ROTHAMSTED RESEARCH

Report for 1957

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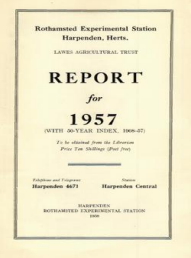

Insecticides and Fungicides Department

C. Potter

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INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

During the current year Miss Janet Samuel left the department and K. A. Jeffs has been appointed in her place. A. J. Arnold has been seconded to the West African Cocoa Research Institute for 3 years. M. Das was awarded the Ph.D. degree of the University of London and has returned to India. R. A. Harrison completed a stay of 6 months and returned to New Zealand. H. J. Smith, who had just joined the Pyrethrum Board of Kenya, spent approximately 4 months in the department with M. Elliott, being trained in the chemistry of the pyrethrins. E. Mastrandreou arrived from Greece to work in the department.

M. Elliott attended the 16th International Congress of Pure and Applied Chemistry in Paris, and A. H. McIntosh, P. H. Needham and C. Potter attended the 4th International Congress of Plant Protection in Hamburg. After the Congress A. H. McIntosh and C. Potter visited a number of laboratories in Germany and Holland.

In collaboration with the Entomology Department a course on insecticides and applied entomology was given on behalf of the British Council. Representatives from Denmark, France, Germany, Italy, Lebanon, the Netherlands, Portugal and Sweden attended the course.

Two of the new constant-temperature–constant-humidity rooms came into operation during the year and have proved very useful. There are difficulties in operating the other two, and work has not yet begun on the constant-environment plant-growing rooms.

The new glasshouse accommodation has nearly been completed.

INSECTICIDES

Time, temperature and toxicity of insecticides

M. Das, under the supervision of A. H. McIntosh and with his help, has finished his work on this subject.

More results have been obtained by the general method, which is described in *Rep. Rothamst. exp. Sta. for* **1956**.

With toxaphene on *Tribolium castaneum*, the temperature coefficient between 10° and 28° C. was $> +12$ on the first day after treatment; it decreased to zero on the 9th day, and to $-3{\cdot}9$ on the 24th day after treatment. This is similar to the previous results with toxaphene on *Tenebrio molitor*.

With " Valone " on *Musca domestica*, the temperature coefficient between 10° and 28° C. was $> +2{\cdot}6$ two hours after treatment; it had decreased to zero by the 12th hour, and to $-1{\cdot}6$ on the 5th day after treatment. This is similar to the previous results with *T. castaneum*.

With α-chlordane on *M. domestica*, the temperature coefficient

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between 10° and 28° C. was +6·1 on the first day after treatment; it decreased to zero on the 3rd day after treatment, but did not become negative, as in the previous tests with *T. castaneum* and *T. molitor*.

With "Dimetan" on *M. domestica*, the temperature coefficient between 10° and 28° C. was —1·6 on the first day after treatment. After this it remained unchanged, even on the 5th day. This is similar to the previous results with *Oryzaephilus surinamensis*.

The negative coefficients previously found for DDT on various species in the range above 10° C. were either constant in size (*T. castaneum* and *M. domestica*), or increased in size as time passed (*T. molitor* and *O. surinamensis*). The positive temperature coefficient found for DDT on *T. molitor* in the range below 6° C. has been found to change in size as time passes. Thus, the temperature coefficient between 2° and 6° C. was +45 on the 12th day after treatment, +3·8 on the 16th day and +1·5 on the 20th day. It has not been possible to find a positive temperature coefficient for DDT on other species; *T. castaneum*, *O. surinamensis* and *M. domestica* are all much more sensitive to cold than *T. molitor*.

In considering the results given above, and in last year's report, it may be said by way of summary that when single measured doses are applied in contact tests there is a general trend: the temperature coefficients are either negative or change, as time passes, in the direction positive to negative. This applies to tests of 2-bromomercurithiophen, α-chlordane, DDT, "Dimetan", toxaphene and "Valone", tested on adults of *T. castaneum*, *O. surinamensis*, *T. molitor* and *M. domestica*. It does not apply to tests with rotenone. The temperature coefficient of any one poison on any one species seldom has any single value. In many cases it is not even enough to say that the temperature coefficient is "positive" or "negative". The effect of time on temperature coefficient must be specified. If the time between treatment and counts of kill is long enough, the temperature coefficient may reach a steady value. This may be said to be the true temperature coefficient of the poison, and should be used when making comparisons between poisons.

Tests with DDT as a mosquito larvicide

Some tests have been made to find the temperature coefficient of DDT as a mosquito larvicide. Such tests have, in the past, usually been made by immersing the larvae in suspensions of DDT for the duration of the test. Temperature affects the activity of the larvae and causes them to take up more DDT at a higher temperature. This favours a positive temperature coefficient.

However, Das's tests have shown that if the larvae are immersed in the suspensions for a short time at 15° C. and are then removed to clean water at 15° and 28° C., a large negative temperature coefficient is obtained. As with other species, the effect of DDT on mosquito larvae is to some extent reversible. Larvae which are badly affected by treatment at 15° C. recover to some extent if placed in clean water at 28° C., but not at 15° C.

The results of these tests are in line with those in which single measured doses are applied to non-aquatic insects.

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Effect of drugs on action of DDT

Sublethal doses of various nerve-stimulant and nerve-depressant drugs were injected into adult *T. molitor*, either before or after topical application of DDT. None of the treatments had any effect on the course of poisoning by DDT, nor on its temperature coefficient in the range 10–28° C.

The isolation and properties of insect esterases

K. A. Lord has continued his work on this subject. The acetyl choline hydrolysing enzyme obtained from *Blatella germanica* has been further purified by fractional precipitation with acetone in the cold, and an examination of its properties begun.

Although the preparation hydrolyses phenyl acetate to some extent, heat inactivation and mixed substrate experiments indicate that it is the cholinesterase which is responsible and not a separate enzyme.

The cholinesterase from the German cockroach is qualitatively similar to that obtained from houseflies and bees and the so-called true mammalian cholinesterase. It hydrolyses acetyl choline, propinyl choline, acetyl β-methyl choline but not butyryl choline or benzoyl choline. Further it is inhibited by high concentrations of choline-esters. Activity substrate concentration curves using both the Warburg and electrometric methods of titration resemble those obtained with human and housefly cholinesterase.

