ROTHAMSTED RESEARCH

Report for 1961

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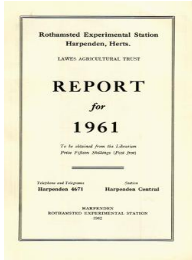

Insecticides and Fungicides Department

C. Potter

C. Potter (1962) *Insecticides and Fungicides Department ;* Report For 1961, pp 130 - 144 **- DOI: https://doi.org/10.23637/ERADOC-1-94**

The ecological effects of applying DDT and aldrin are being studied on Highfield, with plots kept completely free from plants. Some Collembola prey on nematodes, so effects on nematodes are being studied by C. Doncaster (Nematology Department); the rate at which the insecticides disappear is being measured by K. Jeffs (Insecticides Department). At the end of the first 6 months all the DDT treatments (at 6, 20 and 60 lb. a.i./acre) again decreased the numbers of predacious mites, and increased Collembola, which were five times as numerous as in the untreated plots. DDT at 6 lb. a.i./acre increased the Orobatid mites. Aldrin did not affect the numbers of predacious mites, but Symphyla, Pauropoda, Protura, Diptera, Coleoptera, Hymenoptera, Lepidoptera and Hemiptera were all diminished greatly by all treatments except the lowest rate of DDT. (Edwards, Raw and Lofty.)

The effect on the soil fauna of accumulations of aldrin from regular applications, at rates commonly used in agriculture, is being studied at Levington Research Station by courtesy of Messrs. Fisons Ltd. Aldrin is applied annually, to crops in a rotation of sugar beet, barley, potatoes and winter wheat, from 1 to 2 lb. a.i./acre according to the crop; 6-monthly samples of soil will show faunal changes, and crop yields will be taken. Already, after 1 year, the total arthropod population in the treated fields was only 57% of that in the controls; Acarina, Collembola and other arthropods were respectively 44%, 62% and 68% of their levels on the untreated plots. (Edwards, Raw and Lofty.)

Ecological effects of soil fumigants

Soil fumigants are commonly used to control nematodes and pathogenic fungi, but their effects on other soil-inhabiting organisms have been almost ignored. A programme to study the short- and long-term effects of some nematicides on soil arthropod populations was begun in greenhouse beds with a high arthropod micro-fauna, using "D.D. Mixture" (50% 1 : 2-dichloropropane, 50% 1 : 3-dichloropropene). Changes in the horizontal and vertical distribution of the populations caused by fumigation, and the rate and form of recolonisation of treated soil, are being studied. (Buahin and Edwards.)

The arthropod fauna of different soil habitats

Because different species of arthropods are concerned with litter breakdown in different habitats, the composition of the fauna is being studied in heathland, woodland moss, woodland litter, sphagnum moss and in six plots on Park Grass, Rothamsted, that carry different plant species. In a 2-year census of the first four habitats over a quarter of a million arthropods recovered consisted of Arachnida (75%), Collembola (23%) and other arthropods (2%). 90% of the Arachnida were orobatid mites and 7% and 3% Parasitiform and Trombidiform mites. All these proportions were approximately the same for all four habitats.

Over 90% of the mites and Collembola were restricted to the litter and humus layers or to a soil depth of 2 cm. There was no obvious vertical migration during the year, probably because of the spring. Like leaf burial, fragmentation was related to weather and depended on the moisture content of the litter, beginning after heavy rain and stopping in dry weather. Oak and beech woods had similar populations of worms, an approximate fresh weight of 1,000 lb. earthworms/acre, with *Lumbricus terrestris* more common than *L. rubellus*.

Leaf breakdown is not always done by earthworms, however; in arable land worms are assisted by small arthropods. So far the importance of various species of soil arthropods in leaf breakdown has been obscure, but a new method enables these activities to be analysed. Leaf disks were encased in nylon bags of various meshes and buried in the soil. The mesh regulates the entry of different-sized animals reaching the leaf disks. This method, coupled with studies on changes in the composition of the soil fauna after treatment with different insecticides and nematicides, indicated that Collembola and Dipterous larvae are important in the initial breakdown of leaf disks (worms being excluded by some bags) and that numbers of Collembola in plots treated with different insecticides were correlated with the amount of feeding as shown by the erosion of the disks.

This work was supplemented by studying changes in the total soil carbon levels and the proportion of undecomposed plant material extracted by flotation on concentrated $ZnSO_4$ solution. The degree of fragmentation of disks is also being followed by estimating the changes in hydrolysable carbohydrate. These processes of leaf breakdown are correlated with changes in the metabolic activity of soil organisms and attempts are being made to assess this by measuring changes in the CO_2 of the atmosphere of blocks of soil. (Edwards, Raw, Heath and Lofty.)

The effect of chemical control on pests and on other arthropods and worms in the soil

The extensive use of chemicals to control insects and other pests often leads to toxic substances accumulating in the soil, and it is important to know how such treatments affect predators of pests and soil organisms concerned with litter breakdown and soil fertility.

