ROTHAMSTED RESEARCH

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Insecticides and Fungicides

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

T. Jephcott and Mrs. Gertrud Green left, and Margaret May, D. C. Griffiths and N. F. Janes were appointed to the staff. I. Henderson joined the department to work on the chemical control of slugs, with a grant by the Department of Agriculture and Fisheries for Scotland. Visiting workers included Professor E. P. Lichtenstein, of the University of Wisconsin, and Dr. J. S. Grewal, of the Indian Agricultural Research Institute, New Delhi.

At the request of the Government of the United Arab Republic, C. Potter visited Egypt to advise on the problem of suspected resistance to insecticides of the Egyptian Cotton Worm *Prodenia litura*. By invitation, R. Sawicki lectured in Paris on the "Synergistic Activity of Sesoxane" to the Comité Français pour l'Etude des Applications des Pyréthrines.

Insecticides

The action of organophosphorus insecticides and the causes of resistance

Concentrations of inhibitor in the haemolymph of resistant and susceptible houseflies. Earlier work (*Rep. Rothamst. exp. Sta.* for 1961, p. 132) showed no detectable difference in the concentrations of diazoxon, the oxygen analogue of diazinon [*O*,*O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate], required to inhibit cholinesterase in the isolated thoracic ganglia of susceptible and resistant flies. On the assumption that the thoracic ganglion is representative of the central nervous system and that inhibition of the cholinesterase of the central nervous system is the cause of death, this suggests that, for a given amount to reach the central nervous system, more poison must be applied to the resistant fly than to the susceptible fly. The poison would reach the central nervous system through the haemolymph, so techniques to estimate inhibitor in the haemolymph were studied.

The first method tried was to draw samples of haemolymph and assay the inhibitor by finding its capacity to inhibit esterase activity. By cutting off the wings near their bases, haemolymph was obtainable *via* the wing veins of the housefly without gross contamination by poisons applied to the thorax or abdomen. Freedom from such contamination was checked with fluorescent dyes. Using as an enzyme source an extract of an acetone powder of *Blatella germanica*, with phenyl acetate as a substrate, and measuring the phenol liberated, a method was devised that, with as little as 1–5 μl of haemolymph, can estimate concentrations of cholinesterase inhibitors of the order of 10^{-6} *M* diazoxon.

This technique shows no obvious differences between the amounts of anticholinesterase activity in the haemolymph of susceptible and resistant

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flies at similar stages of poisoning, even when very different amounts of poison had been externally applied to produce the same symptoms.

The comparisons are not yet reliable, because the measurements are complicated by the presence in the housefly haemolymph of an esterase that hydrolyses phenyl acetate and is not inhibited by organophosphorus compounds. No satisfactory way has yet been found to inactivate this enzyme without interfering with the estimation of the inhibitor present. (Lord and Solly)

Relation between amino acids and resistance to organophosphorus insecticides. The susceptibility of adult houseflies to poisoning by organophosphorus insecticides depends on both their age and sex. Some preliminary experiments showed that the amino-acid content also differs with age and sex, but whether changes in amino-acid content are in any way related to changes in resistance to organophosphorus insecticides needs further study. (Lord)

Distribution and inhibition of cholinesterase in the peripheral tissues of houseflies, susceptible and resistant to diazinon. This work is a continuation of the study of the location of cholinesterase in susceptible and resistant strains of houseflies, and of how the amount of inhibition produced by externally applied diazinon varies with the strain and the location of the enzyme.

Previous work with the central nervous system was extended to a study of the peripheral tissues, including the peripheral nerves. Female flies were examined 1–2 days after emergence from the pupa and at maturity, i.e., 4–5 days old. The alimentary canal with the salivary glands and malpighian tubules, the abdominal fat body, ovaries and thoracic flight muscles were removed from the flies and incubated in Koelle's thiocholine medium (as modified by Gomori 1952) for 20 hours. Approximately 2×10^{-2} *M* acetyl thiocholine iodide or butyryl thiocholine iodide were used as substrates. A cholinesterase inhibitor, 10^{-5} *M* eserine, was added to the control solutions. Enzymes hydrolysing both acetyl and butyryl thiocholine were abundant in the peripheral nerves to all the tissues examined, except in the finer nerve branches of the rather specialised indirect flight muscles, which had very little enzyme activity.

The cholinesterase is not confined to the nervous system, but occurs in non-nervous tissues, sometimes together with other esterases which were not specifically studied. These tissues contain much less cholinesterase than the nervous system, so that longer incubation with substrate is necessary to detect it. The enzyme was found in most regions of the alimentary canal, especially in the hind gut. The proximal part of the malpighian tubules is especially rich in the enzyme. It was also found in the fat body and oenocytes and in parts of the ovaries, where its distribution is localised and very distinctive. In the newly emerged flies, spherical larval fat body cells also contain cholinesterase. In the salivary glands cholinesterase is localised in single epithelial cells somewhat haphazardly. In the ovaries the enzyme is confined to the germinal epithelium at the apical tip of the ovarioles and to the follicle cells and membrane surrounding the developing oöcytes. The oöcytes themselves seem free from cholinesterase.

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Few differences in the localisation of cholinesterase were found between 1–2- and 4–5-day-old flies, except in the ovaries, where the enzyme becomes concentrated during development in a narrow band of tissue surrounding the primary, i.e., the most mature, oöcyte and is left behind when this is shed. The cholinesterase-rich larval fat body cells of the young fly are absorbed in the older flies. The differences in distribution and activity of cholinesterase between susceptible and resistant strains were slight and considered unlikely to affect resistance.