Determinations in the Warburg did not indicate any considerable activation by 0·33*M*-NaCl or 0·033*M*-$MgCl_2$ but rather a shift in the position of the curves to higher acetyl choline concentrations. In the presence of 0·33*M*-NaCl there was an indication of an optimum substrate concentration at $10^{-3}M$-AcClCl, but in the absence of added NaCl maximum activity was displayed at substrate concentrations ($10^{-4}M$) when determinations became unreliable. Using the electrometric method in the absence of salts, the cockroach cholinesterase showed maximum activity at substrate concentrations so low ($10^{-5}M$) that reliable readings were not possible.

It is estimated that the cockroach cholinesterase has an affinity for acetyl choline about ten times that of housefly cholinesterase.

Esterases in relation to the development of eggs of Pieris brassicae *and* Gryllus domesticus *and the toxicity of organophosphorus compounds*

Following up the work reported last year on the toxicity of TEPP to eggs of *Pieris brassicae*, C. Potter, K. A. Lord, D. V. Holbrook, S. R. B. Solly and F. Molloy have carried out more extensive investigations on the properties of the esterases of insect eggs, and the toxicity of further phosphorus insecticides has been examined under a range of conditions. Information on the ratio between the amount of poison which will allow development but will not allow hatching and that which will prevent development and on the changes of resistance as development proceeds has been sought, since when it is correlated with the physicochemical properties of the poisons it may give an indication of the esterases involved in the poisoning.

A simpler technique of applying poisons to the eggs has been evolved as a result of finding that the wetter Tween 20 was non-

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toxic to *P. brassicae* eggs. The eggs are simply left standing in a solution of the poison in 0·1 per cent Tween for 5 minutes.

This method has been used to determine the toxicity of "Dipterex" and paraoxon to newly laid eggs. Both of these compounds, like TEPP and many other insecticides, may allow the embryo to develop and yet prevent the egg from hatching. However, the ratio of the dose to prevent visible development to that which just stops hatching is very much greater in the case of "Dipterex" and paraoxon (>100 ×) than in the case of TEPP (<30 ×). The dose of paraoxon which prevents development is, however, less than that required for TEPP. It has not been determined for "Dipterex" owing to lack of solubility.

"Dipterex" and paraoxon have also been applied at various stages of development and the concentrations determined which prevent hatching. These show only small changes (2–4 ×). Perhaps it is significant that the eggs were most susceptible half-way between laying and hatching. This contrasts with the susceptibility to TEPP, which increases slowly until the egg is half-way through development, when it increases more rapidly. The overall change is about thirty times.

Again, with a view to finding out whether the reaction responsible for death may occur at an early stage of development, we have attempted to examine the penetration and reaction of organophosphorus insecticides by treating newly laid *P. brassicae* eggs with lethal amounts of paraoxon and then washing with water or a cholinesterase reactivator. Diacetyl monoxime (DAM) was chosen because of its low toxicity to eggs. Washing the eggs soon after poisoning results in a large reduction in toxicity. Our results indicate that the bulk of the poison is no longer readily removed by washing after the first 10–20 hours. Up to 3 days at 15° C. washing with water causes the same reduction in toxicity as a DAM solution. Subsequently, however, the effect of washing with water slowly decreases, whilst the DAM has about the same effect throughout.

Further observations on esterases hydrolysing acetyl choline and phenyl acetate during the development of *Pieris brassicae* have been made, and the investigations have been extended to include the hydrolysis of triacetin. The results confirm those previously reported and show that enzymes hydrolysing triacetin are present throughout all stages of development.

Although work with crude breis may be misleading, it has been considered worthwhile to make some rather more extensive studies on the hydrolysis of triacetin, phenyl acetate and acetyl choline at two selected stages, soon after the eggs are laid and shortly before hatching (0–12 hours and 5 days old at 20° C.).

We have investigated the effects of salts (sodium chloride, magnesium chloride, calcium chloride), substrate concentration and pH. These results, together with those obtained from development studies, lead us to believe, that in the main, different enzymes are responsible for the hydrolysis of each of the three substrates. It also seems likely that the enzymes responsible for the hydrolysis of both phenyl acetate and triacetin change as development of the egg proceeds. These impressions are supported by inhibition data obtained using three insecticides, TEPP, paraoxon and "Dip-

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terex ". The three poisons behave in qualitatively similar ways except for certain anomalies which may be explained on the basis of their differing chemistry.

The three compounds inhibit cholinesterase non-competitively, and the extent of the inhibition increases with the time elapsing before the addition of substrate. The same phenomenon has been observed with the inhibition of the phenyl acetate hydrolysing enzyme present in the young eggs, but in the older eggs the inhibition of this esterase appears to be competitive and independent of time. The enzymes hydrolysing triacetin are much less susceptible to inhibition than the others.

Similar experiments have been commenced with *Gryllus domesticus* eggs, but have not progressed so far owing to lack of adequate supplies of eggs.

In addition to the biochemical studies on the effect of organophosphorus compounds on insect eggs, histochemical techniques have been investigated for the localization of cholinesterase in insect eggs and the determination of the effect of treatment of the eggs with organophosphorus compounds on cholinesterase *in situ*. Miss Molloy has been chiefly concerned in this work.

Biochemical methods of studying *in vivo* enzyme inhibition suffer from certain disadvantages owing to the mixing of tissues, so that it is uncertain whether the inhibition measured is occurring *in vivo* or *in vitro*. Histochemical methods do not give rise to the same degree of cross-contamination.

Histochemical methods of demonstrating cholinesterase activity depend on the production of a coloured compound at the enzyme site. Very precise localization may be obtained, and the method may be used to detect very small amounts of the enzyme.

When the techniques were applied to insect material, difficulty was encountered in cutting frozen sections. Some material, however, was kindly freeze-dried and embedded by Dr. Pratt at the Maudsley Hospital, and it has proved possible to cut satisfactory sections of this.

Preliminary work was carried out on mouse muscle tissue, so as to gain experience of the staining procedures and frozen-section cutting. Sharply localized staining of the sites of cholinesterase activity was obtained by a simplification of the technique of Koelle (*J. Pharmacol.* 1957, **103**, 153).

In the application of this method to *Pieris brassicae* L. eggs, these were not sectioned, but the embryos were incubated whole, after the removal of the choria, vitelline membranes and residual yolk.

The younger embryos (0–5 days old) were very fragile. The best staining results were obtained with embryos 6–7 days old. The staining is apparently entirely confined to the central nervous system, and is often noticeably darker in the " inner core " or " neuropile " of the ventral nerve cord. Staining of older embryos (7–9 days) is patchy, and is often associated with damage, suggesting increasing impermeability of the cuticle and the nerve sheath.