Thus, in addition to studying how cultivation and manuring affect earthworm populations, attention was also given to the effects of spraying with copper fungicides. One orchard with a long history of such spraying had accumulated 1,500–2,000 p.p.m. of Cu in the surface litter. Laboratory studies were therefore made to see the effects of feeding a mat of partially decomposed litter with this high copper content to earthworms. *Lumbricus terrestris* seemed unharmed and pulled the mat into its burrows, but several other species died after feeding on it. (Raw and Lofty.)

Preliminary work showed that single applications of widely used soil insecticides can greatly affect both the numbers and the proportions of various species of arthropods in arable land; in general, population decreases, but the various groups of animals are not all equally affected. DDT decreased the numbers of Mesostigmatic mites, but simultaneously *increased* the numbers of Collembola, presumably because it removed the mites which normally prey on the Collembola. (Edwards.)

ENTOMOLOGY DEPARTMENT

C. G. JOHNSON

K. Mellanby left to become Director of The Nature Conservancy Field Station, St. Ives, Hunts, and T. Lewis was appointed in October; C. J. Banks returned from a secondment to the Institute of Nuclear Science, Central Treaty Organisation, Iran. Mr. G. K. A. Buahin of the Division of Agriculture, Ghana, and Mr. R. MacGregor-Loaeza of The National Institute of Agricultural Research, Mexico, joined the Department as visiting members. Dr. E. Dimelow of Mount Allison University, New Brunswick, came in the summer to study the annelid and mollusc food of hedgehogs, and Mr. S. Agwu (Eastern Nigeria) also visited the Department.

K. Mellanby, who was appointed to the Inter-University Council for Higher Education Overseas, visited Hong Kong in March at the invitation of the Hong Kong Government, to advise on the establishment of a second University there; he also lectured in the Republic of the Philippines. C. G. Johnson visited Ceylon in October to advise on aphid research, at the invitation of the Ceylon Tobacco Company.

A. J. Cockbain and D. S. Madge were awarded the Ph.D. degree of London University.

SOIL FAUNA

There are three main lines of study: the biology of earthworms and soil arthropods in relation to soil fertility; the effects of insecticides and other chemicals on pests and on beneficial (or at least innocuous) soil animals; and the biology of pests which live in the soil.

The breakdown of vegetable matter in the soil by soil animals

Part at least of the contribution of earthworms to soil fertility is their burying of plant material, so beginning the process of its incorporation into the soil. This activity occurs at different rates according to the weather. Recent work on orchards, where leaf burial has been studied for some years (*Rep. Rothamst. exp. Sta.* for **1959**, **1960**), showed that from December to March rainfall had little effect on the rate *Lumbricus terrestris* bury leaves, presumably because the litter never dried out and soil moisture was never a limiting factor at this time; but burial rate was positively correlated to soil temperature.

Once the leaves are buried, worms also help to break them down. Disks of leaf placed at three levels in the litter layer of oak and beech woods showed that fragmentation was almost entirely by earthworms, and only about 2% of disks showed signs of skeletonisation by Collembola and other small arthropods. Fragmentation, which occurred almost exclusively in the lowest litter layer, was at its peak in February and March, decreased in April and occurred only sporadically till August; later it increased again to a peak in the

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but significantly increased their height. The response to formalin varied less than the response to chloropicrin.

Formalin was applied at 250 ml. of 18% formaldehyde per sq. yd. soil, by watering-can in 4–5 litres of water, depending on soil moisture. Chloropicrin was applied by soil injector to a depth of 6 inches at the rate of 16 shots/sq. yd. each of 2 ml. of approximately 100% active ingredient. Both chemicals were applied in December 1960 and January 1961 and seed was sown in late March.

This series of experiments at five sites was modified to test the effects of single and repeated doses of formalin. At Old Kennington, Sitka grew significantly better on plots treated once than on plots given repeated doses annually. Formalin greatly decreased numbers of fungi per unit of plot soil, but had comparatively little effect on numbers in rhizospheres. In both locations the different types of fungi responded very differently to treatment. Treatment decreased the incidence of species of *Cylindrocarpon*, *Fusarium* and *Mortierella* and increased the prevalence of *Penicillium canescens* Sopp, which seems to be an important coloniser of partially sterilised soils.

The effects of several chemicals and methods of application were tested at Old Kennington and Ringwood. These included pre-sowing treatments with metham-sodium (165 g./sq. yd. in 4–5 l. of water, applied in the same way as formalin, in January), thiram (112·5 g. of 15% dust/sq. yd., broadcast, in February), quintozene (112·5 g. of 25% dust/sq. yd., broadcast, in February), and formalin (rate as above, in January); and a post-sowing drench of maneb (2·0 g. of 80% dust in 2–4 l. of water, applied 5 times at monthly intervals). At both sites all treatments except thiram increased the numbers of surviving seedlings, maneb giving consistently large increases. Formalin increased the height most, with metham-sodium next. Maneb increased height appreciably at Ringwood, but not at Old Kennington.

