SUPPORTING PAPERS

LIST OF PAPERS PRESENTED

Papers Supporting Thesis (Sole Authorship)

14. Connective tissue strands in blowfly larvae.
Aust. J. Sci. 13: 25-26. 1950.

15. Histochemical detection of barium and strontium.
Nature 167: 358. 1951.

16. Temperature preference in the Australian sheep blowfly, Lucilia cuprina Wied.
Aust. J. Sci. 2: 31-32. 1939.

Co-operative Papers Supporting Thesis

17. Insectary tests with insecticides to protect sheep against body strike.
Aust. J. Agr. Res. 1: 440-455. 1950
(With M.T. Scott.)

18. The use of borax for the prevention of fly breeding in trap baits.
C.S.I.R. Journal, 19: 321-329. 1947.
(With M.E. Fuller.)

19. An account of experiments undertaken to determine the natural population density of the sheep blowfly, Lucilia cuprina Wied.
C.S.I.R. Bulletin No. 195: 1-39. 1946.
(With D. Gilmour and G.A. McIntyre.)

Supporting Papers Unrelated to Subject of Thesis (Sole Authorship)

20. The effect of colour on the numbers of houseflies resting on painted surfaces.
Aust. J. Sci. Res., Ser. B. 1: 65-75. 1948.

21. An examination of the Peet-Grady method for the evaluation of household fly sprays.
C.S.I.R. Bulletin No. 216; 1-24. 1947.

22. Spray tests against adult mosquitoes. 1. Laboratory spray tests with Culicine (Culex fatigans) adults.
C.S.I.R. Bulletin No. 219: 1-27. 1947.

Co-operative Papers Unrelated to Subject of Thesis

23. Spray tests against adult mosquitoes. 2. Spray tests with Anopheline (Anopheles punctulatus farauti) adults.
C.S.I.R. Bulletin No. 219: 29-40. 1947.
(With D.O. Atherton.)

24. Laboratory and field tests of mosquito repellents.
C.S.I.R. Bulletin No. 213: 1-28. 1947.
(With R.N. McCulloch.)

25. Notes on the terrestrial ecology of the five islands.
Proc. Linn. Soc. N.S.W., 63: 357-388. 1939.
(With C. Davis and M.F. Day.)

26. The present status of DDT as an insecticide.
J. Aust. Inst. Ag. Sci. 11: 172-178. 1945.
(With G. Nelson.)

INTRODUCTION

The thirteen supporting papers are introduced by the following brief account of the fields they cover.

Paper 14 records the presence of blowfly larvae of fine strands of tissue, which maintain the malpighian tubules in their characteristic positions. The optical properties and staining reactions of these strands are typical of connective tissue, which has been seldom reported to occur in insects.

Paper 15 describes a histochemical staining method which was developed for the detection of barium and strontium in tissues.

In paper 16, the temperature preference of adult Lucilia cuprina is discussed, the zone of preferred temperature being shown by a simple method to lie between 31.5° and 38°C.

An evaluation of the application of insecticides to the surface of the fleece as a means of protection against body strike is presented in paper 17. It was found, under controlled insectary conditions, that both DDT and BHC conferred valuable protection for many weeks and that this protection was not affected by artificial rain. The current recommendations for the prevention of body strike in Australia are largely based on this work.

Some attention was paid in paper 18 to the effect of trapping on the blowfly population. It was found that many flies may be produced by untreated baits in blowfly traps, the number bred sometimes exceeding the total number of flies caught by the trap. Borax sprinkled over the surface of the bait was the most effective inhibitor of larval development tested, but suffered from the disadvantage that it generally reduced the numbers of flies caught. On general population density considerations it was concluded that the addition of borax to baits was not warranted.

In paper 19, an estimation of the natural population density of L. cuprina in an area of 50 square miles of grazing country near Canberra is described. The method employed was that of releasing a known number of stained flies and of recording the ratio of stained to unstained flies in the catches of 102 traps

placed within the area. The results of four experiments indicated that the natural population varied between 0.3 and 5.7 adult flies per acre at different times of the year. The maximum distance from the point of release at which flies were taken was 4.7 miles, this distance being covered in less than 30 hours. Data of value in assessing the practicability of trapping as a measure for the reduction of the sheep blowfly population was obtained in these experiments.

In paper 20 a simple technique was devised to determine the effect of colour on the numbers of houseflies resting on painted surfaces. They were found to react to intensity of light reflected from a surface rather than to its colour, lighter colours being less attractive than darker colours.