Pre-treatment of the embryos with trypsin improves the staining. Further enzymes are in the course of investigation in this respect.

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General, diffuse staining, indicating the presence of non-specific esterases, has been obtained in whole embryos of *P. brassicae* after incubation with indoxyl esters.

Preliminary experiments on the eggs of *P. brassicae* treated with TEPP at 0 days old indicate that some inhibition of acetylcholinesterase or a precursor had taken place.

Toxicity of organophosphorus compounds to various strains of Musca domestica

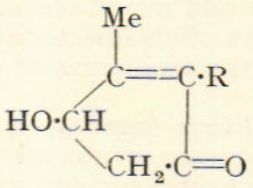
K. A. Lord, D. V. Holbrook and T. Doherty have worked on this subject. In view of the lack of resistance of the strains of housefly (79 and 150) supplied by Dr. Keiding reported last year, an exchange of material was arranged with Dr. Keiding. The resistance of the adults of the strains in both countries appear to have lost the resistance which was originally reported.

The attempt to breed resistant houseflies by incorporating paraoxon in the larval medium has been abandoned owing to breeding difficulties which were encountered with the selected flies. In connection with this a simple larval dipping test was evolved. This test has been applied to the larvae of the various strains of flies in the department.

No great differences were noted in the average level of resistance of the larvae from the different strains. It was, however, noted that whilst the larvae from the strain supplied by Dr. Busvine and strain 150 supplied by Dr. Keiding were all killed by 0·003 per cent paraoxon, 20–30 per cent of the larvae from the normal strain and 79 strain survived concentrations ranging from 0·003 to 0·1 per cent paraoxon. 0·001 per cent paraoxon had no effect on the larvae from any of the strains tested.

Pyrethrins and related compounds

M. Elliott has studied some of the properties and reactions of pyrethrolone (I), the alcoholic constituent in pyrethrins I and II, which supply most of the insecticidal activity in preparations from *Chrysanthemum cinerariaefolium* flowers.



(I) R, $CH_2CH\overset{c}{=}CH\cdot CH=CH_2$

(II) R, $CH_2CH\overset{c}{=}CH\cdot Me$

(III) R, $CH=CH\cdot CH=CH\cdot Me$

(*a*) Previous workers have obtained a mixture of pyrethrolone (I) and cinerolone (II) by shaking the semicarbazones for a long time with aqueous potassium hydrogen sulphate. The two closely boiling alcohols were separated by distillation of their acetates, followed by hydrolysis or, less satisfactorily, by fractionation of the free alcohols. In both these methods pyrethrolone, which is an unstable compound, rapidly decomposed in air and light, was obtained in an uncertain state of purity. Quantities of pure, naturally derived pyrethrolone were required for making relatively large amounts of pyrethrins I and II, of which sufficient were not available by synthesis or by chromatographic separation.

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It has been found that pyrethrolone (I) and cinerolone (II) may be regenerated from their semicarbazones in 24 hours with dilute sulphuric acid, rather than by shaking for 6–18 weeks with potassium hydrogen sulphate solution. Moreover, both keto-alcohols form monohydrates. That from (+)-pyrethrolone is crystalline and may be purified by low-temperature recrystallization from ether. The hydrate is apparently stable for some days at room temperature and indefinitely at −20°. It has therefore been possible to determine the physical (including infra-red and ultra-violet spectra) and chemical properties of the keto-alcohol more precisely than before.

Pure (+)-pyrethrolone (from the monohydrate by removal of water *in vacuo*) has been esterified with naturally derived, purified, chrysanthemic and pyrethric acids, to provide supplies of pure pyrethrins I and II limited only by the amount of extract available for processing. These esters have been characterized by their physical (including absorption spectra and optical rotations), chemical and biological properties (toxicities to adult *Musca domestica* [by R. Sawicki] and to *Phaedon cochleariae* [by P. H. Needham]). In all cases the reconstituted esters have been found identical with fractions isolated from the natural material by displacement chromatography on alumina.* Although pyrethrins I and II have been reconstituted before, the methods used for isolating the keto-alcoholic fraction were more likely to produce molecular changes than those described here, and they have not previously been compared with esters obtained from natural sources under conditions unlikely to have produced any change in their constitution.

Using the reconstituted esters, a study of their decomposition in air and light has been started. Preliminary results indicate that, under these conditions, pyrethrin I ($C_{21}H_{28}O_3$) sets to a glass of composition $C_{21}H_{28}O_5$.

(*b*) Brown, Phipers and co-workers † showed that compounds of unknown constitution ($\lambda_{max.}$ 270 mμ) were formed when pyrethrum extract ($\lambda_{max.}$ 230 mμ) was heated to 200°. Through the kindness of Dr. R. F. Phipers and Mr. N. C. Brown, of the Cooper Technical Bureau, Berkhamsted, this phenomenon has been investigated in this department. In agreement with the prediction of these authors, it has been found to involve migration of the double bonds in the side chain of the pyrethrolone constituent of the esters into conjugation with the double bond in the *cyclo*pentenone ring (III). Development of a small-scale technique for following the reaction spectrophotometrically enabled it to be proved that only pyrethrolone and its derivatives produced the characteristic triple absorption maximum at 260, 270 and 280 mμ. The infra-red spectrum of the heated mixture of natural pyrethrins showed that the terminal methylene group frequency had disappeared. Thermally isomerized pyrethrolone methyl ether ($\lambda_{max.}$ 260, 270 and 280 mμ; $\lambda_{min.}$ 215 mμ) still contained a keto group (semicarbazone and 2 : 4-dinitrohydrazone) in a five-membered ring (infra-red spectrum); hydrogenation under mild conditions indicated two double bonds, and acetaldehyde was obtained by ozonolysis. Since (+)-pyrethrolone on isomerization

* Ward, J. (1953). *Chem. & Ind.*, 586.

† Brown, N. C., Hollingshead, D. T., Phipers, R. F. & Wood, M. C. (1957). *Pyrethrum Post*, **4**, No. 2, 13.

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gave (—)-"*iso*pyrethrolone", double-bond migration to the optically active centre at C_4 cannot have taken place. Structure (III) is therefore proposed for the parent alcohol of the thermally isomerized compounds; the stereochemistry is at present unknown. It is the first example of a cross-conjugated ketone of this type (*Rep. Rothamst. exp. Sta. for* **1956**, p. 131); the carbonyl group appears to depress the intensity and not to affect the position of maximum absorption of the triene system. A study of the spectroscopic properties of these compounds, and of their derivatives, has been made.