Work on chemical control of Sitka diseases has largely been concerned with the soil-borne organisms. Seed-treatment experiments started in 1960 were continued and indicate that seed-borne fungi may cause appreciable losses. Application of thiram, captan or organo-mercury fungicides increased numbers, but not heights, of seedlings, even with apparently clean seed. Dieldrin mixed with fungicides had no effect on seedling development. (Last and Ram Reddy.)

Work on attempts to control take-all of cereals by chemicals is described in the report of the Plant Pathology Department.

The silicone preparation used was " Dri-Sil 37 " (Messrs. Midland Silicones Ltd.). This is a stable aqueous concentrate of sodium methyl siliconate which is normally used to make masonry water-repellent. Films formed from such solutions react with the atmospheric carbon dioxide to form the corresponding silanol, which polymerises at ordinary temperatures to form a water-insoluble and highly water-repellent deposit.

The following remarks refer to 1 : 20 solutions of " Dri-Sil 37 ". The pH is high (about 11·5); but the high pH probably does not persist for long in the thin chemically changing film on a treated surface. The surface tension (about 66 dynes/cm.) is only slightly less than that of water, i.e., too high for such a solution to spread really efficiently over a potato leaf. However, the siliconate is compatible with some surface-active agents; the function of each of the two components in such mixtures seems to be unaffected by the presence of the other. The mixtures are surface active and spread efficiently over potato leaves, yet dry to water-repellent films. The surface tensions of freshly formed surfaces of siliconate solutions containing 0·01% of " Ethomeen S/25 " (Messrs. Armour Hess Chemicals Ltd.) or " Manoxol OT " (Messrs. Hardman & Holden Ltd.) were 39 and 27 dynes/cm.; the corresponding figures for the surface-active agents in water alone were 38 and 30 dynes/cm.

However, the proportion of surface-active agent need not be so low as 0·01%. When detached potato leaves were sprayed with siliconate solutions containing 0·1% of either surface-active agent and kept for 24 hours the leaves were undamaged, and the advancing contact angle on the leaves was over 90°; the corresponding angle on untreated leaves was 58° (Majestic) or 85° (Ulster Supreme).

When detached Ulster Supreme leaves were sprayed with a siliconate solution containing 0·01% " Ethomeen S/25 " and kept for 24 hours, the leaves were undamaged and the advancing and receding contact angles were, on sprayed leaves, 94° and 67°, and on unsprayed leaves, 84° and 12°. When water was applied to the sprayed leaves it did not form a film on them, but formed itself into drops which rolled off, leaving the surface dry.

The deposits left by simple siliconate mixtures are rather brittle, and the water-repellent effect did not persist for very long on field plants. Single Ulster Supreme plants in the field were sprayed with siliconate solutions, with and without 0·01% " Ethomeen S/25 ", on five occasions from 10 August to 29 August. Damage was slight, except when the weather was very hot at the time of spraying. Leaves were sampled on six occasions after spraying. The advancing contact angle on sprayed differed from that on unsprayed leaves for only 8 days after spraying; the mean angles during this time were $>90°$ and 84°. No measurements were made this year on the effect of the increased water-repellency on blight infection. (McIntosh.)

The effects of chemicals used to control seed- and soil-borne diseases in forest nurseries

Both in 1960 and 1961, soil treatment with formalin and chloropicrin usually had no effect on numbers of Sitka seedlings surviving,

https://doi.org/10.23637/ERADOC-1-94

Work, primarily on the use of seed dressings to control wireworm, continued in collaboration with the Entomology Department. Details are given in the report of the Entomology Department. (Raw, Bardner and Potter.)

Fungicides

The control of potato blight

Potato leaves are infected by spores of *Phytophthora infestans*, the cause of " blight ", only when there is free moisture on their surfaces. Infection therefore might be checked if leaf surfaces could be made sufficiently water-repellent. To study this possibility, tests were started with wax " emulsions " and a water-soluble silicone preparation, on detached leaves and on single growing plants. The results were assessed by measuring the advancing and receding contact angles of distilled water on the upper surfaces of the leaves. The angles give a measure of the water-repellency of the surface. The advancing angle determines how easily water spreads over the surface; the receding angle determines how easily water is shed by the surface. The two angles are seldom the same; the advancing is nearly always larger than the receding angle.

The wax preparations, known commercially as " emulsions ", were made by emulsifying hot waxes directly in water, but at the time of use are more accurately called suspensions. The preparations used were: (*a*) 1% carnauba wax emulsified with 0·35% "Armac T" (Messrs. Armour Hess Chemicals Ltd.); (*b*) 1% carnauba wax emulsified with 0·88% "Armac T", with the addition of 1% " Stockalite ", a finely divided kaolin (Messrs. English Clays, Lovering, Pochin, & Co. Ltd.) to increase the surface roughness; the effect of this is to increase the advancing contact angle; (*c*) 1% " Cardis 319 ", an oxidised microcrystalline wax (Messrs. Cornelius Produce Co. Ltd.), emulsified with 0·4% "Armac T"; (*d*) 0·35% "Armac T" only; (*e*) 4·3% paraffin wax emulsified with 1·3% bentonite and 1·3% ammonium linoleate (U.S. Patent 2,013,063; 1935).

