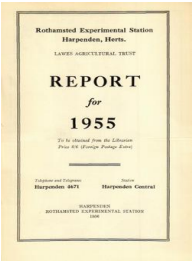
ROTHAMSTED RESEARCH

Report for 1955

Full Table of Content



Insecticides and Fungicides Department

C. Potter

C. Potter (1956) *Insecticides and Fungicides Department ;* Report For 1955, pp 112 - 130 **- DOI: https://doi.org/10.23637/ERADOC-1-77**

INSECTICIDES AND FUNGICIDES DEPARTMENT

C. POTTER

During the current year the following appointments have been made: Helen Salkeld to work on the effect of insecticides on pollinating insects, Janet Samuel to work on analytical methods for insecticides and R. Solly to work on biochemical effects of insecticides. Mr. Das from India has arrived in the department and started research for a higher degree. Mr. D. G. Glynne-Jones and Miss E. Hayton of the Kenya Pyrethrum Board, Mr. Clinch of Boots Pure Drug Co., Mr. J. P. P. Amaro from Portugal, Mr. D. Taylor from the Gold Coast, Mr. M. A. Nour from the Sudan, Mr. V. D. Krentos from Cyprus and Mr. Da Silva from Portugal have worked in the department during the current year.

The department has moved into fresh accommodation during the course of the year. The move has seriously interfered with laboratory work, although the field work was continued uninterrupted. The facilities in the new accommodation are not yet complete, and the work will be handicapped until they are. This is particularly true of the constant-environment facilities.

PHYSICAL CHEMISTRY

Effect of temperature on relative contact toxicity of different sizes of particle

A. H. McIntosh and Margaret Macfarlane have finished their work on particle size and toxicity of contact insecticides. The following is a summary of the most recent work.

Some assumptions have been made about penetration of insect cuticle by solid contact poisons. The three most important assumptions are: (*a*) that a contact poison begins to penetrate insect cuticle by dissolving in the epicuticle wax layer; (*b*) that the poison must saturate the wax layer locally before any insects die; and (*c*) that if counts of kill are made before the "end point", the kill brought about by a fixed dose of one poison at one after-treatment temperature is decided by the length of time the wax has been saturated. In such tests of contact action, small particles of poison are more toxic than large ones, weight for weight; the after-treatment temperature of the insects may affect the absolute and the relative toxicities of the two sizes of particle. The assumptions lead to the conclusion that the effect of temperature on the relative toxicity depends on the temperature coefficient of internal action of the poison on the insect. This can be measured by injection tests. If the coefficient is negative or zero, the ratio of toxicities of the two sizes by contact action, measured at some time before the end point, will increase if the after-treatment temperature is lowered; but if the coefficient is positive, the effect of temperature on relative toxicity cannot be foretold.

These ideas have been tested by using aqueous suspensions of

https://doi.org/10.23637/ERADOC-1-77

DDT and two of its analogues ("Perthane" and "BNP" or "Prolan"), rotenone, 2-bromomercurithiophen, dieldrin and endrin. Suspensions of colloidal poison were used for "small particles", and suspensions of uniform crystals for "large particles". Their toxicities were measured on as many as possible of three species. The temperature coefficients of internal action were found by injection tests (*Tribolium castaneum* Herbst and *Tenebrio molitor* L.); tests of contact action were by the dipping method (*Oryzaephilus surinamensis* L. and *T. castaneum*), or the measured drop method (*T. molitor*). All counts of kill were made 24 hours after treatment. The results seem to confirm the assumptions.

This work is now being published.

Time, temperature and toxicity of insecticides

Mr. M. Das has started work on the effects of after-treatment temperature and time on the toxicity of insecticides. If a poison has, for example, a "positive temperature coefficient of kill", the kill is higher as the temperature after treatment is raised. This difference in kill will be permanent if the speed of action is unaffected by temperature. But if the speed of action is affected by temperature, any difference in kill may just be transient.

With DDT on *T. castaneum* at 28° C. and 10° C., the temperature coefficient of kill is negative; but it is the same 12 days after treatment as it is 1 day after treatment. The kills increase at the same rate at each temperature; each of the rates of increase in kill becomes less and less as time goes on, and about 12 days after treatment there are no further increases in kill at either temperature.

With "Valone" on *T. castaneum* at 28° C. and 10° C. the temperature coefficient is positive if counts of kill are made less than 20 hours after treatment. But if counts are made 20 hours after treatment the temperature coefficient is zero; and if counts are made more than 20 hours after treatment the temperature coefficient is negative. There are no further increases in kill after about 16 hours after treatment (28° C.) or 48 hours after treatment (10° C.).

This work is being continued.

Biochemistry

Isolation and properties of insect esterases

K. A. Lord and R. Solly have continued work on the separation of esterases from *Blattella germanica* L. adult males, and considerable progress has been made in the separation of esterase activities. The acetyl choline hydrolysing activity has been largely separated from enzymes which hydrolyse phenyl acetate but not acetyl choline. Paper electrophoresis indicates that at least two enzymes have been separated from choline-esterase activity during the course of ammonium sulphate fractionation.

A satisfactory method of detecting small quantities of choline-esterase on paper has been evolved. The method is based on the hydrolysis of acetyl thiocholine by the enzyme and the detection of the thiocholine liberated by its ability to decolorize phenol-indo-2 : 6-dichlorophenol. Using this method and also a test method involving weakly buffered solutions of acetyl choline with a suitable

https://doi.org/10.23637/ERADOC-1-77

indicator, it has been possible to locate choline-esterase activity on paper electrophoretograms of extracts of *B. germanica*. Under conditions which do not inactivate the enzyme, the bulk of the proteins and the activity do not move appreciably from the point of application. It seems likely from other experience with the enzyme that it is strongly adsorbed to the paper. So far it has not been established whether the hydrolysis of acetyl choline in extracts of *B. germanica* is due to one or more enzymes.

A number of choline esters and analogues have been collected, and preliminary investigations indicate that the properties of the choline esterase activity from *B. germanica* are similar to those of the mammalian enzymes classified as true or erythrocyte choline-esterase. These do, however, appear to be different, and the insect enzyme appears to have a higher affinity for acetyl choline than is usual with mammalian enzymes.

