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Insecticides and Fungicides Department

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DEPARTMENT OF INSECTICIDES AND FUNGICIDES

By F. TATTERSFIELD

During the war period there were considerable changes both in personnel and type of work in this department. Drs. J. T. Martin and S. H. Harper, who for a number of years had been engaged on detailed studies of plant insecticides such as pyrethrum (*Chrysanthemum cinerariifolium*) and derris, left (1942) to take up other appointments.

During the three years (1939-42) Dr. Martin undertook a considerable amount of urgent work, particularly upon the dermatitis factor in pyrethrum and, with Dr. Brightwell of the Imperial Institute, of the chemical evaluation of pyrethrum-in-oil sprays, which is likely to be permanently valuable. Dr. Harper continued his important researches on the constitution of the active principles of derris and other plant insecticides.

New appointments have been Messrs. K. A. Lord (1942), A. H. McIntosh (1944) (chemists), Mr. M. J. Way (entomologist) (1943), Mrs. E. M. Gillham (1942 and Miss R. I. Stoker (1943) (horticulturists). Mr Lord has been chiefly engaged in researches on the following subjects; (a) the preparation of dermatitis-free pyrethrum extracts; (b) the revaluation of the factor for determining Pyrethrum I; (c) the synthesis and insecticidal valuation of D.D.T. analogues; (d) the stability of D.D.T.; (e) with Mrs. Dion of the Plant Pathology Department on the fungistatic properties of the triphenylmethane dye-stuffs. Mr. McIntosh has worked on a micro-method for determining D.D.T., and the effect of particle size and shape on its insecticidal value. Dr. C. Potter has been in general oversight of the entomological side of the Department's work and with Dr. Tattersfield has investigated what was in the early days of the war the urgent problem of the biological evaluation of pyrethrum-oil preparations. With Mrs. Gillham he has studied the effects of environmental conditions on the susceptibility of insects to a number of insecticides including D.D.T., and had, with Mr. Perkins of the Plant Pathological Department of the Ministry of Agriculture, general charge of the field experiments with D.D.T. Mr. Way, in addition to the investigation of methods for determining the insecticidal potency of stomach poisons, has carried out with Miss Stoker investigations of the effect of leaf growth upon the insecticidal value of D.D.T. and the effect of D.D.T. upon parasites and predators. Mrs. Gillham and Miss Stoker have provided the extensive stocks of plants needed to rear the very large number of insects required and have carried out investigations on the phytocidal action of D.D.T., in addition to helping in the spraying trials and assessing the results. Throughout this period, therefore, the work of the Department has been concerned with

- (a) laboratory techniques for evaluating insecticides,
- (b) insecticides of plant origin,
- (c) the new synthetic insecticide known as D.D.T. (2, 2-bis-parachlorophenyl 1, 1, 1-trichloroethane).

At different periods of the war such insecticides as pyrethrum, derris and D.D.T. were withdrawn from agricultural and horticultural use to other more urgent purposes and could only be obtained in experimental quantities. A good deal of the work carried out was not published, but incorporated in confidential reports. It was considered important that before D.D.T. was released in large-scale amounts for general purposes more should be learnt of its possible uses and dangers in the horticultural and agricultural field. Part of this work has been undertaken here at the instance of a sub-committee of the Agricultural Research Council.

This review, presented in yearly periods, is in general divided into three main categories; (a) biological, (b) biological-chemical, (c) chemical investigations, but all three are closely interlocked.

PERIOD 1938-39

Biological investigations. (a) Insect rearing

During this period and, in fact, during the whole period under review a considerable degree of attention was devoted to the study of the facilities and the techniques needed for rearing insects under controlled conditions and the selection of such insects as were suitable for testing insecticides. A constant-temperature-and-humidity room together with an incubator was set up and found to be suitable for rearing a number of stored-products pests, which being readily available throughout the year have often tided us over periods when plant feeders were not available. In addition, *Macrosiphoniella sanborni* (chrysanthemum aphid) was investigated and found to be available for many months of the year. It is a useful insect for the preliminary testing of new insecticides, as it is one of the more susceptible species. *Plutella maculipennis*, the caterpillar of which was suitable for stomach-poison trials, was closely investigated: it is readily reared at all periods of the year, and has proved a great stand-by in working out a laboratory technique for the quantitative assessment of the toxicity of this class of insecticide.

(b) *Contact insecticides.* During this period the elaboration of the larger spray tower was completed (14). It is a development of the Tattersfield-Morris spraying apparatus, but more suitable for quick-moving insects, as an even distribution of spray over a greater area with control of doses is secured, but difficulties of replication are being met with. We have available now two machines—the original Tattersfield-Morris apparatus, for which only small amounts of insecticide materials are required, and the larger Potter apparatus for normal use.

Biological-chemical investigations

(a) A prolonged study (9) was undertaken of the relative toxicities of the fractions of derris resin to *Aphis fabae*. Rotenone was thus compared with the so called deguelin concentrate (neutral resin not extracted by alkali) and with the sumatrol-toxicarol fraction (extracted by alkali), and the toxicities of these expressed in terms of their rotenone equivalent. Thus the deguelin concentrate was $\frac{1}{4}$ — $\frac{1}{8}$ and the sumatrol-toxicarol complex $\frac{1}{3}$ — $\frac{1}{5}$ as toxic as rotenone. Rotenone and these fractions could be determined

chemically. Thus from the chemical analysis of four samples of derris root of different varieties it was predicted that the relative toxicities would be 1, 1.3, 1.7, 2.7. The biological determination of the toxicities gave 1, 1.5, 1.7, 2.6. In addition, the toxicities of the available pure active principles were determined. Elliptone was found to have a potency of one-third to one-fifth that of rotenone, toxicarol and sumatrol being less toxic.

(b) Preliminary investigations of the effect of media on the toxicity of insecticides were made. It was found that *Calandra (Sitophilus) granaria*, the grain weevil, was highly resistant to derris resin when applied in an acetone sulphonated lolol medium, but incorporated in a heavy oil sulphonated lolol emulsion, derris extract was an effective insecticide. Heavy oils at the same concentration and deposit were non-toxic, but were so at higher deposits. The wetter used had a pronounced importance, as was shown by the greater toxicity of derris extracts applied in alcohol sulphonated lolol over the same extract in 10 per cent. alcohol saponin medium.

Other insecticidal plants (8). An Indian sample of *Mundulea sericea* (Supli) was found not so toxic to insects as the rotenone-rich species of derris roots now obtainable, but it was of interest as it was found that the leaves, bark and roots all possessed insecticidal properties, whereas in the African strain of this plant the bark is the only toxic part. Cashew-nut shell oil was found to possess some toxicity. The chief investigation, however, concerned four species of *Annona* received through the Colonial Office from Ceylon. They all proved aphiscidal in varying degrees, but *Annona reticulata* seed, leaf, stem and root, particularly the seed and root, had a considerable measure of toxicity of a delayed-action type to both the chrysanthemum and bean aphid. The action is specific to certain species, some insects being unaffected when extracts are administered by contact or as a stomach poison.

Chemical Investigations. The determination of rotenone (17)

This was a conjoint piece of work with the Imperial Institute, the Department of Agriculture of the Federated Malay States and the Cooper Technical Bureau. Completely concordant results were not obtained by two methods even when details of procedure were minutely defined. A modified technique was detailed for study upon another batch of samples of specified varieties.

The active principles of derris. A new derivative, which has been named elliptone, closely related to rotenone, was isolated by Dr. Harper (6(a), 6(b), 7); it has an *iso*-rotenone structure, but is devoid of the propyl side-chain. This structure was confirmed by degradation experiments. Elliptone also differs from rotenone in not forming a carbon-tetrachloride complex. The hydroxyl derivative, previously found by two Dutch workers, was also isolated and was found to bear the same relationship to elliptone as sumatrol does to rotenone (10).

A sample of "White-milk" Haiari, a species of *Lonchocarpus* from British Guiana, was found to contain 2.3 per cent. of rotenone, which was also shown to be present to the extent of 0.5 per cent. in *Tephrosia toxicaria*.

PERIOD 1939-40

Biological investigations

These were a continuation of those of the previous year, but no new insect test-subject superior to those already in use was discovered. Much time and attention were given to the problem of obtaining in large numbers the eggs of *Ephestia kühniella* (Mediterranean flour moth), *Sitotroga cerealella* (Angoumois grain moth) and *Pieris brassicae* (cabbage white butterfly), needed for investigations to be detailed later. A commencement was made in the long-term study of the relative susceptibilities of insects to insecticides.

The difficulties met with in replicating the amounts of deposit obtained in the Potter spraying tower were overcome after prolonged effort. Evenness of distribution and facility of repeating the amounts of deposit appear to be mutually exclusive. The reason is that the apparatus depends for its effectiveness upon the spray being thrown into a state of turbulence in the upper part of the spray tower used. A turbulent stream is largely out of control and susceptible to abrupt variation in character with minute changes in environment. A careful analysis was made of the factors involved—e.g. the turbulence, its subsequent subjection to streamlining as induced by the shape of the tower, and the effect of the size of the vents at its top and bottom upon both. A study was made of the pressure and regularity of flow of air to the nozzle, the latter's structure, and the electrification of the droplets of spray as they issue from the nozzle. Finally, there was evolved the present apparatus—an earthed, metal tower of circular section, open at the top except for a centrally placed, adjustable, atomising nozzle and reservoir. The tower tapers to the centre and then becomes cylindrical. A platform upon which the insects are placed, can be rapidly moved into position, leaving an accurately spaced gap between it and the bottom of the tower. A good distribution with good replication was thus obtained, and the whole apparatus and technique are flexible enough to allow of wide variation in deposit of the insecticide sprayed and of considerable speed in operation (14).