Single field-growing Ulster Supreme plants were sprayed with each of these on two to four occasions from 8 August to 4 September; the plants, except those sprayed with (*b*), were almost undamaged. Leaves were sampled on six occasions after spraying. The changes in water-repellency were small but very consistent. The length of time for which the advancing contact angles on sprayed leaves differed from those on unsprayed leaves, with the mean angles during this time, were: with (*a*) 25 days, 76°; with (*c*) 8 days, 65°; with (*d*) 37 days, 69°; with (*e*) 27 days, >90°; and on unsprayed leaves, 84°. Thus, the effect of these sprays, except the paraffin wax–bentonite–ammonium linoleate suspension, was to lower the advancing contact angle of water on the leaf surfaces, i.e., to make them slightly more easily wetted than the unsprayed leaves. The effect of these treatments on blight infection was not measured, but any effects must be very small. However, wax emulsions are said to reduce transpiration by the plants, and may therefore be of value, in conjunction with copper sprays, in checking the increased transpiration and loss in yield which sometimes follows copper spraying of potatoes.

The laboratory of the Government Chemist found the following insecticide residues in tubers harvested from each treatment: phorate, 0·15 p.p.m.; dimethoate, 0·13 p.p.m.; menazon and "Disyston", less than 0·05 p.p.m. (Burt, Dr. L. Broadbent (Glasshouse Crops Research Institute), Heathcote.)

Seed dressings with insecticides

Glasshouse tests compared the toxicity to aphids and the persistence of the following thirteen systemic insecticides when applied in a slurry to the seed: American Cyanamid 18133 (0 : 0-diethyl-0-2-pyrazinyl phosphorothioate), demeton-S-methyl, demeton, demeton-methyl, dimethoate, "Isolan", menazon, morphothion, phorate, phosphamidon, schradan, "Disyston" and "Trithion". Tests were made with wheat seed and *Rhopalosiphum padi*, kale seed and *Brevicoryne brassicae* and rubbed sugar-beet seed and *Aphis fabae*. Rates of application in terms of grams of active ingredient per 100 g. of seed were 0·2 and 0·4 for wheat, 2 and 4 for kale and 0·44 and 0·88 for sugar beet. Some differences in degree of toxicity and persistence could be related to the species of seed used, but the differences were not great.

The toxicity and persistence of the chemicals differed considerably, and the degree of initial toxicity to the aphids was unrelated to the persistence of toxicity. The maximum period over which aphids were all killed was about 40 days, although populations were affected for a further 10–30 days. Dimethoate best combined high insecticidal activity with a long persistence, but demeton, phorate and "Disyston" were also good. "Isolan" was tested only on kale, and was very effective over a long period. Menazon and American Cyanamid 18133 were very persistent, and would probably have been more insecticidal at higher rates. Schradan and "Trithion" were ineffective.

The uptake by mustard and wheat of phorate applied as a slurry to the seed was studied. Removing the seed coat of mustard at intervals after treatment and then sowing the embryos showed that the insecticide does not penetrate the seed coat into the embryo before germination. The seedling obtains nearly all the insecticide from the soil through its roots, but a little is picked up by the cotyledons and hypocotyl as they force their way through the soil. When phorate is applied as a slurry to wheat seed, some insecticide passes directly from the endosperm into the seedling, but most of the insecticide is absorbed by the roots from the soil. Only insignificant amounts of insecticide are picked up by the coleoptile as it emerges through the soil. Thus, it seems that with one insecticide and technique of application, the mode of uptake may differ with different species of seed. Mode of uptake is also likely to differ with different chemicals, or with changes in the formulation or technique of applying a given chemical. (Bardner.)

An experiment with dry powder dressings containing 70% of γ-BHC on seeds of cabbage and white mustard confirmed unpublished findings by workers of Plant Protection Ltd. that small particles (13–39 μ diameter) of seed dressing protected seedlings from flea-beetle attack better than large ones (260–390 μ diameter). (Bardner.)

more slowly than those from untreated tubers, but no such differences in growth occurred with tubers from the 1960 experiments. The difference in measurable residual effects was probably because dimethoate was applied at lower rates (2–5 lb./acre) in 1960 than in 1958 (10·5 lb./acre).

Another experiment at Efford this year tested the effectiveness of treating seed tubers or soil with systemic insecticides in checking virus spread. Four chemicals were used:

1. Menazon as a dispersible powder suspended in water and sprayed on to the seed tubers immediately before planting at the rate of 1·3 lb. active ingredient/acre = 0·8 lb./ton of seed.
2. Phorate applied in the drill at planting as a 10% granular formulation at 4·5 lb./acre active ingredient.
3. Dimethoate in the drill as a 6·9% granular formulation at 4·7 lb./acre active ingredient.
4. " Disyston " (diethyl *S*-[2-(ethylthio)ethyl]phosphorothiolothionate), in the drill as a 5% granular formulation at 1 lb./acre active ingredient.