After applying diazinon externally the amount of inhibition of cholinesterase was assessed in the peripheral tissues of dead and living flies of both susceptible and resistant strains. The inhibition was measured 24 hours after applying technical diazinon topically to 0–1- and 3–4-day-old flies. The interval before examination was the same for the dead and living flies, but as the treatment killed within a few hours, the dead flies were less mature than those that survived. Cholinesterase was not inhibited in the nerves to the fat body and ovaries, except in flies of either strain that were killed by an LD80–90 dose. Cholinesterase activity was largely inhibited in the nerves to the alimentary canal of dead flies of both strains, even after an LD40–50 dose, which is 0·0085% for the susceptible and about 0·5–0·8% for the resistant strain. With the survivors, inhibition in these nerves was slight, even with an LD80–90 dose, which is approximately 0·02% for the susceptible and 0·7–1·6% for the resistant strain. Inhibition also occurred in the fine nerve branches in the thoracic flight muscles, although the small amount of enzyme normally present in these sites made interpretation difficult.

In killed flies of both strains the cholinesterase in the fat body, oenocytes and proximal parts of the malpighian tubules was partially inhibited, and almost completely inhibited in the walls of the alimentary canal and in the salivary gland epithelium. In the ovaries the enzyme is more readily inhibited in the germinal epithelium than around the mature oöcyte.

The degree of inhibition of cholinesterase in the peripheral tissues differed among flies of the resistant and susceptible strains in ways that might have a bearing on the resistance problem. For instance, inhibition was greater in the fat body and oenocytes of older susceptible flies killed by 0·0085%, and in living or dead flies after 0·02% diazinon, than in any resistant flies, even those killed by a dose of 1·6% diazinon. The enzyme in the gut, malpighian tubules, salivary glands and ovaries of young flies was slightly more inhibited in the resistant strain when killed by 0·72% diazinon (LD80) than in susceptible flies killed by 0·0085% (LD80). In the older flies of both strains a large dose gave almost complete inhibition in all these tissues except the ovaries, whereas a small dose (approx. LD40) caused slightly less inhibition in the resistant than in the susceptible strain. Cholinesterase can, however, be almost completely inhibited in the wall of the gut, malpighian tubules and salivary glands, particularly in the resistant strain, without necessarily killing the insect. (Szeicz)

Correlation between cholinesterase inhibition and loss of nerve function in insects. If insects poisoned by organophosphorus insecticides die because cholinesterase of the nervous system is inhibited it is presumably because

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the lack of the enzyme causes loss of function by the nerves. Histochemical studies have provided information on the amount and location of the inhibition in the nervous system when houseflies are killed with organophosphorus insecticides, but there is no direct evidence to show whether this inhibition causes loss of function of the nervous tissue. The correlation between inhibition, demonstrated histochemically, and loss of function, demonstrated electrophysiologically, can not easily be made with houseflies because of the difficulty of setting up a suitable electrophysiological preparation, and attention was turned to the cockroach *Periplaneta americana*. The cholinesterase inhibitors are applied to the 6th abdominal ganglion, and nerve function is tested by stimulating the cercal nerve and observing the nerve impulse generated in the giant fibres in the abdominal nerve cord anterior to the ganglion. After the functional state of the preparation has been recorded the distribution and amount of active cholinesterase in the nerve tissue is determined histochemically.

So far an approximately linear correlation has been found between log of the time taken by the inhibitor to block nerve impulses passing through the ganglion and the log of the concentration of the inhibitor applied. The functional state of the ganglion and the degree of inhibition of its cholinesterase also relate to one another fairly consistently over a range of conditions.

The effects of *in vivo* application of the poison are being studied to seek information on the relationship between death of the insect, pattern and degree of inhibition of cholinesterase and loss of nerve function. This may help to explain the normal mechanism of poisoning and eventually elucidate the causes of resistance. (Burt and Szeicz)

Technique of selection of houseflies* (Musca domestica L.) *to increase resistance to diazinon. The resistance factor of a diazinon-resistant strain (SKA) of houseflies was increased from about 25 to over 100 by changing from Sacca's method of exposure to residual films to a topical-application method of selection. The adult flies were chilled and sexed when less than 24 hours old. To prevent mating the two sexes were then kept separate in 100-ml glass or plastic containers and treated on the following day by tropical application of a 1·0-µl drop of diazinon dissolved in acetone. The survivors of both sexes were counted and released, 24 hours after treatment, in a cage where they mated. Usually only flies that survived an LD50 or higher were kept for breeding. The high resistance factor was maintained only by selecting each generation. When the flies were not selected for one generation the resistance factor of the succeeding generation fell to 50–60. (Sawicki and Green)

***Changes in resistance to diazinon with age and sex of adult houseflies* (Musca domestica L.).** In a normal susceptible and the SKA-resistant strain of houseflies the adults were most susceptible to diazinon immediately after emerging from the pupae. The resistance increases for 1 or 2 days after emergence and remains steady for the next 4 days. The increase in resistance with age was proportionately higher in the SKA strain.

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In both strains the two sexes were equally susceptible to diazinon during the first day, but later the females were more resistant than the males. The resistance factor of the SKA strain changed with age and was least immediately after emergence. There was no direct correlation between changes in susceptibility and changes in the weight of the flies on ageing. (Sawicki and Green)

Isolation and estimation of organophosphorus compounds and their metabolites by gas–liquid chromatography. Gas–liquid chromatography offers possibilities for isolating and estimating very small amounts of organophosphorus compounds relatively quickly and easily, and tests were made with a range of column packings at three different temperatures, using several different organophosphorus insecticides and possible metabolites.