Action of organo-phosphorus compounds on insects

C. Potter, K. A. Lord, Daphne Holbrook and Helen Salkeld have continued their examination of the esterases of insect eggs in relation to embryonic development and the ovicidal action of organo-phosphorus compounds. The possibility that films of pure TEPP inhibit embryonic development at an early stage by a suffocation mechanism has been ruled out by measurements of oxygen uptake. It has been established that the embryonic development of *Pieris brassicae* L., which have a chorion more permeable to water than *Diataraxia oleracea* L., can be stopped at an early stage of development by aqueous solutions of TEPP. When this is correlated with earlier work which indicated that choline-esterase activity only occurred with the later stages of development this provides some evidence that the poison may be obtaining its effect by a mechanism other than that of inhibition of choline-esterase.

It has been decided to include eggs of *Gryllus domesticus* L. in further tests of this nature, since they are highly water permeable, and problems of penetration will be somewhat different. A method of collecting large quantities of eggs by inducing the females to lay their eggs on damp filter-paper has been elaborated. A start has been made in studying their embryology. Tests have shown that these eggs will develop normally when exposed to solutions as acid as pH 3·0 or even lower, so that toxicity of unbuffered solutions of TEPP as strong as 0·1 per cent w/v must be due to effects other than the acidity of the hydrolysis products of TEPP. Preliminary tests with these eggs show the presence of at least three esterases which hydrolyse acetyl choline, triacetin and phenyl acetate respectively.

An attempt has been made to localize the esterase enzymes inhibited by TEPP *in vivo* by use of indoxyl acetate, which is hydrolysed by a wide range of enzymes and then gives indigo, in the presence of molecular oxygen. Three species were used, *T. molitor*, *B. germanica* and *Dysdercus fasciatus* Sign. The insects were dissected and then bathed in an aqueous solution of indoxyl acetate until sufficient colour had been produced in the various tissues. Both normal insects and insects poisoned by topical application of TEPP were examined. In poisoned insects colour had not developed in a number of tissues, notably thoracic muscles. Colour was visible in the nervous system of insects treated with 1 μl. of low

https://doi.org/10.23637/ERADOC-1-77

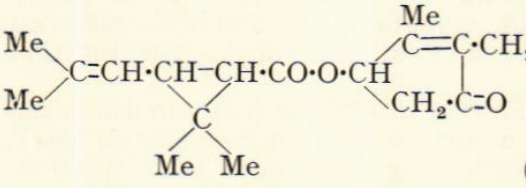
concentrations of TEPP and only ceased to appear with high concentrations (1–5 per cent).

When tissues from normal insects were bathed in 2×10^5 M (approx.) TEPP before the application of indoxyl acetate solution the colour development was inhibited in the nervous, muscular and parts of the reproductive system.

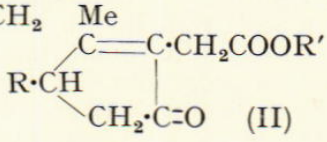
Organic Chemistry

M. Elliott has continued his study of compounds related to the pyrethins.

The most highly active synthetic compound related to the pyrethrins that has been made so far is allethrin (I), the ester of (±)-*cis-trans*-chrysanthemic acid with (+)-2-allyl-3-methyl*cyclo*pent-2-en-4-ol-1-one (allethrolone).



(I)



(II)

Since allethrolone is comparatively expensive to manufacture, an investigation has been carried out by M. Elliott to explore the feasibility of using derivatives of 3-methyl*cyclo*pent-2-enone-2-acetic acid as the alcoholic component in esters related to allethrin. Insecticidal data on such compounds would supplement information already obtained on the part played by the side chain of the alcohol in the insecticidal activity of compounds related to allethrin.

3-Methyl*cyclo*pent-2-enone-2-acetic acid (II; R = R′ = H) is obtained from the readily accessible furfurylidene acetone,* and its preparation has been simplified. Reaction of the esters (II; R = H; R′ = ethyl, allyl or phenyl, respectively) with *N*-bromosuccinimide gave compounds whose structure was (II; R = Br; R′ = ethyl, allyl or phenyl, respectively) by analogy with results of previous investigations. Without isolation the bromides were treated with the silver salt of (±)-*cis–trans*-chrysanthemic acid to give the corresponding esters (II; R = (±)-*cis–trans*-chrysanthemyl). The ethyl ester was *ca.* $\frac{1}{10}$ and the allyl ester *ca.* $\frac{1}{4}$ as toxic as allethrin (I) to *Phaedon cochleariae* Fab. adults, but the phenyl ester was much less active. The importance of ethylenic unsaturation in the alcoholic side chain of esters related to (I) was thus again demonstrated. These compounds are the first examples of esters with alcoholic side chains containing a functional group of this type.

The amide, and various substituted amides derived from (II; R = R′ = H), were prepared but failed to give 4-bromo*cyclo*pentenones (II; R = Br) with *N*-bromosuccinimide. The amide was dehydrated, best with acetic anhydride, to the corresponding nitrile, from which the crude chrysanthemyl derivative was obtained in low yield by the *N*-bromosuccinimide–silver salt route. Unfortunately, it has so far proved impossible to purify the compound sufficiently to give significant insecticidal results.

* Hunsdiecker, *Ber.*, 1942, **75**, 459.

https://doi.org/10.23637/ERADOC-1-77

Toxicity and Persistence of Insecticidal Deposits

J. Ward and Eileen M. Gillham have continued their work on DDT deposits. Comparisons between the toxicity of crystalline deposits and of deposits of DDT with Arochlor resin (which remain liquid) have shown that, when tested for contact activity to *T. castaneum*, the ratio of the toxicity of the two types of deposit depends on the time for which the test insects crawl on the deposit. With a 4-hour exposure period, the liquid deposit is 3–4 times as toxic as the crystalline, but with 24 hours, the ratio is only 1·6–2·5. The explanation may be that crystals of DDT which have been picked up by the insects are easily lost, while the viscous liquid deposit is more adherent. During exposure, the crystals lost are readily replaced by others. Once the insects have been removed from the deposit, however, and the adhering crystals have been lost, only the insecticide which has already penetrated into the cuticle remains. With a liquid deposit, a greater proportion of the adhering deposit remains on the insects, and can penetrate before the time for mortality counting.

A programme of work on films of insecticide from emulsions has been begun. After spraying a surface with an emulsion of DDT, the acqueous phase evaporates leaving droplets of DDT solution; in the course of a few hours these begin to crystallize. The form which the crystals take is influenced by the addition of non-volatile, oil-soluble materials to the emulsion before spraying. The effect of these additives can be very great if the emulsion is sprayed on to a glass surface, but is less if the surface is first coated with wax; it is less still if commercial DDT is substituted for pure *pp'*-DDT. The nature of these differences is being recorded by photomicrography, and their influence on the toxicity of the films will be studied.

