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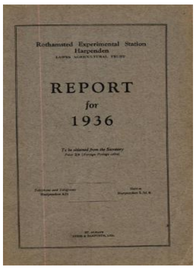

Department of Insecticides and Fungicides , 1918-1936

Rothamsted Research

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THE WORK OF THE DEPARTMENT OF INSECTICIDES AND FUNGICIDES 1918-1936

F. Tattersfield, D.Sc., F.I.C.

Work on Insecticides and Fungicides began at Rothamsted in 1918, with investigations of the possible control by chemical means of wire-worms and certain nematode pests existing in the soil. A study of the toxicity of organic compounds to wire-worms isolated from the soil (1) led to the conclusion that the relationship between chemical constitution and the toxicity of their vapours was of a dual nature depending on the type of compound and on its volatility. Thus the aromatic hydrocarbons and halides were, on the whole, more toxic than aliphatic hydrocarbons and halides, but within these types there was a fairly close relationship between toxicity, vapour pressure, and volatility. Compounds of high boiling point were thus likely to be more toxic than those of low. The fact that the adsorbability of vapours can be correlated with these physical properties led us to the view that adsorption was the fundamental explanation of the toxic effect produced (2) ; a view which has had increasing support in recent years. The decreasing vapour pressure allows a limit to be put to the value of a series of homologous compounds as fumigants, also such compounds as chloropicrin and allyl isothiocyanate were much more toxic than their physical properties would lead one to expect. Both physical and chemical properties have therefore to be taken into account in endeavouring to assess the toxicity of fumigants from first principles.

The limiting factors, however, to the successful use of fumigants in the soil are (*a*) the protecting action of the soil (*b*) their chemotropic effect on the insect (*c*) economic factors (*d*) means of evaluating results.

(*a*) In the early researches carried out at Rothamsted it became evident that pests existing in the soil are protected in several different ways from the action of the soil fumigants. The penetration of the insecticide may be inhibited by solution, by adsorption, by the tilth of the soil, and its effectiveness by decomposition of the chemical through the action of the soil bacteria. Such inert materials as toluene were shown by Mrs. Matthews (3) and naphthalene by the writer (4) to be used as sources of energy by the specific soil bacteria. In the case of naphthalene the rate of decomposition, which was determined by chemical means, depended upon the organic richness of the soils and upon a repetition of the treatment. Thus a thorough admixture of naphthalene with a relatively poor soil led to a complete if slow toxic effect on wire-worms, whereas in rich soils the control was less complete and after one or two treatments with the chemical a further administration was ineffective. The specific bacteria, greatly enhanced in numbers by the previous treatments, led to so

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(1) F. Tattersfield and A. W. R. Roberts—" The influence of chemical constitution on the toxicity of organic compounds to wireworms." J. Agric Sci., (1920), X, 199.

(2) F. Tattersfield—" The relationship between chemical constitution of organic compounds and their toxicity to insects." J. Agric. Sci., (1927), XVII, 181.

(3) Mrs. A. Matthews—" Partial Sterilisation of soil by antiseptics." J. Agric. Sci, (1924), XIV, 1.

(4) F. Tattersfield—" The Decomposition of Naphthalene in the soil and the effect upon its insecticidal action." Ann. App. Biol., (1928), XV, 57.

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rapid a decomposition of the naphthalene that it had no toxic effect. The substitution of a chlorine atom in naphthalene stabilised the molecule against bacterial attack, but α-chlornaphthalene spread less rapidly in the soil and was more phytocidal than naphthalene. (*b*) Insects are attracted or repelled by chemical substances and unless they are stupefied or killed rapidly, they may retreat before the enlarging zone of action of the chemical, until the latter becomes so attenuated as to have little effect. The use of positive chemotropic compounds or attractants for purposes of concentrating the soil pest and limiting the volume of soil to be subsequently treated with the fumigant, may lead to more economic means of control, but although this possibility was visualised and apparatus constructed for its study, the call to other work did not permit of it being again actively pursued at Rothamsted until recent years. (*c*) Except in the case of high priced crops, the control of soil pests has proved of difficulty, partly for financial reasons, and until recent years only meagre success can be said to have attended the efforts of many workers throughout the world. (*d*) Mechanical means of quantitatively separating insects from the soil and the statistical technique of determining the significance of the results obtained in field experiments have now led to methods of assessing accurately the relative utility of various chemicals as soil fumigants.

Ladell (5) at Rothamsted has used a flotation apparatus for separating the fauna from the soil, which is a great improvement upon the methods hitherto in use. The sampling error in his plot experiments on the control of wire-worms has been reduced from 30 per cent to 19 per cent and thus the significance of any particular treatment can be more precisely stated. By taking account of the number of cysts (*Heterodera Schactii*) and the crop yields he has also been able to simplify the study of the control of this pest. The ideal soil fumigant has, however, still to be discovered.

The difficulties attending the successful treatment of the soil diseases by chemical means are also illustrated by the studies of Roach, Glynne, Brierley and Crowther (6) (7) on the control of wart disease of potatoes. Experiments during the years 1922-24 gave evidence that under the experimental conditions of those years, sulphur treatment at the rate of 1 ton per acre or less on the light land at Ormskirk, and of two tons per acre on the heavy land at Hatfield greatly reduced the incidence of wart disease on the succeeding crop of susceptible potatoes. Under the experimental conditions of 1925 a reduction in the amount of disease was produced by treatment with 10 and 15 cwts. per acre at Ormskirk, but at Hatfield little if any, effect was produced. The phytocidal effect of sulphur depended on the type of soil, being more severe on the light than the heavy land, and the question arises as to what extent the results obtained depended on this fact or upon unascertained soil conditions prevailing at the time of the experiments. Subsequent experiments in the soil, (8) infected artificially with *Synchytrium endobioticum*,

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(5) W. R. S. Ladell—" A new apparatus for separating insects from the soil." Ann. App, Biol., (1936), XXIII, 862.