The layout and procedure were similar to those of the 1960 experiment. The granular materials were applied by a specially built, light, hand-propelled machine, incorporating a Gandy Row Crop Chemical Applicator. The machine was fitted with an electric speedometer to ensure accurate application rates, and was convenient and efficient in practice.

Potato aphids were fairly numerous, reaching 950/100 leaves in the control plots on 8 June. Table 4 shows aphid counts at the different plots and includes for comparison counts on nearby potatoes sprayed with DDT and demeton-methyl.

TABLE 4

Potato aphids per 100 leaves (Haulms burned off 5 July)

	20/4	4/5	24/5	30/5	8/6	23/6
Menazon	0	0	6	5	61	25
Phorate	0	2	2	0	1	3
Dimethoate ...	0	1	13	24	115	26
" Disyston " ...	0	0	0	0	1	1
Control	5	1	70	436	950	175
Demeton-methyl ...	—	—	—	58 *	1 †	—
DDT	—	—	—	39 *	5 ‡	—

* Sprayed 24 May. † Sprayed 6 June. ‡ Sprayed 7 June.

Phorate and " Disyston " were most effective, controlling aphids throughout the life of the crop. Menazon was equally effective until 11 weeks after planting, but its efficiency then decreased, possibly because the small quantity applied remained close to each seed tuber, and therefore out of reach of many of the late-developed roots of the plants. Dimethoate, very efficient in two previous experiments, was relatively ineffective, perhaps because the weather during the 1961 experiment was much drier than during the two earlier experiments. Dimethoate slightly delayed the emergence of the plants, and menazon caused slight initial discoloration of their leaves, but no treatment decreased yield significantly.

commonly used as seed dressings, lindane, dieldrin or ethyl mercury chloride, was lethal to bees by fumigation, whereas phenyl mercury acetate was not.

The honey samples were from hives where bees had been poisoned with dieldrin. The source of the dieldrin was probably a wasp bait, hence it was thought that some of the bait may have been stored with the honey. Extraction and bioassay indicated the presence of dieldrin at approximately 0·04 p.p.m. Chromatographic confirmation was not possible because of the small size of the sample and the low concentration of dieldrin.

Weedkillers. Each year bees are received suspected of being poisoned by weedkillers. Therefore, the approximate oral LD50s of four common weedkillers were estimated by feeding bees with the chemicals in sucrose solution and keeping the insects after treatment at 28° C. and approx. 75% R.H. The approximate oral LD50 dose under these conditions was between 5 and 10 μg./bee for monochloracetic acid, 300 μg./bee for 2, 4-D, less than 1 μg./bee for dinoseb (DNBP) and 1,500 μg./bee for MCPB.

A technique developed for identifying MCPA, MCPB and 2, 4-D by paper chromatography was successfully used with bees poisoned in the laboratory by a stomach-poison technique. As it is unusual to receive freshly killed bees, the insects were weathered for 10 days after poisoning, but these three weedkillers were recovered and identified at levels that would be likely had poisoning occurred in the field.

The samples received this year which showed no other cause of death were examined for MCPA, MCPB, 2, 4-D and DNOC, but none was found. The techniques outlined would not detect dinoseb or monochloracetic acid. (Needham and Solly.)

Control of the spread of potato viruses

To improve the timing of sprays used to check the spread of viruses in potato crops, detailed information is needed about when spread occurs. The time of spread was again studied by spraying with insecticide at different times during the life of the crop. Potato aphids were few, and the most counted in the crop was 194 per 100 leaves on 29 June. Numbers then declined, but rose slightly in September. The insecticide treatments controlled the aphids, but effects on virus spread will not be known until next year.

Dimethoate, applied in the soil at 4 and 5 lb./acre of active ingredient in an experiment at Efford in 1960 (*Rep. Rothamst. exp. Sta.* for 1960, pp. 117 and 148), almost prevented the spread of leaf roll; at 2 lb./acre, like fortnightly sprays of DDT, it decreased spread to one-fifth of that in the control plots. Spread of rugose mosaic was halved by the dimethoate treatment, but unaffected by DDT.

A previous experiment (*Rep. Rothamst. exp. Sta.* for 1959, pp. 127–128) showed that shoots from potato tubers harvested from plants treated with soil insecticides when infested with adult *Myzus persicae* carried fewer aphids a week after infestation than shoots from control tubers. Shoots from tubers harvested from the 1960 experiment at Efford tested similarly gave no consistent difference in aphid populations between control and treated tubers. In the 1958 experiments the shoots from treated tubers grew initially following systemic insecticides was compared: mevinphos, morphothion, phorate, "Isolan", dimethoate, phosphamidon, demeton-methyl, fluoroacetamide, menazon, "Trithion" and schradan. The insecticides were sprayed on to sugar beet and field beans at 0·05% in water containing 0·05% wetting agent. The plants were kept in a glasshouse at about 18°, and persistence of insecticidal activity was assessed by confining aphids on the leaf surfaces by means of light plastic cages and recording mortality. Aphids (*Myzus persicae* and *Aphis fabae*) were first put on the plants 24 hours after spraying and again at intervals until no kill was recorded. *M. persicae* were usually more resistant than *A. fabae*, though not to all the chemicals. *A. fabae* were never more resistant than *M. persicae*. Toxicity persisted for from 1 to 15 days, depending primarily on the insecticide. The order of decreasing persistence was as follows: (1) phosphamidon; (2) demeton-methyl; (3) "Trithion"; (4) morphothion; (5) dimethoate; (6) fluoracetamide; (7) menazon; (8) schradan; (9) phorate; (10) "Isolan"; (11) mevinphos.

