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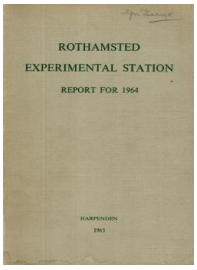

Insecticides and Fungicides Department

C. Potter

C. Potter (1965) *Insecticides and Fungicides Department ;* Report For 1964, pp 156 - 176 **- DOI: https://doi.org/10.23637/ERADOC-1-57**

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

R. Solly left and G. E. Gregory was appointed to the staff. C. Potter and P. H. Needham attended the 12th International Congress of Entomology in London, and K. A. Lord the 6th International Congress of Biochemistry in New York. By invitation of the Kenya Pyrethrum Board, M. Elliott visited their laboratories in Nakuru, Kenya. P. E. Burt gave a two months' course at the Escuela Nacional de Agricultura, Mexico, by invitation of the British Council. At the invitation of the International Atomic Energy Agency, I. J. Graham-Bryce took part in a discussion on "The use of isotopes and radiation in studies on plant nutrient supply and movement in soil systems" in Vienna.

The work of the department ranges widely from field trials with different chemicals to control specific pests, through studies of the enzyme systems of houseflies, to the synthesis of molecules allied to the pyrethrins. Although it may seem diverse, it all aims to increase the efficiency of chemicals against pests, while decreasing the chances of unwanted other effects. There are still some pests that cannot be fully controlled, and for these adequate measures are sought. Many can be controlled by current insecticides, but improvements are needed, for some of the insecticides are not only toxic to other organisms than the pests but their continued use can lead to strains of the pest that resist their action. How insecticides act is still far from certain, and our work with resistant and susceptible strains is done both to see what features convey resistance and to gain more information about their action, in the expectation that this will help in designing chemicals more specific than those currently in use. Greater specificity is also sought in field trials, which assess effects of changing treatment, i.e., formulation or method and time of applying the insecticide, not only on the pest but also other organisms, particularly bees or other pollinating insects, and insects and other animals that prey on pests.

Insecticides

The causes of resistance to organophosphorus insecticides. Houseflies (*Musca domestica*) of different susceptibilities to organo-phosphorus insecticides were compared for three features that might affect their susceptibility: (1) the permeability of their cuticles; (2) their ability to metabolise the poison; (3) the correlation between susceptibility and inhibition of cholinesterase in the central nervous system.

Diazinon labelled with ^{14}C (kindly supplied by Messrs. Geigy, New York) entered susceptible houseflies faster than resistant (SKA) flies and was decomposed faster in resistant flies. Using a calibrated capillary, 1 μl of the labelled diazinon in acetone solution was placed on the ventral side of the thorax of flies, and at various time intervals the flies were immersed

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in cyclohexane to remove insecticide still on their surface. The flies were ground, first in cyclohexane, then water and finally in a mixture of two parts of chloroform and one part methanol, to extract the diazinon and radioactive metabolites. Each of the four solutions was examined separately by two paper-chromatography processes, one using No. 11 Whatman filter-paper with methyl cyanide:water:ammonia = 40:9:1, and the other No. 11 Whatman paper dipped in 10% liquid paraffin in ether with ethanol:water = 3:5. The solution in organic solvent extracts contained almost exclusively unchanged diazinon, and the aqueous extract contained decomposition products, which have not yet been fully identified.

Immediately after the diazinon is applied to a fly almost all is removed by immersion in cyclohexane. As time passes, less can be washed from the outside and more can be extracted from inside the insect. The proportion recoverable from the surface after any given time increased with increases in the amount applied, so that although more enters, the increase is not directly proportional to the amount applied. Thus, to overcome any increase in internal resistance to the action of the insecticide, whatever the cause, may require a disproportionately greater increase in the amount applied externally.

Diazinon moves faster into newly emerged (younger than 1 day) flies than into flies 3 days old, and this is probably related to the increase in resistance with age (reported by Sawicki and Farnham). It also disappears faster from the surface of susceptible than from resistant flies of the same age; with newly emerged resistant flies it disappears at about the same rate as with 3-day-old susceptible ones. These results indicate that permeability of the cuticle plays a part in determining resistance, but it is not the only cause, because newly emerged resistant flies are more resistant than 3-day-old susceptible flies (see next section).

The ratio of decomposition products to unchanged diazinon was similar with both newly emerged and 3-day-old susceptible flies, suggesting that metabolism did not change greatly with age. With resistant flies, the ratio was larger in the older flies, suggesting an increased metabolism of poison. With both ages of fly of both strains only about half of the radioactivity lost from the surface of the flies could be recovered in extracts. Some of that not recovered was in the solids after our extraction procedures, but techniques are not accurate enough to measure it accurately. Some radioactivity was lost from the fly as vapour and could be collected in liquid paraffin, probably as unchanged diazinon, or in alkali, probably as radioactive CO_2 resulting from the metabolism of the ethoxyl groups of diazinon. (Lord)

The response of susceptible and resistant houseflies to diazinon and diazoxon applied externally and injected. Comparing the effects of applying a poison externally with those when it is injected gives some indication of the effect of the cuticle and of how the rate the poison is metabolised affects toxicity. Newly emerged and 3-day-old female houseflies of susceptible and resistant strains were treated topically with 1·0-μl drops of diazinon or diazoxon in acetone on the thorax, or injected with 0·3 μl in 70% aqueous acetone, and the proportion killed was determined 24 hours later. All tests

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followed the general design of probit assay. From the results in Table 1 the following inferences can be drawn: (*a*) old flies of both strains are more resistant than newly emerged flies to the two poisons, whether topically applied or injected; (*b*) injected diazoxon was much more toxic to all flies than injected diazinon, especially to the resistant flies. When applied

TABLE 1

Weighted mean LD50s of susceptible and SKA flies created by topical application and injection with diazinon and diazoxon. (*μg/fly*)

	Diazinon			
	Topical		Injection	
	Young	Old	Young	Old
Susceptible	0·040 ± 0·001	0·070 ± 0·002	0·038 ± 0·001	0·052 ± 0·003
Resistant	3·11 ± 0·15	7·03 ± 0·24	0·51 ± 0·03	1·51 ± 0·10
	Diazoxon			
	Topical		Injection	
	Young	Old	Young	Old
Susceptible	0·030 ± 0·002	0·053 ± 0·002	0·012 ± 0·007	0·013 ± 0·005
Resistant	1·57 ± 0·13	2·44 ± 0·19	0·052 ± 0·004	0·090 ± 0·004

topically, diazoxon was only slightly more toxic than diazinon to susceptible flies, but 2–3 times as toxic against resistant flies; (*c*) injection increased the toxicity of diazinon to susceptible flies only by 1·3 times, but of diazoxon by 3–4 times. Diazinon was 5–6 times, and diazoxon 27–30 times, more toxic when injected than when applied topically to resistant flies; (*d*) the way either poison was applied greatly affected the degree of resistance shown by the SKA strain. In young SKA flies the resistance factors of topically applied diazinon and diazoxon were 78 and 52, and in old flies 100 and 46. Injection decreased the factors in young flies to 13 and 4 and in old flies to 29 and 7.