(6) W. A. Roach, M. D. Glynne, W. B. Brierley and E. M. Crowther. Ann. App. Biol., (1925) XII, 152.

(7) W. A. Roach and W. B. Brierley. Ann. App. Biol., (1926), XIII, 301.

(8) E. M. Crowther, M. D. Glynne and W. A. Roach. Ann. App. Biol., (1927), XIV, 422.

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revealed the fact that there was complete freedom from infection when the acidity of the soil had been raised to a very high value, (*p*H 3.4) but the conclusion was drawn that sulphur in controlling wart disease shows some other form of action, but whether this property could be enhanced sufficiently to render sulphur treatment of practical value needed further investigation. Later, Roach and Glynne (9) examined the toxicities of several simple sulphur derivatives towards winter sporangia of this organism ; they showed that acidified thiosulphate and sodium hydrosulphite were ten times as toxic as sulphuric acid owing, probably, to the presence of free thiosulphuric acid. In addition, di-, tri-, tetra- and penta-thionic acids were shown to have only feeble toxic actions, when pure, and toxicity could be explained as due to their hydrogen-ion concentrations. The polythionic acid theory advanced by Young (10) to account for the fungicidal properties of sulphur was thus shown to be unsound, a conclusion confirmed by Wilcoxon and MacCallan (11) Later, Roach (12) suggested that the changes of sulphur in the soil take place in the following stages, sulphur—thiosulphate-polythionate(s)—sulphate, and considered that the effectiveness of sulphur as a soil fungicide to wart disease may well be due to the temporary accumulation of the relatively unstable thiosulphuric acid depending on specific soil or fortuitous climatic conditions.

Roach during these investigations carried out an interesting experiment which demonstrated the formation of a particulate cloud when sulphur was gently heated in air. A layer of sulphur was placed in a box containing windows on opposing sides and an observation window at the top: It was allowed to stand for 24 hours in a dark room, a beam of light did not betray its passage through the box, when viewed from the upper window, until the sulphur was gently heated and a critical temperature was reached. This result tends to add force to the view that sulphur partly exerts its fungicidal action at a distance in this way. (13)

INVESTIGATIONS ON CONTACT INSECTICIDES

The investigations of insecticides, to be used as sprays or dusts, have chiefly occupied the department during recent years. They were mainly concerned with the class known as contact poisons although some attention was given for a short time to certain stomach poisons, the motive behind the work being the need for plant sprays highly toxic to insects but cheaper and less dangerous to man than nicotine and the arsenicals. In order that more rapid progress could be made than field experimentation allowed, the development of laboratory technique for purposes of assessing insecticidal values (14) (15) was undertaken. The spraying apparatus elaborated, the first of its kind, was so arranged that successive

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(9) W. A. Roach, M. D. Glynne. Ann. App. Biol., (1928), XV, 168.

(10) H. C. Young—" The toxic property of Sulphur." Ann. Miss. Bot. Gard., 1922, IX; 403.

(11) F. Wilcoxon, and S. E. A. McCallan. Contrib. Boyce Thomson Inst., (1930), II, 389.

(12) W. A. Roach. J. Agric. Sci., (1930), XX, 74.

(13) W. A. Roach. 5th Internat. Bot. Congress, (1930), 393. B. T. P. Barker, C. T. Gimingham ; S. P. Wiltshire, Long Ashton Stat. Repts., 1919, 57-75.

(14) F. Tattersfield, F. M. Morris.—" An apparatus for testing Insecticides." Bull. Ent. Res., (1924), XIV, 223.

(15) F. Tattersfield—" An apparatus for testing contact Insecticides." Ann. App. Biol., (1934), XXI, 691.

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batches of insects were sprayed under conditions as uniform as possible, so that on using various substances at different concentrations the results were directly comparable. When different concentrations of a solution or emulsion of a compound were sprayed upon aphids in the apparatus and the percentages of the resulting moribund and dead insects plotted against them, S-shaped curves resulted. A number of such curves for different compounds were graphed together and gave a diagrammatic demonstration of their respective toxicities. The shape of the curves showed, however, that comparisons of toxicity at the so-called minimum lethal dose, that is the dosage just killing 100 per cent of the test subjects, was not strictly possible, as the curve approached the 100 per cent kill asymptotically. It was suggested by Dr. R. A. Fisher that comparisons could be made at the concentration giving 50 per cent kill. This suggestion has been almost universally approved and the so-called median lethal dose is now very widely employed in toxicological work. The dosage-mortality curves, obtained in our work by the use of this machine, have been widely used by various authors in critical examinations of the action of drugs and insecticides, and the dosage-mortality results for nicotine were the first to be employed by Bliss (16) and by O'Kane (17) and his co-workers for the reduction of these types of data to straight lines, in order to simplify comparisons between insecticides.

The results in these studies can best be considered under the following headings :

(*A*) Synthetic organic compounds.
(*B*) Plant products of the alkaloid class.
(*C*) Fish poison plants.
(*D*) Pyrethrum.

(*A*) SYNTHETICS. A systematic survey (18) (19) (20) was conducted for a number of years on groups of organic chemical compounds and an attempt made to ascertain the relationship between their chemical constitution and physical properties and their toxicity to *Aphis rumicis*, the adult wingless form of which was used as one of the test subjects. Some comparatively simple relationships were found. If the symbols < and > are used respectively to mean " less toxic than " and " more toxic than," when molar concentrations are considered, they can be summarised as follows :

(1) Benzene < toluene < xylene < naphthalene > tetrahydronaphthalene > decahydronaphthalene.

(2) Benzene < chlorbenzene < o-dichlorbenzene < 1:2:4 trichlorbenzene > 1:2:4:5 tetrachlorbenzene and hexachlorbenzene.

Naphthalene < α-chlornaphthalene.

(3) Benzene < nitrobenzene < meta-dinitrobenzene. One experiment showed that to eggs of *Hadena oleracea m*-dinitrobenzene >trinitrobenzene.