No differences in persistence were found with the two different host plants with any of the test chemicals. (Etheridge.)

Bee poisoning in the field

Insecticides and fungicides. Forty-five samples of bees suspected of being poisoned in the field were received for post-mortem examination from Mr. P. S. Milne of the National Agricultural Advisory Service. Twenty samples were poisoned by cholinesterase-inhibiting substances, i.e., organophosphorus or carbamate insecticides. Two samples, in which no other poison could be detected, gave inconclusive evidence of the presence of a cholinesterase inhibitor. Nine samples contained dieldrin and were considered to have been poisoned by this insecticide or aldrin; two of these were further samples from an earlier case of poisoning, and indicated that damage was still occurring 2 weeks later. Three samples were considered to have been poisoned by lindane.

No insecticide was found in the ten remaining samples, so these were examined for mercury and copper fungicides. One contained 8 μg. Hg per bee. A 20% aqueous sugar syrup containing phenyl mercury acetate gave an oral LD50, equivalent to 3·9 μg. Hg per bee, so it was concluded that these bees may have been killed by an organo-mercury fungicide.

Copper was present in all samples, but only in trace amounts.

Paper chromatographic techniques were developed and used to confirm the presence of insecticides detected by bioassay and to assist in their identification.

In addition to the bees, one sample of sacking and two of honey were received for examination. The sacking was thought to have been responsible for the death of bees in a hive covered with it. Tests showed the sacking to be lethal to bees both by contact and fumigant action. The information received with the sample indicated that the sacking might have been contaminated with a seed-dressing, so attempts were made to extract the toxicant and identify it by bioassay and chromatography, but these failed because of interfering substances present. However, we found that sacking impregnated with a 1% acetone solution of chemicals *marus pulex*) to solutions of pure insecticides. The actual LD50 can, of course, always be altered by changing the conditions of test.

TABLE 3

Median lethal doses (parts per million) of insecticides applied to Aëdes aegypti *larvae,* Daphnia obtusa *and* Gammarus pulex *in aqueous solution and applied to* Drosophila melanogaster *adults by exposing them over potato pulp containing the insecticide*

	A. aegypti	*D. obtusa*	*G. pulex*	*D. melanogaster*
γ-BHC	0·03	0·7	—	—
DDT	0·002 †	0·03	—	—
Demeton-methyl...	4·0	—	0·04 ‡	22
Dieldrin	0·003	0·3	0·4	0·7
Parathion... ...	0·002	0·0005 †	0·006	0·2
" Sevin " * ...	1·0	0·02 †	—	> 100

* 1-Naphthyl N-methylcarbamate.
† Assessment after 2 hours; otherwise after 24 hours.
‡ Assessment after 48 hours; otherwise after 24 hours.

Attempts to use the newly hatched nauplii of the brine shrimp (*Artemia salina*) for assays failed, because too few of the eggs obtained from several sources were viable. The use of the guppy (*Lebistes reticulatus*) was also abandoned, because too few fish could be reared. Extensive inquiries failed to disclose other species that might be promising test organisms for bioassay of insecticide residues. *Drosophila melanogaster* and *Daphnia obtusa* are now the most promising test organisms. Their culture is not likely to present difficulties to analytical chemical laboratories, assay procedures can be made fairly simple and reproducible, and they are sensitive enough to the poisons tested. (Stevenson.)

Factors influencing the insecticidal activity of systemic insecticides applied as foliage sprays

(a) *Fumigant effects.* An attempt was made to assess the relative toxicity, by vapour action alone, of the following commonly used systemic insecticides: mevinphos, phorate, morphothion, menazon, " Isolan " (1-isopropyl-3-methylpyrazolyl-(5)-dimethylcarbamate), phosphamidon, " Trithion " (0, 0-diethyl S-*p*-chlorophenylthiomethyl phosphorodithioate), dimethoate, demeton-methyl, fluoroacetamide and schradan. Initial tests were made in sealed Petri dishes, using 0·05% concentrations of insecticide in diethyl ether; 0·5 c.c. of solution was applied to filter-paper in the bottom of the dish by a pipette and the solvent evaporated. Aphids (*Megoura viciae*), used as test organisms, were confined in shallow crystallising dishes by coating the sides of the dishes with polytetrafluoroethylene (" Fluon ") and placed inside the sealed Petri dish system. After a 2-hour exposure at 20° the aphids were removed to clean Petri dishes and the knockdown assessed; after a further 20 hours at 20° toxicity was finally assessed. Pronounced fumigant action in these tests was shown only by mevinphos, phorate and " Isolan ", but the possible contribution of fumigant action to the overall toxicity of systemic insecticides is being further explored.