Biological-chemical investigation

These followed up the work carried out in 1938-9. (a) The extracts derived from the seed of *Annona reticulata* were fractionated and tested biologically. One of the fractions was found to have an insecticidal value of the same order as some of the derris extracts. (b) Trials were made to determine the most suitable solvent for extracting pyrethrum for analytical purposes. The value of petroleum ether for this purpose was confirmed (15). (c) The toxicity values and rotenone equivalents of various crystalline derivatives from derris were re-determined (16). (d) The effect of media and adjuvants upon the toxic action of constituents of derris was determined. Phenol accentuated, cyclohexylamine depressed, the insecticidal value of rotenone. Terpeneol increased the toxicity of this compound to *Oryzaephilus surinamensis*. (e) Advantage was taken of a considerable invasion of cabbage-white butterflies to test in a preliminary way the effect upon their eggs of a variety of plant insecticides. Rotenone and pyrethrum extracts were each found to have a powerful ovicidal action (25).

Dermatitis factor in pyrethrum (13). Being susceptible to the dermatitis effects of pyrethrum, and this insecticide being of considerable importance for the control of insect pests during the war, Dr. J. T. Martin volunteered to undertake with Dr. K. H. C. Hester, a local medical man with experience of dermatitis problems, an investigation of the causal factors of this painful complaint affecting certain susceptible people who handle or are exposed to pyrethrum flowers, sprays and dusts. Much valuable information was obtained and a report submitted to the Medical Research Council, who suggested a medium for publication. In the course of the work Dr. Martin prepared a concentrate containing 93 per cent. of pyrethrins that had only a very slight irritant effect, thus the active principles are not responsible for dermatitis. On the other hand a constituent, volatile in steam, gave a most powerful and painful reaction, although other preparations and fractions in varying degrees gave rise to characteristic skin effects. The work, it was hoped, might lead to the preparation of dermatitis-free extracts.

Chemical investigations

(a) *Determination of rotenone* (17, 22). The co-operative work was continued. A committee, composed of a group of investigators in this country, suggested a new method of analysis and six samples of derris root of different types, but of known origin, were analysed by the participating laboratories. The results showed a good agreement among the workers in this country, but the figures obtained by chemists in Malaya were of a lower order, probably because of climatic conditions.

(b) *The active principles of derris*. The constitution of the new phenol compound (malaccol) was established. Five out of the six derivatives of derris have now been isolated in an optically active state. Deguelin has eluded separation so far, and has only been obtained in a racemic form. A compound from *D. malaccensis* with insecticidal properties that seems to be new was isolated, but the amount obtained was too minute for critical work. Studies of *l*-a-toxicarol, first isolated by Tattersfield and Martin, showed that its anomalously high methoxyl content was due to the presence in minute amounts of a derivative with a high methoxyl content. Dr. Harper (11, 21) suggested that it may have a flavone or *iso*-flavone structure and be the precursor of one or other of the rotenone-like compounds isolated. The determination of its structure would need the separation of much larger amounts than have been, so far, available.

(c) *Annona species*. The fractionation of the extractives has shown that the fraction soluble in ethyl ether *but not in petroleum ether* has a high toxicity to aphides. It is of the same order as that of some derris extractives.

PERIOD 1940-41 : 1941-42

During this period pyrethrum insecticides assumed a major role owing to their use for the rapid control of many pests of importance to the war effort and to public health. Their use in warehouse sprays for controlling pests of stored food, in air-raid

shelters and on men for the control of pests of medical interest made pyrethrum a subject of some importance to the Ministry of Food, the War Office and the Medical Research Council. Finally all supplies were taken over by the Government and use restricted for certain limited purposes. During this period, however, we were consulted on pyrethrum preparations by a number of interested bodies, such as the Entomology Department of the School of Hygiene, Dr. Page of the Ministry of Food on certain aspects of their use, and for a short time Dr. J. T. Martin co-operated with Dr. Mellanby in his experiments on scabies control. We were requested by the Agricultural Research Council to take up certain pyrethrum investigations as matters of urgency.

The investigations undertaken and continued in this period were ;—

(a) *Chemical analysis of pyrethrum-in-oil preparations.* In conjunction with the Imperial Institute a suitable method has been worked out for determining pyrethrin I in these preparations. The method was shown to give accurate results with two types of commercial preparations used for warehouse and air-raid shelter spraying. The method was the subject of a report to the authorities concerned and has since been extensively used.

(b) *Biological testing of pyrethrum-in-oil preparations (23).* One of the commonly used methods of applying pyrethrum is in the form of an oil spray. Our work involved the study of two techniques of evaluation in which the insect was subject (i) to direct spray, (ii) to the effect of a film formed after spraying. In addition, the methods of preparing standard solutions of pyrethrum-in-oil for purposes of comparison had to be undertaken.

It was found that provided the insect test-subject, in this case *Tribolium castaneum*, were of good quality, free from parasites and disease, and reared and handled in a uniform way, both methods (i) and (ii) were satisfactory for quantitative insecticide tests, but the factor of insect quality was most important if reliable results were to be obtained. The Potter spraying apparatus, after a little adaptation to meet the special case of pyrethrum-in-oil sprays, was found suitable for the application of the two methods. A special cupboard ventilated by fan and incorporating electric warming equipment was built to house it.

For both techniques (i) direct spray and (ii) film, the surface upon which the spray falls or the oil film is formed very largely determines the degree of toxicity : the less absorptive the surface the higher the kill and the steeper the characteristic toxic curve. In addition, the weight of the deposit per unit weight of fluid sprayed is altered by slight changes in the physical properties (e.g., viscosity) of the pyrethrum preparations. Moreover, the period of time the insects are left on the film determines its toxic effect. These difficulties were overcome by using surfaces of constant sorptive properties, (e.g. tricoline or hardened filter paper, Whatman No. 544), by determining the weights of deposits per unit area at frequent intervals, and by keeping the insect on the film for a constant period (five days).

For comparing the two techniques (i) direct spray and (ii) film, the concentrations in terms of pyrethrins and the deposits

per unit area were successively changed and the toxic effects noted. The results plotted as probits against the log concentrations and log deposits were analysed by D. J. Finney who introduced a new statistical concept—that of probit planes for purposes of analysing toxicity data involving the three variables. It was found that the planes were parallel, that the direct-spray technique gave the higher kill, and that so far as homogeneity of data was concerned there was no significant difference between the direct-spray and the film techniques.

(iii) Standard pyrethrum-in-oil preparations of known and repeatable constitution were prepared (24), requiring a considerable amount of work on processes of extraction and blending. Three such preparations were prepared, and their insecticidal values were compared with each other and with commercial preparations.

The application of the direct spray technique showed that a 10 per cent. difference in pyrethrin content could be readily detected, that is, a composition containing 1.0 per cent. of pyrethrins could be differentiated from one of 0.9 per cent. When the film technique was applied to commercial preparations the presence of sludge or slime could introduce heterogeneity into the data. The sludge could probably be safely separated out since its presence had no very marked effect on the toxicity of the pyrethrins. Apparently no constituent of the commercial preparations tested, other than the pyrethrins, had any effect upon toxicity. With oil sprays relative potency was more closely correlated with *total* pyrethrin content than with pyrethrin I, a finding which led to an investigation of the effect of media on the toxicity of the pyrethrins. It was found that pyrethrin I was more toxic than II in aqueous media, but they were of the same order of potency in petroleum oil.

The loss of activity of pyrethrum. Its quantitative determination (23)

Pyrethrum extracts are highly repellent to mosquitoes. At this time considerable attention was being given to the preparation of ointments for this purpose, and the problem of their possible loss of activity at tropical temperatures was referred to us through the Agricultural Research Council, by the School of Hygiene and Tropical Medicine. Two types of ointment were sent in to us, embodying pyrethrum extracts with (i) gum tragacanth and (ii) soft wax. The preparations were exposed to the temperatures of (i) refrigerator (taken as control), (ii) 35° C., (iii) 50-60° C. The tests were thus very drastic. The loss of toxicity was determined by both chemical and biological means. The results agreed within close limits. A gum-tragacanth preparation in 14 days at 50° C. showed a loss of pyrethrin I and II *determined chemically* of 11.8 per cent. w/w. The loss of activity *determined biologically* was 12.8 per cent. The soft-wax preparation kept at 50° C. for 14 days showed loss of pyrethrin I and II *determined chemically* of 12.5 per cent. w/w. The loss of activity, *determined biologically* was 16.5 per cent. (in the latter case the temperature rose for some days above 50° C.). The loss at 35° C. is not significant.

Insecticides applied jointly, the effect of adjuvants and media

These investigations were carried a stage further, but owing to other more immediately important problems, they were somewhat

intermittently pursued. The work upon the toxicity of the active principles of derris applied alone and together was completed. Using a statistical technique developed and published by Mr. Finney of the Statistical Department, Dr. J. T. Martin (16) showed that the constituents of derris had no synergistic effect on each other's toxicity and that therefore his earlier work upon the assessment of the toxic value of derris root by the determination of its rotenone equivalent is not invalidated by this cause.