Biological evidence obtained so far indicates that the ester of chrysanthemic acid with pyrethrolone (I) is 16 times as toxic as that from "*iso*pyrethrolone" (III) to adult *Phaedon cochleariae*.

R. Sawicki and P. H. Needham have carried out biological measurements of the activity of the isolated insecticidal constituents of pyrethrum flowers.

In the absence of a more suitable standard, a pyrethrum extract, which contained pyrethrin I and cinerin I, 13·1 per cent; pyrethrin II and cinerin II, 10·9 per cent: total pyrethrum 24·0 w/v. [P.B.K. modified Seil's method of analysis] was used. The extract served as a constant with which to compare the biological activities of the separated constituents. It was unsatisfactory because the activities relative to this standard depended on the proportions of active constituents in it, and tests against another extract with different proportions of constituents are likely to have given different results. Another disadvantage of using a pyrethrum extract as a standard is that the various methods of analysis of pyrethrum give different results, so that the numerical ratios expressing the relative potencies depend on the method of analysis used.

A comparison between the concentration of active constituent under examination and the concentration of total active constituents of the extract to produce the same biological response was made.

Two test insects were used, adult houseflies (*Musca domestica* L.) by R. Sawicki and adult mustard beetles (*Phaedon cochleariae* F.) by P. H. Needham. With houseflies insufficient knowledge of the influence of the age of the test insect and of the length of the period between treatment and inspection on both the absolute and relative figures for knockdown or paralysis and toxicity has handicapped the work. Considerable information on both these points has been obtained, and is described in the section on bioassay, where the full technique is given.

(*a*) Tests with adult houseflies *Musca domestica* L.:

(1) *Pyrethrin I.* Preliminary determinations were made using small quantities of pyrethrin I obtained by Dr. Spickett of the Tropical Products Institute from the natural material using the chromatographic method of Ward. The main work was carried out on reconstituted material prepared by M. Elliott of this department (see pp. 136–138) after it had been shown that it produced the same biological effects as the material supplied by Dr. Spickett.

Five comparisons were made with after treatment conditions of 20° C. and 80 per cent relative humidity and an inspection time of 48 hours. It is considered that with an inspection

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time of 48 hours the figures indicate relative toxicity rather than relative paralysis or knockdown.

The pyrethrin I was 1·09, 0·87, 0·89, 0·94 and 1·069 times as toxic as the extract.

In tests where the inspection period was 24 hours, i.e., where knockdown was being measured rather than kill, pyrethrin I was found to be 0·72 and 0·69 times as effective as the extract on young flies and 0·93 times as effective on old flies.

(2) *Pyrethrin II.* All the pyrethrins II material was prepared by M. Elliott. Only preliminary figures have been obtained so far. They indicate that under the same conditions as the test with pyrethrin I, pyrethrin II gives a similar kill to pyrethrin I weight for weight. However, in contrast to pyrethrin I, pyrethrin II appears to have a greater knockdown effect than the extract when measured after 24 hours.

(3) *Cinerin I.* The cinerin I used so far was obtained from Dr. Spickett; it is not definitely known how pure this material is. Preliminary experiments under the same conditions as the preceding tests indicated that when measured at the LD50 level, cinerin I was much less toxic than the pyrethrum extract to young flies as shown by a 48-hour inspection.

A comparison after 24 hours during preliminary tests indicated that the knockdown effect was very much less than that of the pyrethrum extract when measured at the LD50 level.

Using young flies, the probit lines for cinerin I were not parallel with those of the extract. The extract gave steeper slopes. In these preliminary experiments with younger flies the insects treated with cinerin I showed little or no recovery from initial knockdown, and the kill increased with time; this is in contrast with pyrethrin I, pyrethrin II and the extract, where considerable recovery from initial paralysis occurred.

(*b*) Tests with adult mustard beetle *Phaedon cochleariae*:

A topical application method was used with this species also; the following were the conditions of test.

A 0·001-ml. drop of an acetone solution was applied to the ventral surface of the thorax of each beetle. Two replicates of twenty-five beetles were treated at each concentration of the poisons used. After treatment storage was in " petri " dishes, with glass lids, at a temperature of 20° C. and 55 per cent relative humidity. The beetles were not fed after treatment. Mortality counts were made 48 hours after treatment. At this time mortality and not knockdown was being estimated.

The reconstituted pyrethrin I prepared by M. Elliott was found to have exactly the same toxicity to mustard beetles as the chromatographed material from Dr. Spickett, the probit lines being parallel and the LD50 under the conditions of the experiment being 0·0008 per cent w/v.

The pyrethrin I prepared by M. Elliott was used for the subsequent tests.

In three comparisons where the data were satisfactory, pyrethrin I was found to be from approximately twice, to approximately three times, as toxic as the pyrethrum extract.

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In two tests pyrethrin I was found to be approximately three times as toxic as pyrethrin II. The exact figures for these tests are given in Table 1:

TABLE 1

	Pyrethrin I		Pyrethrin II		Extract	
Date	LD50, % w/v	Relative potency	LD50, % w/v	Relative potency	LD50, % w/v	Relative potency
15.10.57	0·0007	100	—	—	0·002	35
23.20.57	0·0007	100	—	—	0·0014	50
19.11.57	0·0006	100	0·0018	33	0·0010	60
6.11.57	0·0004	100	0·0015	27	—	—

When the tests on the houseflies are compared with those on mustard beetles it appears that the relative as well as the absolute toxicity of the active constituents depend on the test species. Thus pyrethrin I has approximately the same toxicity as both the extract and pyrethrin II to young houseflies, but it is between two and three times as toxic as the extract and about three times as toxic as pyrethrin II to mustard beetles. The importance of using more than one test species before coming to any general conclusions is here emphasized.

(*c*) Temperature effects and synergism:

R. Sawicki has worked on this subject. A considerable amount of work was done before the factors in the technique that affect the results were worked out sufficiently to give reliable figures.

The object of the present study was to determine how temperature affects synergized pyrethrum extracts in comparison with extracts that have no synergist. At 25° C. it appears that with the extract alone there is a considerable recovery after 48 hours from the knockdown recorded after 24 hours; in the presence of synergist there is little recovery from the initial knockdown.

It also appears that for the extract alone the temperature coefficient of knockdown may be greater than the temperature coefficient of kill.

The time taken for the completion of the knockdown effect and for the completion of the toxic effect (end point) varies with the temperature of after treatment, and the correct inspection times for the different temperatures must be used.