Spray testing methods, which were developed, and spray tests against flies and mosquitoes under laboratory and field conditions are dealt with in papers 21, 22 and 23, and repellent tests against mosquitoes are described in paper 24. The Australian army specifications for both mosquito sprays and mosquito repellents for campaigns in New Guinea and adjoining islands during the recent war were based directly on the results of this work.

Finally, a paper on the general ecology of the Five Islands lying off Port Kembla, New South Wales (No. 25) and a review article published some years ago on DDT as an insecticide (No. 26) are also included.

Reprinted from *The Australian Journal of Science*, Vol. 13, No. 1, August, 1950, p. 25.

Connective Tissue Strands in Blowfly Larvae

Connective tissue is very inconspicuous in most insects and has often been completely overlooked. One of its main functions, namely, the maintenance of the internal organs in their correct position, has largely been taken over by the tracheae.

In some insects connective tissue is known to occur between the epithelial cells and the muscularis of the alimentary canal (Lazarenko, 1925; Riedel, 1946). Connective tissue investing other tissues, or serving to connect muscles with their insertions, has also occasionally been described (Dennell, 1942; Lazarenko, 1925; Ochsé, 1946). In view of the very few reports of long thin strands of connective tissue in insects, however, it is worth recording that the malpighian tubules of *Lucilia cuprina* are held in place not only by tracheae but also by strands which have many of the characteristics of connective tissue.

L. cuprina larvae possess two pairs of tubules. The pair discharging into the left side of the gut is modified distally for the accumulation of granules. This region shows a sharp transition to the normal type of insect tubule when it bends sharply backwards at the level of the brain (for figure see Waterhouse, 1950). Connective strands occur in several places:

(*a*) In the bend region, each anterior tubule is connected by several strands of unstriated tissue to a long fine striated muscle inserted at one end in the tip of a dorsal midgut caecum and at the other in the body wall. After meeting the bend region of the tubules, each strand runs around or along the surface of the cells for a short distance, often breaking up into finer branches.

(*b*) The blind end of each granule-accumulating region is joined by a strand of tissue to the respective blind end of the second pair of tubules. This strand is not attached to any muscle.

(*c*) The posterior half of the granule region is further held in position at four levels by unstriated strands which are attached to heart muscles. The fat body and tracheae in the vicinity of the strands may also receive fine branches.

(*d*) The second pair of tubules are attached near their blind ends to the hindgut by one or more strands.

In other muscoid larvae, striated muscles attached to the bend of each anterior tubule have been described in *Thrixion* (Pantel, (1898) and attached to the blind ends of each anterior tubule in *Drosophila funebris* (Eastham, 1925), although these latter attachments in *Thrixion* are unstriated and no striations could be seen in these strands in *D. buscki*. In *Auchmeromyia* unstriated strands join the blind ends of anterior and posterior pairs of tubules, while muscles attach each posterior tubule near its blind end to the rectum (Roubaud, 1913). The arrangement of the tubules of all of these larvae, however, is somewhat different from *L. cuprina*, so that the attachments may not actually be homologous.

The strands of tissue in *L. cuprina*, as observed with phase-contrast, polarized light, or after staining, have no cross striations, although the material comprising them is oriented longitudinally. They are less strongly birefringent than larval striated muscle; and, if mounted in turn in fluids covering a range of refractive indices, they show a variation in degree of birefringence characteristic of connective tissue, but unlike that of smooth muscle (Claesson, 1947). They stain red in van Gieson's picric-acid/acid-fuchsin, blue or purple in Mallory, and fail to stain in alcoholic orcein; all properties of connective tissue which are not shared by muscle. They have not been observed to undergo spontaneous movements, but serve to hold the tubules in position or to return them to their position following displacement by locomotory activity or peristalsis

of the alimentary canal. Because of the flexible cuticle of blowfly larvae and their type of locomotion, the internal organs tend to be displaced by these activities more than in harder bodied insects.

The unstriated strands in blowfly larvae can be regarded as connective tissue because of their properties and because smooth muscle reputedly does not occur in insects. If, however, the striated strands described in other species are homologous with the unstriated strands which occur in *L. cuprina*, there arises the difficulty that tissue which appears to be connective in one species is replaced in another by striated muscle. It is commonly held to be unusual for muscles to appear and disappear at random during evolution. In fact many of the homologies of insect morphology are based on the assumption that the position and shape of sclerites may vary but that there is relatively little change in the muscles inserted thereon. Perhaps this difficulty may be resolved by regarding the differences, if they are confirmed on re-examination, as being due to a very long or a very short length of connective tissue between the muscle and its insertion.