Ovicidal action of plant insecticides

This investigation was nearly completed. It was shown that both derris and pyrethrum have ovicidal effects in certain cases and at certain stages of egg development, but that derris extract is not toxic to red-spider eggs.

Derris. Dehydro-tetrahydroelliptone was synthesised, confirming the formula ascribed to elliptone from degradative experiments (20). Toxicity experiments showed that *l*-elliptone was more toxic than *dl*-elliptone, and *l*-dihydroelliptone than *dl*-dihydroelliptone, a conclusion confirming that among this class of compounds the optically active (*laevo*) compounds are quantitatively more powerful insecticides than the racemic compounds (19). The results confirmed earlier more detailed and exact results that rotenone was five times as toxic to *Macrosiphoniella sanborni* than elliptone. The contaminant of *l*-*a*-toxicarol having been shown to be an *iso*-flavone, a series of analogous compounds was prepared which were remarkable in giving the Durham test characteristic of rotenone, but they had no material insecticidal potency.

Annona species. Further work demonstrated that both the seed oil and the root contained insecticidal principles with very similar properties. The action was slow, but the order of toxicity was high, and to certain aphides was comparable with that of rotenone. The fractionation of the seed oil gave a toxic portion which a preliminary examination showed to be a glyceride of a hydroxylated acid of unusually high molecular weight. The slow but cumulative paralytic action on insects is of interest. An investigation of *Pongamia glabra* from India proved the insecticidal properties to be concentrated in the flavone fraction of the extracts of the seed. Cashew-nut shell oil was examined and cardanol (its active principle) shown to be ovicidal. The cardanol distillate was separable into an easily reducible and a non-reducible portion.

Biological work

Work continued on the rearing of plant-feeding test insects. Those so far reared have proved very useful, but the search for others continued with the result that the aphis, *Macrosiphum solanifolii* was kept going throughout the year. For successful rearing, it requires a succession of host plants. It has been a promising test-subject and a welcome discovery, since the aphis *Macrosiphoniella sanborni* during the year fluctuated both in quantity and quality.

Relative susceptibility of insects to insecticides

Effect of external conditions. The problem of the effect of environmental factors upon insect resistance was examined and the tem-

perature at which the insect was kept after spraying was found to have a pronounced effect on the subsequent mortality induced by pyrethrum sprays—recovery being helped by higher temperatures.

General susceptibility. Preliminary exploratory work was started upon the relative toxicities of the pyrethrins, rotenone and derris resin, and lauryl thiocyanate to a number of insects under approximately similar conditions with the object of ascertaining :—

(i) How far the results obtained with a given insecticide on one insect are applicable to other species.

(ii) To what extent the order of toxicity of a series of insecticides will vary with the insect test-subject.

Although a number of experiments were carried out, this long-term research had to remain in partial abeyance owing to pressure of other work.

PERIOD 1942-43

During this period large changes in the future programme of work had to be arranged. Funds were made available for the employment of two new assistants, and an entomologist (M. J. Way) and a horticulturalist (Miss E. M. Mills, later Mrs. Gillham) were appointed. The proposals are of a long-term character and have meant a careful review of our programme of work, which during the year was discussed with and approved by Messrs. Fryer and Gimingham of the Ministry of Agriculture. Although the main outline was drawn, much interruption in carrying it out supervened. Drs. Martin and Harper left in 1942, and although Mr. Lord was appointed in August 1942, and he rapidly familiarised himself with many of the details of the several lines of investigation and has done valuable work, there was inevitably a slowing-down in the carrying out of our programme.

Chemical investigations were chiefly concerned with pyrethrum. The possibilities of separation of the pyrethrins I and II by means of preferential sorption of the chromatographic type were explored in a preliminary way by Mr. Lord, and progress was made. The method of pyrethrin determination in pyrethrum-in-oil preparations, which had been worked out conjointly by this Station and the Imperial Institute, was found to give erroneous results for very dilute solutions. A satisfactory solution of the difficulty was found by increasing the time of reaction, the concentration or volume of sodium hydroxide in the saponification process.

Fungicidal properties of synthetic dyes

Mr. Lord (in collaboration with Mrs. W. M. Dion of the Plant Pathology Department) (26) compared the toxicity of salts and bases of a number of dyestuffs to spores of *Fusarium culmorum* and *Cercospora herpotrichoides*. Mr. Lord prepared a certain number of the salts and bases. The findings were as follows. (a) The basic dyestuffs of the triphenylmethane series were highly toxic and/or highly fungistatic, irrespective of the acid radicle associated with the dye. (b) Malachite Green base (tetramethyl-p-diaminotriphenylcarbinol) showed high toxicity which was, if anything, progressively lowered by substituting the methyl groups

by ethyl, propyl, and butyl radicles. (c) The *leuco* bases were not toxic. (d) In triphenylmethane compounds the substitution in the benzene nucleus of acid groups lowers toxicity, of basic groups increases activity. Sulphonation or carboxylation of any of the nuclei lowers toxicity to the vanishing point. (e) The introduction of aniline or dimethylaniline radicles into the methane radicle progressively increases toxicity. (f) Naphthylamine and diphenylamine radicles can partially replace dimethylaniline radicles without loss of activity. (g) Bridging between benzene nuclei by oxygen or nitrogen tends only to slight increases in toxicity. (h) The central carbon atom can be replaced by nitrogen, the diphenyl-ammonium compounds were, however, somewhat less active than the triphenylmethane dyes. (i) Some dyestuffs, e.g., Eriogreen S.F.L., Erichrome Azural and Erichrome Cyanine, and Tetraethyl-p-amino-p-sulphonate stimulated the growth of the hyphae of *Fusarium culmorum* and hastened sporulation, an observation which has aroused interest.

Biological work

The means of providing a range of insects suitable for use as test-subjects were further and more extensively studied. The object is one of importance since the range has to be wide enough to enable us to ascertain the degree of specificity of an insecticide, if any. Moreover, as many species as possible should be available throughout the year. Insects successfully reared were *Macrosiphoniella sanborni*, *Aphis* (*Doralis*) *fabae*, *Macrosiphum solanifolii*, *Macrosiphum pisi* amongst the aphides; *Plutella maculipennis*, *Euxoa segetum*, *Tortrix promubana*, *Orgyia antiqua* among the lepidopterous insects; and *Otiorrhynchus sulcatus*, *Ceutorrhynchus quadrimaculatus* and *Phaedon cockleariae* among the coleoptera. Such insects were devoted to a number of investigations, among them the following:

1. The elaboration of apparatus and technique for the study and evaluation of insect poisons. Thus the apparatus for testing contact poisons was extensively used for examining new insecticides against insects and their eggs. But where it was inapplicable—as for example with red-spider eggs—a dipping technique was evolved and used. An investigation of methods for testing stomach poisons was commenced—a seriously felt want, but difficult to meet—and considerable progress was made during the year.

2. *Work on the environmental factors affecting the susceptibility of insects to various insecticides.* The effect of the host plant and the physical conditions ruling before, during and after spraying were examined as well as the relative susceptibility of a number of species to different insecticides. It was clear that certain elaborate apparatus and equipment would be needed for the adequate prosecution of these researches, including constant temperature and humidity chambers and rooms with facilities for rearing, spraying and storing insects and their plant hosts under controlled conditions.

3. *Ovicidal Tests.* Red-spider eggs on apple twigs and the dipping technique with temperature accurately controlled were used. The twigs were dipped for a definite time in thermostatically controlled reservoirs containing the insecticide at appropriate concentrations. When dry the eggs in marked-out areas were immediately counted, and again some months later. Thus the unhatched eggs were deter-

mined. 3 : 5 dinitro-*o*-cresol was toxic to the eggs both in benzene and heavy petroleum oil, but the most interesting effect observed was that the susceptibility of the eggs was greater in May than February: pyrethrum, for example, not normally regarded as ovicidal, had little effect at the earlier, but was definitely toxic at the later, date.

4. *Indigeneous Plants*. A number of these received from the Royal Botanic Gardens, Kew, occupied a good deal of our attention. A few with suggestive names like Flea-bane showed some slight effect as contact insecticides to aphides—but only one, *Euonymus europaeus*, the Spindle or Louseberry was worth extended investigation. The fruits of this plant had some toxicity, but were not anything like as toxic as pyrethrum flowers, readily grown, but not indigeneous to this country.

5. *Ad hoc* investigations were referred to the department, among which the most important was a preliminary survey of the insecticidal potentialities of D.D.T. to horticultural and agricultural insect pests. A report was sent to the Ministry of Agriculture and the Agricultural Research Council.

This department had very early contacts with D.D.T. and had directed the attention of the appropriate authority to its reputation in Switzerland for louse control. On this side its use was developed by Professor Buxton and others to such good purpose that all but experimental supplies were appropriated for war service. Beyond reports sent out by Messrs. Geigy of Basle, little was known of its range of usefulness in agricultural and horticultural fields. This investigation was now undertaken. D.D.T. was, therefore, tested in the laboratory on the following natural orders; Lepidoptera (larvae, five), Hymenoptera (fully grown larvae, two), Coleoptera (adults, two), Rhyncota (apterous viviparous females, five species).