It has been reported by Martin and Batt (*Rep. agric. hort. Res. Sta., Bristol for 1953*) that when a deposit of DDT from an emulsion or a wettable powder is sprayed on to a leaf (potato or apple), a proportion of the DDT enters the leaf, so that it cannot be removed by washing with solvents, but can be recovered only by drying the leaf, grinding and extracting exhaustively. This was at variance with our own earlier findings with cabbage leaf. On investigation our method of analysis for DDT in extracts of dried cabbage leaf was found to be unsatisfactory. Attempts to remove the interfering substances by the method used by Martin and Batt were unsuccessful, perhaps because of the very waxy nature of cabbage. The interfering substances, however, could be removed by the procedure commonly used for determining DDT in fats, which involves passing a solution in carbon tetrachloride through a column packed with kieselguhr wetted with concentrated sulphuric acid. Using this analytical step, it was found that deposits on cabbage behaved in the same way, a proportion of the DDT being found to be inside the leaf. The amount inside increased with time when the deposit was allowed to remain on the leaf; when the deposit was lost by evaporation, the DDT inside the leaf was lost more slowly. Work has been begun on penetration of leaves from *Graptopetalum paraguayense*, a succulent from which the leaf cuticle can readily be stripped. With this plant, it is hoped to gain information on the depth to which the DDT penetrates the leaf. To simplify this work, a further modification of the analytical method for DDT has been

https://doi.org/10.23637/ERADOC-1-77

worked out, using glass cells which give a 1-cm. light-path through 0·3 ml. of liquid; the SP500 spectrophotometer has been adapted to take these cells. The effect of this modification is that the analytical method is now ten times more sensitive, and quantities of DDT down to $\frac{1}{2}$ μg. can be estimated. This makes it possible to work with single leaves of *G. paraguayense*.

Work on the effect of insecticides in the soil, both at Rothamsted and elsewhere, have made it desirable to develop techniques to follow the rate of loss of insecticide from the soil. Janet Samuel was appointed in September to undertake this work. The first requirement is for a method capable of determining lindane (γ-BHC) down to a lower limit of 1 part in 10^8 parts of soil.

The stability of deposits of pyrethrins has been studied by means of a new bioassay technique, depending on knockdown of *Drosophila melanogaster* Meig. The insecticide is sprayed on to squares of cellophane in the Potter tower; these are rolled into cylinders which are slipped into flat-bottomed glass tubes. After putting in the insects, the tubes are closed with glass caps, and are illuminated from above. At intervals the number of insects resting on the bottoms of the tubes is counted. It was found that in the presence of hydroquinone or pyrogallol as an anti-oxidant there was no measurable loss of insecticidal activity after 2 days' storage at 30° C. in the dark. When the deposits were artificially illuminated at an intensity of 600-foot candles about three-quarters of the activity was lost in 2 days. About the same loss occurred when the light was screened through a sheet of plate-glass to filter out any short ultra-violet radiation. The effect of adding various materials to the pyrethrins, aimed at absorbing the long-wave ultra-violet, was studied. Benzene azo-β-naphthol was found to reduce the rate of loss of activity, as has already been reported by Blackith, but *o*-nitrophenol and 2 : 4-dinitro-α-naphthol had no effect, and *p*-nitroaniline increased the rate of loss. So far only purified and decolourized pyrethrum extracts have been used. Work has been temporarily discontinued because of lack of time.

Bio-assay Technique

Margaret Macfarlane has worked out a method for injecting small amounts of aqueous suspensions of insecticides into adult *T. castaneum*. Each CO_2-gassed insect receives 0·125 μl. of suspension by way of a 31-gauge needle inserted through the abdominal tergites. Control kills, measured 24 hours after treatment, seldom exceed 10 per cent.

Pyrethrum and Related Compounds

The constituents of Pyrethrum flowers

Work on the separation of the four known insecticidal principles of extract of pyrethrum flowers by means of displacement chromatography has been continued by J. Ward. An improved column, consisting of four sections of regularly decreasing diameter joined by ground-glass joints, has been made and has been shown to give rather better separations than the earlier continuously tapered pattern. Dinitrophenylhydrazones of the separated constituents

https://doi.org/10.23637/ERADOC-1-77

have been prepared. As a chemist has now been appointed by the Kenya Pyrethrum Board to work in London on this problem, it is intended to bring the work here to a conclusion as soon as possible.

The relationship between insecticidal activity and chemical structure in compounds related to the pyrethrins

Esters of allethrolone and chrysanthemic acid. The department is associated with the screening programme sponsored by the Chemical-Biological Co-ordination Centre of the National Research Council, Washington, U.S.A. Fourteen esters, derivatives of either (±)-*cis–trans*(?)-chrysanthemic acid or of (±)-2-allyl-3-methyl*cyclo*pent-2-en-4-ol-1-one (allethrolone) were supplied by the Centre and were tested by M. Elliott and P. H. Needham. The compounds (0·001 ml.) were applied as 2 per cent w/v solutions in acetone to the ventral surface of the thorax of male and female *Phaedon cochleariae* Fab. adults (mustard beetles). None of the derivatives of allethrolone showed any insecticidal activity, but the piperonyl, *o*-chlorobenzyl, benzyl and *p-iso*propylbenzyl esters of chrysanthemic acid gave kills of 100, 78, 63 and 61 per cent, respectively. Since the LD50 for allethrin to these insects under similar conditions is *ca.* 0·02 per cent w/v, the toxicity of the benzyl compounds is relatively low, but the results are of interest, since they indicate that the nature of the alcoholic component in esters related to the pyrethrins is somewhat less critical for insecticidal activity than is that of the acid. The piperonyl ester has been patented as an insecticide and synergist, and a further test has shown it to be one-tenth as toxic as allethrin.

The α- and β-forms of (±)-trans-*allethrin.* M. Elliott and P. H. Needham have investigated the relative potency of the so-called α and β isomers of (±)-*trans*-allethrin (the ester of (±)-*trans*-2 : 2-dimethyl-3-*iso*butenyl*cyclo*propane-1-carboxylic acid with (±)-2-allyl-3-methyl*cyclo*pent-2-en-4-ol-1-one) to adult male and female *P. cochleariae* (mustard beetles). When (±)-*trans*-allethrin is cooled, about half of it separates as a crystalline solid,[2] and this crystalline α-fraction consists of the racemic pair (+)-allethronyl (−)-*trans*-chrysanthemate and (−)-allethronyl (+)-*trans*-chrysanthemate. The liquid remainder is (+)-allethronyl (+)-*trans*-chrysanthemate and (−)-allethronyl (−)-*trans*-chrysanthemate (β-fraction).[3, 4] Gersdorff and Mitlin [5] found the liquid β-fraction to be 4·83 times as toxic as the crystalline α-form to adult houseflies by the Campbell turntable method.