Toxicity and persistence of insecticidal deposits

Effect of additives on DDT emulsions. J. Ward has worked on this subject. The rate of evaporation of DDT from deposits on glass plates was measured using the ventilated cabinet described in *Rep. Rothamst. exp. Sta. for* **1956**.

The evaporation from deposits of various types, produced by spraying plates with emulsions containing resinous or oily additives, was compared in this way. No large differences in rate of evaporation were found. In particular, formulation of DDT with Arochlor resin did not reduce its rate of evaporation, though Arochlor is reported to reduce the rate of evaporation of BHC. It is intended

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to use the apparatus to study the evaporation of insecticides other than DDT.

Much of the work on insecticidal deposits was devoted to the study of bioassay methods for the determination of toxicity and persistence which is described in the next section.

Bioassay Techniques

Bioassay of contact toxicity of insecticide deposits. (1) Adult *Tribolium castaneum.* Efforts by J. Ward and E. M. Gillham to trace the causes of the unreproducible results obtained when deposits of DDT on glass plates are tested for contact activity, using *Tribolium castaneum* (Herbst), have continued. In experiments in which the insects have been confined on the deposit singly instead of in groups of ten, the variability of the results was much reduced. This may have been because a larger area of the deposit was included in a single sample, and therefore irregularities in the deposit had less effect on the result, or it may have been because the variability is connected with an effect of the insects on one another when exposed in groups. More reproducible results were obtained when deposits of liquid droplets were used, instead of crystals; this indicates that the variability is at least in part due to irregularities in the nature of the deposit. A very large amount of data has now been collected, and work with *T. castaneum* has been suspended until the results have been examined statistically.

(2) Adult *Musca domestica.* The method of assessment mentioned in the last Annual Report, in which flies are confined between two glass plates so spaced that the flies are forced to crawl on the one bearing the deposit, has several defects. In particular, the flies are held on the deposit in an unnatural attitude, and the method is not applicable to deposits on irregular surfaces. Alternative methods of persuading the flies to remain on the surface under test are being examined; of these, the most promising involves the use of a temperature gradient.

Bioassay of contact toxicity of pyrethrum type molecules with adult Musca domestica

For the purpose of his work on the insecticidal action of the constituents of pyrethrum flowers R. Sawicki adopted a topical application method with adult houseflies (*Musca domestica*) as test subject. He has studied some of the factors on the technique influencing the results. The technique finally elaborated is as follows. Female flies only are used for the tests; the insects were sorted out 24 hours before dosing in a cooling cabinet, and were kept in Petri dishes (fifteen flies per dish) which were covered with terylene gauze. The flies were preconditioned for 24 hours at the temperature and humidity at which the experiment was going to be carried out. During that period they were given a paste made of dried milk with water and a sugar solution. The flies were then knocked down by cooling for a very short period during dosing, and a measured drop (1 microlitre) of Analar acetone containing the substance dissolved, was then applied to the thoracic sternum of each insect. Food was withdrawn at the time of dosing, and no food was given during the host treatment period. The insects were replaced into the cabinet in

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which they had spent the preadaptation period, and were subsequently inspected 24 and 48 hours after dosing. Sometimes when mortality in controls did not exceed 10 per cent, inspections were carried out on the third day after dosing.

In order to find not only the knockdown power of the substances investigated, but also the final kill, it was necessary to keep the flies alive for as long as possible. This was not very easy, because in order to prevent complications the insects had to be kept without food during the experiment. Flies usually do not live very long at high temperatures when starved, and it was found that they had to be fed after sorting. Flies would not survive 24 hours at 25° C. if they were not fed after having been in the cold cabinet for periods of up to 4 hours. It was therefore decided to supply the insects with food during the preadaptation period and remove it at the time of dosing.

Apart from those standardized in the technique as described above, two factors were found to have a marked influence on the results. The first was the inspection time, and the second was the age of the fly. If short inspection times were given, knockdown was measured and not kill. The relation between knockdown and kill varied with the chemical under investigation. If the inspection time increased sufficiently to give a reliable record of final kill the flies are liable to die naturally. The addition of food during the after-treatment period would prolong the life, of the flies, but would provide a considerable added complication in assessing the results. After investigation it was found that the life of the flies without food could be prolonged by giving the shortest possible exposure to cold in the sorting cabinet and by keeping the flies after treatment at a high humidity.

For a time it was believed that young flies 1–2 days old would have a longer post-treatment life, but this does not appear to be necessarily true, and it seems that older flies may be preferable because, for a given degree of standardization, their resistance is less variable.

Using pyrethrum extract with after treatment conditions of 20° C. and 80 per cent relative humidity and an inspection period of 48 hours, 1-day-old flies were 1·68 times as resistant as 2–3-day-old flies and 2·45 times as resistant as 3–4-day-old flies. At present the age of the flies that it is best to use has not been finally decided.

Detection and identification of toxic substances in poisoned bees

P. H. Needham and J. Ward collaborated in this work. During the summer, ten samples of poisoned bees were received from the National Agricultural Advisory Service for testing for the presence of insecticide. Of these, three were found to contain benzene hexachloride, two contained an organophosphorus type of insecticide, one contained insecticidal material which could not be identified, and in four samples no insecticidal material could be found. Four of the samples, all despatched between 12 June and 5 July, were thought by the beekeepers to have been poisoned by organophosphorus insecticides.

Some improvements in the methods of detection and identification were made so that they were less laborious than those used last

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year. The insecticides were separated from the bulk of the lipoid material in the bee-extracts by evaporating the extracts to dryness in the presence of a coarse grade of Celite, so as to deposit the involatile matter in a thin film on the grains. The Celite was then washed on a porous glass filter with increasing strengths of aqueous alcohol or acetone. All insecticides could be washed out in this way without removing much of the waxy material. The presence of organophosphorus insecticides was shown by a spot-test in which the insecticide, after suitable chemical treatment where necessary, inhibited esterase applied to filter-paper. Unaffected esterase was then made visible by a colour reaction. The problem of further identification of the organophosphorus compounds by their physical and chemical properties is difficult, because many of them are known to be metabolized by the insect into various esterase-inhibiting compounds.

Bioassay tests were used as a general method of detection of the presence of insecticidal material. The mosquitoes necessary for this work (*Aëdes aegypti*) are now being reared in the department, and a continuous supply of larvae is available.