In collaboration with Professor Lichtenstein, the method was tested to study the uptake of disulfoton [*O*,*O*-diethyl *S*-2 (ethylthio) ethyl phosphorodithioate] by plants. A suitable extraction procedure was evolved, but the initial attempts to find a system that would separate the metabolites failed. A possible system has now been found. (Lord and Solly)

Physico-chemical characteristics of residual insecticide films. Dieldrin and aldrin crystals on non-sorptive surfaces (glass) at 20° in still air volatilised at constant rates, which were very sensitive both to air movement and temperature change. On a glass surface in still air at 20° decreasing crystal size of dieldrin to about one-hundredth doubled the volatilisation rate. Thus, dieldrin crystals forming needles 1–10 mm long (average, 5 mm) at an initial deposit density of 2 μg/cm² gave a rate of volatilisation of 0·06–0·08 μg/cm²/day; dieldrin crystals forming a milky white deposit of needles <0·01–0·05 mm long (average length approx. 0·02 mm) at initial deposit densities of 2 μg/cm² or 5 μg/cm² gave rates of volatilisation of 0·12–0·14 μg/cm²/day. Under the same conditions rod-shaped crystals of aldrin (average length approx. 0·01 mm) at an initial deposit density of 2 μg/cm² gave a volatilisation rate of approximately 2 μg/cm²/day. Aldrin thus volatilised at about 14 to 16 times the rate of a similar deposit of dieldrin.

Radioactive materials were employed in these experiments, and the films were spread on the surface with the edge of a glass coverslip. This technique is not suitable for many surfaces, including plant surfaces, and normal spraying techniques are not suitable for radioactive materials. A special spraying technique for use with radioactive materials was developed. An apparatus that will spray very small quantities of solution is essential, and it is desirable that a high percentage of the material reaches the spray target and deposits evenly there. In addition, the sprayed material must be confined to avoid contaminating the surroundings.

Basically, the apparatus consists of a spray nozzle as used in the Potter tower, with the central liquid jet surrounded by an air annulus. The modification introduced is a needle valve used to allow a controlled amount of air at atmospheric pressure to leak into the liquid jet. On the carburettor principle some of the solution is then atomised before it

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reaches the tip of the jet. The solution is sprayed down a 9-in. long, $2\frac{3}{4}$-in.-diameter, straight-sided glass tube which rests on a platform carrying the target surface. There is no peripheral gap between tube and platform as there is in the Potter Tower. The air pressure used is 10 lb/sq in. Some spray is lost by deposition on the side of the tube, and there is some entrainment of spray out of the top of the tube. The entrained spray is trapped by sealing the top with a plate with a central hole just fitting the nozzle and an outlet tube leading from the plate to an absorption tube.

Spraying aqueous dye solution on filter-paper shows that this apparatus can give a very fine deposit and an even coverage. When water was sprayed into a 50% solution of molten vaseline in kerosene the diameter of the atomised spherical droplets formed was in the range 0·01–0·05 mm (average 0·02 mm). As little as 0·05 ml of solution has been successfully sprayed by this method. (Phillips)

Pyrethrum: biological activity and pyrethrin content of extracts of fresh and dried flowers. Plants of pyrethrum (*Chrysanthemum cinerariaefolium*) were grown in an unheated glasshouse and in the open. During the summer, plants from the open yielded an average of 79·7 flowers per plant with an average fresh weight of 0·801 g. The pyrethrin content of the dried flowers varied, but was usually between 1·4 and 1·8%. These plants not only provided the material for our experiments but also for the work at the Tropical Products Institute on biosynthesis of the pyrethrins using radioisotopes.

Preliminary experiments were made to determine the biological activity of extracts of fresh and dried flowers and to obtain information on the effect of different drying conditions on the biological activity and pyrethrin content of extracts of dried flowers. Random samples of fresh flowers were obtained from bulk by a riffling procedure. Twenty flowers from each sample were cut in half, and one group of twenty halves was immediately extracted, the other group was dried over silica gel at laboratory temperature to a constant weight, then extracted. Drying took approximately 10 days. Both samples were extracted by grinding for 20 minutes in a ball mill containing 40 ml of 10% v/v methanol in acetone, then centrifuging and washing the ground flowers in acetone until no further colour was extracted. The activity of extracts of fresh and dried flowers was assessed by bioassay by topical application to adult *Tribolium castaneum* Hbst. and by the Birchfield–Harris technique with larvae of *Aëdes aegypti*. The results are not fully analysed, but activity seems to be little affected by drying the flowers in this way.

Some preliminary experiments tested the effect of drying temperature on loss of activity. Comparable samples were dried on trays to a constant weight at temperatures of 100°, 80°, 40° and 25°, when they were extracted as described above. Preliminary bioassay with adult *Tribolium castaneum* indicated some loss of activity in the samples dried at 100°, and this correlated well with the figures for total pyrethrins obtained with the spectrophotometer, and pyrethrin I content obtained by gas–liquid chromatography* shown in Table 1. (Stevenson)

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TABLE 1

Concentration of pyrethrins and pyrethrin I in flowers dried at different temperatures

Drying temperature	Drying time	Moisture content (%)	Concentration total pyrethrins (% dry wt)	Concentration pyrethrin I* (% dry wt)
A. 100°	6 hours	77·2	1·99	0·45
80	13 hours	77·1	2·25	0·56
40	52 hours	78·1	2·28	0·56
25	7 days	78·0	2·18	0·53
B. 100	5 hours	77·1	1·60	0·44
80	11½ hours	77·0	1·93	0·50
40	52 hours	77·8	2·16	0·55
25	7 days	77·5	2·08	0·49

* Analysis of pyrethrin I using gas–liquid chromatography made by Dr. P. Godin of Tropical Products Institute.