Any of the three available explanations for the situation in muscoid larvae ((*a*) non-homologous connectives, (*b*) the substitution of connective tissue for striated muscle, or (*c*) greatly varying lengths of connective tissue between muscle and insertion) raises interesting problems which require further investigation.

The work described in this paper was carried out as part of the research programme of the Division of Entomology, C.S.I.R.O.

D. F. WATERHOUSE.

Division of Entomology,
C.S.I.R.O.,
Canberra, A.C.T.
July 1950.

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Histochemical Detection of Barium and Strontium

SMALL amounts of barium and strontium can be detected in the tissues of many insects by means of the extremely sensitive rhodizonate reaction. This reaction was described for microchemical use by Feigl[1], who has since investigated its limitations[2,3]. In the present histochemical method, tissues are fixed in carefully neutralized 10 per cent formalin in 70 per cent alcohol and immersed for 30–60 min. in a freshly prepared 1 or 2 per cent solution of sodium rhodizonate, in distilled water or in phosphate buffer at *p*H 7. Excess sodium rhodizonate is afterwards removed by washing in dilute alcohol. Stained tissues can be sectioned in the usual manner and permanent preparations obtained.

Both barium and strontium produce intensely coloured reddish compounds following this treatment. By means of chromate, barium can be distinguished from strontium. Unlike the corresponding strontium salts, barium chromate is less soluble than barium rhodizonate. Treatment with aqueous potassium chromate before staining with rhodizonate prevents the reaction with barium, and treatment after staining causes the red colour due to barium rhodizonate to be discharged. Spectrographic analyses have confirmed the presence of barium and strontium in the intensely staining tissues of several insects. Experiments with radioactive barium and strontium[4–6] provide confirmatory evidence that these elements are characteristically distributed in the insect body, as demonstrated by rhodizonate staining. Barium and strontium are absorbed in the midgut of most insects and deposited most frequently in the Malpighian tubules, less often in the midgut and reproductive organs, and only occasionally in the hindgut and fat body.

Both barium and strontium can be detected in bone. This is interesting in view of the recent report that both are indispensable for calcification in rats and guinea pigs[7]. The role of the deposits detected in insects, where calcification is usually unimportant, is at present unknown. One of their functions may be that of storage excretion.

Fuller details of the methods and results obtained will be published elsewhere[8].

D. F. WATERHOUSE

Commonwealth Scientific and
Industrial Research Organization,
Division of Entomology,
Canberra. Nov. 2.

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Reprinted from *The Australian Journal of Science*, Vol. II, No. 1, Aug., 1939, pp. 31-32.

Temperature Preference in the Australian Sheep Blowfly, Lucilia cuprina Wied.

DURING a series of laboratory studies last winter on *L. cuprina*, some observations were made which showed that this species has a definite temperature preference. Several hundred flies were free to move about in a large muslin cage, 12 ft. × 12 ft. × 8 ft. high, which was hung in one of the laboratory rooms. As part of the heating apparatus, a radiator was directed from a distance of 2 feet towards one side of the cage. This source of heat was found to give a temperature gradient, as measured by thermometers hung on the inner side of the muslin wall, ranging from the ordinary temperature of the room (about 12° to 15°C.) to about 55°C.

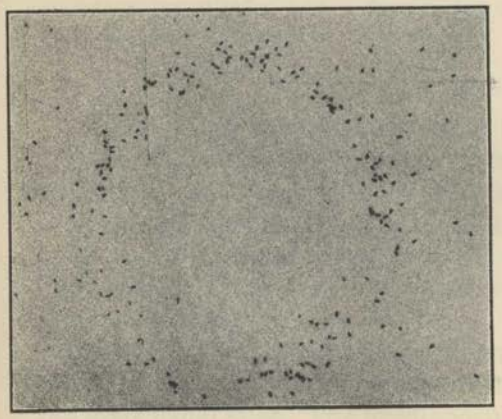

Figure 1.