The separation of the α- and β-forms has been repeated, and the β-fraction (still containing some dissolved α) is 9·14 times as toxic as the α-fraction to mustard beetles. This is a remarkably higher difference in toxicity, since all previous values for the relative potencies of esters related to the pyrethrins determined in this department on mustard beetles have shown good agreement with those of the American workers with houseflies.

When these relative potencies of the α- and β-forms to one another and to the mixed α- and β-forms in (±)-allethronyl (±)-*trans*-chrysanthemate are used to estimate biologically the relative proportions of the α- and β-forms present, it is found that the α-form constitutes a little over half the mixture, in agreement with what is

https://doi.org/10.23637/ERADOC-1-77

found in the chemical separation. However, Gersdorff and Mitlin [5] estimated similarly that the α-crystalline form constituted only 27 per cent of the mixture, although the necessarily somewhat inefficient separation showed at least 50 per cent of the α present.

Through the generosity of Dr. LaForge, (+)-allethronyl (+)-*trans*-chrysanthemate and (−)-allethronyl (−)-*trans*-chrysanthemate have been available to us, and the pure β-form has been constituted by mixing equal amounts of these two esters. It has been found to be 11·9 times as toxic as the α-form. The possibility that the (+)-(+) ester might synergize the activity of the (−)-(−) ester, and vice versa, has been eliminated by estimation of their potencies separately against the α-form; it is calculated thus that the β-form should be 12·9 times as toxic as the α-form, in good agreement with the experimental result. It is still possible, however, that the (+)-(−) and (−)-(+) forms together in the α-fraction depress each others' activity towards mustard bettles.

The factor rendering this high relative activity of the β- over the α-form remarkable is that from the relative toxicities of (*a*) the α- to the β-isomer and of (*b*) (+)-allethronyl (+)-*trans*-chrysanthemate to (−)-allethronyl (−)-*trans*-chrysanthemate, it should be possible to calculate the effect on potency of changing the optical form of the acid and of the alcohol; the estimation would be invalid, however, if (*a*) synergism or antagonism between the optical isomers of the esters operated and (*b*) if the effect on potency were dependent on the acidic or alcoholic component with which the two forms being considered are esterified. When the results of Gersdorff and Mitlin [3] are analysed in this way the values calculated agree well with those found, viz., that changing the optical form of the alcohol increases or decreases the toxicity of the ester by six times, and a similar change in the acid alters the toxicity by twenty-five times. In our work it has been found that the corresponding values against mustard beetles are 3–4 times and 30–40 times. These figures predict that the β-form should be 3·78 times as toxic as the α-form. Until further data are available, therefore, it is concluded that the most probable explanation of the discrepancy between theory and practice is that depression of insecticidal activity to mustard beetles of the (+)-(−) and (−)-(+) esters occurs when these are mixed.

References

[1] Synerholm, U.S. Patent 2,458,656.
[2] Schechter, LaForge, Zimmerli & Thomas (1951). *J. Amer. chem. Soc.* **73**, 3541.
[3] Gersdorff & Mitlin (1953). *J. econ. Ent.* **46**, 999.
[4] Elliott, M. (1954). *J. Sci. Fd Agric.* **5**, 505.
[5] Gersdorff & Mitlin (1952). *J. Wash. Acad. Sci.* **42**, 313.

Pyrethrum synergists (C. Potter, K. A. Lord, Daphne Holbrook)

The work of comparing the synergistic action of piperonyl butoxide and sulphoxide using adult *T. molitor* as test subject was completed. In order to determine how much specificity of effect occurred with the synergists, the work is being repeated using adult houseflies (*Musca domestica* L.) as test subjects.

Curves on houseflies have now been obtained with piperonyl butoxide. As with *T. molitor*, so with *M. domestica*, the amount of

https://doi.org/10.23637/ERADOC-1-77

pyrethrins required to give a selected percentage kill is at first greatly decreased by the addition of synergist, but the effect decreases with increasing amounts of added synergist.

Apart, however, from this general similarity it has been found that much larger proportions of synergists are required to effect a given reduction in the amount of pyrethrins with *T. molitor* than with *M. domestica*.

Insect Resistance to Insecticides

F. Tattersfield and Jill Kerridge have continued their work on this subject.

Work carried out on the possibilities of selecting strains of *D. melanogaster* adults resistant to DDT showed that such was possible, if the selection were carried out for a sufficient period of time. This was shown not to be due to conditioning by carrying out a series of trials upon the adults and their progeny by repeated treatments with sublethal doses of DDT. The effect showed, if anything, that this insect becomes more susceptible under these conditions.

The results obtained, however, suggested that the effect of pre-treatment of the adult insects upon their resistances to DDT was worth quantitative study. The preliminary results showed that insects reared from eggs to adult in the usual way upon a maize-meal porridge containing honey, dead yeast and live yeast, then transferred as adults for a further 6 days to a honey solution alone, absorbed on cotton-wool, were only about one-half as resistant as adults fed for the same period on honey solution and baker's yeast or on the original medium.

A repetition confirmed the greater resistance of the adults fed on honey plus baker's yeast when contrasted with the adults fed on honey alone or left on the old maize-meal porridge medium upon which they were reared. Malt extract added to the yeast and honey had no effect.

In view of these results a series of tests were carried out under similar conditions with a 25 per cent glucose solution with and without yeast (2·5 per cent) and with and without soluble casein (6·25 per cent). Insects reared in the normal way until adult when they were fed on glucose alone, absorbed on cotton-wool, had the same resistance with both males and females as those which continued feeding on a medium of the maize meal, honey and yeast, but the adult females fed on glucose solution and yeast and glucose solution and casein were so resistant that an expression of the comparative result was not obtained. In the case of the male adults the addition of fresh baker's yeast increased the resistance over the glucose medium alone by 2 times, and the addition of casein by $2\frac{1}{2}$ times. The most noticeable feature was, however, that whereas with the addition of yeast, large, healthy larvae and adults were produced from ♂ and ♀ adults fed on this medium, where glucose alone was used, and also with the addition of casein no adults were produced and the larvae, if any, were so minute as to be observable with some difficulty.