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

PLANT FEEDING

Hemiptera	*Acyrthosiphon pisum* (Harris)
	Aphis fabae (Scop.)
	Brevicoryne brassicae (L.)
	Megoura viciae (Buckt.)
	Myzus persicae (Sulz.)
	Rhopalosiphum padi (L.)
Lepidoptera	*Diataraxia oleracea* (L.)
	Pieris brassicae (L.)
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.)
	Ptinus tectus (Boieldieu)
	Tenebrio molitor (L.)
	Tribolium castaneum (Herbst.)
	Tribolium confusum (J. du V.)
	Trogoderma granarium (Everts)
Diptera	*Aëdes aegypti* (L.)
	Drosophila melanogaster (Meig.) (3 strains including a wingless mutant)
	Musca domestica L. (4 strains)
Hymenoptera	*Apis mellifera* L.

Guppies (*Lebistes reticulatus*) were also reared

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Bee poisoning in the field. Twenty-four samples of bees suspected of being poisoned in the field were received from Mr. P. S. Milne, Chief Bee Advisory Officer of the Ministry of Agriculture, Fisheries and Food. Three samples contained sufficient carbamate of the "Sevin" type to cause death. This is the first season that a colorimetric test for carbamate was included in the analyses. Eight samples were poisoned by cholinesterase-inhibiting substances and were probably killed by organophosphorus insecticides. One other sample gave inconclusive evidence of this type of poisoning. There was evidence of poisoning by dieldrin or aldrin in three samples and by lindane in one.

The remaining eight samples showed no evidence of poisoning by insecticides, mercury or by phenoxy-acetic acid type weedkillers. L. Bailey of the Bee Department concluded that the bees in three of the samples may have been suffering from paralysis.

The detection and identification of the chlorinated hydrocarbon insecticides in extracts of bees prepared for bioassay was helped by the use of gas–liquid chromatography. It is hoped that techniques will be found to allow this method of analysis to be extended to other insecticides, including organophosphorus compounds.

The efficiency of the extraction of dieldrin, aldrin, lindane, DDT and parathion from bees is being checked, both by bioassay and gas–liquid chromatography. So far, only dieldrin has been tested by bioassay, but the agreement between this method and that of gas–liquid chromatography is good. (Needham and Solly)

Effect of chemicals on aphid behaviour. Chemicals can affect insects in other ways than killing them; for example, they may attract, repel or irritate insects. Such effects may be important in influencing the spread of virus diseases, for chemicals that stimulate aphid vectors to probe leaves and wander are likely to increase spread, whereas those that inhibit these reactions are likely to decrease it.

As a preliminary to studying the effects of chemicals on the behaviour of *Myzus persicae* on potato plants, a standard laboratory technique is being developed for making tests in a glass-sided box at constant temperatures. Flight-mature, alate aphids are given a period of tethered flight and then released on to potato leaves, or other surfaces, where their settling responses are observed. Attempts are being made to fly the aphids on small suction tubes from which they can be released directly on to the test surface. The effect of the duration of flight period on behaviour is first being examined, using untreated plants, and methods of recording the aphids' probing and wandering behaviour are being developed. If a technique giving reproducible results with untreated plants can be worked out the effects of chemical treatments will be studied. (Griffiths)

The insecticidal activity of systemic insecticides applied as foliage sprays

(a) ***Effect of light on persistence.*** Demeton methyl (*O*,*O*-dimethyl *O*-2(ethylthio) ethyl phosphorothioate), dimethoate (*O*,*O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate) and phosphamidon (dimethyl 2-chloro-2-diethylcarbamoyl-1-methyl vinyl phosphate) in

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aqueous medium containing 0·05% v/v active ingredient and a wetting agent were sprayed on bean plants afterwards put in a glasshouse at approximately 20°, where half were kept in darkness and half in the normal summer conditions of daylight and dark. Insecticidal activity, measured by confining aphids (*Megoura viciae*) in perspex cages on the lower surface of the leaves and observing the proportion killed after 24 hours, persisted longer with the plants kept in darkness.

(b) ***Effect of the stage of growth of the plant on translocation.*** Demeton methyl, dimethoate or phosphamidon at 0·1% v/v in aqueous medium, with 0·01% "Lissapol N" as a wetter, were painted on a pair of leaves in the middle region of actively growing and mature bean (*Vicia faba*) plants, and movement of the insecticides into the leaves above and below the treated pair was detected by confining aphids on them. In young plants demeton methyl and phosphamidon moved within 24 hours into both pairs of untreated leaves and killed all the aphids confined on them; the leaves remained highly toxic for several days, when their toxicity decreased slowly. Dimethoate took a day longer to make the untreated leaves fully aphicidal, and the activity was less persistent than with the other two poisons.

Translocation was much slower in mature plants, and aphids confined on leaflets above or below the treated ones were not killed until about 6 days after the treatment. In both young and old plants there was some indication that the three insecticides move upward more readily than downward.

