (*O*,*O*-diethyl *O*-2-pyrazinylphosphorothioate) and fenthion (*O*,*O*-dimethyl *O*-4-(methylmercapto)-3-methylphenyl phosphorothioate), which last year showed promise for wireworm control, was included in a small-plot field trial with spring wheat. The trial was on an organic silty clay loam in Lincolnshire which had a high initial wireworm population. The plots, 12 ft × 14 ft, were arranged in four randomised blocks. The insecticides, applied on 9–10 April as emulsions at 2·7 lb/acre active ingredient, were harrowed into the soil on 15 April and the field was drilled on 23 April. Plants were examined during the season and the plots were harvested on 18–19 September, when soil samples were taken to assess late wireworm populations.

The grain yields (85% dry matter), cwt/per acre were: control, 16·66; fenthion, 18·75; "Zinophos", 25·24; Bayer 38156, 24·15 (SE ± 1·91). There were significant increases from the "Zinophos" and Bayer 38156 treatments. In fact, these two treatments gave a greater increase in yield than a standard aldrin spray treatment (treatment 23·5 cwt/acre, control 18·6 cwt/acre) applied in an immediately adjacent experiment in the same field. A larger experiment is being planned to see whether Bayer 38156 and "Zinophos" are effective at more economic rates of application and in other formulations. (Griffiths)

Fungicides

Laboratory and field testing of potato blight fungicides was continued.

Laboratory methods. In all laboratory tests foliage was used from 4–8-week-old King Edward plants, grown under glass. Fungicides were at field strength, viz. copper oxychloride at 0·25% Cu, and fentin acetate

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(triphenyl tin acetate) at 0·03%. The methods described last year are still used, with some changes in detail.

Initial retention was measured by Somers' method (*J. Sci. Fd Agric.* (1957), **8**, 520).

Tenacity. We now use a new machine for applying artificial rain to sprayed detached potato leaflets. The leaflets are fixed, with rubber bands, to flat panels at the bevelled rim of a 30-in. wheel rotating at 6 rpm. The panels pass through a fan-shaped jet of tap water coming at maximum pressure from a coarse T-jet (size 650067), held 3 in. away from the panels. The wheel holds 30 leaflets, each of which receives about 0·3 in. of "heavy rain" in 30 revolutions (about 5 minutes). Most of the tenacity figures given below, however, were obtained by using 30 minutes of "light rain", as in 1962.

Bioassay. The bioassay test was improved and simplified. Four logarithmically graded volumes, in the range 0·5–20 ml, are sprayed in a Potter tower on to the *under* surfaces of freshly detached leaflets, with 14 leaflets per volume. The deposits are proportional to the volumes. The dry deposits are given 5 minutes of "heavy rain" before infection. The test is a 4-point "all-or-none" assay, suitable for probit analysis. The standard error of the log EV50 (the logarithm of the volume needed to prevent infection on 50% of the leaflets) is usually about 0·085; this means that differences in effectiveness of 1·7 times or more are usually significant. We hope to improve the test further by spraying, "rain-washing" and infecting the *upper* surfaces only. For this, the "rain-washing" time will have to be increased, because tenacities are often greater on the upper surfaces, which in any case need less fungicide to protect them. In this form the test will be more realistic. However, nearly all of the results given below are from an earlier form of the test, in which *both* surfaces were sprayed and "rain-washed", and the *under* surfaces infected. The standard errors are no smaller by this method, which is not now used.

Results of laboratory tests. In the copper oxychloride–1% wax formulation used in 1962 the wax had mp 54° C. Changing its mp did not improve the tenacity. In "light rain" tests tenacities of mixtures made with three grades of wax were 63% (mp 49° C), 73% (mp 54° C) and 66% (mp 66° C). In bioassay tests the wax formulation, with wax of mp 54°, was over 10 times more effective than a copper oxychloride wettable powder. There was no particle size difference between the formulations.

"Dri-sil 37" (containing the water-soluble silicone, sodium methyl siliconate) can be added to spray materials to make deposits water-repellent, and possibly more effective. It increased the tenacity of the copper oxychloride-wax formulation. In "light rain" tests tenacities were 79% (plain wax formulation) and 95% (same with 1% "Dri-sil 37"). Under these conditions the copper oxychloride wettable powder had a tenacity of about 70%. No bioassay test was done with the wax–"Dri-sil 37" formulation.