(4) Benzene < phenol (hydroxybenzene) > 1:2 dihydroxybenzene > 1:2:3 trihydroxybenzene.

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(16) C. I. Bliss. Science, (1934), LXXIX, 38 and 409.

(17) W. C. O'Kane, W. A. Westgate, L. C. Glover. (1934). New Hamp. Tech. Bull., 58.

(18) F. Tattersfield, C. T. Gimingham, H. M. Morris—" Studies on contact insecticides." Pt. III. Ann. App. Biol, (1925), XII, 218.

(19) F. Tattersfield, C. T. Gimingham—" Studies on Contact Insecticides." Pt. V. (1927), XIV, 217. Pt. VI. *ibid*, (1927), XIV, 331.

(20) F. Tattersfield. J. Agric. Sci., (1927), XVII, 188.

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(5) Benzene < anisole (methoxybenzene) < 1:2 dimethoxybenzene < 1:2:3 trimethoxybenzene.
phenol (hydroxybenzene) > anisole (methoxybenzene)
1:2 dihydroxybenzene < 1:2 dimethoxybenzene.
1:2:3 trihydroxybenzene < 1:2:3 trimethoxybenzene.

(6) Phenol slightly < o-nitrophenol < *m* and *p*-nitrophenol < 2:4 dinitrophenol > 2:4:6 trinitrophenol
o-cresol slightly > 3-nitro-o-cresol < 5-nitro-o-cresol < 3:5 dinitro-o-cresol.

(7) Aniline (phenylamine) < diphenylamine > triphenylamine
aniline (phenylamine) < benzylphenylamine > tribenzyphenylamine
benzylamine < dibenzylamine > tribenzylamine
aniline < α-naphthylamine < phenyl-α-napthylamine = approximately diphenylamine
α-naphthylamine and its derivatives > β-naphthylamine and its derivatives.

(8) Pyrrole < pyridine < picoline (α-methyl pyridine) < lutidine (dimethylpyridine) < quinoline and isoquinoline < acridine.
pyrrole < pyrrolidine. pyridine < piperidine
pyridine and pyrrolidine < l-nicotine (pyridine-N-methylpyrrolidine).

(9) In the straight chain fatty acids toxicity increases from acetic acid up to the 11-carbon atom acid and then declines.

Methylation of the carboxyl group reduces toxicity throughout the whole group.

The sodium salts of the acids, with the exception of sodium oleate are less toxic than their corresponding acids.

Neutralisation with ammonia reduces toxicity of the lower fatty acids; from nonoic to tridecoic acid the effect is small and for myristic and oleic acid toxicity is enhanced.

The unsaturated oleic acid is more toxic than the saturated stearic acid.

Although an abbreviated list of this kind does not bring out the quantitative relationships in the toxicities of the different compounds it allows certain conclusions to be drawn. A complete analysis, however, cannot be attempted here.

(*a*) On the whole, there is an increase in insecticidal activity with increase in molecular weight, but in each series there is an upper limit to the molecular size, beyond which toxicity shows a decline.

(*b*) Some substituent groups increase insecticidal activity more than others, thus when introduced into the benzene ring, the effects produced by the following groups on toxicity are approximately in the descending order: Phenylamine and benzylamine, nitro, dimethylamino, methylamino, hydroxyl, amino, chlorine, methoxyl, methyl.

(*c*) The effect of substitution depends upon the nature of the ring. (In general, radicles substituted in the aniline molecule increase toxicity more than when substituted in to α-naphthylamine.) Hydrogenation tends to decrease the insecticidal activity

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of naphthalene but to increase that of such heterocyclic compounds as pyrrole and pyridine.

(*d*) The presence of more than one grouping affects the result by their interaction and by their relative positions in the ring. Thus o-dichlorbenzene was more toxic than p-dichlorbenzene, 3:5 dinitro-o-cresol than 3:5 dinitro-p-cresol.

(*e*) Certain groups have specific toxic properties ; thus tetramethylammonium salts are more rapidly toxic than tetraethylammonium salts, the former resembling nicotine in their action.

(*f*) The magnitude of the effect of a polar group may depend upon the nature and the size of the chain or ring to which it is attached. (Fatty acids and esters).

(*g*) Toxicity may depend upon the presence of an asymmetric carbon atom, thus nicotine is more toxic than its constituent rings and to a degree not explicable by its greater molecular weight. *

Although these conclusions may apply to a number of insects and to insect eggs, particular exceptions are frequently to be noted, and a compound highly toxic to an adult may be less so or even non-toxic to certain insect eggs. Also the phytocidal properties of some chemicals limit their use to dormant trees, 3:5 dinitro-o-cresol being an example. This compound was found to be highly toxic to both adult aphides and eggs and field trials showed that it was an effective winter wash. (21) (22) Certain disagreeable properties prevented its practical use.

Certain facts emerged from this series of investigations. It is extremely difficult to find any simple or comprehensive generalisation to account for the variations in toxicity of these compounds, and this is to be expected when it is considered that the death of insects may result from different physiological causes when treated by different compounds and that the physiological effect may be the consequence of a complicated chain of chemical reactions. Nevertheless, it is fairly clear that in certain of the series where a polar grouping is present (e.g. fatty acids) there may be increasing orientation leading to enhanced adsorption by, and penetration into, cells as the series is ascended, until a maximum effect is reached. This may well be conditioned by the two factors of water solubility and surface activity, a decline in toxicity resulting when the solubility in water has fallen so low as to bring about a decline in the surface activity. Thus it would appear that a toxic polar grouping may have its toxicity enhanced by attachment to a relatively massive hydrocarbon chain or ring ; this important point is illustrated by the high insecticidal properties of the fatty thiocyanates, with an optimum toxicity at lauryl thiocyanate,(23) and by the powerful insectide (24) obtained by substitution of a cyclohexyl group for methyl in 3:5 dinitro-o-cresol.

It would appear that for an organic compound to have high insecticidal power it needs a toxic spearhead attached to a heavy but not too heavy a shaft.