The reproductive effect was confirmed by experiments in which glucose solution with addition of (1) live yeast, (2) yeast heated

https://doi.org/10.23637/ERADOC-1-77

1 hour in a water bath and (3) biotin, absorbed on cotton-wool were compared. The live baker's yeast and the yeast heated to 100° C. produced many larvae, but with the glucose alone and the glucose plus bioltin no larvae were observed. Many larvae were produced when the adults were kept for a similar period (5 days) upon the usual medium composed of a maize-meal porridge, plus agar, glucose, dead and live yeast, but the larvae seemed larger than in the case of glucose plus live yeast and glucose plus yeast heated in water bath. The differences in resistance to DDT under these circumstances were observable but not nearly so marked.

A series of experiments in which the adult insects (♀ ♂) kept six days on media containing glucose alone, glucose plus live baker's yeast and glucose plus heated yeast were compared. In one series the media were absorbed on cotton-wool and in the other gelled by the addition of agar. It was clear that the nature of the medium has a pronounced effect on the comparative resistances of the adult insects. With cotton-wool as base, although they run in the same order, the differences were not so marked as when agar was used. The differences when cotton-wool and agar were compared at the LC50 were scarcely noticeable for glucose alone and glucose and dead yeast, but were marked when live yeast was added to the glucose, being significantly greater with the agar medium.

These experiments suggested that possibly other factors might prove of some importance. Attempts were made to ascertain whether the freshness of the medium had any pronounced effect, as we had noted in our experiments that insects from old cultures were often less resistant to DDT, and it was by no means clear that this was determined solely by the age of the insect, as adult insects reared on certain yeasts other than baker's yeast, showed a marked decline in *susceptibility* to DDT if transferred every 2 or 3 days to fresh food, when compared with the parent culture on which the insects had remained for some time. The experiment was repeated using baker's yeast on our ordinary medium in which the adult Drosophila were transferred to fresh food 2, 4 and 6 days after emergence. Tests were carried out 6 days after the first transference, the males and females being separated on the day of spraying. The results showed that there was a progressive increase in the LC50, the later the transference or the fresher the food before actual application of DDT. There appeared to be some indication with the females that insects of higher resistance were relatively fewer in number in the case of those insects fed on the older food. The numbers of insects, however, varied ranging from 475 (252 females 223 males) in the first transference, 641 (329 females 312 males) in the second, and 392 (212 females 180 males) in the third. The log LC50 for the females were first transference 2·38, second 2·49, third 2·58 (log mg./1,000 ml.). The log LC50 for the males first transference 2·16, second 2·32, third 2·52 (log mg./1,000 ml.). Age may have proved an important factor in this experiment and it was followed up by one in which the original stock of insects was transferred to fresh food on three separate occasions, separated by 2 days, but at each transference the one previous was also transferred to a fresh food medium. Thus on the day of spraying when the sexes were separated, each batch of insects had had food of the same age. The numbers transferred were :

https://doi.org/10.23637/ERADOC-1-77

(1) 677♀ and 596♂ with three transfers, 6–7 days old when sprayed;
(2) 454♀ and 420♂ with two transfers, 4–5 days old when sprayed;
(3) 340♀ and 360♂ with one transfer, 2–3 days old when sprayed.

The log concentration–probit lines were determined.

In the case of the females the log LC50 was (1) 2·69 (2) 2·52. No. 3, however, was so much less susceptible that the LC50 could not be determined. In the case of the males the order for the log LC50 were (1) 2·27< (2) 2·36< (3) 2·43.

In the above cases the sexes were kept together until the day when they were sprayed; in a further experiment they were separated every 24 hours. This, however, did not prevent fertilization, and the numbers of offspring and parents were of the order :

(*a*) Days 1–2 combined parents 422♀ 396♂—Offspring 1,174;
(*b*) Days 3–4 combined parents 769♀ 724♂—Offspring 8,201;
(*c*) Days 5–6 combined parents 571♀ 516♂—Offspring 4,118;

showing an average per female of (*a*) 2·8, (*b*) 10·7, (*c*) 7·2. The female adults gave for the log LC50 in log mg./1,000 c.c. :

(*a*) 2·57> (*b*) 2·37 = (*c*) 2·36 Mean ((*b*) and (*c*)) 2·36.

The male adults gave for the LC50 in log mg./1,000 c.c. :

(*a*) 2·32> (*b*) 2·23 = (*c*) 2·26 Mean ((*b*) and (*c*)) 2.24

The differences are not great, but the order has been reversed from that given by tests where the males and females were separated only on the day spraying was carried out. It is clear in both cases that the population density of the adults was a factor that might affect the results. Different numbers per culture of adult insects were therefore enclosed with their normal food.

(1) 800 (400♀ + 400♂), viable eggs laid 730;
(2) 400 (400♀ only), viable eggs laid 591;
(3) 400 (400♂ only);
(4) 400 (200♀ + 200♂), viable eggs laid 1,242;
(5) 200 (200♀ only), viable eggs laid 1,233;
(6) 200 (200♀ only);
(7) 200 (100♀ + 100♂), viable eggs laid 2,103;
(8) 100 (100♀ only), viable eggs laid 1,818;
(9) 100 (100♂ only).

They were left on the same food for 4 days and sprayed with DDT. The relative susceptibilities as expressed by the log LC50 (mg./1,000 ml.) were :

Females (1) 1·844 > (2) 2·291 > (4) 2·40 = (5) 2·40 = (7) 2·42 = (8) 2·39
Males (1) All killed (3) 2·331 (4) 2·121 (6) 2·34 (7) 2·15 (9) 2·31

It would appear that the population effect was different in the case of the females from that of the males. With the females it appears to depend on the total population of the females and males, if it is

https://doi.org/10.23637/ERADOC-1-77

very high, and at a certain level to become independent of the presence of the male insects (No. 4) and to reach a relative constancy (4), (5), (7) and (8). Whereas with the male insects there is a measure of dependence upon the presence of female insects, thus in above table (3) is less susceptible to DDT than (4) and (7) and of the same order as (6) and (9).

There is, however, a change in the steepness of the regression lines. In the case of the females the slopes (b) being (1) 2·1 < (2) 6·7 < (4) 8·5 (5) 8·7 (7) 10·2 (8) 8·2; " b " in nos. (4), (5), (7), (8) is not significantly different.

In the case of the males the slope (b) is as follows: (1) 0 < (4) 3·97 < (7) 4·63 much less than (3) 7·98 < (6) 10·29 > (9) 7·22. Nos (4) and (7) are scarcely significantly different, neither are (3), (6) and (9).