At least three mechanisms seem concerned in the resistance of the SKA flies to diazinon: (1) their cuticle is less permeable than the cuticle of the susceptible strain; (2) diazinon is converted into diazoxon more slowly; (3) both compounds are detoxicated faster by the resistant flies. The rate the poisons penetrate the cuticle and are detoxicated seem the major factors, judging from the LD50s of topically applied and injected diazoxon in SKA flies. Both permeability of the cuticle and the resistance to injected poisons change with changes in the age of the insects. It is interesting to note that SKA flies still show resistance factor of 7·0 when injected with diazoxon. (Sawicki and Farnham)

Effect of abrasion of the cuticle on the susceptibility of houseflies to diazinon. The effect of the cuticle on resistance makes a biological study of the mechanisms responsible for internal resistance of the SKA strains difficult, because the usual techniques of treating houseflies with insecticides are unsuitable. Topical application is unsatisfactory for obvious reasons, and injection because the insecticide and carrier are liberated *en masse*, possibly close to where they act, and little time is allowed for the detoxication mechanism to protect the insect from poisoning. These difficulties were overcome by abrading the cuticle and applying the insecticide to the abraded areas.

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In the first series of experiments the thoracic tergites of the houseflies were abraded with a carborundum-tipped, miniature hand-drill. This was unsatisfactory because the drill abraded only the outermost layers of the cuticle, and the pressure needed to remove deeper layers cracked the cuticle. This method probably removed the epicuticle and wax layer, because the flies died unless kept in a humid atmosphere. The abraded and normal flies were treated topically on the thorax with a 0·5-μl drop of diazoxon or diazinon in acetone, and the insects in batches of 5 were checked for signs of poisoning at various time intervals. Preliminary tests showed that abraded flies from either resistant or susceptible strains were more affected than unabraded ones by diazinon. However, this increase in susceptibility was not caused by the removal of the epicuticle, because when abraded flies of either strain were treated on unabraded areas or injected with diazinon, more were killed than with unabraded flies. Abrading itself probably caused a physiological change reflected in increased susceptibility to diazinon. In the only experiment with diazoxon this effect was not observed, and equal numbers of abraded and unabraded flies died, suggesting that the physiological reaction to the abrading may increase the rate diazinon changes into diazoxon without influencing the response to diazoxon. In later experiments the effect of the physiological change was measured by applying the drop of insecticide on the thorax of one half of the abraded flies and on the abdomen of the other, and comparing the kill of abraded and normal flies treated on the two sites. When the kill of the abraded flies was corrected for the physiological change the differences between the abraded and unabraded flies disappeared, indicating that the outermost layers of the cuticle, presumably the epicuticle and wax layers, do not determine the rate diazinon penetrates the cuticle of either strain of flies.

Experiments have now started with another abrading apparatus, S. S. White's "Airbrasive", which abrades with a high-speed jet of compressed air and abrasive powder. It can remove the cuticle down to the endocuticle over an area of the thorax large enough to receive the major part of a small measured drop (0·1 μl). Ethyl cellosolve was chosen from several solvents tested instead of acetone, because it evaporates slowly enough to make delivery accurate, but fast enough to prevent excessive spread over unrubbed cuticle. Several initial difficulties were overcome, and preliminary experiments now show that abraded SKA flies are much more susceptible than unabraded flies of the same strain to diazinon, even when the results are corrected for the physiological changes produced by abrading. In preliminary tests with diazoxon 10–12 times more was needed to kill the same proportion of unabraded as of abraded SKA flies. With susceptible flies only 4 times as much poison was needed to kill unabraded flies. Flies of neither strain showed any effects of physiological changes from abrading.

It seems that the cuticle of the susceptible flies differs from that of SKA flies in the exocuticle, which in the resistant flies is a more effective barrier to the entry of diazoxon. The epicuticle seems unimportant in this context, but the SKA strain still shows considerable resistance to diazinon when the exocuticle is removed. (Sawicki and Farnham)

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Recovery of houseflies poisoned with diazinon or diazoxon. The inspection of houseflies treated with diazinon during abrasion tests showed that some that seemed dead later recovered and became apparently normal a few hours after treatment. This was unexpected, because organophosphorus insecticides are generally assumed to inhibit cholinesterase irreversibly, thereby causing death.

Newly emerged and 3-day-old flies of susceptible and resistant SKA strains were treated topically in the usual way with diazinon or diazoxon, examined for symptoms of poisoning at intervals up to 5 hours and 24 hours after treatment, and the ED50 (effective dose to paralyse 50%) was calculated for each treatment. When treated with either of the two poisons at ED50 level the flies remained normal for variable lengths of time, then some showed signs of excitation followed by partial or complete paralysis, from which some recovered and others died. The pattern of change of response to the poison with time depended on the age of the flies, the strain and the poison.

Table 2 shows the time ED50 was first reached by the most concentrated solution used in each test. Susceptible flies reached 50% paralysis rapidly,

TABLE 2

Minimum time to reach 50% paralysis by young and old susceptible SKA flies treated with diazinon and diazoxon. (Dose in μg. of poison/fly)

	SKA flies			
	Young		Old	
	Dose	Time to reach ED50	Dose	Time to reach ED50
Diazinon	10	*c.* 30 min.	20	70–240 min.
Diazoxon	2·5	*c.* 15 min.	10	10–15 min.
	Susceptible flies			
	Young		Old	
	Dose	Time to reach ED50	Dose	Time to reach ED50
Diazinon	0·15	*c.* 10 min.	0·20	20 min.
Diazoxon	0·60	<10 min.	0·15	<15 min.

and the differences between the age groups was small. Resistant flies reached 50% paralysis slowly with diazinon, especially the old flies, but rapidly with diazoxon. Only young flies recovered from paralysis; the proportion of old flies paralysed by either poison increased with time to reach a steady value at death end-point. The ratios of the smallest dose to give ED50 to LD50, which indicate the extent of recovery, were: young resistant flies—diazinon 1·5, diazoxon 4·1, young susceptible flies, diazinon 1·3, diazoxon 1·9. Maximum paralysis occurred fastest with young resistant flies treated with diazoxon (*c.* 20 minutes), followed by young susceptible flies treated with diazoxon (*c.* 40 minutes) and diazinon (*c.* 60 minutes), and then young resistant flies treated with diazinon (*c.* 80 minutes). The flies that recovered did so within 2 hours after the number paralysed was maximal, so that about 3 hours after treatment the visible signs of toxic action on flies of both strains and ages treated with both poisons reached end-point, except with old resistant flies treated with diazinon, when it was 5 hours or more. Paralysis end-point (ED50) for diazoxon was consistently shorter than for diazinon, indicating that activation occurred before the full action of diazinon was visible. The

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reasons are not yet known for the young flies recovering, but some relevant information may come from the experiments on the inhibition of cholinesterase, described below. No reasons can as yet be given for more recovering from diazoxon than from diazinon poisoning, or for young resistant flies reaching ED50 so fast with diazoxon. (Sawicki and Farnham)