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* It has since been shown by C. H. Richardson and his co-workers that l-nicotine is more toxic to insects than d-nicotine.

(21) C. T. Gimingham, A. M. Massee, and F. Tattersfield. Ann App. Biol., (1926), XIII, 446.
(22) C. T. Gimingham, and F. Tattersfield. J. Agric. Sci., (1927), XVII, 162.
(23) E. W. Bousquet, P. L. Salsberg and H. F. Dietz. Ind. Eng. Chem., (1935), XXVII, 1342.
(24) C. H. Richardson, J. E. Kajy. J. Econ. Ent., (1936), XXIX, pp. 52, 62, 393, and 397.

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(*B*) Plant Alkaloids. A number of plant alkaloids and alkaloid-bearing plants were tested for their insecticidal properties. (25) The *Genus* Lupinus showed no marked toxic properties, but species of *Genista*, *Ulex*, *Sophora*, *Baptisia* which contained the alkaloid cytisine shown by H. H. Dale to have a physiological action of the nicotine type, proved to have toxic properties. Cytisine itself was relatively highly toxic. Lobeline, another alkaloid which physiologists had (26) found to have nicotine-like properties, was less toxic. Of the many other alkaloids and alkaloid-containing plants tested, only eserine was of the same order of toxicity as nicotine, and again this alkaloid had been found to have physiological effects similar to those of nicotine. The study of this group clearly demonstrated that alkaloids toxic to the higher animals are not necessarily highly insecticidal; coniine, for example, had only feeble toxic properties to *A. rumicis*. It is also of interest that in our experiments cytisine did not prove materially toxic either as a contact poison to the eggs or as a stomach poison to the larvae of *Selenia tetralunaria*.

(*C*) Fish Poison Plants. It has been a common and widespread practice, from very early times, for fish to be caught through the agency of the stupefying properties of many natural orders of plants. Species of *Verbascum* were used in the time of the Romans, and in isolated parts of Europe are still in use. Spurge has been a standby to poachers. In tropical countries the practice has been wide-spread, and it is curious that certain leguminous plants, containing the same group of chemical compounds, have been employed as an aid to the trapping of fish in the East Indies, Africa, and South America. Roots, stems, leaves or seeds containing either rotenone or other of its closely related compounds have been used. The practice has now been prohibited by the more enlightened governments of these countries. Early in the nineteenth century the root of one of these plants—of the derris species—was found to have insecticidal properties. The observation was apparently forgotten, and it was not until the early years of this century that this root found its way into the markets of the western world. In more recent years these rotenone-containing plants, of which many now have been discovered, have excited world-wide interest. Through the intense investigations of groups of workers the structure of their active principles has now been almost completely elucidated.

Work at Rothamsted began in 1920, when an investigation of derris root was undertaken in conjunction with the Plant Pathological Department of the Ministry of Agriculture. (27) Extracts of the root prepared in several different ways were found highly toxic to insects but there was a difference in insect susceptibility. The colourless crystalline derivative rotenone (then known as tubatoxin) was isolated and shown to have highly toxic properties as had the resins, freed as far as possible from rotenone. The yellow-coloured crystals, then grouped together under the name anhydroderride, were found to be not a single, but several compounds, but they were without exception without material toxicity, the conclusion being

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(25) F. Tattersfield, C. T. Gimingham, H. M. Morris—"Studies on Contact Insecticides." Pt. IV. Ann. App. Biol., (1926), XIII, 436.

(26) H. H. Dale, P. P. Laidlaw. J. Pharm. Exp. Therap., (1912), III, 205.

(27) J. C. F. Fryer, R. Stenton, F. Tattersfield, W. A. Roach. Ann. App. Biol., (1923), X, 18. F. Tattersfield and W. A. Roach. *Ibid*, X, 1.

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drawn that they did not exist as such in the root, and this has been confirmed by subsequent work. It was shown that rotenone with certain solvents (e.g. benzene) gave rise to crystalline complexes, containing the solvent. When exposed to ultra-violet light rotenone was degraded to non-toxic derivatives. This property has been confirmed and has proved a limiting factor to the use of rotenone as a stomach poison to codling moth.

The problem of the evaluation by chemical means claimed our attention and it was found that the rotenone content did not account for the whole of the toxic properties of the root. Although for some years this view was questioned it is now almost universally held. Our suggestion for assessing value by the total ether extract with a subsequent determination of methoxyl content has not met with general consent, and for several years now the market considers not only the ether extract but rotenone content as well.

In the years subsequent to this investigation many tropical leguminous plants were examined (28), samples being collected by colonial officers from many parts of the British Empire. We gladly express our indebtedness to these officials for the thorough way in which the search for native fish poison plants was carried out. Many of the plants, belonging to a number of natural orders, showed only feeble insecticidal properties. The more interesting are shown in the following table.

Name	Country of Origin	Active Part	Active Principles
Derris elliptica	Malaya & East Indies	Root	Rotenone, deguelin
Derris malaccensis	,, ,, ,,	,,	,, ,, toxicarol
Derris (sumatra type)	,, ,, ,,	,,	Rotenone, deguelin toxicarol
White Haiari* (*sp. Lonchocarpus*)	British Guiana	,,	Rotenone, deguelin
Black Haiari (*sp. Lonchocarpus*)	,, ,,	Root and Stems	,,
Tephrosia toxicaria	,, ,,	Root	Toxicarol
Tephrosia Vogelii	The African Continent	Leaves and Seeds	Rotenone, deguelin
Tephrosia macropoda	Natal	Root	Rotenone
Neorautanenia fisifolia	S. Rhodesia	Tuberous Root	—
Mundulea suberosa	India, Africa	Leaves † and Bark	Rotenone
Dolichos Psendopachyrizus	Kenya	Root	—
Unknown Vine probably derris sp.	British Solomon Island	Leaves	

* Since shown to be conspecific with Cubé of Peru.

† One specimen of Mundulea from India possessed toxic leaves.