(c) ***Fumigant action.*** Most of the commonly used systemic insecticides have some fumigant action when aphids are confined above them in sealed petri dishes, but only a few are highly toxic in this way (*Rep. Rothamst. exp. Sta.* for 1961, p. 137). Toxicity in a closed system does not necessarily mean there will also be toxicity in the open conditions of the plant surface, and an experiment was done with mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate), which has a strong fumigant action, to test for fumigant action under conditions approximating to those in practice. A 0·1% v/v solution in water containing 0·01% "Lissapol N" was painted on leaf surfaces, and aphids were confined close above the surface. A 2·5-cm-diameter ring, 0·2 cm thick, separated the aphids from the leaf surface. The aphids were kept in a perspex cage also 2·5 cm diameter with walls 0·8 cm high, and a piece of "Kleenex" tissue, placed between the disk and the cage, formed the floor of the cage and prevented the aphids making contact with the leaf or feeding on it. The top of the cage was covered with 0·1-cm-mesh terylene net so that the vapour was not confined. The maximum distance of the aphid from the plant was about 1·0 cm, the minimum distance 0·3 cm. Under these conditions mevinphos killed all *Megoura viciae* caged within 20 hours after leaf treatment. (Etheridge)

Vapour-phase movement and root uptake of systemic insecticides. Of four systemic insecticides applied in the soil to control the aphid vectors of potato viruses, all were effective in wet seasons, but in dry seasons menazon (*O*,*O*-dimethyl *S*-(4,6-diamino-1,3,5-triazin-2-yl) methyl phosphorothio-

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lothionate) and dimethoate were less effective than disulfoton or phorate (*O*,*O*-diethyl *S*-(ethylthiomethyl) phosphorodithioate).

To be effective, systemic insecticides must move through the soil from the region where they are placed to the roots of the crop; this may happen either in solution in the soil moisture or by diffusion in the vapour phase. The observed effects could be explained by the different physico-chemical properties of the four chemicals. Menazon and dimethoate are fairly water soluble, so could move readily in wet soils, but their vapour pressure might be too low for them to move readily in the gaseous phase when soils are dry. Disulfoton and phorate, which have a very low solubility in water, could move in the gaseous phase in both dry and wet soil. Phorate has a much greater fumigant activity than dimethoate (*Rep. Rothamst. exp. Sta.* for 1961, p. 137), indicating that it more readily produces toxic quantities of the vapour phase.

The relative ability of the four chemicals to move in the vapour phase and be taken up by plants was further explored. Wheat seedlings were kept at 20–25° with their roots dipping in a few ml of water in a small sealed vessel. A little insecticide, enough to provide a saturated atmosphere, was kept in the air space, above the water and away from the roots. The mortality of aphids (*Rhopalosiphum padi* (L.)) confined on the aerial part of the plant was measured to assess the insecticide taken up by the plants. After 3 days all the aphids were killed by phorate and disulfoton but none by menazon and dimethoate, so phorate and disulfoton seem sufficiently volatile for toxic quantities to move in the vapour phase and either enter the roots directly, or the water in which they dipped, whereas menazon and phorate are not. (Bardner and Burt)

The control of aphids on field beans. *Aphis fabae* can be controlled by spraying with demeton methyl when the aphids have just finished migrating into the crop. This procedure has disadvantages; the beans are often tall and are damaged by the passage of the sprayer; the time may coincide with flowering and thus make spraying dangerous to pollinating insects, and it may not coincide with the entry of *Acyrthosiphon pisum*, which is thought to be the principal vector of pea leaf-roll virus.

Using field beans sown in mid-March, a field experiment was done to test the effectiveness of methods of treatment without these disadvantages. The following treatments were applied: Control; menazon applied as a slurry dressing to the seed with methyl cellulose sticker at the equivalent of 1 lb active ingredient/acre, and at 3 lb a.i./acre; 5% w/w menazon granules combine-drilled with the seed at the equivalent of 1 lb a.i./acre and at 3 lb a.i./acre; 5% w/w menazon granules broadcast on the foliage by means of a Gandy applicator at the equivalent of 1 lb a.i./acre and at 3 lb a.i./acre on 5 June when the beans were about 18 in. high; 5% w/w disulfoton granules applied to the foliage at the equivalent of 1 lb a.i./acre in the same way and at the same time as the menazon granules; 40% w/v menazon emulsifiable liquid sprayed at the equivalent of 1 lb a.i./acre in 60 gal; 50% v/v demeton methyl emulsifiable liquid sprayed at the equivalent of 12 fluid oz/acre in 60 gal. The full experiment was done at Rothamsted, and most of the treatments were repeated at Woburn.

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The migration of both aphid species into the crop was abnormally late and sparse. By late June only 5·6% of the bean stems on untreated plots at Rothamsted had any aphid colonies of either species, and the infestation remained small until the end of July, when there was a secondary migration into the crop and untreated plots had 70% of stems infested. The aphids arrived too late and were too few to damage the untreated plants, and the insecticide treatments had no effect on yield, although they all decreased the infestation.

The very late appearance of the aphids provided a severe test of the toxicity and persistence of the treatments other than the sprays. Spraying with demeton methyl made plants toxic to aphids for 4 weeks and with menazon for at least 6 weeks. Applying insecticides as granules to the foliage was less effective than applying them to the seed or soil, although done very much later. Toxicity persisted longest with the seed and soil treatments, which decreased infestations until early July.

The dry weather in late spring produced deep-rooted plants with few lateral roots. In plots watered heavily in mid-June plants developed many lateral roots just below the soil surface, and this increased the toxicity to aphids of plants in plots where insecticides had been applied to the seeds or soil. (Bardner, Etheridge and Gibbs, Plant Pathology Department)

Control of potato viruses. This co-operative work with G. D. Heathcote and L. Broadbent continued. In 1961 a potato crop containing 0·8% plants with leaf roll and rugose mosaic was sprayed on one or more of four occasions, with demeton methyl on 16 June, DDT and demeton methyl on 28 June and DDT on 19 July and 9 August. Aphids were few, and plants grown in 1962 from seed tubers saved from the variously treated plots showed that viruses spread very little (Table 2).