Cholinesterase in thoracic ganglia of houseflies affected by diazoxon. Houseflies that recover from paralysis may do so because the cholinesterase of the thoracic ganglia is not inhibited. To check this, the thoracic ganglia of flies poisoned with diazoxon were examined for inhibition of cholinesterase, using the Gomori (1952) modification of the Koelle thiocholine technique. The cholinesterase of the thoracic ganglia of houseflies that recovered within 24 hours after poisoning with diazoxon was nearly normal, whereas in flies killed by the poisons it was inhibited. We do not know yet whether the thoracic ganglia of all the paralysed flies during the "paralysis stage" are inhibited, because technical difficulties, recently overcome (see below), interfered with the tests for cholinesterase in the ganglia of these flies. (Gregory, Farnham and Sawicki)

Examination of whole housefly thoracic ganglia stained for cholinesterase by the Gomori (1952) modification of the Koelle thiocholine method suggested that, of the cholinesterase present throughout the tissues of unpoisoned ganglia, only that in the outermost layers of the ganglia was stained. This suggestion was confirmed by examining paraffin sections of ganglia previously stained whole. In ganglia of flies poisoned with diazinon the outer limit of uninhibited cholinesterase was marked by a narrow band of staining; the cholinesterase in the centre of the ganglia was unstained. This poor penetration of the Koelle method was improved by transferring the ganglia directly from the acetone used for fixation to a substrate solution containing six times the standard concentration. This modification eliminates the intermediate treatment of washing in saturated sodium sulphate. (Gregory)

Effect of removing the abdomen on the resistance of houseflies. There are statements in the literature that insecticides can be detoxified in the gut, malphigian tubes and the fat body, and that the fat body converts phosphorothionates into corresponding phosphates, which are potent cholinesterase inhibitors. Most of these organs are in the abdomen, which also contains half the total aliesterases and arylesterases, enzymes thought to be important in making houseflies resistant to organophosphorus insecticides. To find whether the organs in the abdomen are important in this context, the response to diazinon and diazoxon of susceptible and resistant flies with and without abdomen was compared. The tests followed the general design of probit assay. The abdomen was cut off from 2-day-old susceptible and SKA flies, which were treated topically on the thorax with 0·6-μl drops of diazinon or diazoxon, and intact flies were treated with 1·0-μl drops. Observations continued for only 3 hours because flies with their abdomen removed then started to die. The LD50s of the operated flies were about half the LD50s of intact flies in both strains, and the resistance factor of the operated and intact SKA flies was about the same. This

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indicates that for the first 3 hours after treatment the abdomen was not a special site of resistance and its loss did not alter resistance, because the LD50s of the operated resistant and susceptible flies decreased in the same proportion. The halving of LD50s by the operation may indicate either that the abdomen plays some part in detoxication and a proportionately similar part in each strain, or that the operation increases the sensitivity of the insects. (Sawicki and Farnham)

Effect of some additives on the toxicity of diazinon and diazoxon to houseflies. The toxic action of diazinon and diazoxon was studied when combined with the following compounds: piperonyl butoxide, S421 (octochlorodipropyl *ether*), SKF 525A (*p*-diethylaminoethyl diphenyl propyl acetate), and Toxogonin[R] (bis [4-hydroxyiminoethyl-pyridinium-(1)-methyl] ether dichloride). Piperonyl butoxide, S421 and SKF 525A are pyrethrum synergists, and the first two compounds synergise some organophosphorus insecticides. SKF 525A and piperonyl butoxide antagonise the toxic action of malathion, and SKF 525A prevents the conversion *in vitro* of parathion into paraoxon. Toxogonin[R] is analogous to 2-PAM (pyridine-2-aldoxim methiodide), both compounds reactivate mammalian cholinesterase, but 2-PAM does not reactivate house fly cholinesterase *in vitro*.

The additives were mixed with diazinon or diazoxon at 1:1 w/w and the mixtures were either applied topically in acetone (1·0-μl drop) or injected in 70% v/v aqueous acetone (0·3 μl drop/fly). Toxogonin[R], which is insoluble in commonly used organic solvents, was injected in water (5·0 μg/fly SKA strain; 0·5 μg/fly susceptible strain), and the flies were then immediately treated topically with solutions of diazinon or diazoxon. Piperonyl butoxide and S421 synergises diazinon only slightly by either method of application (synergistic factor (SF) 2·0). SKF 525A synergised diazinon and diazoxon when applied topically on SKA flies (S.F. diazinon *c.* 2·0; S.F. diazoxon *c.* 3·0), but not when injected. However, applied either way, it synergised diazinon and diazoxon with susceptible flies (S.F. diazinon 2·5, diazoxon 1·9–3·5 by topical application. S.F. for both insecticides by injection 1·6). Toxogonin acted as a synergist with both strains of flies (S.F. diazinon 1·3; diazoxon 1·8 against SKA flies, S.F. diazinon 1·2, diazoxon 1·4 against susceptible flies). Toxogonin alone in large doses (12·5 γg/fly) was considerably more toxic to susceptible than resistant flies. The small differences in synergistic factors between the various additives with both strains of flies show that these compounds probably interfere in some way with minor defence mechanisms common to both strains. (Sawicki and Farnham)

Resistance of SKA houseflies to other insecticides than diazinon. The SKA strain of houseflies was compared with the susceptible strain for susceptibility to several insecticides applied topically in measured drops, and the resistance factor $RF = \frac{LD50\ (SKA)}{LD50\ (S)}$ calculated for each. The SKA flies are very resistant (RF over 50×) to ethyl "Chlorthion" > diazinon > "Chlorthion" > parathion; moderately resistant, (RF from 10–49×) to

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ethyl fenchlorphos (ethyl "Ronnel") > dicapthon > ethyl malathion > "paraoxon" > VC 1–13 > "Sumithion", and slightly resistant (RF less than 10×) to methyl "paraoxon" > malathion > "maloxon", ethyl "Chlorthion" ethyl phosphonate > fenchlorphos ("Ronnel") > dichlorvos. The flies, which have been selected only for resistance to organophosphorus insecticides, also resist chlorinated hydrocarbon insecticides, e.g., lindane RF 85×, dieldrin RF 58× (20% of the flies were immune to dieldrin), and DDT RF > 600×. They have little resistance to "Isolan" (RF 3·5×), the only carbamate insecticide tested.

The resistance of the strain to any given insecticide cannot be predicted, because it can differ greatly even to very closely related organophosphorus compounds. For example, the RF with "Chlorthion" is 71×, but only 24× with dicapthon, an isomeric substance. The only difference between the two chemicals is the position of one chlorine, in position 2 in the benzene ring of dicapthon and position 3 in "Chlorthion". The strain is also considerably more tolerant of diethoxy than of dimethoxy organophosphates, e.g., the RF with ethyl "Chlorthion" is 450× and 71× with the methyl "Chlorthion". The relative toxicity of the oxygen analogues and the parent sulphur compounds differed: diazoxon was 2·3 times more toxic than diazinon; "paraoxon" and methyl "paraoxon" were nearly as toxic as the parent compounds parathion and parathion methyl; "malaoxon" was much less toxic than malathion.