Of these the most important are the species of *Derris* and *Lonchocarpus*. The others may probably have a local use, and LePelley has suggested that *T. vogelii* may be found valuable for increasing the killing properties of pyrethrum-kerosene sprays used in Kenya. Some of them have been used as parasiticides by the natives of their country of origin. Unless, however, they are improved by selection, they are not likely to compete in the markets of Europe and the United States in competition with derris, cubé, and timbó roots, of which potent specimens have already been

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(28) F. Tattersfield, C. T. Gimingham, H. M. Morris.—"Studies on Contact Insecticides." Pt. II. Ann. App. Biol., (1925), XII, 66. Pt. IV. *ibid*, (1926), XIII, 426. F. Tattersfield, C. T. Gimingham, Ann. App. Biol., (1932), XIX, 253. F. Tattersfield. "Fish Poison Plants as insecticides." Emp. J. Exp. Agric., (1936), IV, 136.

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discovered and which are likely to be very extensively planted in the East Indies, Africa and South America.

The haiari plants are of considerable interest, those found growing in the forests of British Guiana as lianes by R. A. Altson being recognised definitely as insecticidal and they contained rotenone in fairly high amounts. Some little time afterwards cubé was discovered by American investigators and recognised as *L. nicou* and this variety, richer in rotenone than the haiari plants so far found or cultivated, is now produced on an extensive scale and is in active competition with derris. In recent years a specimen of the white haiari has been found in flower—a very rare phenomenon—and it has been recognised as conspecific with cubé of Peru. The black haiari with its darker roots is possibly more closely related to timbó of Brazil.

It has been by no means an easy matter to decide what are the factors making for the production of roots rich in active principles. There is an optimum time of harvesting when the toxic principles are present in a maximum amount, but for many years it was considered fortuitous whether roots were rich or poor when harvested. Obscure factors of soil and climate were called in to explain rather curious differences in potency of what were regarded as roots of the same variety, when grown in different parts of Malaya. Indeed, it is sometimes difficult to account for the differences in the rotenone content between cultivated and wild specimens of haiari and also for the fact that a genuine sample of *D. elliptica* from Burma was found by us to contain no rotenone and to have no toxicity. Recent work has shown, however, that the varieties and strains of derris are numerous and that they differ widely in quality. Selections made in Malaya and Java have increased the prospects of the production of *D. elliptica* with a content of rotenone reaching the 10 per cent. mark and that this will be possible over a wide range, for rich roots are now being grown in Malaya, Java and at different altitudes in Tanganyika.

The chemical evaluation of these plants has exercised a considerable amount of thought in many laboratories and a study of this phase of the work has again been undertaken at Rothamsted ([29] [30]). It was found that none of the methods suggested gave an accurate relative assessment of the value of different varieties of derris root, though, when comparisons were made within the boundaries of a definite species, both the rotenone content and the ether extract gave a close measure of their relative insecticidal powers. It became clear that chemical means of differentiating species and varieties were needed ([30] [31]), and this was all the more necessary in that it was realised that the native names used, such as tuba tedong, tuba rabut and various Chinese terms were adding to the confusion of the terminology of varieties of derris, and that the term " barbasco " was employed indiscriminately in S. America for any fish poison plant. Three types of root were chosen—*D. elliptica*, *D. malaccensis*, from both of which rotenone could be separated by

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(29) F. Tattersfield and J. T. Martin—" The problem of the evaluation of rotenone containing plants." Pt. I. Ann. App. Biol., (1935), XXII, 578.

(30) J. T. Martin and F. Tattersfield—" The problem of the evaluation of rotenone containing plants." Pt. II. *ibid.* (1936), XXIII, 880.

(31) F. Tattersfield and J. T. Martin—" The problem of the evaluation of rotenone containing plants." Pt. III. *ibid.* (1936), XXIII, 899.

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the usual methods, and a third type known as the " Sumatra-type " root, from which it could only be separated by a special technique. It was found that the last two varieties contained a large proportion of matter extractable and precipitated by caustic potash—this precipitate on acidification and extraction by ether gave a resin laevo-rotatory in benzene, from which later a crystalline derivative was obtained. This crystalline derivative, also laevo-rotatory, was apparently a precursor of inactive toxicarol. Two things were noteworthy (1) that this potash-extracted resin was more toxic than inactive toxicarol (2) that after its separation rotenone crystallised out from the Sumatra-type resin on solution in carbon-tetrachloride. Ether solutions of *D. elliptica* resins only gave a small amount of potash-extractable material. The benzene solutions of the resins of these three types were also examined polarimetrically; they were all laevo-rotatory. The solutions prepared from *D. malaccensis* and the Sumatra-type were however, distinguished from those of *D. elliptica* by the instantaneous change-over from laevo- to dextro-rotation on adding normal caustic potash in methyl alcohol. This change was followed by a decline in rotation of an approximately monomolecular type. These features characterised the potash precipitated resins from *D. malaccensis* and the Sumatra-type roots. The crystalline derivative isolated from the potash-precipitated resin behaved in a similar way. It was thus obvious that by simple chemical and polarimetric means *D. malaccensis* and the Sumatra-type could be differentiated from *D. elliptica*, and that the Sumatra-type root was more nearly related to *D. malaccensis* than to *D. elliptica* and was probably a sub-variety of it. Later a crystalline derivative (32) was obtained from a resin of the Sumatra-type by direct crystallisation from ethyl acetate, the crystals melted at a temperature of the same order as those obtained from the potash-treated resin (95°-99° C) and showed the same characteristic change-over and subsequent decline in rotation on the addition of potash in methyl alcohol. On boiling with alcoholic potash both gave rise to optically inactive toxicarol in high yield. As it had been previously found that the rate of decline in the dextro-rotation depended on the amount of methyl alcohol present, it seems probable that the inactive toxicarol does not occur as such in the root and that its precursor has undergone change in the usual process of separation. Moreover, our experiments would show that since the precursor of toxicarol is frequently present in considerable amounts in such roots as *D. malaccensis* and the Sumatra-type and the precursor resin is more toxic than toxicarol itself, the presence of this constituent cannot be ignored in assessing the value of these roots. From American work this would also seem to be true of the precursor of deguelin, another optically inactive crystalline material isolated from the root. It has probably been changed in the process of extraction with consequent loss of toxicity.