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However, bioassay tests showed that addition of 1% "Dri-sil 37" did not improve the efficiency of a fentin acetate wettable powder ("Brestan 60", kindly given by Hoechst Chemicals Ltd).

Fentin acetate can be formulated in wax, but the increase in efficiency is less than with copper oxychloride. The fungicide was dissolved in melted paraffin wax (mp 54° C), and the hot solution emulsified. Suitable emulsifiers are: (*a*) a mixture of "Ethofat 60/25" and "Ethomeen 18/12" (kindly given by Armour Hess Chemicals Ltd.); or (*b*) a mixture of "Brij 76" and "Brij 72" (kindly given by Honeywill & Stein Ltd.). These emulsions were made at 20% wax, but were diluted for use to 1% wax, 0·3% emulsifiers and 0·03% fentin acetate.

In a water-repellency test contact angles were >90° (wax formulation (*a*)) and 86° (unsprayed); on another occasion, contact angles were 71° (wax formulation (*b*)) and 75° (unsprayed). In four comparative bioassay tests wax formulation (*a*) was twice as effective as "Brestan 60". Wax formulation (*b*) was roughly as effective as formulation (*a*).

We have continued testing, as additives for copper sprays, the alkali salts of organic thiols, i.e., compounds of the general formula RSNa, where R is a medium or long chain. Some of these compounds increase the tenacity of copper fungicides, but many can be rejected because of cost or smell, or because they react too quickly with copper oxychloride, or because they are too easily oxidised, or are phytotoxic or ineffective as stickers. For such reasons we rejected nabam and the alkali salts of 2-mercaptobenzothiazole, toluene-*p*-thiol, dodecanethiol and various xanthates; but we had some success with sodium thiobenzoate.

The mixture we used contained 0·25% Cu as copper oxychloride; 0·05% of "Belloid TD" (a dispersing agent, kindly given by the Geigy Co. Ltd.); and 0·1% of sodium thiobenzoate (kindly given, as a 40% aqueous solution, by Robinson Brothers Ltd.). No surface active agent was added; initial retention was decreased to $\frac{1}{3}$, although tenacity was not affected, by adding only 0·025% of "Triton X-100" (kindly given by Lennig Chemicals Ltd.).

In a water-repellency test, contact angles were 89° (thiobenzoate formulation), 67° (wettable powder) and 87° (unsprayed). Maximum tenacity of copper oxychloride was reached at 0·1% sodium thiobenzoate. In a "light rain" test tenacities were 96% (thiobenzoate formulation) and 58% (same mixture without thiobenzoate); comparable figures for other materials (on different occasions) were 70% (wettable powder) and 83% (Bordeaux mixture). In "heavy rain" tests tenacities were, after 5 minutes, 44% (thiobenzoate formulation) and 26% (wettable powder); and, after 60 minutes, 25% (thiobenzoate formulation) and 19% (wettable powder). Thus the advantage of the thiobenzoate is only temporary. In bioassay tests the thiobenzoate formulation gave "flat" probit lines, but was roughly as effective as the wax formulation.

The action of sodium thiobenzoate, in improving the tenacity of copper oxychloride, may be twofold. It may be oxidised to the insoluble dibenzoyl disulphide, which would stick the fungicide to the leaves; or it may slowly react with copper oxychloride to form cupric thiobenzoate in a "tank-mix" form. Probably both of these reactions occur to some degree. Under our

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conditions, however, 94% of the added sodium thiobenzoate was recovered unchanged from the jets of a field sprayer.

Field tests. A 6 × 6 Latin square experiment was done on King Edward potatoes to compare these formulations with commercial formulations. Treatments were: A, commercial copper oxychloride wettable powder; B, copper oxychloride–1% wax formulation; C, copper oxychloride–sodium thiobenzoate formulation; D, fentin acetate–1% wax formulation (*a*); E, commercial fentin acetate wettable powder ("Brestan 60"); and F, unsprayed.

Differences in the amounts of copper found on the leaves were slight. The experiment failed as a test of blight control because a severe attack by aphids in July–August killed many of the plants, and because harvest was delayed until 5–6 December. (McIntosh and Eveling)

The physical effect of spray particles on leaves. Pesticides sprayed on to leaves may have both chemical and physical effects; there seems to be no method for distinguishing between these two effects using pesticides as such. However, by the use of dusts of little chemical activity, the physical effect of spray particles can be determined. The work, so far, has been confined to determining the effect of sprays on transpiration.