The SKA flies, which were selected with diazinon, differ in their resistance to malathion from resistant strains selected by exposure to malathion, for they are only slightly resistant to malathion (RF > 10×). Piperonyl butoxide has no effect on toxicity of malathion to SKA flies, but synergises ethyl malathion and "malaoxon". Other workers have shown that piperonyl butoxide antagonises the action of malathion in malathion-selected strains and diazinon-selected strains, also that tri-*o*-cresylphosphate and tributyl phosphorotrithioate synergise the action of malathion in malathion-selected strains, whereas we found that tri-*o*-cresylphosphate antagonises both ethyl and methyl malathion, and tributyl phosphorotrithioate synergises malathion only very slightly. (El Basheir)

The mechanism of resistance to DDT in the SKA strain. The SKA flies are very resistant to lindane, dieldrin and especially to DDT (RF > 600×), although they and the parent strains (203a and Saccà a) have never been in contact with chlorinated insecticides in this laboratory (i.e., for over 6 years). Because both parents came originally from areas where chlorinated insecticides had been used, the parents may have been resistant to chlorinated insecticides. Unfortunately, the resistance of the parent strains to DDT (both parents resistant to diazinon) had not been determined. The SKA strain, which is heterogeneous, is unlikely to have maintained great resistance to DDT without continued selection, so selection with diazinon has probably at least maintained and may have increased resistance to DDT; indeed, exposure to diazinon may have selected forms resistant to DDT. To compare the mechanism of resistance to DDT in the SKA strain with that in a resistant strain selected with DDT (F58W), the effect of WARF anti-resistant (N,N-di-*n*-butyl-*p*-chloro-benzene sulphonamide)

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on the susceptibility of the two strains was first tested, and the relative capacity of the two strains to metabolise DDT was measured.

The two strains differed in the response to mixtures of DDT and WARF. WARF anti-resistant nearly eliminated the resistance of the F58W strain to DDT (R.F. from 53 × to 3 ×), but not of the SKA strain, although the resistance factor dropped from > 600 × to 87 ×. WARF anti-resistant synergised DDT more against F58W flies (S.F. synergistic factor ≃ 18), than SKA flies (S.F. ≃ 8·0), and did not synergise DDT against susceptible flies. SKA flies seem to have at least two resistance mechanisms to DDT, one similar to that in the F58W strain and another that is unaffected by WARF anti-resistant. (El Basheir)

To see whether flies of different susceptibilities metabolise DDT at different rates, they were injected with sublethal doses of DDT, extracted at intervals and the extracts examined by electron capture gas chromatography. Preliminary results show that the F58W strain degrades DDT to the non-toxic metabolite DDE faster than does either the SKA or the susceptible strain. (El Basheir and Lord)

Resistance in aphids. After reports that aphids were showing resistance to insecticides, samples of *Aphis fabae* and *Myzus persicae*, sent by workers at Broom's Barn, were examined for their susceptibility to dimethoate. The aphids, collected from areas growing sugar-beet crops in various parts of the country, were increased at Rothamsted to get populations large enough to test. The different samples of *Aphis fabae* were all similarly susceptible to dimethoate applied topically, but those of *Myzus persicae* differed considerably. Further tests with *Myzus persicae* showed that to kill some populations required ten times as much insecticide as to kill others. Those with most and least resistance came from places where sugar beet is commonly sprayed to control aphids, apparently successfully. The work started only late in the year, and the experience gained will be applied in future surveys. (Needham)

Persistence and toxicity of residual chlorohydrocarbon insecticide films. Tests were made to see how the persistence and toxicity of a standard DDT wettable powder formulation is affected by various amounts of "Lovo 192", a proprietary product consisting principally of a mixture of amine stearates, which produces a film of stearic acid round the particles of wettable powder and is said to prolong the life of the deposit. Deposits of different DDT concentrations, on glass plates and the under surface of excised leaves, were obtained by spraying in a Potter tower, and were sampled and analysed for their content of DDT. The glass plates and leaves were then kept in a constant-temperature room in still air at 20° C for 8 (occasionally 12) weeks.

Other tests were done using leaves on living cotton plants, also sprayed in the Potter tower. When the plants had four mature leaves formed the first-formed leaf was removed, as were all those that formed later. After 8 weeks, when the three remaining leaves were nearly the same size, one of the top two was sprayed with the standard wettable powder, the other with the powder containing "Lovo 192" and the third was not sprayed.

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The plants were kept in the glasshouse (temperature range 17°–30° C, mostly 20°–25° C and 60–80% R.H.) for up to 5 weeks.

Toxicity was measured in both series of tests by exposing houseflies to the deposits, and quantities of DDT were measured chemically. Table 3 shows that increasing the concentration of "Lovo 192" above 2½% lessened the toxicity of the deposit to flies. After ageing for 8 weeks deposits

TABLE 3

The effect of different concentrations of "Lovo 192" on the toxicity to houseflies of DDT deposited on a glass surface

% DDT in spray solution	No. of deposits analysed	DDT analysis Mean deposit density (μg/cm² DDT)	% kill
(i) *Standard Wettable Powder*			
0·05	28	1·3	50
0·1	26	2·6	70
0·2	12	4·8	90
0·4	—	—	100
(ii) 2½% "*Lovo* 192" *Wettable Powder*			
0·025	1	0·62	33
0·05	26	1·47	47
0·1	23	2·8	61
0·2	—	—	84
(iii) 5% "*Lovo* 192" *Wettable Powder*			
0·025	—	—	8
0·05	10	1·33	20
0·1	18	2·82	35
0·2	7	4·27	40
0·4	2	7·07	60
(iv) 10% "*Lovo* 192" *Wettable Powder*			
0·05	2	1·24	7
0·1	3	2·52	7

on either glass or excised leaves were still toxic, whether or not they contained "Lovo 192". Deposits on glass were about twice as toxic as deposits on leaves throughout the experiment. Toxicity did not diminish measurably for three weeks, but then did. Activity was lost at the same rate with or without 2½% "Lovo 192". The deposit containing 5% "Lovo 192" was only half as toxic as the others, but it persisted longer.

The deposits from 0·4% DDT suspensions on the upper surface of leaves of plants kept in the glasshouse lost only a little of their toxicity after 5 weeks, when deposits containing 2½% "Lovo 192" were the most toxic and killed 60% of the test flies. During this period the leaves expanded very little, so the amount of deposit per unit area of leaf did not alter.

The main purpose of adding "Lovo 192" is to slow the loss of poison by weathering, particularly rain. Therefore, some deposits were "rain-washed" by the equipment designed by McIntosh (*Rothamsted Report* for 1963, p. 142). After exposure to the equivalent of 0·3 in. of heavy rain fresh deposits of standard wettable powder on glass, excised leaves or leaves on plants lost nearly all their toxicity, whereas deposits containing 5% "Lovo 192" retained half theirs. "Lovo 192" protected old deposits against rain at least as well as it protected young deposits.