(b) *Persistence.* The persistence of insecticidal activity of the

The decomposition of DDT and aldrin in soil was studied as part of an experiment testing these insecticides on soil organisms done in collaboration with the Entomology Department (see p. 147). Using a modification of the method for DDT worked out by the Joint BHC/DDT panel (*Analyst*, **85**, August 1960), analyses were made 3 months and 6 months after applying DDT at different rates; after 6 months the loss was 15% with 60 lb. DDT/acre and 20% with 6 lb. DDT/acre.

Samples of the aldrin-treated soils are being held until a satisfactory analytical technique is developed. (Jeffs.)

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 product, 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.	3 colonies were maintained as a source of control bees for the field poisoning studies

Guppies (*Lebistes reticulatus*) were also reared.

Bioassay of insecticide residues

The Analytical Methods Committee of the Society for Analytical Chemistry, after sponsoring a survey of existing methods for bioassay of insecticide residues by Needham (*Rep. Rothamst. exp. Sta.* for 1959, p. 117) financed a study of these methods to determine their ease of working, accuracy and sensitivity.

Table 3 shows the relative sensitivity of larvae of yellow-fever mosquito (*Aëdes aegypti*), water flea (*Daphnia obtusa*), adult fruit flies (*Drosophila melanogaster*) and the fresh-water shrimp (*Gam-*

to crystallise. The amount deposited per unit area depends on several factors, some of which are difficult to control. Considerable precautions against radioactive contamination have to be taken.

Spraying methods. Even coverage and a satisfactory deposit of needle crystals ranging from 0·1 to 5 mm. long (average 1 mm.) were obtained by spraying dieldrin in a hydroxylic type solvent (" Cellosolve ") in a Potter tower at 18°–20°. The small percentage of the total sprayed material depositing on the test surface and the contamination risks make it difficult to use this method without modification, but an attempt is being made to produce a special atomising nozzle for spraying radioactive materials on to a small surface area (5 sq. cm.) in such a way that all, or nearly all, the sprayed material is deposited evenly on the test surface.

Spreading methods. When dieldrin in " Cellosolve " was applied in droplets at the centre of a glass plate, previously wetted with solvent, the results were not reproducible and the physical state of the poison after evaporation of the solvent varied. When droplets of dieldrin in " Cellosolve " were placed on dry glass and spread with the edge of a glass cover-slip, results were more reproducible and the deposits were the nearest approximation to those obtained in the Potter tower, but the needle crystals were larger (range 1–10 mm. long). This method is being used for the time being to study the loss of dieldrin from glass surfaces.

A deposit of 2 μg./sq. cm. was obtained by spreading 40 μg. of radioactive dieldrin in 10 μl. of " Cellosolve " over an area of 20 sq. cm. A central circular area of 5 sq. cm. was measured radiometrically at intervals using a masked Geiger end-window counter. With the glass plates placed very close to the counter (3 mm. from the mica end-window), a counting efficiency of better than 20% was obtained using dieldrin labelled with ^{36}Cl. Three replicates were used and the plates kept at 18°–22° shielded from draughts. Table 2 shows the rate of loss of dieldrin under these conditions. Initially volatilisation occurred at a constant rate, the loss being directly proportional to the time of exposure. After 15 days, when the deposit was down to 0·4 μg./sq. cm., the rate of loss started to decrease and the curve tended to approach the time axis asymptotically.

TABLE 2

Rate of loss of dieldrin crystals 1–10 mm. long from a glass surface kept at 18°–22° in still air

Exposure (days)	0	4	8	11½	15	20	25	30	35
Deposit (μg. dieldrin/cm.² remaining on plate) ...	2·0	1·6	1·2	0·8	0·4	0·15	0·075	0·05	0

(Phillips.)

Analyses

We again collaborated in work, organised by the joint BHC/DDT panel and the demeton-methyl residues panel of the Scientific Sub-committee on Poisonous Substances used in Agriculture and Food Storage, testing analytical methods for BHC and demeton-methyl. The work on BHC was completed. (Jeffs, Lord and Solly.)

day was 0·093 γ/insect, significantly less than the 0·132 γ on and in the dead insects. The mean amounts inside live and dead insects differed more (0·073 γ/living insect and 0·116 γ/dead insect). However, the living insects had lost more poison (0·014 γ/insect) than those that had died (0·010 γ/insect). The amounts outside the bodies after 11 days were about the same in each group, averaging 0·0063 γ on each live and 0·0068 γ on each dead insect. The amounts picked up and the percentages lost to the containers were negatively correlated.

In a similar experiment, with insects exposed for 45 minutes and kept for 9 days, 93% died. The amounts picked up ranged from about 0·110 γ to 0·410 γ, with a mean of about 0·230 γ/insect. The distribution curve was again skewed towards the higher values. The insects dying within 4 days contained more dieldrin (0·265 γ/insect) than those still surviving (0·200 γ/insect). The difference was larger after 9 days, when the mean amount of poison contained by the dead insects was 0·227 γ and by the survivors 0·128 γ.