The change in the slope of lines with a tendency to meet at a point at or near the 100 per cent kill would indicate a relatively rapid decrease in the more susceptible insects, with selection. Under the conditions of our tests the population pressure, however, appears with this insect to differ as between males and females; with the former, it depends on the presence of females and with the latter more on the general population.

Some data recently obtained indicate that the presence of larvae in the medium slightly increases the resistance of adults; possibly it is due to an effect they may have upon relative humidity or in breaking up the surface of the medium.

A repetition of the work on the effect of the addition of yeast and of casein to a glucose agar medium, upon the resistance of the adults to DDT was carried out. The change in the resistance was studied using a slightly larger deposit of insecticide and a slightly higher content of acetone in the medium than in the former case, since under the previous conditions the resistance of the females fed on yeast or on casein was too great to be expressed numerically. In this case the reverse result with the males was obtained, no expression for the LC50 for the insects fed on glucose alone was obtainable owing to the almost complete kill obtained at all concentrations tested. The results with the females confirmed those obtained with the males in the earlier tests. The values for log LC50 (log mg./1,000 ml.) and for b (slope of the lines) obtained were as follows (two days after spraying):

(1)	Glucose agar medium	Log LC50	1·86	b 5·2 ± 1·5
(2)	Glucose agar medium + dead yeast... ...	,,	2·01	,, 7·4 ± 1·4
(3)	Glucose agar medium + live yeast	,,	2·11	,, 5·9 ± 0·8
(4)	Glucose agar medium + casein (vitamin free)	,,	2·16	,, 4·3 ± 0·6
(5)	Glucose agar medium + casein (soluble) ...	,,	2·15	,, 4·2 ± 1·0

Susceptibilities to DDT in descending order were:

Glucose > dead yeast > live yeast > casein (vitamin-free) = casein (soluble)

A series of tests carried out before the above tests, in which 100 males and 100 females (per bottle) were used for each test showed that only minute larvae, which died before producing pupae or adults, were produced in the glucose agar medium and no pupae or adults in glucose agar and casein medium, whereas with live yeast

https://doi.org/10.23637/ERADOC-1-77

numbers of larvae, pupae and adults were produced after a period of 10 days. A repetition of the experiment with the insects used for testing resistance gave similar results, but there was in this case a greater risk of contamination, and this was somewhat accentuated by the procedure adopted of changing the adults to new food every 2 days, to prevent its exhaustion, and the additional risk of inoculating it by yeasts carried by the insects themselves. There were 3 to 4 replicates for all samples of the medium given in the table above; the results confirmed our previous tests. In one month (25/10 to 25/11) no culture raised on glucose–agar medium produced pupae or adults, although many minute larvae were seen. Only one culture (obviously contaminated) reared on glucose–agar plus casein produced a few adults. Glucose-agar plus baker's yeast [(*a*) alive and (*b*) heated on water-bath for 1 hour] produced many larvae, pupae and adults. It was noteworthy with the baker's yeasts that both larvae and pupae were fewer but much bigger in the case of the heated yeast. Weights were taken. The weights of the adult insects were :

Heated yeast	(1) ♀ 1·3 mg.	♂ 0·83	Live yeast	(1) ♀ 0·49 mg.	♂ 0·36
	(2) ,, 1·3 mg.	,, 0·81		(2) ,, 0·91 mg.	,, 0·65
	(3) ,, 1·3 mg.	,, 0·90		(3) ,, 1·1 mg.	,, 0·68
	(4) ,, 1·3 mg.	,, 0·91			

There were fewer adult insects in the live yeast No. 3, and the pupae took apparently a longer time to emerge. The result is unquestionably due to the great numbers of larvae noted in the cultures containing live yeast.

These results have raised the question whether population pressure amongst the larvae may have a significance for the resistance of resulting adults, and work is now under way to ascertain its significance and the biochemical factors involved.

Insect Rearing

General

Insect species reared during the year included the following species which feed on growing plants : *Acyrthosiphon pisum* Harris (Pea aphid), *Megoura viciae* Buck. (Vetch aphid), *Aphis fabar* Scop. (Black bean aphid), *Myzus persicae* Sulz. (Green peach aphid) and *Tuberolachnus saligna* Gmel.; Lepidoptera—*Pieris brassicae* L. (Large cabbage white butterfly), *Diataraxia oleracea* L. (Tomato moth); Coleoptera—*Phaedon cochleariae* F. (Mustard beetle); Diptera—*Leptohylemyia coarctata* Fall. (Wheat bulb fly) and *Hylemyia antiqua* Meig. (Onion fly).

Stored products and other species reared included : Hemiptera—*Dysdercus fasciatus* Sign. (Cotton stainer); Orthoptera—*Blatella germanica* L. (German cockroach), *Blatta orientalis* L. (Oriental cockroach), *Periplaneta americana* L. (American cockroach) and *Gryllus domesticus* L. (House cricket); Lepidoptera—*Achroia grisella* Fabr. and *Ephestia kühniella* Zell.; Coleoptera—*Oryzaephilus surinamensis* L., *O. mercator* Fauv., *Tribolium castaneum* Hbst., *T. confusum* Duval., *Tenebrio molitor* L., *Calandra granaria* L., *Trogoderma granarium* Everts.; Diptera—*Aëdes aegypti* L., *Drosophila melanogaster* Meig. and *Musca domestica* L.

Main developments this year have been the addition of *H.*

antiqua and *A. aegypti* to the list of insects reared, and the rearing of different strains of *M. domestica* in large numbers. In the New Building two constant-temperature rooms for rearing insects at high temperature are in operation, but rooms for rearing plant-feeding insects and for rearing insects at relatively low temperatures are not yet ready.

Diapause in Leptohylemyia coarctata *Fall.*

M. J. Way has continued work on dispause, which is being done as part of the laboratory rearing programme. This year optimum temperatures for egg development have been fairly closely determined, and it is now possible to store eggs in standard conditions and to control the time of hatching.

Temperature optima vary with the stage of development of the egg. Initially, and before the diapause, a relatively high temperature (about 20° C.) is beneficial. Then a prolonged period of relatively low temperature (below 12° C.) is needed before diapause ends. Hatching will then occur over a wide temperature range (at least between 1° and 30° C.).

Eggs were also kept at constant temperatures from the time they were laid. None hatched at 1° C. (too low for pre-diapause development) or at 20° C. (too high for diapause development), but many of those kept at 7° and 12° C. hatched eventually.

This work is being continued.