It seems that "Lovo" additives may have two useful effects. The obvious

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one is to increase the persistence of deposits exposed to rain and, less obvious, that they may increase the selectivity of DDT. Our results show that "Lovo 192" can diminish toxicity as a contact poison, but it is unlikely to affect toxicity as a stomach poison, so that deposits containing "Lovo 192" would be fully effective against leaf-eating pests, but less harmful to other kinds of insects. (Phillips and Gillham)

Improvements in apparatus. An injection port and column holder for gas chromatography equipment was built that can be contained together with the detector in a commercial stirred-air oven. This greatly improved the performance of the equipment, because all parts are at the same temperature and screened from electrical interference. Columns of a wide range of sizes can be readily interchanged, and the use of short columns (3–6 in.) greatly increases the number of analyses possible in a given time. This equipment is used routinely to measure small amounts of organochlorine and organophosphorus insecticides. (Arnold and Lord)

An efficient and simple micro-applicator was developed and tested. It is a motorised unit that can be operated in any position from mains or battery, and delivers doses from 0·1 to 1·0 μl in steps of 0·1 μl at up to 80 doses a minute. Measurements of its accuracy of delivery, by weighing individual drops of mercury, showed its consistent behaviour, and even with drops as small as 0·1 μl, the coefficient of variation was only 6%. (Arnold)

Toxicity of insecticides to bees

Poisoning of bees in the field. Of thirty samples of honey bees (*Apis mellifera*) received from the Bee Advisory Service of the Ministry of Agriculture, Fisheries and Food, 16 yielded evidence of poisoning by organophosphorus insecticides. Although the organophosphates cannot be identified with certainty, information supplied with the samples suggests that 12 were poisoned by dimethoate and 2 by demeton methyl. Of the 12 poisonings attributed to dimethoate, 6 were thought to have happened when peas were sprayed from the air, when the plane went over a field of clover. Two others were also attributed to aerial spraying, and 4 to the ground spraying of an orchard, potatoes and field beans. One with demeton methyl was also ascribed to aerial spraying.

Of 3 samples that contained dieldrin in lethal quantities, crop spraying was responsible for 2, but the the third was not accidental and came from spraying a swarm with an aerosol. Bees alleged to have been in contact with a sack that had contained dieldrin seed-dressing actually contained lindane.

Two samples contained carbamates in lethal quantities, apparently acquired by carbaryl spray drifting on to clover; from fruit-tree spraying with one sample.

Of the remaining 8 samples that yielded no evidence of poisoning by insecticides, 3 were from the incident concerning the aerial spraying of peas and may be regarded as one sample. (Needham and Stevenson)

Assessment of toxicity to honeybees. Methods of assessing the toxicity of insecticides to honeybees were developed, and the acute toxicities of

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some compared when administered to bees topically and orally. For topical application, bees are anaesthetised with carbon dioxide and 1·0-μl drops of the insecticides in acetone are placed on the thorax. Each insecticide is tested on 2 lots of 10 bees at 5 or 6 concentrations. The bees are fed with unlimited 20% sucrose in water and kept at 27° C in cylindrical cages 4½ in. long and diameter 1½ in. made from ⅛-in. tinned wire mesh, 22 gauge. All the poisons tested reached their end-point effect within a day, so the proportion of bees killed was determined 24 hours after they were treated.

TABLE 4

Toxicity of insecticides in acetone solution applied topically to honeybees. Mortality counted 24 hours after treatment, calculated by the probit method

Insecticide	LD50 ± standard error (μg per bee)	Slope (*b*) ± standard error
Endosulfan	7·0 ± 0·76	3·0 ± 0·86
Menazon	4·5 ± 0·30	2·5 ± 0·40
Disulfoton	4·0 ± 0·17	5·7 ± 0·63
Chlordane	1·4 ± 0·084	6·0 ± 0·83
Carbaryl	1·1 ± 0·092	1·5 ± 0·44
Malathion	0·27 ± 0·0094	8·5 ± 0·34
Diazinon	0·22 ± 0·0078	7·7 ± 1·29
Dieldrin	0·15 ± 0·0034	7·9 ± 0·65

Table 4 shows that commonly used insecticides differ considerably in their ability to kill bees by contact action.

For testing oral toxicity, groups of 10 honeybees were put in the wire mesh cage with 0·2 ml of the insecticide in 5% acetone in 20% sucrose, contained in a small glass tube with a constriction through which the bees' proboscis can enter. The assumption that the 10 bees would share the dose equally (20 μl per bee) was supported by the results. When the dose had been taken the bees were given unlimited 20% sucrose solution and kept at 27° C for 24 hours, when the effects were assessed. Techniques giving individual bees known doses are being tested. Median lethal doses are calculated using the probit method, but too few of the insecticides have yet been tested to know their relative toxicities. (Stevenson)

Work was started to see whether nectar secreted by plants treated with systemic insecticides contains toxic amounts of insecticide, using borage (*Borago officinalis*) and nasturtium (*Tropaeolium* sp.), which give a reasonable flow of nectar when grown in the glasshouse. Preliminary biological assay with mosquito larvae shows that nectar can be toxic, but tests with bees have yet to be made. (May and Stevenson)

The movement and uptake of systemic insecticides. As a preliminary to measuring the movement of insecticides in soil directly, their adsorption by soils was studied. A suitable routine method for determining the adsorption isotherm was developed, making analyses by gas–liquid chromatography. The isotherm for the uptake of disulfoton from aqueous solutions by Rothamsted topsoil is linear for concentrations up to saturation, and when in equilibrium with saturated solution the soil contains approximately 0·3 mg/g disulfoton. (Graham-Bryce)

For studying the movement through soil of four systemic insecticides,

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it was essential to measure their solubilities in water at temperatures obtaining in British soils during spring and summer. Those of dimethoate and menazon were large enough to be measured by conventional methods, and suspensions were shaken continuously at constant temperature, while small amounts of water were added until the material just dissolved. Phorate and disulfoton are much less soluble, and for them a simple method using gas–liquid chromatography was devised. Saturated solutions in water were made at constant temperature, centrifuged and partitioned with dichloromethane. By gas–liquid chromatography, the amount of insecticide in the organic phase was compared with known amounts partitioned between similar volumes of water and dichloromethane, so avoiding the need to determine partition coefficients of the solutes and the mutual solubility of the solvents (Table 5). Within the temperature range

TABLE 5

Solubility in water of four systemic insecticides commonly applied in soil (mg/l)

	5° C	15° C	26° C
Dimethoate	14×10^3	23×10^3	40×10^3
Menazon	56	83	120
Phorate	11	15	20
Disulfoton	9	13	18

chosen, log solubility is directly proportional to temperature for all four compounds. (Burt and Lord)

After laboratory tests comparing the ability of four organophosphorus insecticides to move in the vapour phase and be taken up by plants (*Rothamsted Report* for 1963, p. 138), a small experiment was done in the field to try to correlate movement in soil and uptake by potato plants with moisture content of the soil. The insecticides were applied (2 lb a.i./acre) as granules (menazon 5% a.i., disulfoton 5% a.i., phorate 10% a.i. and dimethoate 6·3% a.i.) below the seed tubers at the time of planting. It was intended to irrigate some plots, but this proved impossible. Natural aphid infestations were counted at regular intervals, and the toxicity of the plants to aphids was further assessed by confining *Macrosiphon euphorbiae* on leaves and seeing how many were killed during the next week. The results supported previous observations in the field. When the soil was moist all four insecticides were equally effective, as during June, when rain totalled 4·02 in., 1·83 in. more than average. In July, when the soil became very dry (rainfall 1·29 in., 1·26 in. less than average) dimethoate and menazon became much less effective than phorate and disulfoton, particularly in the tests with the caged aphids. (Etheridge)

Effect of chemicals on survival and behaviour of wireworms. Laboratory tests of effects of organophosphorus and carbamate insecticides on wireworms were continued. Of ten materials tested, only "Sumithion" [*O*,*O*-dimethyl-*O*-(3-methyl-4-nitrophenyl)phosphorothioate] was toxic enough to warrant inclusion in field trials.