TABLE 1

Mean weight of dieldrin (p.p.m. of fresh weight) found in separate parts of adult Tenebrio molitor *L. at increasing times after exposure to films*

	Mean fresh weight (mg.)	Time after removal from the film: 0 hr. All alive	6 hr. All alive	4 days: Alive	4 days: Total	4 days: Dead	9 days: Alive	9 days: Total	9 days: Dead
No. of insects used ...		*22*	*25*	23	*32*	9	2	*26*	24
Head ...	5·4	*4·6*	*2·7*	1·5	*1·7*	2·2	1·6	*2·3*	2·3
Legs ...	6·2	*17·0*	*6·9*	1·4	*1·7*	2·5	1·4	*2·7*	2·8
Abdomen ...	34·5	*0·9*	*1·9*	2·3	*2·3*	2·5	0·9	*2·0*	2·1
Thorax ...	52·7	*1·4*	*2·1*	1·9	*2·1*	2·7	1·3	*2·1*	2·1
Whole insect	98·8	*2·4*	*2·3*	2·0	*2·1*	2·6	1·2	*2·1*	2·2

Table 1 shows the amounts of dieldrin, detected at various times after treatment, in the dissected parts of the insects and in the "whole" insects (calculated as the proportional means from the separate weights of dieldrin in, and the weights of, the various parts). The insecticide was mainly picked up by the insects' legs and was distributed from there to the rest of the body, reaching an almost uniform distribution throughout the insect on the 9th day. Previous experiments (*Rep. Rothamst. exp. Sta.* for **1960**, p. 142–144) showed that most of the insecticide enters the body of the insect within the first few hours, so the uniform distribution shown in Table 1 can be assumed to result from internal migration of the dieldrin rather than mechanical transference on the surface of the insect. (Rey and McIntosh.)

The effect of temperature and air movement on the rate of loss of residual films of insecticides from surfaces

Because ^{36}Cl-labelled dieldrin is being used for the initial work, contamination hazards limit the techniques that can be used to produce the residual films of insecticide. Three general methods of forming uniform reproducible deposits were studied.

Sublimation of insecticides from a warm to a cool surface. This gives minute super-cooled globules that sometimes take several days under the conditions of test calythrone is likely to be less toxic than valone.

(I) 

(II) $MeO_2C \cdot \overset{t}{C}Me{=}CMe \cdot CO \cdot CH_2 \cdot CO \cdot CH_2 \cdot CHMe_2$. (Elliott, Jeffs and Needham.)

Chemistry and biological activity of the separate active constituents of pyrethrum flowers. The work done in collaboration with the Tropical Products Institute and the Statistics Department, on the separation and reconstitution of the four known active principles of pyrethrum flowers, and on their relative biological effectiveness to houseflies, alone and in combination with piperonyl butoxide, was prepared for publication. (Sawicki, Dr. E. M. Thain,*, Elliott, Gower and Mr. M. Snarey*.)

Effect of esterase inhibition on the insecticidal activity of pyrethrum extract. If the pyrethrins are hydrolysed by the esterases in insects their toxicity might be lessened. To test this possibility the toxicity by topical application of a 1 : 1 w/w mixture of the pyrethrins with an esterase inhibitor (diazinon) was compared with that of the two insecticides applied separately. No synergism was found, hence esterase activity probably has no effect on the insecticidal action of the pyrethrins. (Sawicki.)

Pick-up and distribution of dieldrin from residual films

The mechanism of poisoning of insects by residual films was further studied using ^{14}C-labelled dieldrin instead of the ^{36}Cl material. The ^{14}C-labelled dieldrin, kindly provided by Shell Chemicals, enabled measurements to be made of the pick-up, distribution and penetration of insecticide on individual insects. The dose acquired by individual insects could be determined very exactly, so the relation between dose and susceptibility could be determined reliably.

Adult *Tenebrio molitor* were exposed at 20° for either 30 or 45 minutes to films of ^{14}C-labelled dieldrin on glass (1·5 γ/sq. cm.), removed to separate clean glass containers at 20° for up to 11 days, and then stored at −18° until analysed for radioactivity by scintillation counting. The poison was assumed not to move at −18°. During the storage period at 20° the insects were periodically classified as " alive " (alive and slightly affected) or " dead " (moribund and dead). Radioactivity was assayed on whole single insects or on dissected parts of them. The amount of dieldrin lost from the insects to their containers was also estimated.

About half of the insects, exposed for 30 minutes, died by the 11th day, which is probably near the end-point. The amounts of dieldrin picked up by individual insects ranged from 0·04 to 0·20 γ/insect, with a mean of 0·113 γ. The amounts outside and inside the bodies of 148 insects, and lost to their containers, followed distribution curves, which were skewed towards the higher values. The mean of the amounts on and in insects still alive after the 11th

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https://doi.org/10.23637/ERADOC-1-94