As was mentioned last year, a more satisfactory method has been adopted for conducting the bioassay tests on the extracts from the bees. The technique is a modification of that described by Burchfield *et al.* (*Contr. Boyce Thompson Inst.* **17** (1), 57–86). This makes use of the inhibition of the negative phototaxis of the larvae by poisons in order to sort the affected from the normal individuals. The method is very sensitive, but difficulty is being experienced from the presence in the extracts of toxic substances other than those we are trying to detect. It is thought that these are due to toxic impurities present in some of the solvents used in the extraction. These are concentrated when the extract is concentrated by evaporation. The problem is being investigated, and we hope to overcome the difficulty in the near future.

Insect rearing

The following insects were reared in the department during the year:

Plant feeding

HEMIPTERA	*Megoura vicae* Buck.
	Aphis fabae Scop.
	Acyrthosiphon pisum Harris.
LEPIDOPTERA	*Pieris brassicae* L.
	Diataraxia oleracea L.
COLEOPTERA	*Phaedon cochleariae* F.
DIPTERA	*Leptohylemyia coarctata* Fall.

Stored product, domestic and medical

ORTHOPTERA	*Blatella germanica* L.
	Blatta orientalis L.
	Periplaneta americana L.
	Gryllus domesticus L.
LEPIDOPTERA	*Achroia grisella* Fabr.

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COLEOPTERA	*Oryzaephilus surinamensis* L.
	Oryzaephilus mercator Fouv.
	Tribolium castaneum Hbst.
	Tribolium confusum Duval.
	Tenebrio molitor L.
	Trogoderma granarium Everts.
DIPTERA	*Aëdes aegypti* L.
	Drosophila melanogaster Meig.
	Musca domestica L.

The rearing of cultures of the following insects has been discontinued: *Actias selene* Hubner; *Ephestia kühniella* Zell.; *Calandra granaria* L.; *Dysdercus fasciatus* Sign.

Diapause in Leptohylemyia coarctata *Fall.*

Experiments described in *Rep. Rothamst. exp. Sta. for* **1956** were repeated by M. J. Way, and it was confirmed that diapause in its later stages is terminated rapidly by exposing the eggs to −20° to −24° C. Such low temperatures have no effect during the first 4–8 weeks of diapause. This and other evidence suggest that the very-low-temperature treatment releases a growth hormone which has accumulated during the earlier stages of diapause.

Eggs laid in August 1956 were kept first at 20° C. and then at −2° C. until diapause was complete. Batches were then kept at −2°, −6° and −18° C. Eggs removed from all temperatures to 20° C. hatched normally in May 1957, but subsequently, viability decreased except at −6° C. At this temperature over 90 per cent of eggs hatched in October 1957, 13 months after they were laid. The young larvae were able to attack wheat and apparently develop normally. Thus it is possible to keep eggs viable until those from the next generation can be induced to hatch by the low-temperature treatment.

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

R. Bardner continued to carry out box and field experiments on insecticidal control of wheat-bulb fly.

(*a*) *Box experiments on insecticidal control*

The search for new materials to control wheat-bulb fly was extended to include fifteen more insecticides (barium silicofluoride, calcium arsenate, chlordane, fluoracetanilide, lead arsenate, "Lethane", malathion, mercurous chloride, methoxychlor, paraoxon, "Pyrolan", sodium fluoracetate, "Thimet", toxaphene, zinc fluorarsenate). These were formulated as seed dressings and compared with seed dressings of dieldrin and heptachlor. The technique used for assessing the protection afforded by a dressing against wheat-bulb-fly attack has been described in previous annual reports. As in 1956, no insecticide was significantly better than dieldrin or heptachlor, but chlordane, which contains some heptachlor, was comparable.

A paper on seed dressings for wheat-bulb-fly control has been submitted for publication. The work is continuing.

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(*b*) *Field experiments on insecticidal control*

(1) *Pennells Piece.* The experiment on Pennells Piece was continued. Various formulations of aldrin and dieldrin were tested, the materials being applied either as dusts on the soil surface or combine-drilled with the seed. Unfortunately the numbers of larvae present were very small, only 7 per cent of the tillers being damaged on the untreated plots. Nearly all the measurements made, including yields of grain, failed to show any significant differences, but Table 2 and other results are consistent in indicating that 4 per cent dieldrin dust at 1 cwt./acre broadcast on the soil surface in December gives control equal to or better than a similar quantity of dieldrin combine-drilled with the seed.

TABLE 2

Effect of soil insecticides on wheat-bulb-fly damage

Treatment	* Damaged Tillers (in thousands per acre) on 14 April 1957	† Yield at harvest in cwt./acre (85% dry matter)
Untreated	17·3	50·0
4% dieldrin dust broadcast at 1 cwt./acre 18 December 1956	1·8	54·8
4% dieldrin dust broadcast at 1 cwt./acre 16 February 1957	5·0	50·2
4% dieldrin dust combine-drilled with seed 5 November 1956 at 1 cwt./acre ...	6·8	52·1
1·5% dieldrin dust combine-drilled with seed 5 November 1956 at 1 cwt./acre ...	7·5	54·1
1·5% aldrin dust combine-drilled with seed 5 November 1956 at 1 cwt./acre ...	6·3	51·9
1·2% dieldrin granules combine-drilled with seed 5 November 1956 at 1·5 cwt./acre...	8·8	50·0

* Least significant difference between means (5% level) = 6·9.
† No significant difference.

(2) *Broadbalk Sideland.* Though parathion sprays have been moderately successful at Rothamsted, other workers using differing formulations have found this method of control very unsatisfactory. In an experiment on Broadbalk sideland, seven different spray treatments were compared.

A standard parathion formulation was applied to separate sets of plots on three different dates. On one of these dates a formulation containing " Thimet " and three other parathion formulations were also applied to other plots.

The percentage of attacked tillers was rather higher than in the Pennells Piece experiment, the unsprayed plots having up to 22 per cent attacked tillers, but only two measurements of samples showed any significant differences. Yields were not taken.

Table 3 shows that the only treatments which showed a significant reduction in the numbers of living larvae compared with the untreated plots were the standard parathion spray applied on the earliest date and two other parathion formulations, one of which contained a high percentage of wetter and another which contained a sticker to give better leaf-adhesion. The latter also had a significantly larger number of undamaged tillers than the untreated plots.

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Wheat leaves are very difficult to wet, and it is probable that the effect of these two formulations was to increase the parathion deposit on the leaves, by better wetting or greater adhesion. The formulation with a high proportion of wetter was used in the previous year's experiments on Pennells Piece.