Two disadvantages of the present laboratory tests are that counts of plants damaged are seldom reliable and that insecticides applied at field

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rates require several weeks to kill wireworms. In preliminary experiments it was noted that feeding by wireworms was soon slowed by BHC, so an attempt was made to use their biting ability as a quick test of insecticidal poisoning, and filter-paper discs soaked in potato juice were offered to wireworms living in treated or untreated soils. Wireworms that had been for 4 days in soil containing BHC, thionazin (= "Zinophos"), Bayer 38156 (*O*-ethyl *S*-*p*-tolylethylphosphonodithioate) or aldrin at 2 lb/acre a.i. all bit much less often than wireworms from untreated soil. Individual wireworms are being observed to see whether those that soon stop biting die when removed to untreated soil.

In co-operation with the Entomology Department an experiment was started in New Zealand field to test the ability of organophosphorus insecticides to protect wheat from wireworms, using materials selected from previous laboratory and field experience. The materials used and their effects on wireworms and other soil fauna are given in the report of the Entomology Department. None of the treatments affected the yield of grain. (Griffiths)

Wheat-bulb fly (*Leptohylemyia coarctata* Fall.). The widespread damage done by wheat-bulb fly in 1963 prompted further work on methods of control. Female flies, collected during summer when swollen with eggs, were kept in breeding jars in the laboratory. The 15,000 eggs laid in 1963 were kept at controlled temperatures until diapause was completed, and the larvae were then used in tests of seed dressings and for experiments with possible attractants. Surplus eggs were stored at $-6°$ C for later tests, but failed to hatch after 9 months at this temperature. In 1964 the flies laid only 4,000 eggs, and attempts are now being made to collect eggs directly from the field by washing infested soil and floating off the eggs.

B. M. Stokes (*Nature, Lond.* (1956), **178**, 801) showed that young wheat plants produced an exudate that attracts the larvae. We have confirmed this and modified Stokes's method to compare the attractiveness of different extracts. Attempts made to isolate the attractant substance have failed, both from wheat plants and from alginate gels in which wheat was grown. Carmine added to plain alginate gels was seen inside the guts of larvae, showing they had eaten the gel. The proportion that did so was increased by adding sugar and macerated wheat to the gel. By adding insecticides to such gels it is hoped to develop a rapid test of toxicity. (Scott and Lord)

Laboratory tests of organophosphates and carbamates for use against wheat-bulb fly were renewed. Twelve experimental compounds, applied as seed dressings, were compared with heptachlor, using the methods described by Bardner (*Pl. Path.* (1958), **7**, 125–129). Plant damage was significantly decreased by Bayer 38156 (*O*-ethyl *S*-*p*-tolylethylphosphonodithioate) at 0·04% active ingredient to weight of seed, Bayer 37289 (*O*-ethyl *O*-2,4,5-trichlorophenylethylphosphonothionate) at 0·04% a.i., and VC 1–13 (*O*-2,4-dichlorophenyl *O*,*O*-diethyl phosphorothioate) at 0·15% a.i.

Sprays of dimethoate ("Rogor 40"), Bayer 37289 and thionazin (= "Zinophos") were applied to boxes of young plants on 12 February (a few days after wheat-bulb fly eggs were put in the boxes) or on 24

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March (when wheat-bulb fly attack was well established). On both dates sprays were applied either: (*a*) on the whole surface of each box; (*b*) on the plant rows only; or (*c*) on the soil between the rows (where the wheat-bulb fly eggs had been buried). None of the early sprays nor any of the late sprays applied to the soil between the rows had any effect, but late sprays with "Rogor 40" and Bayer 37289 significantly decreased secondary attack when applied either over the whole surface of the boxes or to the plant rows. Thus, there was no evidence that any substance killed larvae during their initial migration through the soil. The area of plant at the time of treatment with insecticide may be important, and a field trial was started to study further the timing of sprays in relation to stage of plant growth and of larval development. (Griffiths and Scott)

Seed dressings used against wheat-bulb fly on winter wheat are applied in the autumn, but to be effective must act from January to early March, when the fly attacks. The amount of insecticide remaining at the time of attack, and its distribution between soil and plant, was measured on samples of plants and soil from experiments done by the National Agricultural Advisory Service (Eastern Region). Cores of 2 in. diameter and 6 in. deep were taken on three occasions from plant rows on plots at each of two sites (one peaty loam and one clay loam). The plots had been drilled with seed dressed with dieldrin, BHC, Bayer 38156 (*O*-ethyl *S*-p-tolylethyl phosphonodithioate) and Bayer 37289 (*O*-ethyl *O*-2,4,5-trichlorophenylethyl phosphonothionate). The cores were dissected into: (*a*) seed; (*b*) plant minus seed; (*c*) soil from around roots; (*d*) soil in upper 3 in. of core; and (*e*) soil in lower 3 in. of core. Extractions were made with acetone/hexane and analysed by a G.L.C. electron capture method.

Dieldrin and BHC increased yields on the clay soil; on peat dieldrin was superior to BHC, but neither compound increased yield greatly (unpublished results provided by Mr. F. Maskell, National Agricultural Advisory Service). For the chemical analyses, differences in sampling dates, in the number of plants contained within a core and in the amount of dressing each seed received made exact comparisons impossible. However, they showed that BHC and dieldrin persisted in plants and soils at both sites during the likely period of attack. The performance of the insecticides on the peat soil may have been influenced by the greater depth of sowing there, for there was insecticide in the lower part of the core. The insecticide moves only slowly through the soil, for a large proportion of it was in the soil immediately surrounding the plants, even at the latest sampling date. Bayer 37289 persisted in the soil and plant throughout the sampling period on both clay and peat. This compound decreased attack and killed wheat-bulb fly, but its value was lessened because it harmed the plants. Traces only of Bayer 38156 were found at the second sampling, and none in the last sample, but it killed some larvae in the peat soil and it increased yield on the clay. The persistence and behaviour of insecticides on the two sites differed little, not enough to account readily for the differences in biological effects observed, and it seems that different cultural conditions affected the capacity of the crops to recover from attack. Also, differences in degree of control may result from uneven distribution of seeds (from one to ten in a 2-in. core) giving uneven distribution of

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insecticide, or from differences in the amount of dressing each seed received, which depended on the dressing used: the amounts recovered from the surfaces of individual seeds dressed for the experiment were: BHC = 29 ± 1·4 μg, Bayer 38156 = 27 ± 5·8 μg, Bayer 37289 = 24 ± 5·5 μg (all treated at 2 oz/bu of 40% dressing) and dieldrin = 38 ± 4·2 μg (treated at 2 oz/bu of 60% dressing). (Griffiths, Jeffs, Lord and Scott)

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

PLANT FEEDERS

Hemiptera	*Acyrthosiphon pisum* (Harris)
	Aphis fabae Scop.
	Dysdercus intermedius Distance
	Macrosiphum euphorbiae Ashm.
	Megoura viciae Buckt.
	Metopolophium festucae (Theob.)
	Myzus persicae (Sulz.)
	Rhopalosiphum padi (L.)
Lepidoptera	*Pieris brassicae* (L.)
Coleoptera	*Phaedon cochleariae* (F.)