Field Experiments

Control of bean aphid (Aphis fabae *Scop.*) *on field beans* (M. J. Way and R. Van Baer)

Nature of the aphid infestation. Work on *A. fabae* infestations on spindlewood, and on time and density of migration to beans was continued on the Rothamsted farm. This year few migrants reached the bean crop and less than 5 per cent of stems was infested in June. There was, however, a rapid build-up during the summer, giving big populations on beans and other plants in August, nearly one month later than in 1954.

Effect of planting date. In a field experiment field beans were planted at four different dates and, for each planting date, yields of unsprayed plots were compared with those of plots sprayed once on 23 June with " Metasystox " (0·05 per cent active ingredient and 100 gal./acre). Aphid counts were made at weekly intervals on all plots from the beginning of June to the end of August, and detailed work was done on the accuracy of the method used for determining the aphid populations. The following table gives the main results of the experiment :

	" Metasystox " treated		Untreated	
Date of planting	Aphids per stem at peak (3–11.8.55)	Grain yield (cwt./acre)	Aphids per stem at peak (3–11.8.55)	Grain yield (cwt./acre)
19 March	100	19·2	90	16·9
6 April	150	18·3	620	13·1
25 April	620	12·0	4170	6·7
13 May *	25	10·5	2190	2·4

* Sprayed twice—23.6.55 and 22.7.55.

https://doi.org/10.23637/ERADOC-1-77

It can be seen that this year the single spraying did not give perfect control, especially with late-sown beans. However, the figures for peak aphid populations are somewhat misleading, because they do not indicate the full effect of the control measure early in the season when the plants were relatively very susceptible to damage by the aphid.

Relative importance of biological and chemical control. By using small plots and a caging technique an estimate was obtained of the importance of predators in controlling *A. fabae*, and hence increasing the crop yield. Figures were obtained on the extent to which predators reduce the aphid numbers, and many predators were collected and identified. It was concluded that certain Anthocoridae were of particular importance, but nevertheless biological control was economically inadequate and chemical control was necessary, at any rate on the later-sown crops. Evidence was obtained that, this year, insect parasites and predators of *A. fabae* controlled the aphid more effectively on its winter host (spindlewood) than on its summer host (beans).

Control and laboratory rearing of Leptohylemyia coarctata *Fall., the wheat bulb fly*

Field experiment (R. Bardner and M. J. Way). On Pennells Piece a field experiment has been started that is designed to last for several years. The experimental area is divided into a number of blocks, half of which are bare fallow and half sown with winter wheat (var. Cappelle). Wheat is rotated with fallow, and in this way a permanent infestation of bulb fly is maintained, the adults laying their eggs on the fallow blocks. The experiment will be used to test promising methods of control originating from laboratory and microplot experiments. In the season 1954–55, 5 different treatments were tried in 4 randomized blocks of 6 plots each. These were :

(1) Untreated (2 plots per block).
(2) Seed dressed with technical dieldrin at 2·25 per cent w/w of seed, using a cellulose ether sticker.
(3) 4 per cent dieldrin dust combine-drilled with seed at 120 lb. dust/acre.
(4) Sprayed with parathion on 11 February 1955 at 0·05 per cent v/v active ingredient, 100 gal./acre.
(5) Sprayed with parathion on 7 April 1955 at 0·05 per cent v/v active ingredient, 100 gal./acre.

As the infestation was comparatively light (less than 500,000 eggs/acre), damage was assessed mainly by sampling the plants in the early spring and counting the number of infected tillers.

Results of sampling on 5.4.55

	Untreated	Seed dressing	Combine drill	Early parathion spray
Total tillers	840	511	843	863
Damaged tillers... ...	586	199	255	409

All figures in thousands per acre.

Variance analysis showed that at the 5 per cent significance level there were significantly fewer damaged tillers in the dieldrin treat-

https://doi.org/10.23637/ERADOC-1-77

ments than in the untreated plots. The low count of total tillers on the seed dressing plots was probably due to some phytotoxic effect. This is being investigated.

The results of the early parathion spray treatment need confirmation, as there was great variation between replicates. The treatment is being repeated this year. There was, however, a significantly smaller percentage of damaged tillers than in the untreated plots.

A further sample of plants was taken after the application of the late parathion spray. At this time, about a month before pupation, larvae leave the tillers originally attacked and cause further damage by invading tillers as yet untouched. In 1955 this secondary attack was very light, even on the untreated plots, and hence the late parathion spray which was applied to control this attack had little effect.

Grain yields were taken.

	Yield of grain in cwt./acre (85% dry matter)
Untreated	41·0
Seed dressing (dieldrin)	47·2
Combine-drilling (dieldrin)	53·7
Early spray (parathion)	47·9
Late spray (parathion)	38·7

At the 5 per cent significance level all treatments except the late spray treatment gave significantly higher yields than the untreated plots. In addition, the yield from the combine-drilled treatment was significantly greater than that of any other treatment, and was 31 per cent higher than that of the untreated.

In collaboration with the Entomology Department, a microplot experiment was laid down on the Side land, 1st year after fallow, on Broadbalk. It was intended to compare the effect of damage on yields of three wheat varieties. Strips of Holdfast, Squareheads Master and Cappelle were sown, and divided into plots, half of which were sprayed twice with parathion, and half of which were unsprayed. Unfortunately the parathion sprayings did not give complete control, but the experiment showed that the comparative immunity of Broadbalk from serious bulb fly damage is due largely to the sowing of a variety (Squareheads Master) with vigorous early tillering, and that more modern varieties with delayed tillering, such as Holdfast and Cappelle, suffer more seriously from wheat bulb fly attack.

Box Experiments (R. Bardner and M. J. Way). In these experiments known numbers of wheat seeds and wheat bulb fly eggs were planted in a standardized manner in boxes of soil treated with insecticides.

In studies on mode of action, insecticides (dieldrin, aldrin, demeton) were " placed " as bands in the soil in different positions relative to the wheat plants. A band around the stem was most effective, but some kill was caused by insecticides planted well below the seed, which presumably acted by absorption through the roots and translocation to the stem.

Other experiments in which plants grown in insecticide-treated soil were transferred to clean soil before infection suggested that aldrin as well as γ-BHC and parathion acted systematically against

https://doi.org/10.23637/ERADOC-1-77

wheat bulb fly larvae. This was confirmed in water-culture experiments. A series of box experiments was done to compare different insecticides for wheat bulb fly control and to study their persistence. The results, which require confirmation because seed germination was poor, suggest that endrin is no more effective than dieldrin or aldrin, and that DDT and parathion are effective as seed dressings, the latter at low dosage.