Rotenone Estimation. The estimation of rotenone will always be of importance, as it is the most toxic of the derivatives so far isolated and selection experiments have succeeded in raising its content in derris root to a high level. Nevertheless, its quantitative

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(32) F. Tattersfield and J. T. Martin. Jour. Soc. Chem. Ind. (1937) LVI. 77, T.

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determination is proving a rather intractable problem and a recent conjoint effort on the part of a number of laboratories in America, England, Germany and Holland in the analysis of one sample of derris root distributed from the laboratory at Buitenzorg, Java, revealed grave discrepancies in the several results obtained. It is becoming obvious that a standard method for the determination of rotenone is urgently needed, and it is hoped that the participating parties in the above investigation, in which our laboratory is one, will finally be able to agree to standardised procedures for the determination of the rotenone, extractive and moisture contents of these plants. It has been suggested from Rothamsted that the sampling error, which for such difficult material is likely to prove considerable, should be investigated as well and some common method for sampling agreed upon.

(*D*) PYRETHRUM INVESTIGATIONS. The flowers of certain of the pyrethrum species have been known to be potent insecticides for a long period of time, *Chrysanthemum cinerariaefolium*, *C. roseum* and *C. marschallii* having been used in insect powders. The first-named is the only one of commercial importance to-day. Until quite recently, a number of erroneous beliefs were held about the factors upon which the insecticidal powers of these flowers depended, and, it is only through researches during the last ten or twelve years that they have been cleared away. Our present knowledge dates from 1924 when Staudinger and Ruzicka(33) isolated and determined the constitution of the two active principles pyrethrins I and II. Since that date much has been done by research workers in Europe and America to clear up outstanding problems. Work on this plant was started at Rothamsted at the suggestion of the Plant Pathology Laboratory of the Ministry of Agriculture, in order to ascertain whether it could be grown in this country and how the product compared with the flowers obtained from Dalmatia and Japan.(34) It was found that high quality material could be grown, that the flowers were much more toxic than the stalks, that exposure to damp conditions reduced the potency of the flowers and that insects showed rather wide differences in susceptibility to their effects. So good indeed were the flowers produced from the seed of plants grown in the garden of the Ministry's Plant Pathology Laboratory that demands for supplies have been received from all parts of the world, and it can be regarded as the source from which the rich material grown in Kenya Colony largely derives. Later, a number of points were studied : these are discussed below.

(1) A chemical method of evaluation(35) and the relative toxicities of pyrethrin I and II.

The two pyrethrins were isolated by the process of Staudinger and Ruzicka and their toxicities to *A. rumicis* compared. Pyrethrin I was found definitely more toxic than II. It was pointed out, however, that the method of re-synthesis employed gave rise to the possibility of the pyrethrin II being contaminated with an isomer. The general consensus of opinion now is that in an aqueous spray

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(33) H. Staudinger, L. Ruzicka. Helv. Chem. Acta., (1924), 7, 177-201.

(34) J. C. F. Fryer, F. Tattersfield and C. T. Gimingham—" English Grown Pyrethrum as an insecticide." Ann. App. Biol., (1928), XV, 423.

(35) F. Tattersfield, R. P. Hobson and C. T. Gimingham. J. Agric. Sci., (1929), XIX, 266. F. Tattersfield, R. P. Hobson. *Ibid.*, (1929), XIX, 433.

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pyrethrin II is less toxic than I, but the degree of difference may depend upon the insect and the way in which the pyrethrins are applied.

The pyrethrins are two esters, pyrethrin I containing a cyclic ketonic alcohol with a long side chain united to a volatile acid, whilst pyrethrin II contains the same alcohol united to the methyl ester of a dibasic acid, relatively soluble in water. A method of analysis based on the process of Staudinger and Harder,(36) which depends on the determination of these two pyrethrin acids was worked out.(35) It was found that the estimation of the acids could be correlated with toxicity, and that in general the determination of the pyrethrin I acid gave a good assessment of the relative toxic value of the flowers and also of the loss of activity on exposure as estimated by biological trials. The method has been subjected to a certain amount of criticism and improvements and simplifications have been suggested. The most important suggestion has been the elimination, prior to analysis, of the free fatty acids, which in commercial samples may sometimes be present in distinct amounts. It is doubtful whether any of the methods are very accurate, particularly for commercial extracts; the acid method nevertheless, has led to results for the comparative evaluation of pyrethrum flowers which have removed many of the erroneous beliefs previously held.

(2) The best time for harvesting the crop.(37)

At one time it was believed that the closed and half-open flowers were better than the fully-opened flowers. A critical survey(37) showed that the pyrethrin content increases in the flowers from the small bud stage up to the stage when the disc florets are just fully open. A decline in percentage pyrethrin content follows pollination, but this is not due to the actual loss of pyrethrins, but to the fact that increase in weight of the seed as a result of fertilisation is much greater than the increase in pyrethrin content. The best time to harvest therefore is when the flowers are just fully open.

(3) The location in the flower of the insecticidal principles.(38)

The greatest concentration of the pyrethrins was found in the ovaries, a result confirming the findings of Gnadinger and Corl(39) and other workers.