It was clear that to the larvae of the Lepidoptera such as the cabbage-white butterfly, diamond-back moth, tortoiseshell, vapourer moth, D.D.T. was a powerful insecticide. It was less effective against the lesser wax moth and to this insect its toxic action was of a very slow and delayed type. Larvae of such Hymenopterous insects as Solomon-seal sawfly, (*Phymatocera aterrima*) and the social-willow sawfly (*Pteronides pavidus*) were susceptible. D.D.T. was much more toxic than nicotine to the first and comparable with the pyrethrins to the second. To the Coleoptera *Tribolium castaneum* and *Oryzaephilus surinamensis*, both stored-products pests, D.D.T. had a high potency. Applied in heavy oils to the adults of *T. castaneum* it proved effective.

To *Thrips tabaci* on vegetable marrow, applied at 0.1 per cent. w/v, D.D.T. was effective as a control measure but there was some indication of foliage damage.

In the case of the natural order Rhyncota, apterous viviparous females of the aphides *Macrosiphoniella sanborni*, *Macrosiphum urticae*, *Macrosiphum pisi*, *M. solanifolii* and the winged nymphs of *Pterochlorus saligna* were used. D.D.T. did not prove as toxic as was expected from analogy with its powerful effects upon the body louse and some other insect pests. Some difficulty was met in preventing the aggregation of crystal particles when solutions of D.D.T. in water-soluble organic solvents were added to water

containing a wetter and spreader, and although some improvement was obtained when a proprietary dispersing agent of the firm of Messrs. Geigy was used, further difficulty was met in comparative trials in that this product seemed if anything to antagonise the toxic action of the pyrethrins and rotenone: the latter were, therefore, dispersed in an acetone-sulphonated lorol water medium. Quantitative experiments showed D.D.T. to be of the same order of toxicity as nicotine to *M. pisi*, *M. urticae*, *M. solanifolii* and less toxic to *P. saligna*. Using the last named insect as a test-subject it was not in the same category as a contact insecticide with either rotenone or the pyrethrins. The delayed action of D.D.T.—it requires two to three days for its full effect upon certain aphides to be shown—was a complicating factor, since extensive reproduction proceeds even with partially paralysed insects; thus it is probable that in the field it might prove even less successful against such resistant species as *M. solanifolii* (potato aphid), *P. saligna* (willow aphid) and *Aphis fabae* (bean aphid).

Dermatitis factor in pyrethrum. Since Martin and Hester had shown (p. 210) that the pyrethrins were not the causative factors of the serious dermatitis effects in susceptible people, attempts were made to free pyrethrum extracts of the serious effects. Mr. Lord carried out as a matter of urgency a number of extraction experiments with some degree of success. Trials made at the School of Hygiene and by the R.A.M.C., at Aldershot showed that his preparations were much less irritant than normal ones. The work was not proceeded with as D.D.T. began to replace pyrethrum for certain work and synthetic organic compounds took its place as a mosquito repellent.

PERIOD 1943-44

Early in 1944 a sub-committee of an Insecticides Conference, called by the Agricultural Research Council, asked us to undertake together with Long Ashton and East Malling Research Stations both laboratory and field work on the possibilities of D.D.T. for controlling horticultural and market-garden pests, particularly the latter. A great deal of the time of the laboratory was consumed on it. The work we were able to do can be divided under three headings;—

- (a) Laboratory tests for the determination of the relative toxicities of D.D.T. in different media.
- (b) Laboratory tests to compare the toxicities of D.D.T. and other insecticides to a number of species of insects.
- (c) Trials to ascertain the persistence of D.D.T. under weathering.
- (d) Field trials to ascertain the value of D.D.T. as a control measure for certain pests affecting farm and market-garden crops.

(a) *Laboratory tests on the effect of media on the toxicity of D.D.T.*

This work arose from the fact that in 1943 D.D.T. was found not to have any outstanding toxic action to aphides and it was suggested that the effect of the medium might be a deciding factor. Dr. H. Martin of Long Ashton Research Station prepared two concentrates of D.D.T. using different media, (a) in which it was dissolved in acetone carbitol and dispersed as suspensoid on dilution with water containing sulphonated lorol T.A. as a wetter, (b) in

which a benzene solution containing also an organic emulsifier (cyclohexylamine dodecyl sulphate) was dispersed as an emulsion when diluted with water.

Crystal-aggregation difficulties were met with in the higher concentrations of (a), but these were partially overcome by speedy operation of the spraying technique. The slowness of toxic action, however, was not surmounted and this necessitated a careful selection of test-subjects sufficiently hardy not to be affected by the basal media and of a sufficiently long natural life-period to permit of the determination of the toxic action of the slow-acting chemical compound. The results obtained indicated that D.D.T. was a more effective insecticide to the aphid *Macrosiphum solanifolii* when applied as an emulsified solution in a water-insoluble organic liquid (benzene) than as a suspensoid set free from a water-soluble organic liquid.

(b) *Comparative laboratory trials with D.D.T., nicotine, rotenone and pyrethrins to a number of insects*

Comparisons as contact insecticides made at L.D. 50* gave the following results as relative toxicities ;

	Nicotine	Rotenone	Pyrethrins	D.D.T.
<i>Myzus cerasi</i> (Cherry aphid) ..	1	48	—	1
<i>Phaedon cochleariae</i> (Mustard beetle adult)	1	102	—	16.4
<i>Pieris brassicae</i> (cabbage-white butterfly larvae)	1	—	25-26	6.2
<i>Phymatocera aterrima</i> (Solomon-seal sawfly larvae)	1	500-1,000 (approx.)	500-1,000 (approx.)	20-40 (approx.)
<i>Pteronius ribesii</i> (Gooseberry sawfly larvae)	1	450	—	11
<i>Cheimatobia brumata</i> (Winter moth larvae)	1	—	—	114

The larvae of the small ermine moth were relatively highly resistant to both D.D.T. and nicotine. To the bean aphid D.D.T. was somewhat less toxic than nicotine.

Comparative trials were carried out with both lead arsenate and D.D.T. as stomach poisons to the larvae of *Plutella maculipennis*. It was found that at the median lethal dose D.D.T. was approximately ten times as toxic as lead arsenate.

Tested as a spray on *Plutella* eggs it was found that at a concentration of 0.25 per cent. there was a definite toxic action and although the larvae developed normally in the egg, only 47.66 per cent. emerged successfully.

(c) *The persistence of D.D.T. under weathering*

Three series of tests were undertaken in which cabbage plants were both sprayed and dusted with D.D.T. One half were kept in a covered insectary and the remainder were subjected to weathering for different periods. 1.9 inches of rain fell in the 28 days of the experiment. The effects were tested in the following ways: (i) The plants, weathered and unweathered, together with unsprayed controls were enclosed with the moths of *Plutella maculipennis*.

* The concentration giving a kill of 50 per cent.

The moths were not inhibited from egg-laying. Many small larvae were lost, but it was clear that although some loss of toxicity occurred after two to three weeks there was still a marked lethal effect after three or four weeks. (ii) By placing on each of the treated and untreated cabbages 10 mature and 10 young larvae. Marked toxicity and repellency persisted for four weeks but with some decline in the weathered plants after two weeks. The mature larvae were the more resistant. (iii) By placing portions cut from the leaves in closed dishes with 5 mature and 5 small larvae. The mature larvae were not materially affected by the foliage of plants exposed to weathering for four weeks, among the small ones a few were still affected, but there was a definite decline in toxicity in the case of the sprayed and exposed plants after three weeks' exposure.

The growth of new foliage in these experiments seriously affected the protective power, and a critical series of stomach-poison tests in Way's apparatus, while confirming that some insecticidal action persisted after three weeks of weathering, showed that the inner (new) leaves had no toxic effect after 14 and 31 days. The decline in toxic activity of the treated cabbages with time does not appear to depend entirely on rainfall. Whether there is absorption of D.D.T. or loss by volatility in addition to attenuation by leaf growth requires investigation. It is clear that for some little time protection against caterpillars is afforded by D.D.T. when sprayed or dusted on to foliage, even when exposed to relatively heavy rainfall.

(d) *Field trials*

By arrangement with Dr. Mann a number of experiments with D.D.T. were carried out on vegetable plots at Woburn, others were arranged at outside centres. Dr. C. Potter and Mr. J. F. Perkins of the Plant Pathological Laboratory of the Ministry of Agriculture were responsible for the application of the dusts and sprays and for the determination of the effects. The experiments covered a variety of both crops and pests and the information obtained, although not based on statistical examination, was very comprehensive. There was very marked evidence that, so far as farm and market-garden pests are concerned, D.D.T. is very selective in its action. It is not a cure-all. Thus whereas cabbage caterpillars of all kinds were susceptible and readily controlled by it the cabbage aphid proved more resistant. Mustard beetle (*Phaedon cochleariae*) and pollen beetles (*Meligethes* spp.) were controlled by D.D.T., but the weevil *Ceutorrhynchus assimilis* was not affected. Good control of collar aphides and good protection against carrot fly (*Psila rosae*) were obtained on carrots. Good protection against bean weevil (*Sitona* spp.) was obtained, but poor control of bean aphid (*Aphis fabae*) was achieved by D.D.T. *Aphis sambuci* on elder was, however, susceptible. With the glasshouse red spider (*Tetranychus telarius*) there was neither any control nor any protection: the brown scale (*Lecanium corni*) seemed equally resistant. Among insect parasites and predators Coccinellid adults and numerous parasitic Hymenoptera were killed, but Syrphid larvae (Hover fly) were unaffected. Egg-laying by *Pieris rapae* (small cabbage-white butterfly) was not prevented.