TABLE 2

Percentages of infected tubers from different plots

	Leaf roll	Rugose mosaic
Unsprayed	4·0	5·7
Sprayed 9 August only	3·7	8·9
Sprayed 19 July and 9 August	3·6	7·3
Sprayed 28 June, 19 July and 9 August	2·8	9·8
Sprayed 16 and 28 June, 19 July and 9 August	1·0	5·3

The incidence of virus in tubers taken from plants growing near to infected ones was: unsprayed, 11·3% leaf roll, 25% rugose mosaic; sprayed four times, 2·4% leaf roll, 14·5% rugose mosaic. Thus, spraying four times almost stopped the spread of leaf roll but not of rugose mosaic. Over half the spread of leaf roll occurred before 28 June and almost 90% by 19 July.

Of a sample of 558 tubers taken from the field containing the experiment, 1·4% was infected with leaf roll and 4·1% with rugose mosaic, showing that there was considerable general spread of virus, either from the experiment or from sources elsewhere.

In an experiment at Efford Experimental Horticulture Station in 1961 phorate granules, applied in the soil at 4½ lb/acre of active ingredient, and disulfoton, at 1 lb/acre, almost stopped the spread of leaf roll.

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Dimethoate granules applied to the soil at 4½ lb/acre and menazon wettable powder applied to the seed tubers immediately before planting at 1 lb/acre decreased spread to about one-third that in the untreated plots. Spread of rugose mosaic, which was very great, was slightly decreased by the menazon seed treatments, but was unaffected by the other treatments.

Shoots produced by tubers harvested from this experiment were tested as previously described (*Rep. Rothamst. exp. Sta.* for 1959, pp. 127–128); shoots of tubers from plots treated with dimethoate grew a little more slowly and carried slightly smaller aphid populations than those of tubers from untreated plots, but other treatments had no consistent effects.

In 1962 another experiment at Efford tested the ability of systemic insecticides applied to seed tubers or the soil to check the spread of viruses. Three chemicals were used in four treatments:

1. Menazon as a dispersible powder suspended in water and sprayed at equivalent of 1 lb a.i./acre on to the seed tubers immediately before planting.
2. Menazon in 5% granular formulation applied at 1½ lb a.i./acre in the drill at planting.
3. Dimethoate in 7·1% granular formulation applied at 3 lb a.i./acre in the drill at planting.
4. Disulfoton in 5% granular formulation applied at 1 lb a.i./acre in the drill at planting.

The method of application has been described before (*Rep. Rothamst. exp. Sta.* for 1960, p. 148, and for 1961, p. 140).

The plants emerged during the second and third weeks of May; no treatment delayed emergence, but on 30 May plants on all treated plots were slightly shorter than on the untreated ones.

Because aphids were few and arrived very late, the early insecticidal activity was tested by infesting each of 20 plants per treatment with 10 wingless adult *Myzus persicae* reared artificially. The aphids on these plants were counted 4–5 days later (Table 3). The natural infestation started to increase from 12 June (Table 4).

TABLE 3

Number of aphids on potato plants 4–5 days after introducing 200

Date of infestation:	17 May	21 May	25 May	7 June	21 June
Untreated plants	204	178	387	836	776
Menazon on tubers	43	63	119	405	521
Menazon granules	100	115	213	324	561
Dimethoate	68	34	150	245	578
Disulfoton	21	16	33	126	310

TABLE 4

Potato aphids/100 leaves (haulms destroyed 25 July 1962)

	7 June	12 June	21 June	28 June	4 July	19 July
Untreated plants	0	2	100	174	838	433
Menazon on tubers	—	—	—	—	171	310
Menazon granules	—	—	—	—	246	574
Dimethoate	—	—	—	—	144	455
Disulfoton	—	—	—	—	10	24

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As is usual, the insecticides affected the aphids confined on plants less than the natural population. Taking both series of tests into account, although all treatments decreased aphid populations initially, probably only disulfoton did so sufficiently and for long enough to prevent virus spread, but this will not be known until 1963. As the aphid vectors arrived so late, there may have been little spread even in the untreated plots.

The Laboratory of the Government Chemist found that residues of insecticides in tubers harvested from the experiment were less than 0·05 ppm, the limit of sensitivity of the methods used. (Burt)

Effects of chemicals on the survival and behaviour of wireworms. Collaborative field studies on the control of wireworms (*Agriotes* spp.) are described in the report of the Entomology Department. Some preliminary laboratory experiments were done to obtain more detailed information on the mechanism of toxic action than can be obtained in field trials.

1. The effect on wireworms of testing soil with γ-BHC was studied. Groups of 10 wireworms of similar sizes were put in 100-ml beakers containing John Innes No. 1 Compost that had been intimately mixed with γ-BHC applied at rates of 0·5, 2, 8 and 32 lb/acre surface area. The insecticide was applied as an aqueous emulsion of 0·5% insecticide, 4% xylene and 0·1% "Agral L.N.", which was dribbled on to the soil while it was agitated in a food mixer. Soil to approximately 1½ in. depth was put in the beakers, which were covered with watch glasses to prevent rapid evaporation of water and desiccation of the wireworms, and kept in a constant-temperature room at 15°. There was a ventilation space at the top of the beakers. No food was added.

Few wireworms died in the beakers containing only soil. The γ-BHC soon affected the insects, but several weeks elapsed before any died. Affected insects remained on the surface of the soil after 24 hours in beakers with BHC at 2 lb/acre and after 1½ hours at 32 lb/acre. At 2 lb/acre, a usual rate of application in the field, 47 days elapsed before 50% of the wireworms were killed; at 32 lb/acre half were killed in 12 days and all in 40 days.