(4) The effect if any, of climatical factors on the production of flowers and their pyrethrin content.(38)

Attempts to grow pyrethrum in different parts of the world have shown that in the lowlands of tropical countries, although the plant flourishes vegetatively, it will not flower. Above certain altitudes, *e.g.*, in Kenya, it is in flower for ten months of the year. A carefully devised series of experiments at Rothamsted, in which the effects of duration of illumination, of temperature and dormancy were studied, showed that partial shading of the plant during five months preceding flowering resulted in the production of smaller flowers with a reduced pyrethrin content, but that success in flowering was largely dependent upon the relative temperatures experienced throughout the year. Plants which, following a dormant period, were subjected

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(36) H. Staudinger and H. Harder, Ann. Acad. Sci. Fennicae (1927) A. 29, no. 18, 1-14.
(37) F. Tattersfield. Ann. App. Biol., (1931), XVIII, 602.
(38) J. T. Martin and F. Tattersfield. Ann. App. Biol., (1934), XXI, 670, *ibid.*, 682.
(39) C. B. Gnadinger and C. Corl. J. Amer. Chem. Soc. (1930), LII, 680.

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to a high summer temperature gave large numbers of flowers, and flowering took place at an earlier date than for moderate summer temperatures. In no case did pyrethrum plants subjected to higher temperatures during the winter months produce more than a few flowers in the following summer.

(5) The response in yield of flowers and pyrethrins to manures. (38 40)

A series of pot experiments (38) were carried out with a clone of one plant grown in heavy Rothamsted soil (unmanured for many years) mixed with 10 per cent of sand, for three years and with a replicate for two years. Different manures were applied with the result that, in the first flowering year, an increase in weight of heads was observed where nitrogen and phosphate were added without potash. The increment though just significant was unimportant. After the first year no significant difference due to manurial treatments could be observed in yields of flowers or in the time of maturation. The pyrethrin content did not seem to be influenced by manurial treatment. A field experiment (40) carried out on poor sandy soil at Woburn for several years tends to confirm the conclusions that the fertilizer requirements of pyrethrum are small. The examination (38) of individual plants showed that plants, initially rich or poor in pyrethrins, would continue to produce relatively rich or poor flowers in subsequent years and that rooted shoots would be similar to their parents in this respect, in relative earliness or lateness of flowering and in length of petals. These properties would appear to be of a genetical character.

(6) The stability of extracts and emulsions. (41)

It was found (41) that although pyrethrum powder exposed in a thin layer to the atmosphere, lost its toxicity, the alcohol and petroleum extracts were relatively stable. It was shown that relatively permanent concentrated emulsions could be prepared in various ways in which the pyrethrin content did not rapidly decline with time, and that in the spray tank the diluted emulsions under conditions of a relatively high *p*H value deteriorated but more slowly than expected. Hobson (41) made an interesting study of the factors making for the permanence of aqueous emulsions of pyrethrum extracts, and found that the addition of pyrethrum extract to a petroleum solvent lowered its interfacial tension against water, the interfacial tension decreasing with increasing alkalinity of the aqueous phase. The presence of calcium salts in the aqueous phase raised the interfacial tension, but this could be counteracted by alkaline salts, the resulting interfacial tension being correlated with the ratio of calcium to hydroxyl ion concentration in the case of hard waters.

(7) The cause of the loss of activity of dusts when exposed to sunlight and air and the possibility of stabilisation. (42)

The loss of activity of pyrethrum was formerly ascribed to the volatility of the active principles, until it was shown that the

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(40) Ann. Repts. Rothamsted Exp. Stn., (1934), 203-5, (1935), 201, 202.

(41) F. Tattersfield and R. P. Hobson. Ann. App. Biol., (1931), XVIII, 203. R. P. Hobson, J. Agric. Sci., (1931), XXI, 101.

(42) F. Tattersfield. J. Agric. Sci., (1932), XXII, 396. F. Tattersfield and J. T. Martin, *ibid.*, (1934), XXIV, 598.

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volatile constituents of pyrethrum possessed no insecticidal properties. Nevertheless, the rapid loss of toxicity of finely powdered flowers and of insecticide dusts prepared from them has reacted against their use. We(42) were able to show that dusts prepared by incorporating pyrethrum extract with talc, when spread in a thin layer and exposed to sunlight or strong artificial light lost their activity very rapidly. The loss in the dark was very slight in the time required by sunlight to discharge all the activity. The reaction was largely a surface one. It was found, however, that such dusts, when exposed in the presence of inert gases such as nitrogen or carbon dioxide, lost their potency much more slowly than those exposed to either wet or dry oxygen. The rapid loss of activity is apparently largely due to an oxidising process and it was found by experiment that it could be greatly inhibited by mixing antioxidants with the dusts. Such antioxidants as the dihydroxybenzenes, pyrocatechol, resorcinol and hydroquinone, and the trihydroxybenzene, pyrogallol, were potent stabilisers, but the trihydroxybenzene phloroglucinol was ineffective, probably due to the fact that it undergoes the keto-enol transformation. Tannic acid also stabilises these dusts, an action unquestionably due to the presence of polyhydroxy nuclei.

A critical examination was undertaken in which artificially prepared pyrethrum-talc dusts and flowers, both ground and unground, were exposed to air in a variety of ways in both sunlight and artificial light and also in the dark. It was shown that the loss of pyrethrins took place much more slowly in the dark than in the light and that with the whole heads the loss was much slower than with powders. Pyrethrinised dusts exposed to sunlight in nitrogen lost their pyrethrin content less rapidly than when exposed in air, but a slow loss did take place, which was probably due to some cause other than the presence of free oxygen. There was apparently also a temperature co-efficient, the loss of pyrethrins in the presence of nitrogen was more rapid at high temperatures than low temperatures. The loss of activity could be followed, but not with an absolute degree of accuracy, by the determination of pyrethrin I. The fading of the colour of pyrethrinised dusts, associated with the contraction of the broad band in the violet end of the spectrum, was observed to run roughly parallel with the loss of pyrethrin I, loss of colour and loss of activity apparently being due to the same causes. The loss of pyrethrins was retarded by such antioxidants as hydroquinone and tannic acid, but the addition of these compounds did not increase the initial toxic properties of the pyrethrins. These results seem to have had considerable economic importance.