TABLE 3

Control of wheat-bulb fly by organophosphorus sprays

Treatment	Undamaged tillers (17 April 1957)	Increase	Live larvae per sample (square root transformation) 28 March 1957	Decrease
Unsprayed	1254·3	—	4·0	—
0·1% "Thimet" (w/v) applied as 0·24% emulsion of liquid containing 47·5% Thimet (sprayed 15 March 1957) ...	1284·2	+ 29·9	3·3	0·7
0·1% parathion + 0·18% xylene + 0·4% wetter (sprayed 27 February 1957)...	1305·7	+ 51·4	3·0	1·0
Ditto, sprayed 15 March 1957...	1064·8	−189·5	3·4	0·4
Ditto, sprayed 12 April 1957 ...	1606·5	+352·2	—	—
0·1% parathion + 0·26% xylene + 0·014% wetter (sprayed 15 March 1957) ...	1030·2	−224·1	3·5	0·5
0·1% parathion + 0·4% wetter (sprayed 15 March 1957) ...	1740·0	+485·7	2·8	1·2
0·1% parathion + 0·183% xylene + 0·014% wetter + 0·6% methyl cellulose ether (sprayed 15 March 1957) ...	1751·3	+497·0	2·5	1·5

All parathion measurements are by volume. The wetter used was Atlox 3335 (Honeywill Atlas Co. Ltd.).

Least significant difference of means (at 5% level) = 493·6.

Least significant difference of means of untreated and any treatment (at 5% level) = 1·0.

Bean aphid Aphis fabae *Scop.*

Work on the biological, ecological and chemical factors concerned in the infestation and control of *Aphis fabae* Scop. has been carried out by M. J. Way assisted by T. Doherty.

Chemical control of Aphis fabae *Scop. on spring-sown field beans by low- and medium-volume sprays*

In a field trial a comparison was made of five insecticides each applied once (on 13 June) at a fixed rate of active ingredient in 60 gallons (medium volume) and in 10 gallons of water/acre (low volume). Analyses showed that more insecticide was retained on the plant by the low-volume application. Table 4 gives details of aphid populations and crop yields for the different treatments. It can be seen that the efficiency of *A. fabae* control was not affected by the volume sprayed except with malathion, which did significantly better at the medium volume. The systemic insecticide

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treatments stopped the aphid numbers from rising above a maximum of 8 per plant compared with 3,550 per plant for check treatments sprayed with wetter only. The single spraying by a systemic insecticide raised the crop yields from around 4 to 27 cwt./acre.

TABLE 4

Aphid counts and grain yields on plots treated with insecticides at medium and low volumes

Insecticide	Wt. of action ingredient, oz./acre	Peak aphid, numbers/stem Medium volume	Peak aphid, numbers/stem Low volume	Yield, cwt./per acre Medium volume	Yield, cwt./per acre Low volume
Lindane	6	2,817	2,398	4·9	8·0
Malathion	12	119	233	19·3	16·0
Demeton-methyl ...	6	4	3	27·3	26·3
Compound 4741 ...	3	8	5	28·1	27·4
Fluoroacetamide ...	3	1	1	26·7	26·8
Check	—	3,466	3,547	5·3	3·3

Effect of planting date on infestation and control of Aphis fabae *Scop.*

This year's field trial completed the work on this problem. Field beans were planted at three different dates, and for each planting date yields of unsprayed plots were compared with those of plots sprayed once and twice with demeton-methyl (" Metasystox ") at 6 oz. of active ingredient/acre.

It was interesting that the type of aphid population which developed was at the opposite extreme to that of 1955. In 1955 there was a small primary migration in June and numbers on the crop rose to a peak in August; early sown plots were little affected,

TABLE 5

Effect of chemical control of A. fabae *on field beans sown at different dates*

Time of spraying	Peak aphid numbers per stem for different sowing dates: 20 March	30 April	15 May	Grain yields for different sowing dates, cwt./acre: 20 March	30 April	15 May
Untreated	6918·0	575	38·0	0	9·4	12·3
13 June	3	47	40	16·7	15·3	13·8
13 June and 5 July	—	—	—	18·0	16·9	13·5

but untreated late-sown ones were severely damaged. In 1957 there was a big migration in early June, and numbers on the crop rose to a peak in late June and early July. Table 5 shows that unsprayed early sown plots were devastated by *A. fabae* but late-sown plots were little harmed. A single early application of aphicide stopped damage on early sown plots, but was relatively less effective on late-sown ones. Peak numbers were reached on all plots before 5 July; the second spray treatment was therefore applied to a declining population, and it is surprising that it significantly increased the grain yield of plots sown in March and April.

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Natural control in relation to chemical control of Aphis fabae *Scop.*

This work was continued in collaboration with C. J. Banks of the Entomology Department.

Overwintering eggs of *A. fabae* began hatching in mid-February compared with late March in 1956; the warm winter was seemingly responsible. Continuing favourable conditions caused the primary migration from *Euonymus europeus* to the crop host to begin as early as mid-May.

As expected (see *Rep. Rothamst. exp. Sta. for* **1956**, 144), big populations of aphids developed on spring-sown beans in the Harpenden area during June. This was followed by a sharp fall in numbers in early July. Numbers remained low until late August, when populations developed on wild hosts, notably *Chenopodium album*. Many aphids returned to *Euonymus* in September and October, and more eggs were laid than in 1956. It is expected therefore that in the Harpenden area spring-bean crops will be damaged severely by *A. fabae* unless control measures are adopted.

Studies on natural and on artificially established populations of the aphid and its natural enemies were continued on *Euonymus*, beans, sugar beet and *Chenopodium*, using the caging method as in 1956. Sticky traps were also used to estimate the activity and abundance of flying predators throughout the year. The results have not yet been examined in detail; predators were especially abundant from mid-June to July, and the cage experiments showed that they were important in keeping the aphid numbers at a low level during July. This was confirmed by experiments in which attempts were made to keep plots free of predators by hand picking.

In 1957 natural enemies had become abundant and the general aphid population had begun to fall before aphids became well established on their crop hosts. Adult predators attacking the newly established colonies were probably responsible for *A. fabae* populations not developing to damaging numbers on some bean and sugar-beet crops in Eastern England. There was no evidence, however, that where insecticides were used they upset the natural control.

The distribution of high- and low-volume sprays on field beans

As an adjunct to experiments on the control of *Aphis fabae* Scop., some data were collected by R. Bardner on the distribution of spray deposits on field beans, using a colorimetric method for estimating deposits of a water-soluble dye included in the spray mixture. The method is due to Hebblethwaite (1956: *Plant Path.* **2** (3), 93), who used it for examination of spray deposits on potato foliage.