2. The effect of treating seed with γ-BHC was studied by sowing treated seeds (0·04 g insecticide per 100 g seed) in John Innes No. 1 Compost or sand in 6-in.-diameter dishes, in each of which wireworms were put when the seeds were sown. The dishes were kept at 12–15° in a glasshouse and the soil or sand kept moist by watering. Half were examined after 3 weeks and half after 43 days. Results in compost and sand differed greatly. Plants were not damaged by wireworms in sand, even in the dishes without BHC, which killed about 80% of the wireworms within 43 days, although all were alive after 22 days. In compost about two-thirds of the plants were damaged in dishes without BHC and one-third in those with, and most of the wireworms were still alive after 43 days. Similar results were obtained with dieldrin applied to seed at the same rates as BHC.

3. Chemicals were sought which were effective against wireworms, but without the disadvantages of γ-BHC and dieldrin. The experiments were done in plastic boxes 9 in. × 4 in. × 3 in. deep, containing 1,400 g moist John Innes No. 1 Compost, which was kept at constant weight with water.

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The boxes were kept in a constant-temperature room at 15°. The test chemicals were intimately mixed with the soil as in the first series of experiments with γ-BHC. Eight wheat seeds were sown and eight wireworms were placed in each box immediately after the soil was treated. Fenthion (*O*,*O*-dimethyl *O*-4-(methylmercapto)-3-methylphenyl phosphorothioate), "Isolan" (1-isopropyl-3-methyl-5-pyrazolyldimethylcarbamate), carbophenothion (*O*,*O*-diethyl *S*-*p*-chlorophenylthiomethyl phosphorodithioate), "Zinophos" (*O*,*O*-diethyl *O*-2-pyrazinyl phosphorothioate) and carbaryl ("Sevin") (*N*-methyl-1-naphthyl carbamate) were compared with γ-BHC at rates equivalent to 0·2, 2 and 20 lb/acre active ingredient.

After the first examination at 2½ months the same soil was resown with wheat seed and a fresh batch of wireworms added; after a second examination 2 months later the procedure was repeated, to estimate the toxicity and persistence of the insecticide.

The most effective chemicals, judged by the protection given to the wheat and the kill of wireworms, were γ-BHC, fenthion and "Zinophos". Fenthion at 2 lb/acre did not kill wireworms after 2 months and at 20 lb/acre not after 4 months. "Zinophos" ranked with γ-BHC in persistence; at 20 lb/acre both continued to kill all wireworms 6 months after treatment, and at 2 lb/acre killed two-thirds. (Bardner)

Chemical control of slugs. In collaboration with the Entomology Department, work was started on the chemical control of slugs, using *Agriolimax reticulatus* (Müller), a major pest of winter wheat, as a test species.

Current techniques for assessing the toxicity of chemicals to slugs are inadequate, and methods are being sought to measure their effects as contact poisons, stomach poisons and fumigants. As it is not easy to decide when a slug is dead, the initial work includes the development of electrophysiological tests to determine this. (Henderson)

Fungicides

Work was solely concerned with the control of potato blight. Materials were tested which can be added to water-based copper fungicides used to control blight and which might increase the ability of the deposits, formed when sprays dry on the leaves, to repel water. The underlying idea is that if leaves could be covered with a film of fungicide that is uniform and water-repellent, they might be doubly protected. The water-repellency might, by itself, decrease the likelihood of infection (*Rep. Rothamst. exp. Sta.* for 1961, pp. 142–143) and the tenacity, or rainfastness, of the deposit might be increased. The water-repellent material must be one that does not decrease the toxic action of the fungicide.

Laboratory tests. To select materials for field trials, simple laboratory tests were made on foliage from glasshouse-grown King Edward (KE) or Ulster Supreme (US) potato plants. All copper fungicides were tested at 0·25% w/v Cu in tap-water.

Water-repellency. The upper surfaces of freshly detached compound leaves were sprayed to run-off and dried at room temperature with their

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petioles standing in water. When the deposit was dry, the advancing contact angle of distilled water on the surface was measured by the drop-diameter method of Mack (*J. phys. Chem.* (1936), **40**, 159), using two 0·5-μl drops on each of five leaflets, and the two major axes of each drop.

Tenacity. The upper surfaces of freshly detached leaflets were accurately sprayed, to less than run-off, in the Potter tower. The deposits were dried at room temperature and one disc taken from each leaflet for analysis (Martin, J. T. (1957), *Rep. agric. hort. Res. Sta. Bristol*, for 1956, p. 125); the leaflets were then washed with 0·65 in. of "rain" (tap-water), applied over a period of 30 minutes by means of a specially devised spraying apparatus. After drying at room temperature a second disc was taken from a similar position on each leaflet. At least 10 replicate analyses were used to calculate each deposit level. The ratio of the after-washing to the before-washing level, expressed as a percentage, was the "tenacity".

Distribution. Leaflets were sprayed, as in the tenacity test, and leaf prints were made, either before or after rain washing, by the rubeanic acid method.

Bioassay. Both surfaces of freshly detached leaflets from 4–6-week-old KE plants were sprayed with either 1 ml or 5 ml of fungicide in the Potter tower and dried at room temperature. The petioles were sealed with warm vaseline and a fixed volume of a freshly made suspension of sporangia of *Phytophthora infestans*, containing about 3 sporangia/cu mm of distilled water, was applied to the under surfaces with a throat spray. The leaflets, still wet with spore suspension, were placed at 15° and 100% relative humidity on plastic grills in separate petri dishes. After a week the leaflets were examined; those with any sporangiophores at all on their surfaces were counted as "infected" and those without as "not infected". The test is thus a two-point "all-or-none" assay, each leaflet being a unit. Twenty-five leaflets were used for each of the two spray volumes. The tests were used to compare one formulation with another.

The assessment of the results, using the physical and biological tests, with the materials found to be most promising, is given below.