(8) The artificial drying of flowers.(43)

The drying of harvested pyrethrum flowers is a matter of great importance in countries with unsettled climates or where morning fogs may be prevalent, as in some parts of Kenya. Not only so, but the natural drying of such bulky material in the sunlight or shade demands much space and is relatively slow. An experiment in artificial drying was started in 1936 in conjunction with Mr. S. G. Jary, of the South Eastern Agricultural College.(43) The flowers

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(43) S. G. Jary, J. T. Martin and F. Tattersfield. J. S. E. Agric. Coll., Wye, Kent (1937).

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were laid in a thin layer in an experimental kiln, in which both temperature and air current could be regulated. The flowers were dried at different temperatures, a separate control sample taken from the same bulk being dried in a loft at ordinary temperatures for every kiln-dried sample. The colour of all the kiln-dried material was excellent. Two effects were noted (1) Drying at a temperature of 45°C (113°F) for a prolonged period resulted in a slight loss of pyrethrins. (2) Drying at too high a temperature led to loss of pyrethrins. Temperatures between 50°C (122°F) and 60°C (140°F) for 10 and 6 hours respectively were comparatively safe, results which agree with those obtained by Beckley(44) in Kenya.

(9) The possibilities of improving quality by hybridisation.

Genetical studies of a composite plant like pyrethrum is one of outstanding difficulty; a hybridisation study was, however, commenced some years ago in conjunction with the Plant Pathology Laboratory of the Ministry of Agriculture. Four years harvests have so far been taken from the progeny and the results await analysis.

A colourless extract of pyrethrum is of value where staining by the usual green-coloured extracts is objectionable. It has been found that colourless preparations can be made without serious loss of pyrethrins by extracting the powdered flowers mixed with good decolourising charcoal with petroleum ether. Solvents other than petroleum ether were not so successful. (45)

Stomach Poisons. Only a comparatively small amount of work was possible in this important field of investigation during the period under review. One paper(46) of importance was published in which it was shown that the silicofluorides were potent stomach poisons to mandibulate insects. American workers at the time showed with these compounds that a new group of stomach poisons was available and that they might for many purposes replace the arsenicals. The silicofluorides of sodium and barium and the fluoaluminates (cryolite) have since become articles of commerce as insecticides. Unfortunately it is now known that traces of fluorine have a pathological effect upon the teeth and this fact has tended to limit the field of usefulness of these important compounds. When extracts of the fish-poison plants were sprayed upon the foliage, it became so repellent to young larvae that they died of starvation, the leaves being uneaten.

Acaricidal action of pyridine and ammonia. An infestation of laboratory fungi by three species of mites led to an investigation of means of controlling these pests which would not permanently injure the fungus. (47) It was found that pyridine did so and a critical examination of the toxicity of pyridine, aniline and ammonia showed the vapours of these compounds to be highly toxic to *Tyroglyphus longior* but that the use of aniline was limited by its relatively

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(44) V. A. Beckley—"Pyrethrum drying." Kenya Weekly News. (1936), Apl., 24. East African Agri. Journ. (1937), II, 327.

(45) J. T Martin, C. Potter—"A colourless extract of pyrethrum flowers." Chem. and Ind. (1937), LVI, 119.

(46) C. T. Gimingham and F. Tattersfield—"Laboratory experiments with the non-arsenical insecticides." Ann. App. Biol., (1928, XV, 649. Ind. and Eng. Chem., (1925), XVII, 323.

(47) S. T. Jewson and F. Tattersfield. Ann. App. Biol., (1922), IX, 213.

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low vapour pressure, and that ammonia vapour although useful for deinfesting utensils was highly toxic to fungi. Pyridine above certain concentrations was toxic to cultures of fungi (*Aspergillus niger*) and this could not be explained by an alteration in *p*H value of the culture medium. The effect however was purely inhibitory and on neutralising the free pyridine with acid, the growth of the cultures proceeded normally. Pyridine seemed to have a specific toxic effect to the mites *Tyroglyphus longior* and *Aleurobius farinae.*

Miscellaneous investigations. The immunity to wart disease of certain varieties of potatoes led Roach(48) to explore this field by means of a delicate grafting technique developed by him. All the eight possible types of plants were built up by grafting together root, shoot and tuber systems from either immune or susceptible plants. In none of the experiments was the reaction of the tubers to wart disease changed. The field was thus narrowed since immunity or susceptibility could not be referred to a factor capable of being translocated and Roach suggested that the examination of the proteins by immuno-chemical methods presented a hopeful line of attack. An attempt to follow up this suggestion led to the elaboration of an anaerobic wet grinding apparatus(49) by which means a labile blue compound occurring in certain varieties of potato was observed for the first time.(50) The apparatus presented a number of possibilities; besides the examination of the plant constituents unstable in air, separation of cell-wall material for chemical examination and the estimation of starch content of potato tubers by mechanical means seem feasible by its means.

Roach's technique was applied by him to the grafting of plants upon foreign root-stocks,(51) thus when woody nightshade, (*Solanum Dulcamara*) was grafted on potato (*S. tuberosum*), growth was stimulated and the nightshade attained twice the weight it did on its own roots. Lupin grafted on broad bean was of greater girth and height than when grown on its own roots. In reciprocal grafts the root stock was dwarfing in its effect.

THE WORK OF THE ENTOMOLOGICAL DEPARTMENT AT ROTHAMSTED 1915-1936

By C. B. Williams and Staff

This report is divided into the following sections :—Introductory, Soil insects, Parasites and biological control, Aphid problems, Chemical relationship between insects and plants, Tropic reactions, Relation of insects to climate, Population studies, Studies on Cecidomyidae, Studies on species of economic importance, Migrations and aggregations of insects, Insect morphology, Miscellaneous activities.

At the end of each section a bibliography is given.

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(48) W. A. Roach. Ann. App. Biol., (1923), X, 142, *ibid.*, (1927), XIV, 181.
(49) W. A. Roach. Biochem. Journ., (1925), XIX, 783.
(50) W. A. Roach. Annals of Botany, (1925), XXXIX, 883.
(51) W. A. Roach. Annals of Botany, (1930), XLIV, 859.