A laboratory method was evolved to determine the effects of spray deposits on excised leaflets. Runner-bean leaflets (*Phaseolus vulgaris*) were sprayed in the Potter tower on their lower surfaces, with aqueous suspensions of dusts, and controls were sprayed with distilled water. After spraying the leaflets were placed in a drying chamber, kept at 20° C with a relative humidity between 8 and 12%; the leaflets were weighed after 45, 75 and 105 minutes.

The initial tests were concerned with the possibility that variation in transpiration was caused by a chemical action of the dust suspension. In each of two experiments 10 leaflets were each sprayed with 10 ml of distilled water (controls) and 10 with a 1% w/v suspension of "Stockalite" (a colloidal kaolin). "Stockalite" had 81% of the particles smaller than 2 μ; the suspensions had a pH = 6·2, compared with 4·9 for distilled water. The mean water losses in mg/cm²/h in the two experiments were 2·1 and 1·0 ("Stockalite" sprayed) and 1·1 and 0·4 (controls); both differences between treatments were significant ($P = 0{\cdot}1\%$).

To confirm that the increased water loss was not associated with the relatively small difference in pH, a 1% w/v suspension was centrifuged. The deposit was resuspended in 100 ml of distilled water and sprayed as above; the control leaflets were sprayed with the supernatant liquid. The pH of the resuspended deposit, in the two experiments, was 6·1 and 6·1 and the supernatant liquid 6·5 and 6·7. The mean water losses for leaflets in mg/cm²/h sprayed with the resuspended deposit, was 1·9 and 1·7 and sprayed with the supernatant liquid, 1·2 and 1·2. Both differences between treatments were significant ($P = 1{\cdot}0\%$). These results indicate that the "Stockalite" particles rather than any direct chemical action increased water loss.

The effect of particle size on increased transpiration was then examined.

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Suspensions (1% w/v) of a range of particle sizes of silica were sprayed on leaflets as above. Using particles 1–2·5 μ diameter, the mean water losses in mg/cm²/h were 1·1 and 1·2 and for the controls 0·5 and 0·6; the differences between treatments were significant ($P = 0{\cdot}1\%$). Using particles 2·5–5 μ, the mean water losses were 1·1 and 1·3 and for the controls 0·9 and 1·0; the differences were significant ($P = 1\%$). Using particles 5–10 μ, the mean water losses were 0·9 and 0·5 and for the controls 0·8 and 0·4; the differences were not significant ($P = 5\%$). The pH values of the suspensions were between 6·0 and 6·5.

The Research Department of Messrs English Clays Lovering Pochin Co. Ltd. kindly provided the "Stockalite" and graded silica together with information about their characteristics. (Eveling)

Molluscicides

Chemical control of slugs. The initial object of the work is to develop laboratory methods to assess and compare the toxicity of chemicals to slugs. The methods should enable the assessment to be made of the fumigant activity, stomach-poison activity and contact activity of the compound.

A method of measuring the fumigant activity of chemicals was developed and the toxic effect of metaldehyde vapour measured.

Various methods of administering contact-acting poisons were tried. Little success was obtained in forcibly injecting known doses of poison in solution, and voluntary feeding methods were rejected as they might not distinguish between unpalatable and non-toxic compounds.

The physical characteristics of the slug make the estimation of contact action difficult. Direct-application methods are not suitable because the chemical is sloughed-off in the slimy exudate. Methods involving crawling over a treated substrate gave very variable results. The only satisfactory method was a dipping technique involving the total immersion of the slugs in aqueous solutions or dispersions of poison. Using this method, the contact activities of copper sulphate ($L.C._{50}$ 79·7 ppm), sodium pentachlorophenate ($L.C._{50}$ 12·6 ppm), metaldehyde ($L.C._{50}$ 31·3 ppm), and the sodium salt of 3,5-di-iodo-4-hydroxy-benzonitrile ($L.C._{50}$ 2·4 ppm) were compared.

The appearance of treated slugs differs with different poisons, so death-point was determined as failure to respond to a standard electrical stimulus. (Henderson)

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