Plant insecticides

We received a small consignment of Chinese plants from Professor Chiu of the Sun Yat Sen University through Dr. Needham, of the British Council. The work was undertaken with the approval of the Secretary of the Agricultural Research Council (the late Dr. Topley). The correct nomenclature of all but one of the plants, which was in powder form, was confirmed at Kew, through the kindness of Sir E. J. Salisbury. The samples were; *Rhododendrum molle* (flowers and root), *Milletia pachycarpa* (root and seed), *Tripterygium exesum* (root—bark powder), *Chrysanthemum cinerariifolium* (pyrethrum flowers), *Derris fordii* (root). The samples were too small for us to do more than preliminary sorting-out tests, but we were able to carry out a sufficient number of tests to indicate which of the plants were worth further examination and detailed study. These proved to be *Milletia pachycarpa* (both root and seed), and *Derris fordii* (root), both containing principles of the rotenone class. They would, however, need much improvement by selection to compete in the world's markets with derris and lonchocarpus roots of pre-war standard. *Tripterygium exesum*, despite a certain reputation as a Chinese insecticide, proved disappointing, but as we learned from Sir E. J. Salisbury that a plant or two of *T. wilfordii*, probably the correct title for this plant, are kept at Kew, if anything further of interest arises in connection with it, a small supply is available. The pyrethrum was of medium quality: it is not a Chinese plant, but in view of the world shortage of this insecticide and a possible need in India and Burma its increased production might be worthwhile. It contained 0.44 per cent. Pyr.I and 0.37 per cent. Pyr.II 0.8 per cent. total pyrethrins against 1.3 to 1.6 per cent. for Kenya material.

Effect of environment on the susceptibility of insects to insecticides

Owing to pressure of other work little opportunity for carrying on this work intensively presented itself. But using the pyrethrins, lauryl thiocyanate, nicotine and dinitro-*o*-cresol, D.D.T. and Wakefield white oil against the test-subject *Tribolium castaneum* Hbst., it was shown that while a short period of cooling before spraying had little or no effect on this insect's resistance, cool conditions after treatment increased the toxicity of all the poisons except petroleum oil, in which case there was some increase in insecticidal potency with rise of temperature. Trials were carried out in both oil and aqueous media and although, in the absence of facilities for controlling both temperature and humidity, no very definite differentiations between the effect of these two factors could be made, it was clear that conditions after treatment had an important effect on both the absolute and relative toxicity of contact poisons, and that if any advance is to be made in this important subject a more detailed investigation will have to be made, in the course of which relative humidity as well as temperature will have to be brought into the picture.

Laboratory methods for testing insecticides

Investigations of techniques suitable for evaluating stomach poisons.
After devising small wooden frames in which larvae could be con-

fined with a strip leaf, but in such a way that only the mouth parts of the insect could be brought into contact with its food and any poisons associated with it, attention was given to means for depositing on the food, as evenly as possible, a layer of poison. This was accomplished by means of a tall spray tower, into which the insecticide was atomised. After an interval of time during which the larger droplets were deposited, a shutter was pulled out and the deposit of fine droplets (settling mist) fell on the strips of foliage underneath. The amount of deposit per unit area was determined by the increases in weight of strips of aluminium of known area and weight placed on the stage with the foliage. The treated strips of leaf were cut into sections of known area and presented to the larvae, previously weighed, in the apparatus described. The area of leaf eaten by the larvae was determined by inspection under the microscope, and the insects were set aside in tubes in a constant temperature and humidity chamber for a period of time. The number dying and pupating was determined and correlated with the dosage per body weight. Using the instrument Mr. Way estimated that D.D.T. is about ten times as toxic as lead arsenate to *Plutella maculipennis*.

At intervals during the year a preliminary study was made of a laboratory dusting technique. A certain amount of progress has been made, but it is clear that the uniformity of the deposit and its replication will not be very easily achieved. Modifications in the technique may have to be introduced, depending on whether foliage or insect has to be dusted for use in evaluating contact or stomach-poison dusts.

In both of the above methods it is clear that particle size, and probably shape, are of considerable importance, and although no serious experimental attention has been given here to this matter up to now, it is clear that for substantial progress to be made the investigation of this important subject will require one research worker's individual attention.

Provision of insects

A considerable number of insects of various species have been raised during the year. This has meant the provision of large numbers of plants. Aphides of different species proved to be somewhat difficult to rear in anything like the numbers required owing to the presence and persistence of predators. Attention, however, has been continuously given to the possibilities as test-subjects of a variety of insects in the hope that additions could be made to our growing list. Standardised rearing under electrical fluorescent lighting is being actively experimented with.

Chemical investigations

During the year 1943-44 it was shown in America that the factor used for determining pyrethrin I in pyrethrum flowers and extracts was seriously in error. This was so grave an issue to us and other insecticide workers in this country that we approached the Imperial Institute with the proposal that a conference of workers actively engaged on pyrethrum analysis should be called to consider the matter. This was done in the early

part of 1944 and a programme of work was drawn up in which the four separate laboratories, the Imperial Institute, Messrs. Stafford Allen's, University College, Southampton (Dr. Harper), and Rothamsted should independently determine the factor from a sample of the pure pyrethrin monocarboxylic acid supplied by Messrs. Stafford Allen, who had a stock in hand. The material was distributed in the form of a standard solution by the Imperial Institute. Sufficient evidence was obtained to show that the original factor proposed by Wilcoxon was some 30 per cent. in error, but a statistical analysis carried out by the Rothamsted Statistical Department demonstrated that the discrepancies between the figures obtained by the individual co-operating laboratories were so great as hardly to warrant the publication of a new factor based upon them. It was decided at a second conference at Rothamsted to repeat the co-operative experiment, and a very carefully drawn-up code of regulations for carrying out the method was drawn up.

PERIOD 1944-45

Evaluation of pyrethrin-I factor.—The programme of investigations arranged for at the conference held in the previous year was carried out by the co-operating laboratories, and the results examined by Mr. Finney of the Rothamsted Statistical Department. His finding that, despite some discrepancy, the results were in sufficiently good agreement to warrant a conference to decide upon the factor to be used, led to a further meeting at the Imperial Institute in July, 1945. After a prolonged discussion it was decided to fix the factor for routine analysis at 1 c.c. of N/100 KIO_3 solution = 5.7 mg. of pyrethrin I at normal laboratory temperatures. As, however, there was very distinct evidence of a temperature coefficient, for research and special work the temperature gradient should be taken into account.

The effect of media on toxicity.—For some years intermittent work upon the relationship of media, in which an insecticide is incorporated and applied, to its toxicity has been the subject of research. Obviously particle size in both dusts and sprays may be a correlative factor, and from this angle investigations have once more been undertaken. As D.D.T. is a potent insecticide which can be obtained pure and in definite crystalline forms, it is being used, in the first instance, as the test insecticide. A dipping method has been worked out in which the insects are subjected to the action of the test fluid by end-over-end shaking at constant temperature, but not afterwards handled. D.D.T. by appropriate means can be prepared in two distinct crystalline forms and indications have been obtained that the needle-shaped are more toxic than the plate-like crystals.

Investigations on the insecticidal value of D.D.T. (2, 2-bis-p.chlorophenyl 1, 1, 1-trichloroethane)

The greater part of the work of the Department was again concerned with the insecticidal value of this compound and its analogues—both in the field and laboratory.

Fieldwork

Field trials—particularly on cabbage pests—of a more thorough nature than those of the previous year were allocated to us by the sub-committee of the A.R.C. Insecticides Conference. Through the kindness of Dr. Mann plots of cabbages, of both autumn and winter varieties, were handed over to our Department for plot experiments. Unfortunately, no infestation occurred, despite the presence of cabbage caterpillars in nearby gardens. Large plots, however, laid down at the Oaklands Farm Institute, St. Albans gave a clear verdict that D.D.T. exercises a high degree of control of cabbage caterpillars. The cabbage aphid (*Brevicoryne brassicae*) is only controlled by relatively high concentrations of D.D.T. which, owing to its toxic effect on parasites and predators, may possibly encourage this pest. The control of woodlice by D.D.T. was demonstrated at Woburn Abbey.

Laboratory investigations

Chemical determination of D.D.T.—For many researches on D.D.T. the determination of the amounts present is very important. Various colorimetric techniques have been suggested, but none have been so far employed in our work, since various other factors upset their accuracy. Resort had to be made both on a macro- and micro-scale to the Volhard technique for the determination of chlorine ions after their liberation by the action of alcoholic alkali from D.D.T., one chlorine ion being liberated per molecule. The macro-method has been employed to ascertain the stability of D.D.T. in the presence of a variety of chemical compounds, the micro-technique is now being used to determine the amounts of D.D.T. on foliage after the lapse of different periods of time.

Stability of D.D.T.—A knowledge of this is required if D.D.T. is to be incorporated with certain media or other insecticidal principles. It seems to be quite stable in most of the common organic solvents, and with many wetters and spreaders. Stability, however, depends chiefly on the pH of the medium, and it was carefully studied for alkalinities rising from 7 to 10. Even at so low an alkalinity as pH 7.1 there is some decomposition after 23 days, at pH 8.0 one-half, and at pH 9.10 nearly the whole, is decomposed in that time, but there is a large measure of stability over a period of 2 days for pH's ranging from 7 to 11. The organic solvent used has an important bearing on the speed and certain metals, iron, manganese and copper, catalyse decomposition. A basic insecticide like nicotine and a number of amines give rise to decomposition like other alkalies to $(\text{Cl C}_6\text{H}_4)_2\text{C}=\text{CCl}_2$ which has a low insecticidal value.