OTHERS

Orthoptera	*Acheta domesticus* (L.)
	Blaberus discoidalis (L.)
	Blatella germanica (L.)
	Blatta orientalis L.
	Periplaneta americana (L.)
Lepidoptera	*Anagasta kühniella* (Zell.)
	Plodia interpunctella Hübn.
Coleoptera	*Oryzaephilus mercator* (Fauv.)
	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)
	Drosophila subobscura (Coll.)
	Musca domestica L. Strains. Normal susceptible; SKA (diazinon resistant); Orlando regular; F 58 W (DDT resistant)
Hymenoptera	*Apis mellifera* (L.)
Crustacea Cladocera	*Daphnia magna* (Straus)
Guppies	*Lebistes reticulatus*

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Molluscicides

Chemical control of slugs. Work continued on developing laboratory methods to assess the toxicity of chemicals to slugs, and a method was found of introducing measured amounts of chemicals into the digestive tract of slugs. The relative toxicities of chemicals can now be measured when administered either as fumigants, contact poisons, stomach poisons or when injected into the haemocoel. Using these methods of evaluation, work was started on the assessment of several compounds, including copper sulphate, metaldehyde, acetaldehyde, ioxynil and sodium pentachlorophenate. (Henderson)

Fungicides

Laboratory tests. The water-repellency (contact angle), tenacity and bioassay methods of testing fungicides used to control potato blight, described in the last two years' reports, are still used, but with some small changes. In measuring tenacity, 2·5 in. (40 minutes) of "heavy rain" are now used as routine. In the bioassay method upper surfaces only are now sprayed; this is followed by 2·5 in. of "heavy rain" before inoculation. Figures for relative effectiveness given below are weighted mean ratios of EV50s from at least two replicate comparisons by the bioassay method; all were significant ($P \ngtr 0{\cdot}05$).

As a test of the bioassay method, two formulations of triphenyl tin (fentin) acetate were compared with each other in two ways, by using leaflets from two sources: glasshouse- and field-grown King Edward plants. The comparison was done twice with each type of leaflet. The two types gave almost the same figure ($2{\cdot}8 \pm 0{\cdot}9$ times and $2{\cdot}9 \pm 0{\cdot}6$ times) for the relative effectiveness of the two formulations. This means that leaflets from glasshouse plants, which can be obtained throughout the year, give results applicable to field grown plants.

Various formulations of copper oxychloride and fentin acetate were tested, with the following results. Increasing the concentration of wax in our copper oxychloride–paraffin wax formulation did not significantly change its behaviour. The formulation contained 0·25% Cu, and paraffin wax emulsified with 30% of its weight of emulsifiers (see 1962 report). Mean values for initial retention in μg Cu/cm^2, and for tenacity, were: with 1% wax, 11·5, 45%; and with 3% wax, 12·3, 37%. The difference in tenacity (about 2·3 times) between our copper oxychloride–wax formulation and a commercial wettable powder was scarcely affected by increasing the amount of "heavy rain" from 0·3 to 2·5 in. The corresponding mean tenacities were: for the wax formulation, 66%, 29%; and for the wettable powder, 27%, 13%. In bioassay tests the wax formulation was 2·6 times more effective.

Copper oxychloride was formulated by adding to it 1% of lanolin emulsified with (*a*) "Armac HT" or (*b*) "Brij 76"; or (*c*) by adding 3% of "Indopol" polybutene, grade H–100, emulsified with "Renex 690". (These materials were kindly given by Armour Hess Chemicals Ltd., Honeywill & Stein Ltd., and Kingsley & Keith Ltd.) Contact angles

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and tenacities were: for (*a*), 83°, 64%; for (*b*), 29°, 16%; for (*c*), 48°, 12%; and for a wettable powder formulation, 63°, 13%. Thus formulation (*a*) seemed promising; however, in bioassay tests it was less than a third as effective as the wettable powder.

In all the above tests the particle size of the copper oxychloride was the same. However, effectiveness increased when particle size was decreased. In bioassay tests a suspension with 95% of the particles smaller than 1μ was about 7 times more effective than one with only 14% smaller than 1μ. Adding 1% of emulsified paraffin wax to the more finely divided suspension increased the effectiveness by a further 2·0 times.

Fentin acetate was formulated by dissolving it in melted lanolin and emulsifying the hot solution with (*d*) "Armac HT" or (*e*) "Brij 76". The contact angles given by these formulations (at 0·03% fentin acetate and 1% lanolin), and the numbers of times by which they were more effective, in bioassay tests, than a commercial wettable powder ("Brestan 60", kindly given by Hoechst Chemicals Ltd.) were: for (*d*), 85°, 3·6 times; and for (*e*), 34°, 2·0 times. Formulation (*d*) slightly damaged glasshouse-grown King Edward plants.

Tests of acaricide formulations. As the effectiveness of copper oxychloride and fentin acetate could often be increased by formulating them with wax or lanolin, we tested similar formulations of an acaricide. Technical dicofol [2,2,2-trichloro-1,1-di-(4-chlorophenyl) ethanol] was dissolved in (*f*) melted paraffin wax and the hot solution emulsified with "Ethofat 60/25" and "Ethomeen 18/12" (kindly given by Armour Hess Chemicals Ltd.); or in melted lanolin, and emulsified with (*g*) "Armac HT" or (*h*) "Brij 76". These formulations were tested by a variant of the conventional residual film method, using adult red spider mites on French bean leaves; the sprayed leaves were "rain-washed" before the mites were put on them. As a standard, we used a commercial dicofol emulsifiable concentrate ("Kelthane EC", kindly given by Lennig Chemicals Ltd.). Our formulations were ineffective; for example, at equal rates of dicofol the percentages of mites killed after 4 days at 20° C were: for (*f*), 0%; for (*g*), 2%; for (*h*), 5%; and for "Kelthane EC", 80%.

Anti-transpirant fungicide formulations. The addition of small amounts of phenyl mercury compounds to copper oxychloride sprays has been suggested for two reasons: (1) the compounds may have an eradicant action on the potato blight fungus; (2) they may cause stomata to remain closed when they would normally be open, thus decreasing water loss from the plants and, in some circumstances, increasing yield. However, when applied at conventional spraying times the compounds cannot act as eradicants. The following experiments showed that phenyl mercury acetate affected stomata, but did not increase yield (Table 6).