A mixture of 1·25% "Dri-sil 37" and 0·01% "Manoxol OT", without a fungicide added (*Rep. Rothamst. exp. Sta.* for 1961, p. 143), had no protective action in a bioassay test. However, the use of "Dri-sil 37" is probably the easiest way of making deposits of spray material water-repellent. Another possibility is to add long-chain sulphur (or selenium) compounds of the type used in industrial steam condensers. These materials form very tenacious water-repellent monomolecular films on copper or brass condenser pipes.

Both these methods were used to make deposits of copper fungicide water-repellent by adding either 1% "Dri-sil 37" or 0·1% of dodecanethiol (n-$C_{12}H_{25}SH$), as a 10% solution of the potassium salt in alcohol, to spray materials. With freshly made 10 : 12½ : 100 Bordeaux mixture, the contact angles and tenacities were ~45°, 88% without additive, and >90°, 90% with "Dri-sil 37"; in another experiment the figures were ~45°, 77% without additive, and 62°, 81% with thiol. Similarly, with suspensions of copper oxychloride (0·25% Cu) in 0·05% "Belloid TD" (kindly given by the Geigy Co. Ltd.), the corresponding figures were 71°, 45% without

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additive, and >90°, 84% with "Dri-sil 37"; and in another experiment 74°, 54% without additive, and >90°, 74% with thiol. The additives certainly increased the contact angles, and the tenacity of copper oxychloride, but did not affect the already high tenacity of Bordeaux mixture. Neither additive harmed the plants at the concentrations used; however, they are both expensive, and neither is very efficient at lower concentrations. Dodecanethiol is not very stable.

A cheaper method of making deposits water-repellent is to add wax emulsion; but not all wax emulsions are stable enough, nor do they all give water-repellent deposits. The most suitable emulsion found was paraffin wax emulsified with "Ethofat 60/25" and "Ethomeen 18/12" (both kindly given by Armour Hess Chemicals Ltd.). A 20% wax emulsion in tap-water can be made that is stable on dilution with tap-water. A 10% emulsion showed no visible phytotoxic effects when sprayed to run-off on both surfaces of detached compound leaves of US kept with their petioles standing in water.

A copper oxychloride–wax mixture containing 0·25% Cu, 1·0% wax and 0·36% mixed emulsifiers was compared with a commercial copper oxychloride wettable powder (0·25% Cu), with the following results. The contact angles were: on KE, 84° (wax formulation), 57° (wettable powder) and 65° (unsprayed); and on US, 77° (wax formulation) and 66° (unsprayed). The tenacities were: on KE, 81% (wax formulation) and 69% (wettable powder); and on US, 84% (wax formulation) and 63% (wettable powder).

Leaf prints from both potato varieties showed that the wax formulation gave a more even distribution of copper on the leaflets, before and after rain washing.

In four parallel bioassay tests the numbers of uninfected leaflets out of 25 were: after 1 ml of spray, 22, 13, 1, 6 (wax formulation) and 18, 6, 0, 5 (wettable powder); after 5 ml of spray the figures were 25, 25, 22, 25 (wax formulation) and 24, 14, 10, 18 (wettable powder).

Field studies. Two 6 × 6 Latin square experiments were done with KE and US, to compare wax formulations of copper oxychloride with the commercial formulation, and also with a commercial formulation of triphenyl tin acetate.

Each square was sprayed twice: 20 July and 9 August (KE), and 25 July and 22 August (US). The treatments (all at 100 gal/acre and the same for each square) were: A, commercial copper oxychloride wettable powder at 0·25% Cu; B, copper oxychloride–wax formulation at 0·25% Cu and 1·0% wax; C, as B but with 0·5% wax; D, as B but with 0·25% wax; E, commercial triphenyl tin acetate wettable powder at 0·075% triphenyl tin acetate ("Brestan 60", kindly given by Hoechst Chemicals Ltd.); and F, unsprayed.

Table 5 shows: the delays in destruction of foliage by blight (50% level); the amounts of copper on foliage taken from plots sprayed with A, B, C and D at about the times when infection was just beginning; the increases in yield of total tubers; and the percentages of blighted tubers about 1 week after harvest, which was on 11 October (KE), 15 October

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(US). Increases in yield smaller than 0·72 (KE) or 1·19 (US) tons/acre were not significant.

TABLE 5

Results from field trials on control of potato blight

		King Edward		
Treatment	Delay (days)	Cu (μg per sq cm of leaf)	Yield increase (tons/acre)	Blighted tubers (w/w %)
A	8	0·9	0·23	6·1
B	10	2·1	0·46	3·6
C	10	1·6	0·78	4·2
D	10	1·7	0·61	5·0
E	17	—	1·61	2·5
F	—	—	—	4·2

		Ulster Supreme		
Treatment	Delay (days)	Cu (μg per sq cm of leaf)	Yield increase (tons/acre)	Blighted tubers (w/w %)
A	15	0·8	1·18	8·3
B	15	0·9	1·93	11·5
C	15	0·7	1·77	12·0
D	15	0·6	1·55	12·6
E	25	—	3·43	7·4
F	—	—	—	9·8

The results from treatment E (triphenyl tin acetate) were clearly outstanding. Yields from the copper treatments were not directly related to amounts of copper on foliage. Treatment A did not significantly increase yield of either variety. Yields of KE from the copper oxychloride–wax treatments (B, C and D) were only slightly larger, despite the greater amounts of retained copper; yields of US from treatments B, C and D were all larger than from A, despite the fact that no more copper was retained.

US has broader leaves than KE, and losses in yield, from increased transpiration caused by copper damage, may be offset by the wax more with US than with KE. (McIntosh and Eveling)

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