Insecticidal values of the analogues of D.D.T.—A number of chemical compounds closely related to D.D.T. have been examined quantitatively in the laboratory to determine their insecticidal potency relative to D.D.T. Fluorine, bromine and iodine have been substituted in the benzene ring and combined with chloral to give compounds analogous to D.D.T., a number of chlorine compounds of varying complexity, with the halogen in the ethane bridge and in addition a number of alkyloxy benzene tri-chloroethanes have been examined, as has the hydrocarbon corresponding to D.D.T.

The relative toxicities appear to depend somewhat upon the insect used as test-subject, but it is clear that among the halides D.D.T., the chlorine compound, is the most toxic, but the bromophenyl tri-chloroethane is close to it. Differences, however, in the slope of the probit-characteristic lines make the usual methods of comparative assessment invalid. The steepness of slopes seems to be inversely correlated with molecular weight for compounds of the same molecule type; very exceptional and at present inexplicable results have been obtained with the iodine compound, iodo benzene trichloroethane. The methoxy and ethoxy benzene trichloroethanes are relatively powerful insecticides, but the propoxy compound is less so, while the butoxy and amyloxy compounds are practically devoid of insecticidal action.

The effect of leaf growth upon the toxicity of D.D.T.—As a protective insecticide D.D.T. has a considerable reputation, it is therefore of some importance to know how its toxicity persists on foliage. In 1944 it was shown that despite leaf growth and weathering D.D.T. was still relatively potent upon cabbage after some three weeks. A number of experiments with mosquitoes (a sensitive insect) have introduced a variety of queries as to the fate of D.D.T. sprayed upon leaf surfaces. Experiments were therefore carried out in the glasshouse, in which the leaf growth of cabbages and the toxicity of D.D.T.-sprayed leaves at various stages of growth were determined. Chemical determination of the deposits upon the foliage is still required for completeness, but from the experiments to date the growth of the cabbage leaf, when protected from the weather, has a less depreciative effect upon toxicity than one would expect. If anything, toxicity appears to be enhanced. This may possibly be due to the waxes associated with brassicae foliage.

Effect of particle size and shape upon toxicity.—The cabbage caterpillar *Plutella maculipennis* was used as in the previous experiment to test these factors. It was found that the needle-shaped crystals ($100 \times 2 \mu$) were more effective than the plate-like crystals ($40 \times 8 \mu$) and that when D.D.T. was applied emulsified in olive or heavy-petroleum oil its toxicity was materially increased.

Experiments on the effect of D.D.T. on entomophagous insects.—Some concern has been expressed concerning the possible ill effects upon beneficial insects, parasites, and predators. Field observations in 1944 had shown that the results varied with the species, and laboratory experiments were therefore undertaken particularly with those entomophagous insects likely to be of importance in the control of cabbage pests, the aphid *Brevicoryne brassicae* and various caterpillars. Cabbages sprayed and dusted with D.D.T. preparations were enclosed in cages with the appropriate insect. In general, the insecticide exerted very little repellency and the final results of day-to-day examinations were;—

Various Syrphidae mainly *Syrphus ribesii*, adults.

	D.D.T.	Killed
Spray suspension ..	0.01%	7%
Spray suspension ..	0.1%	100%
Commercial spray ..	0.2%	21%
Commercial dust ..	5.0%	71%

Although the data are incomplete, little effect on the Syrphid larvae seems to have been observed, but all the *Eristalis* adults

were dead in two days. There is thus some reason for believing that although high concentrations of D.D.T. will exercise some control of *B. brassicae*, the cabbage aphid, if an attack of such predators as the above has already well developed, spraying and dusting with D.D.T. against this pest might well do harm.

In the case of the lady-bird species of Coccinellidae, the suspension of pure D.D.T. had only a relatively low toxic effect, but the commercial dust and spray were highly toxic. The hymenopterous parasites of *B. brassicae*, mainly adults of *Aphidius brevicornis*, were relatively susceptible, particularly to the suspension of pure D.D.T. micro-crystals, and the adults of the parasite of *Pieris brassicae*, *Apanteles glomeratus*, were completely wiped out by all the D.D.T. preparations, even at low concentrations. Thus there is some risk as far as cabbage pests are concerned that the partial control exercised by natural insect enemies might be interfered with.

Effect of D.D.T. on plants.—The phytocidal effect, if any, of an insecticide may seriously limit its usefulness, such an effect may also be accentuated by the medium in which it is incorporated and thus restrict means of application. In view of this a number of trials were made upon a few selected market-garden plants. A carefully devised series of experiments on tomatoes showed that this plant was relatively resistant although some foliage scorch occurred and an apparent drop in yield after the application of a commercial spray, but no effect on size of fruit, ripening or flavour was observed. Cabbages were quite immune, and hearted normally, but if dipped in an emulsion containing benzene showed severe scorching although the yield was normal.

Cucumbers and vegetable marrows showed definite injury. In the case of the cucumbers there was scorch, and with the marrows leaf curl, and both showed interveinal yellowing. Cucumbers, however, appeared to yield normally. Lettuces, radishes, turnips, peas, runner beans, French beans, broad beans and carrots showed no injury.

Toxicity to bees.—Conjoint work with the Bee Department indicated that apparently the effect of D.D.T. upon bees varies with the conditions and mode of application; as a contact poison D.D.T. is fairly highly toxic, but its stomach action is less marked than was to be expected, nor does it appear to be highly injurious to foragers on sprayed blossoms, but as no information was obtained as to effects on bee larvae, the status of D.D.T. as a bee poison needs establishing by further work.

PUBLICATIONS

1. MARTIN, J. T., MANN, H. H. and TATTERSFIELD, F. 1939. *The manurial requirements of pyrethrum (Chrysanthemum cinerariaefolium Trev.)*. Ann. Appl. Biol., 26, 14-24.

A small field experiment upon the manurial requirements of the insecticidal pyrethrum plant, grown upon sandy soil of low fertility, is described. Lime had the effect of producing slight, but not significant increases each year in the yield of flowers and their content of the pyrethrins, and decreased the percentages of plant failures in the fourth and fifth years of the experiment. There was a significant depression in the yield of flowers in the year after the single application of double dressings of the manures, but no effect

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in later years. The yearly application of moderate dressings of manures gave significant increases in the yield of flowers in the second and fifth years, and significant increases in the pyrethrin I content of the flowers in the fourth and fifth years of the experiment.

2. TATTERSFIELD, F. 1939. *Biological methods of testing insecticides*. Ann. Appl. Biol., **26**, 365-384.

A detailed review of the biological methods used in the quantitative laboratory evaluation of insecticides. Contact insecticides, stomach poisons and fumigants are briefly dealt with and some consideration is given to the methods of assessing results. A bibliography of 87 references is appended.

3. MARTIN, J. T. and TATTERSFIELD, F. 1939. *The trend of progress— insecticides*. Chem. Ind., **17**, 635-640.

A contribution to the Symposium on the Trend of Progress at the Annual Meeting of the Society of Chemical Industry, 1939. A brief review is given of the work carried out in the several fields of research, including stomach poisons both inorganic and organic, contact insecticides of vegetable origin or derived by synthesis. Recent work on the moth-proofing of fabrics is briefly dealt with. An outline is given of present tendencies in research.

4. MARTIN, J. T. 1939. *Agricultural insecticides*. Manufacturing Chemist, **10**, 41-46.

A general account is given of the sources of supply and chemistry of the species of Derris and Pyrethrum now widely used as insecticides. Instances of the successful use of these materials against specific pests of crops and livestock are cited.

5. HARPER, S. H. 1939. *The active principles of leguminous fish-poison plants. Part I. The properties of l-a-toxicarol isolated from Derris malaccensis Kinta-type*. J. Chem. Soc., 812-816.

The optically active precursor of toxicarol is obtained by direct crystallisation of an ethereal extract of *D. malaccensis* (Kinta-type). After freeing from a small proportion of sumatrol by the method of Cahn, Phipers, and Boam (1938), the *l-a*-toxicarol was identical in properties with that described by Tattersfield and Martin (1937). It is concluded that the optical data of Cahn, Phipers, and Boam are incorrect, and their criticism of Tattersfield and Martin unjustified.

- 6(a) HARPER, S. H. 1939. *The precursor of Buckley's compound*. Chem. Ind. **58**, 292. A letter announcing discovery.

- 6(b) HARPER, S. H. 1939. *The active principles of leguminous fish-poison plants. Part II. The isolation of l-elliptone from Derris elliptica*. J. Chem. Soc., 1099-1105.

An optically active substance, *l*-elliptone isolated from the neutral rotenone-free resin from *D. elliptica* (var. Sarawak creeping), is shown to be the precursor in the resin of Buckley's substance. From a study of its reactions and by comparison of these with the reactions of isorotenone, a structure is suggested for elliptone.

7. HARPER, S. H. 1939. *The active principles of leguminous fish-poison plants. Part III. The structure of elliptone*. J. Chem. Soc., 1424-1427.