When the heating apparatus was not in use and the room temperature was about 12°C., the flies were very sluggish. If the radiator was then turned on, only the few flies in its immediate vicinity became sufficiently active to react to its warmth. By the time the temperature of the room had risen to 20°C. or over, the flies were very active, and only a small proportion remained settled for any length of time. No clear temperature preference was exhibited, although the central zone in front of the radiator was always unoccupied. If now, by opening the windows, the room was allowed to cool, the flies gradually became affected by the drop in temperature and congregated on the muslin wall in the form of a circle about the radiator. Such a circle of flies is shown in Figure 1. The apparent eccentricity of the radiator rim shining through the wall is due to the fact that the camera was not aligned in the axis of the beam.

Within the circle it was too hot for the flies to rest; bordering this 'uncomfortably' hot region there was a well-defined band of flies clearly attracted* by the temperature conditions, whilst outside this again flies were distributed irregularly and no more densely than throughout the rest of the cage. As might be expected, the boundaries of temperature preference aggregations were not very sharp, a few individuals sometimes overlapping into the non-preferred temperatures, whilst patches in the zone of preferred temperatures were often temporarily unoccupied.

Thermometers hung against the muslin walls in the region where the flies congregated gave the readings summarized in Figure 2 from forty individual temperature records.

It is quite probable that the limits of temperature preference may be influenced by other factors affecting the reactions of the fly, such as humidity of the air, age, sex, state of nutrition of the flies, and previous temperature conditions to which they have been subjected. It is also realized that the temperature records

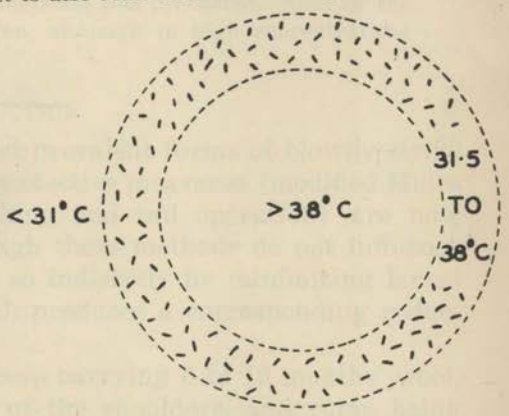

Figure 2.

have not the same meaning as similar records for a gradient in air temperatures, since the readings shown under the present conditions are affected by the properties of absorption and

* By 'attracted' it is not meant that there is a positive tropism acting at a distance. What happens is that the flies are moving and alighting more or less at random. When they settle in the favourable temperature zone they stay there; when they settle elsewhere they move off again, so there is an accumulation in this zone.

radiation of the thermometers, which doubtless differ from those of the flies. The temperatures recorded should, however, approximate to the temperature conditions experienced by the flies. More important than absolute figures is the fact that *L. cuprina* does show a preference for a certain limited band of temperatures.

These observations were made in fairly dim, artificial light. When the blinds were raised and the room was flooded with light, the flies were stimulated to intense flight activity, and, unless the temperature was below about 15°C., did not settle long enough anywhere for zoning to be apparent.

Evidence of preferred temperatures was also obtained by Nicholson (1934)[1] in the course of experiments on the effects of temperature on activity of *L. cuprina*. He noticed that at high and at low temperatures a large proportion of flies rested on the corks of his observation jars, whilst at intermediate temperatures few did so. He considered that the corks, by virtue of their low conductivity, would 'feel' relatively warm at low temperatures and cool at high temperatures, and that a preponderance of flies resting on the cork was an indication of 'uncomfortable' temperatures in the jar. In constant temperature experiments, Nicholson found the zone of 'comfortable' temperatures to be from 25° to 35°C. Considering the very different manner of assessing temperature preference, these figures are quite close to those obtained by the radiator method (31·5° to 38°C.).

D. F. WATERHOUSE.

C.S.I.R.,
Canberra, A.C.T.

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INSECTARY TESTS WITH INSECTICIDES TO PROTECT SHEEP AGAINST BODY STRIKE

By D. F. WATERHOUSE* and MARION T. SCOTT†

(*Manuscript received March* 23, 1950)

Summary

Areas on the backs of sheep were sprayed with several insecticides and a comparison was made of the degree and length of protection thus provided against oviposition, and in one experiment against strike, by *Lucilia cuprina* when the sheep were exposed to a dense population of flies in an insectary. Indole plugs were tied into the fleece to provide conditions suitable for oviposition.