Laboratory rearing of wheat bulb fly (R. Bardner). As in 1954, large numbers of adults captured in the field were kept in lamp-glass cultures in the laboratory to provide eggs for experimental purposes. Between 15,000 and 16,000 eggs were obtained.

Although it has not been possible to shorten the diapause period of the egg appreciably, the life cycle can be staggered. This was done by freezing eggs in blocks of ice after the completion of the diapause period but before hatching would normally occur. The eggs were stored at a temperature of —8° C. or below until required. Hatching occurred several hours after transference to room temperature. Adults were produced in September from eggs which hatched in June, instead of late January. These flies laid some fertile eggs. Larvae also emerged in November from eggs laid the previous year.

By manipulation of the temperatures at which larvae and pupae are kept, some idea of the optimum temperatures for development of these stages has been obtained. Larvae kept at 20° will develop into adults laying fertile eggs provided the pupae are kept at lower temperatures.

Attempts were made to rear larvae on several artificial diets. These were unsuccessful with 1st instar larvae, but 2nd and 3rd instar larvae lived for 1–2 months at 10° C. on a diet similar to that used for *Drosophila*. Though larvae eventually pupated, growth was not observed, even when macerated wheat plants were added to the diet. It seemed that feeding occurred, because dyes added to the food could be seen later in the gut.

Potato virus diseases

In association with L. Broadbent of the Plant Pathology department, the experiments on the control of the spread of potato viruses were continued by P. Burt.

Good results obtained in previous trials with several insecticides suggested that it might be possible to reduce the number of sprays while still controlling virus spread. In an experiment with the same lay-out as was used in 1954 the effects on the natural aphid population, and on virus spread, of spraying 2, 4, 6 and 8 times with DDT was studied.

Whenever a spray application was due the toxicity to aphids of the insecticide remaining from the previous application was tested, the growth of the crop was followed by measuring the foliage area of selected plants at intervals through the season, and the time at which the virus spread was estimated.

It is hoped that a study of the results from this and from later experiments, particularly of the inter-relationship between rate of growth of the plants, persistence of the insecticide and variation in natural population, will make it possible to minimize the total number of sprays by timing them so as to obtain maximum persistence of the insecticides throughout the season.

https://doi.org/10.23637/ERADOC-1-77

Further information about this year's work and about the result of last year's experiment is contained in the report of the Plant Pathology Department.

A small trial was carried out to compare the effectiveness of some high- and low-volume spraying systems in covering potato foliage with insecticide and to compare the persistence of the deposits so produced.

One high-volume and two low-volume systems applied DDT emulsion at a rate of 2 lb./acre of active ingredient to potatoes at 3 stages of growth— just fully emergent (13 June), two-thirds grown (5 July) and mature (15 August). Although at all stages of growth high-volume spraying covered the crop somewhat more completely than spraying at low volume, the persistence of the deposits up to 43 days when tested by confining apterous adult *Myzus persicae* on shoots selected at random from the crop was similar for all systems.

Fungicides

F. T. Last, assisted by Rosemary Hamley and Jane Jackman, has worked on a technique assessing fungicidal action by variations of infectivity rather than germination *in vitro*, and has studied the behaviour of griseofulvin as a systemic fungicide using club-root of brassicae.

Technique to measure infectivity of fungus spores

A local-lesion technique to measure the infectivity of *Botrytis fabae* Sardiña conidia has been developed. A drop of conidial suspension is rubbed over the upper surface of half-leaflets of broad beans (*Vicia faba* L.) with the forefinger, and then the detached plants standing in a test-tube of water are kept in a water-saturated atmosphere at 20–25° C. for 24 hours, when chocolate-coloured lesions are countable.

The number of lesions developing is directly proportional to the concentration of conidial suspension. Suspensions of equal concentration produce more lesions when prepared from young than from old cultures.

The number of lesions developing on different plants varies significantly. Equal numbers of lesions develop on all the leaves of young plants, but significantly more develop on the old than on the young leaves of old plants. The effects of plant variation can be minimized using a suitable experimental design, i.e., Latin square or balanced incomplete blocks.

This technique was used in collaboration with E. W. Buxton (Plant Pathology Department) to determine the effect of ultra-violet irradiation on the infectivity of spores of *B. fabae* and *Uromyces fabae* (Pers.) de Bary and on the susceptibility of broad bean leaves (*Vicia faba* L.) to infection by them (see report of Plant Pathology Department).

Griseofulvin and club-root of cabbages

The effect of griseofulvin, a metabolite of *Penicillium* sp., on the development of club-root is being investigated with I. Macfarlane (Plant Pathology Department). Its effect is linked with the concentration of *Plasmodiophora brassicae* Woron. spores in the soil.

https://doi.org/10.23637/ERADOC-1-77

When added to the soil at the time of inoculation and when a range of inoculum concentrations were used varying by a factor of 625 but all giving 100 per cent infection and clubs of equal size (dry weight) in the controls, the highest concentration of griseofulvin gave the greatest reduction in club size, and at all dosage levels griseofulvin was most effective against the lowest concentration of inoculum. The inhibitory effect of griseofulvin on the development of club-root appeared to lessen after 6 weeks.

The rate of increase in size of already infected roots was retarded by the application of griseofulvin to the soil, and there is evidence to suggest that the same effect could be obtained by spraying the foliage. Application of griseofulvin to the soil 2 weeks after inoculation benefited the plants more than applications made simultaneously with or 4 weeks after inoculation.

The retardation in club-root development was associated with increased dry weights of foliage and healthy roots.

Powdery mildew of barley

That powdery mildew can cause a reduction in yield was confirmed. As spraying with lime sulphur was shown not to affect the yield of mildew resistant cv. Haisa II in three seasons, only the mildew susceptible cv. Plumage Archer was sown in the 1955 experiment. Barley was sown on 30 March and 28 April at $1\frac{1}{2}$ and $2\frac{1}{2}$ bushels/acre. Each plot was split, one half being sprayed four times between 24 May and 27 June with 1 in 80 lime sulphur; the other was not sprayed. Plants infected with *Erysiphe graminis* DC. were planted in the unsprayed sub-plots on 19 May.

The unsprayed plants were severely infected and the sprayed plants were lightly infected. The unsprayed plots yielded significantly less grain than the sprayed, 20 per cent less for the early and 27 per cent for the late sowing. The losses are attributable to reductions in : (*a*) yield of grain per 100 years, and (*b*) numbers of ears per plant.

The yields were unaffected by seed rate.

https://doi.org/10.23637/ERADOC-1-77