The structure advanced for elliptone in Part II is placed beyond doubt by degradation experiments. Dehydroelliptone with zinc and alkali yields elliptic acid, oxidation of which with alkaline hydrogen peroxide gives derric acid. This establishes the identity of rings A, B, and C with those of rotenone and iso-rotenone. *l*-Elliptone with alcoholic alkali gives 4-hydroxycoumarone-5-carboxylic acid, whereas iso-rotenone under the same conditions gives 4-hydroxy-2-isopropylcoumarone 5-carboxylic acid (isotubaic acid). This establishes the nature of rings D and E and hence that of the whole molecule.

8. TATTERSFIELD, F. and POTTER, C. 1940. *The insecticidal properties of certain species of Annona and of an Indian strain of Mundulea sericea (Supli)*. Ann. Appl. Biol., 27, 262-273.

Laboratory insecticidal trials, made with several species of *Annona*, *A. muricata*, *A. palustris*, *A. reticulata*, *A. squamosa*, show that plants of this genus possess contact insecticidal properties to aphides. *A. reticulata* was the most potent of those tested. Seed, root, leaf and to a less extent the stem of this species possessed contact insecticidal properties. The seed of *A. muricata* and the leaves of *A. squamosa* and *palustris* also showed contact toxicity, but were not so potent as *A. reticulata*. The seed of *A. squamosa* was not available for testing purposes. None of the above are superior to the leaf, root and bark of *Mundulea sericea* Wild. of the variety known in India as 'Supli' and are much less toxic than the richer specimens of *Derris elliptica* root such as Changi No. 3.

The toxic action of the *Annona* spp. appears to be rather specific. Aphides are susceptible, but *Oryzaephilus surinamensis* L., which is susceptible to the rotenone class of insecticides, is resistant.

Tests made with extracts of a variety of *Mundulea sericea* Wild. from India showed that leaf, bark and root possessed marked insecticidal properties when tested on *Macrosiphoniella sanborni*, and *Oryzaephilus surinamensis*. This is in contrast with the African variety, the leaf and root of which are reported as distinctly less potent than the bark. The toxicity of none of these parts is of the same order as that of the root of *Derris elliptica*, Changi No. 3.

9. MARTIN, J. T. 1940. *The problem of the evaluation of rotenone-containing plants. V. The relative toxicity of different species of derris*. Ann. Appl. Biol., 27, 274-294.

The relative toxicities to *Aphis rumicis* of different species and varieties of derris root have been determined. A *Derris elliptica*, Changi root, was two and one-half times, a *D. malaccensis*, Sarawakensis root, was one and three-quarter times, and a *D. elliptica*, Sarawak-creeping root, was one and one-quarter times as toxic as a *D. malaccensis*, Kinta, root.

Preliminary trials showed that the relative toxicities of three of the roots to a stored product insect, *Oryzaephilus surinamensis*, were of a similar order. All toxicity data have been subjected to statistical analysis.

Various methods suggested for the chemical evaluation of Derris have been examined and discussed. Methods based upon optical rotation values have been shown to fail in the evaluation of roots and resins of different species, while the method of Jones and Smith (1936), in which a definite toxic value is given to the non-rotenone fraction of the derris extract, has been found to be inadequate when applied to the roots under consideration.

The determination of the percentage "rotenone equivalent" values of the roots, based upon the alkaline fractionation of the resins and the toxicities of the deguelin and toxicarol fractions relative to that of rotenone, has given a reasonably close estimate of the toxicities of the derris roots examined. The application of the method to the assessment of the toxic value of derris resins is described.

10. HARPER, S. H. 1940. *The active principles of leguminous fish-poison plants. Part IV. The isolation of malaccol, from Derris malaccensis*. J. Chem. Soc., 309-314.

An optically active phenol, malaccol, isolated from an ethereal extract of the root of *Derris malaccensis* (Kinta type) is shown to have the formula $C_{20}H_{16}O_7$. From a study of its reaction and by comparison of these with the reactions of sumatrol and of elliptone, the structure 15-hydroxyelliptone, is suggested for malaccol.

11. HARPER, S. H. 1940. *The active principles of leguminous fish-poison plants. Part V. Derris malaccensis and Tephrosia toxicaria*. J. Chem. Soc., 1178-1184.

The resin from *D. malaccensis* root has been fractionated by chemical means and *l-a*-toxicarol obtained in a pure condition. In addition, rotenone, elliptone, deguelin, malaccol, sumatrol, and a new phenol have been isolated. The properties of this phenol, which is isomeric with toxicarol, are discussed and as a working hypothesis an isoflavone structure is suggested.

12. TATTERSFIELD, F., MARTIN, J. T. and HOWES, F. N. 1940. *Some fish-poison plants and their insecticidal properties*. Kew Bull. Misc. Inf., No. 5, 169.

The distribution, native uses, and contact insecticidal properties of a number of fish-poisoning plants are described. None is of the same order of effectiveness as *Derris elliptica*. The most interesting from an insecticidal point of view are a vine from the British Solomon Islands, indistinguishable from *Derris trifoliata*, the leaves of which are toxic, *Dolichos pseudopachyrhizus* from Kenya, *Milletia pachycarpa*, from India, *Tephrosia macropoda* from Natal and *T. vogelii* from Uganda.

13. MARTIN, J. T. and HESTER, K. H. C. 1941. *Dermatitis caused by insecticidal pyrethrum flowers* (*Chrysanthemum cinerariifolium*). Brit. J. Dermatology and Syphilis, **53**, 127-142.

Patch tests of powdered and dissected pyrethrum flowers have been carried out. The causal agent, or agents, of the dermatitis appears to be concentrated in the lower parts of the flower-head. Powdered flowers moistened with water gave a more intense reaction than the air-dried ground flowers. Pollen separated from the flowers gave only a slight reaction.

Ether and petroleum ether extracts of the flowers have been fractionated and a number of products tested for their ability to produce dermatitis. A colourless extract, obtained by petroleum ether extraction of the flowers mixed with charcoal, gave an intense reaction. The pyrethrins, tested in a concentrate containing 93 per cent. of apparent pyrethrins as determined by a modified Seil method, are shown to be devoid of dermatitis-producing properties.

The volatile oil, obtained by steam distillation of powdered flowers, is shown to be highly active. The oil after the extraction of a volatile acid fraction with alkali gave a less intense reaction, while the steam-distilled flowers also gave a positive reaction. The possibility that crystalline constituents of the petroleum ether extract of the flowers are contributory causal agents of the dermatitis is indicated.

14. POTTER, C. 1941. *A laboratory spraying apparatus and technique for investigating the action of contact insecticides with some notes on suitable test subjects*. Ann. Appl. Biol., **28**, 142-169.

An outline of the general problem of the evaluation of liquid contact insecticides is given. A summary of the laboratory methods of evaluation already described and the reasons for the adoption of the procedure described are outlined. An account is given of the development of the spraying apparatus together with experiments on factors likely to cause variation in the replication and distribution of the deposit. The apparatus finally adopted is described and data are presented on its physical performance. The selection and rearing of test insects is described. An account is given of the factors in the technique which may cause errors in the determination of the dosage-mortality data, with some experiments. The technique at present adopted is described, with examples of the dosage-mortality curves obtained.

15. MARTIN, J. T. 1941. *The chemical evaluation of pyrethrum flowers* (*Chrysanthemum cinerariifolium*). *The extraction of the flowers for analysis and the preparation of colourless concentrates of the pyrethrins*. J. Agric. Sci., **31**, 178-185.

Biological trials have been carried out to determine the efficacy of petroleum ether as solvent for the extraction of pyrethrum flowers for analysis. Ninety-five per cent of the toxic material was extracted from flowers one year old after only 3 hr. percolation. An extraction period of 8 hr. with petroleum ether is suggested.

A method of preparing colourless extracts of pyrethrum and analytical data for such extracts are given. They are shown to be of value for the preparation of concentrates of the pyrethrins. The preparation of a colourless concentrate containing 93 per cent of total pyrethrins, as determined by a modified Seil method, is described.

16. MARTIN, J. T. 1942. *The problem of the evaluation of rotenone-containing plants. VI. The toxicity of l-elliptone and of poisons applied jointly, with further observations on the rotenone equivalent method of assessing the toxicity of derris root.* Ann. Appl. Biol., **29**, 69-81.

The assessment of toxicity by the determination of the rotenone equivalent has been shown successfully to apply to four of the derris roots examined earlier in this series of investigations. Observations on the stability of the resins in ground roots stored in tins at room temperatures have been recorded. l-Elliptone has been shown to be one-fifth as toxic as rotenone to *Macrosiphoniella sanborni* when tested in an alcohol-saponin medium. The toxicities of poisons applied jointly have been examined. The observed toxicities of mixtures of rotenone with a deguelin concentrate, l-elliptone and l- α -toxicarol have been compared with those predicted from the potencies of the constituent poisons. No significant synergistic or antagonistic effect has been found. The bearing of this upon the validity of the rotenone equivalent method of assessing toxicity has been discussed.

17. COOMBER, H. E., MARTIN, J. T. and HARPER, S. H. 1942. *The determination of rotenone in derris root.* J. Soc. Chem. Ind., **61**, 110-112.

An improved method for estimating rotenone in derris root, employing the carbon tetrachloride complex separation is described. It has been shown by the three groups of workers to give concordant results with various types of root. Methods of determining moisture content and chloroform extract are also given. Their adoption in the United Kingdom as standard methods is recommended.