The upper surfaces of glasshouse- and field-grown King Edward (KE) and Ulster Supreme (US) plants were thoroughly sprayed with a wettable powder formulation (0·25% Cu) of copper oxychloride without and with the addition of up to 0·01% phenyl mercury acetate. Readings were made daily with an Alvim portable resistance porometer, applied to the upper

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surfaces. The figures given below are the factors by which the spray residues increased the time needed for an arbitrary pressure fall on the porometer, relative to unsprayed leaves on the same plant (glasshouse) or on unsprayed plants (field). With glasshouse KE plants, factors on the 1st, 3rd and 5th days after spraying were, with no and 0·01% phenyl mercury acetate: 3·6, 0·9, 1·0; and 13·2, 14·1, 9·0. With field plants the differences were smaller and rather erratic, but during the hot dry July weather they were significant for about a week after spraying. Mean factors during this time were, with no, 0·003%, 0·006% and 0·01% phenyl mercury acetate: 1·8, 2·9, 2·6, 2·3 (KE); and 2·4, 3·3, 4·2, 5·7 (US).

In similar tests with "Brestan 60" (0·03% fentin acetate) instead of copper oxychloride, there was little indication of any effect on stomata. With glasshouse KE plants (both leaf surfaces sprayed), factors on the 1st and 3rd days after spraying were, with no, 0·003% and 0·01% phenyl mercury acetate: 1·3, 1·6; 0·9, 1·2; 1·0, 2·9.

Possible fungicidal action of systemic aphicides. Forrest *et al.* (*Proc. Br. Insecticide and Fungicide Conf.*, 1963, p. 117) noticed that blight was less on potato plants treated with the systemic aphicide disulfoton than on untreated plants. One of several possible reasons for this is that disulfoton can act as a systemic fungicide, but the following experiments showed that this is not true of disulfoton or of two other systemic aphicides, dimethoate and menazon. The aphicides were applied to the soil round 9-in.-high glasshouse-grown King Edward plants. Nine to 22 days later, leaflets were detached and tested for resistance to blight by McKee's method (*Trans. Br. mycol. Soc.* **47**, 365). So far from providing evidence of fungicidal action, the results suggested the treatments slightly increased susceptibility to blight; the approximate factors of increase were: with disulfoton, 1·1; with dimethoate, 1·4; and with menazon, 1·4. These increases are probably unimportant.

Field trials

Rothamsted. Two 6 × 6 Latin square experiments were done with King Edward (KE) and Ulster Supreme (US). The objects were: (1) to compare some of our formulations of fungicides (see 1962 and 1963 reports) with commercial ones; (2) to see whether adding phenyl mercury acetate to a copper oxychloride spray affected yield; (3) to see whether spraying the soil ridges with wettable powder formulations during late summer affected the incidence of blight in tubers. This third point was not covered by our laboratory tests.

The foliage on whole plots was sprayed twice: 3 and 23 July (KE); 13 and 28 July (US), with 0·25% Cu or 0·03% fentin acetate, at 100 gall/acre. The treatments were: A, a commercial copper oxychloride wettable powder; B, our copper oxychloride–1% wax formulation; C, as A, but with 0·003% phenyl mercury acetate (0·006% in the second spraying of US); D, our fentin acetate–1% wax formulation (sprayed on US on 13 July only); E, "Brestan 60" (fentin acetate); and F, unsprayed.

Two of the six ridges in each plot were sprayed once (31 July–4 August) with wettable powder formulations at about 400 gall/acre. In

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whole plots with copper treatments on the foliage (A, B and C) the sub-plot ridges received copper oxychloride at 0·125% Cu; and in those with fentin acetate treatments on the foliage (D and E) they received "Brestan 60" at 0·015% fentin acetate.

Tubers were harvested on 11 September (KE) and 18 September (US). Table 6 shows the yields in tons/acre; differences in yield smaller than 0·89 (KE) or 1·13 (US) tons/acre were not significant. In the unusually hot dry summer there was almost no blight on foliage or tubers, so these experiments failed as tests of blight control. However, the weather was ideal for

TABLE 6

Results from field trials on control of potato blight, and of transpiration

Treatment	Yields in tons/acre King Edward	Ulster Supreme
A	7·35	9·13
B	7·34	9·07
C	6·93	9·11
D	7·25	8·42
E	7·94	9·53
F	7·59	9·70

testing the anti-transpirant formulation (treatment C: copper oxychloride with phenyl mercury acetate). In neither experiment did this treatment have any significant effect on yield; its effect, if anything, was to decrease it. Thus, although treatment C temporarily affected stomata, as already said, this had no practical value even in a dry year.

With US, treatment D (fentin acetate + wax) was the only one that significantly affected yield, presumably because it harmed this variety, an effect that may have been accentuated by the hot weather.

N.A.A.S. trial. At the suggestion of Mr. S. C. Melville we supplied the N.A.A.S., Starcross, Devon, with our copper oxychloride–wax formulation for a field trial with the variety Majestic at Crediton, Devon, where blight appeared in August. Our formulation was sprayed only three times, whereas the other three were sprayed four times, but it was almost as effective as the best other material used (mancozeb at 1½ lb/100 gall/acre). (McIntosh and Eveling)

The effects of spray particles on leaves. Further work confirmed that sprays with materials of small chemical activity can increase evaporation from and visibly affect leaves. The sprays used were suspensions of "Stockalite" (a colloidal kaolin), silica or talc in distilled water. To remove soluble impurities the "Stockalite" and talc were centrifuged and the deposit re-suspended in distilled water ("resuspended deposit"). The materials are chemically almost inactive, so any effects on leaves are attributable to physical action by the suspension. Water loss from leaves was measured by a method previously described (*Rothamsted Report* for 1963, p. 144). Spraying 1% w/v suspensions of silica, or resuspended deposits of "Stockalite" and talc, on detached leaves of *Coleus blumei* or runner bean (*Phaseolus multiflorus*) significantly increased ($P = 0·01$) their mean water loss and sometimes produced necrotic areas on the leaves. A 1% w/v

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suspension of "Stockalite" also significantly increased ($P = 0{\cdot}05$) water loss from detached, field-grown leaves of potatoes, varieties Ulster Supreme and King Edward. Leaves of runner beans whose petioles were immersed in 1% w/v suspensions were unharmed. Roots developed on petioles in suspensions of "Stockalite", talc, silica and distilled water, but not in a 0·25% w/v copper oxychloride suspension.

To see whether the sprays increase water loss by affecting the cuticle or the stomata, excised *Coleus blumei* leaves were sprayed on either their lower or upper surface with a 1% w/v resuspended deposit of "Stockalite". In one test water was lost equally from both lots of sprayed leaves, but in two others those sprayed on the upper surface lost slightly more. As the lower surface had about a hundred times as many stomata and the spray deposits on both surfaces were similar, the effect of the spray is obviously on the cuticle. Similarly, with bean and potato leaves, water loss was increased by much the same amount whether the upper or lower surface was sprayed.

That spraying with "Stockalite" increases the permeability of coleus leaves to vapour was further indicated by the fact that when held in ammonia vapour their colour changed from red to blue or green faster than leaves sprayed with water.

The concentration and size of the particles in the spray affected the results. Thus, water loss from runner-bean leaves was increased by 0·25% w/v "Stockalite" but not by 0·1%. With beans and coleus leaves, particles of silica smaller than 0·5 μ diameter increased water loss much more than particles larger than 1 μ. When leaves were treated with suspensions of particles of three size ranges, 1–2·5 μ, 5–10 μ or 20–30 μ, and then exposed to ammonia vapour, those sprayed with the smallest particles changed colour fastest, and with the largest particles slowest. (Eveling)

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