Estimates were made of deposits in the "crown" of the bean plant (unopened leaves and flower bracts) as compared with the rest of the plant (stem and opened leaves) for sprays applied at 10 and 60 gallons/acre. A very-late-sown bean crop was used, which at the time of the experiment was only 10–12 inches high. The plants possessed a well-developed crown, and apart from the length of stem were in a similar stage of growth at the time of spraying to plants in other spraying experiments.

Two spray mixtures were used: (*a*) high volume, containing 2

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per cent Nigrosine 865 (by weight) and 0·5 per cent benzoyl-*p*-oxydiphenyl-polyglycol ether (by volume) as wetter; (*b*) low volume, containing 1 per cent Nigrosine and 0·125 per cent benzoyl-*p*-oxydiphenyl-polyglycol ether.

The sprays were applied with the department's tractor-mounted sprayer, with jets mounted vertically above each row of plants. Jets were arranged 2–3 inches above the crop for the low-volume application, and 10 inches above the crop for the high-volume spraying.

Immediately after the spray deposit had dried, a sample of thirteen plants was cut at ground level and removed from each treatment. The Nigrosine deposit was washed off and estimated colorimetrically at 580 μV. The colorimeter was calibrated with serial dilutions of a Nigrosine solution of known strength. Results were expressed as the equivalent of ml. of low volume spray mixtures retained by each plant, it being assumed that 1 ml. of low-volume insecticide mixture would be equivalent to 6 ml. high-volume mixture. Variance analysis showed that highly significant differences existed (Table 6).

TABLE 6

Retention of equivalent spray deposits on plants

Low volume		High volume	
Crown	Rest of plant	Crown	Rest of plant
2·42	46·73	1·66	14·50

Least significant difference between means (5% level) = 6·05.
Least significant difference between means (1% level) = 8·06.

Differences in deposit between the crowns of the plants were not significant. This was so even when deposits on the crowns were analysed separately. The plants sprayed at low volume had much higher total deposits than those sprayed at high volume. It is probable that in the high-volume treatment droplets would coalesce and drip off the leaves before drying.

Evidence for this was provided by an additional experiment, Nigrosine was replaced by Primuline A150, a dye which fluoresces in ultra-violet light. The technique has been described by R. B. Sharp (*N.I.A.E. Tech. Memor.* 119, 1955). Examination of photographs of sprayed leaves showed that with low-volume application the droplets remained discrete on the surface of the plant. With high-volume sprays the droplets coalesced, and the deposit was heaviest along the lamina of the leaves, indicating that run-off had occurred. Deposits in the crown of the plant are the most important for aphid control in the early stages of attack, and in this connection it is of interest that not only were these crown deposits very similar for either method of spraying, but that the degree of aphid control achieved in the experiment on Deacon's field (described elsewhere in this report) in 1957 was very similar for both high and low volume for most insecticides.

This work is being continued.

Mode of action of seed dressings

M. J. Way has carried out a series of laboratory and pot experiments and confirmed the systemic action of dieldrin used as a seed

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dressing against *L. coarctata*. It was shown that systemic action may be solely responsible for killing the larvae when the treated wheat seed is sown deeper than about 1½ inches with the *L. coarctata* eggs less than about 1 inch deep in the soil. Contact action may be important with a shallow-drilled crop and also if the eggs lie below the level of the treated seed. These conclusions were substantiated by a preliminary experiment with treated seed sown at different depths which showed that seed dressings were more effective with the shallow-drilled crop.

Dieldrin seed dressings can apparently stop damage to young onion plants by larvae of the onion fly *Hylemyia antiqua*, which is closely related to *L. coarctata*. Observations were begun on *H. antique* which may, perhaps, lead to an explanation of the comparative ineffectiveness of dieldrin against *L. coarctata*.

The control of the vectors of potato virus diseases

P. E. Burt continued to work in collaboration with L. Broadbent of the Plant Pathology Department on this subject. The joint work is described in the report of the Plant Pathology Department.

Fungicides

Only a small amount of work on fungicides was done in 1957, first, because F. T. Last was away on secondment in the Sudan, and secondly, because A. H. McIntosh had to spend a great deal of time helping and supervising M. Das in his work on the effect of temperature on the toxicity of insecticides.

The work described below was carried out by A. H. McIntosh.

Technique for spore-germination tests

A method has been worked out for making cavity-slides from perspex. The cavities are made by "shock-moulding" perspex sheet, ¼ inch thick. The cavities are cylindrical and of uniform size (1·41 cm. diameter × 1·25 mm. deep); their surface is flat and transparent. No drilling, polishing or cementing is needed. The slides are cheap and easy to make, and are almost unbreakable. They have the disadvantage that they will not withstand washing in strong acids, and in some organic solvents.

These perspex slides are now being used in our spore-germination tests of water-soluble fungicides. They are used in the same way as Böttcher's slides, as described in *Rep. Rothamst. exp. Sta. for* **1956**. They are cleaned by swabbing with cotton-wool and washing in running water, and can be dried at 70° C. without deformation.

Time, temperature and toxicity of fungicides

The work reported last year has been continued, using spores of a strain of *Botrytis fabae*, in slide-germination tests.

Interest has centred on salts of mercury, all of which gave positive temperature coefficients of fungitoxicity. When counts were made 48 hours from the start of the experiment, the temperature coefficients between 10° and 25° C. were: $Hg(CN)_2$, 17; $HgCl_2$, 7·1; $HgBr_2$, 2·8; $Hg(CNS)_2$, 3·4; HgO, 2·5; $Hg(OAc)_2$, 1·9. However, after an interval of some months, we found that the culture of our

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strain of *B. fabae*, although still pathogenic and still growing and sporing well, had changed in an unexpected way: the temperature coefficients are now very much smaller than before. For example, the temperature coefficient of $HgCl_2$ is now only 1·4; the susceptibility at 25° C. has remained almost unchanged, but the susceptibility at 10° C. has increased sharply. The reason for this change is not known. The work on mercury salts is now being extended to other species, and if possible to other strains of *B. fabae*.

The adsorption of mercury from solution on glass and perspex surfaces is a possible source of interference in spore-germination tests, in which very dilute solutions are used. We have found that the loss in strength of solutions of $HgCl_2$ (0·0005 per cent Hg), kept in small perspex pots, is about 6 per cent in 24 hours. This loss is not affected by temperature in the range 10–25° C. The loss on soda-glass tubes is almost identical, and is not affected by temperature.

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