18. HARPER, S. H. 1942. *The active principles of leguminous fish-poison plants. Part VI. Robustic acid.* J. Chem. Soc., 181.

A substance isolated from the ethereal extract of *Derris robusta* has been shown to be a mono-carboxylic acid, $C_{27}H_{24}O_8$ (less probably $C_{28}H_{26}O_8$) containing two methoxyl groups, to which the name robustic acid has been given. It is probably related to lonchocarpic acid $C_{26}H_{26}O_8$ a mono-carboxylic acid containing one methoxyl group isolated by Jones (J. Amer. Chem. Soc., 1934, **56**, 1,247) from a species of Lonchocarpus, as they both give the same sequence of colour change in the Durham test.

19. HARPER, S. H. 1942. *The active principles of leguminous fish-poison plants. Part VII. The reduction of elliptone.* J. Chem. Soc., 587-593.

The course of the reduction of l and dl-elliptone in acetic acid over platinum oxide catalyst has been elucidated, and the following stages characterised: dihydroelliptone, dihydrodeoxyelliptone, octahydrodeoxyelliptone and perhydroelliptone.

The zinc-alkali reduction of the dehydro-compounds has been shown to give in addition to the acids of the derrisic acid type, phenols analogous to derritol.

Oxidation of elliptone with nitrous acid has given elliptonone, a diketone, whose structure has been established by a partial synthesis from elliptol, the phenol obtained in the zinc-alkali reduction of dehydroelliptone.

Biological trials using chrysanthemum aphid as test insect have shown that l-elliptone is, next to rotenone, the most toxic insecticidal substance to be isolated from Derris resin in an optically active form. In the order of decreasing toxicity; l-elliptone > dl-elliptone > l-dihydroelliptone > dl-dihydroelliptone.

20. HARPER, S. H. 1942. *The active principles of leguminous fish-poison plants. Part VIII. The synthesis of dehydrotetrahydroelliptone and of dehydrotetrahydromalaccol.* J. Chem. Soc., 593-595.

Condensation of methyl-2-cyanomethyl-4; 5-dimethoxyphenoxyacetate with 2-ethylresorcinol by the Hoesch method and subsequent hydrolysis gave rise to tetrahydroelliptic acid. Cyclisation of this acid with hot acetic anhydride and sodium acetate furnished through the acetate dehydrotetrahydroelliptone identical with that prepared from elliptone. The formula established for elliptone by degradative experiments (J., 1939, 1,424) has therefore been fully substantiated.

By an analogous condensation with ethylphloroglucinol, tetrahydromalaccolic acid was prepared. This acid on cyclisation gave dehydrotetrahydromalaccol, for which an angular and a linear formula are possible. It has not proved possible however to compare this product with that derived from natural sources.

21. HARPER, S. H. 1942. *The active principles of leguminous fish-poison plants. Part IX. The synthesis of furanoisoflavones related to rotenone.* J. Chem. Soc., 595-598.

By utilising the method of Venkataraman *et al.* (J., 1934, 513, 1,120, 1,769) isoflavones have been prepared from the methyl ethers of derritol, isoderritol and elliptol. In the case of the last by the isolation of a crystalline intermediate, it is shown that the reaction does not proceed directly to the isoflavone but occurs through the formation of an isoflavanonol and its subsequent dehydration. Isoflavones of this type have been postulated as likely to occur in Derris resin along with rotenone and elliptone, with which they are isomeric.

These isoflavones are remarkable in giving a positive Durham test, previously regarded as specific for the rotenoids. Their reactions have been studied, and a method devised for the detection of the formic acid formed in their hydrolysis. This method has been applied to the "toxicarol isoflavone" isolated from crude toxicarol to establish conclusively its isoflavone nature and hence support for the formula previously suggested (J., 1940, 1,178).

22. COOMBER, H. E., MARTIN, J. T. and HARPER, S. H. 1943. *The determination of rotenone in derris root. A reply.* J. Soc. Chem. Ind., 62, 73-75.

The criticism by Edwards of the method established by the authors for determining rotenone is answered. The position with regard to sampling derris consignments is discussed, and the need for the improvement of sampling methods is shown. Objections to the method of analysis given by Edwards are stated.

23. TATTERSFIELD, F. and POTTER, C. 1943. *Biological methods of determining the insecticidal values of pyrethrum preparations (particularly extracts in heavy oil).* Ann. Appl. Biol., 30, 259-279.

Pyrethrum extracts in non-volatile oil carriers are effective insecticides in the field because they act as a direct spray killing the insect and also form a toxic film over which the insect crawls. It is necessary to study both these effects for a complete laboratory assessment of toxicity. Suitable laboratory techniques and methods for the assessment of results are described together with an account of experiments on the effect of various factors on the insecticidal efficiency of pyrethrum-in-oil preparations, both as direct sprays and as toxic films.

24. MARTIN, J. T. 1943. *The preparation of a standard pyrethrum extract in heavy mineral oil, with observations on the relative toxicities of the pyrethrins in oil and aqueous media.* Ann. Appl. Biol., 30, 293-300.

The preparation of a pyrethrum extract in highly refined heavy mineral oil, suitable for use as a basis of reference in the biological evaluation of commercial pyrethrum-heavy oil preparation, is described. The solution was standardised with respect to colour, resin content and equal proportions of the pyrethrins. A standard solution containing pyrocatechol remained stable, when judged by the chemical determination of the pyrethrins, over a period of months.

When applied in heavy mineral-oil medium, pyrethrin II was shown to possess a toxicity to *Tribolium castaneum* Hbst., approaching, if not equal to, that of pyrethrin I. When applied in an aqueous medium the toxicity of pyrethrin II was very much lower than that of pyrethrin I.

25. POTTER, C. and TATTERSFIELD, F. 1943. *Ovicidal properties of certain insecticides of plant origin. (Nicotine, pyrethrins, derris products.)* Bull. Entom. Res., 34, 225-244.

The pyrethrins, nicotine, rotenone and a derris resin have been tested in the laboratory under standard conditions for their ovicidal effect. The test-subjects were: *Pieris brassicae*, *Plutella maculipennis*, *Aphis rhamni*, *Ephesthia kühniella* and *Sitotroga cerealella*. Notes on the technique of egg production are given for these species. In the majority of the tests made a water medium containing acetone together with sulphonated loral as a wetting agent was used.

It has been shown that all these products are toxic to the species on which they were tested. Lauryl thiocyanate, β -butoxy β -thiocyanodiethyl ether and 3 : 5 dinitro-*o*-cresol were included as substances of recognised ovicidal value for the purposes of comparison.

All the above insecticides derived from plants compared favourably in their toxic action with the synthetics. In two instances during tests on eggs of *Ephesia kühniella*, data were obtained enabling a satisfactory statistical comparison of relative potency to be made. In the first of these the pyrethrins were found to be 8.45 ± 1.47 times as toxic as 3 : 5 dinitro-*o*-cresol, while in the second the pyrethrins were found to be 2.66 ± 0.26 times as toxic as *Derris elliptica* resin (rotenone contents 37-40 per cent.). It therefore appears that both the pyrethrins and the derris resin were more toxic, weight for weight, than 3 : 5 dinitro-*o*-cresol which is recognised as one of the most potent ovicides.

The above vegetable poisons were found to be toxic not only to eggs developing in a few days without a diapause, but also to those of *Aphis rhamni*, a species of overwintering egg. The tests on *A. rhamni* were made at the time the eggs were just starting to hatch. The same result might not have been obtained with eggs at an earlier stage of development.

Observations were made on the effect of the different insecticides on the development of the egg. This effect appeared to differ with each substance, either qualitatively or quantitatively, but within the limits of the experiment it seemed to be independent of the species of the egg.

26. DION, W. M. and LORD, K. A. 1944. *A comparison of the toxicity of certain dyestuffs to the conidia of Fusarium culmorum*. Ann. Appl. Biol., **31**, 221-231.

The toxicity of a number of dyestuffs to the spores of *Fusarium culmorum* and *Cercospora herpotrichoides* was determined by the slide-germination technique. No attempt was made to distinguish between fungistatic and fungicidal activity.

The toxicity of basic dyestuffs was unaffected by the acid radicle associated with the dye base.

The high toxicity to *Fusarium culmorum* of malachite green dye base was reduced weight for weight and mole for mole by substitution of ethyl, propyl or butyl groups for methyl groups.

The reduction of malachite green to malachite green leuco base removed toxicity.

The substitution of amino groups and alkylated amino groups in benzene nuclei of triphenyl methane increased toxicity, whereas acid groups reduced toxicity. Sulphonation and carboxylation reduced toxicity to vanishing point.

Alkylation of amino groups increased, but alkylation of benzene nuclei did not affect toxicity appreciably.

When the central carbon atom of the triphenylmethane dyestuffs was replaced by nitrogen (e.g., Bindschedler's green) the diphenyl ammonium compounds were less toxic than the corresponding triphenyl methane compounds.

The prevention of rotation of the aminated benzene rings by bridging, in the *o*-position to central atom, with O or N, and so obtaining a planar molecule only slightly affected toxicity.

Certain acid dyes stimulated fungal growth.

The toxicity of the basic dyestuffs seems to depend not on one specific part but on the molecule as a whole, and within certain limits the structure may be varied without pronounced changes in toxicity.

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