Under these conditions DDT gave better protection than other insecticides. In 2 per cent. concentration it gave excellent protection for 6-8 weeks; in 1 per cent. concentration the protective period was shorter, although valuable protection was still given. A noticeable feature of the DDT treatments was their long partial protection after some oviposition was permitted, an effect which was not as marked with other insecticides. Crude BHC preparations gave valuable protection when applied at 0.5 per cent. gamma isomer. Intermittent artificial rain, amounting to 17 inches, did not affect the protection afforded by 1.0 per cent. DDT or 0.5 per cent. BHC over a 64-day period. Chlordane gave some protection, but it was less effective than DDT, and chlorinated camphene did not give useful protection.

In vitro tests indicated that none of the insecticides was ovicidal. However, in larvicidal tests BHC was extremely toxic, the vapour killing larvae rapidly even when contact with the solid material was prevented. None of the other insecticides had a fumigating action, although in high concentrations they were often lethal on contact.

I. INTRODUCTION

Crutch and body strike are the most prevalent forms of blowfly strike in Australia. Effective and permanent protective measures (modified Mules operation, combined with correct docking and tail operation) are now available against crutch strike. Although these methods do not influence body strike directly, they probably do so indirectly by minimizing larval development from crutch strikes, which produces a corresponding reduction in fly numbers.

Body strike is most frequent in sheep carrying 6 to 12 months' wool, the back of the neck, withers, points of the shoulders, and rump being the most usual sites. The predisposing factors are not well known, although it is generally agreed that "fleece rot", which occurs under summer rainfall conditions, together with certain types of body conformation and certain fleece characteristics provide particularly favourable conditions for the

* Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

† Division of Animal Health and Production, C.S.I.R.O., McMaster Animal Health Laboratory, Sydney.

development of body strike. Hence, effective control of body strike by an attack on the factors that predispose to it, which has been successful with crutch and tail strike, cannot be expected in our present state of knowledge. However, a feasible method of prevention would be to render the fleece toxic or repellent to flies. Whereas some information is available on blowfly repellents, none of the effective repellents is sufficiently persistent or cheap for use as a preventive of body strike. With the development of the modern, highly effective, and persistent insecticides, however, there were grounds for hoping that their application to the fleece might reduce the incidence of body strike. Insectary tests with one method of application, namely spraying the surface of the fleece over susceptible areas, are reported in this paper.

II. Methods

(*a*) *Treatment of the Sheep*

Measured quantities of insecticidal preparations were sprayed on a restricted area of the back of each sheep. The sprayed area was located symmetrically about the mid-line towards either the anterior or posterior end of the body, leaving an untreated area which was protected from contamination during spraying by several thicknesses of absorbent paper. Equal numbers of sheep were sprayed anteriorly and posteriorly, although no significant difference in attractiveness had been found between these sites (Mackerras and Mackerras 1944).

The insecticides were applied in this fashion for two reasons. First, it was desirable to test the treatments on the portion of the sheep most susceptible to body strike and, by restricting the treated area to the dorsal surface of the sheep, it was hoped that transference of insecticide from sheep to sheep, or from sheep to structures in the insectary, would largely be avoided. Secondly, owing to the great individual variability in attractiveness to flies within an apparently uniform group of sheep and the small number of sheep with which it was possible to work, it was important to have on each sheep a treated and a control area for comparison. The extent of the area sprayed varied somewhat according to the size of the sheep; in the lambs used it was 10 in. by 10 in. (Experiment 4), in the ewes and small wethers 10 in. by 12 in. (Experiments 1, 2, 3, 5), and in the larger wethers 12 in. by 12 in. (Experiments 6, 7, 8). In the early experiments (1 to 5) the treatments were applied with an electric spray gun producing coarse droplets, and in later experiments (6 to 8) by a knapsack sprayer with a 3/16 in. nozzle. The amount of insecticide applied per sheep with the electric spray gun was about 75 ml., which wets the surface of the fleece thoroughly without allowing any run-off. With the knapsack sprayer a very much heavier application of spray was made, amounting to about 1100 ml. of fluid per sheep. This saturated the outer portion of the fleece, allowing penetration to the skin between staples, but some spray was lost by run-off from the surface of the fleece and by drainage.

(*b*) *Testing the Effectiveness of Treatments*

To measure protection against oviposition the indole-plug method (Hobson 1936; Mackerras and Mackerras 1944) was employed. Briefly, the method consisted of tying into the centre of treated and untreated areas of fleece a cotton-wool plug saturated with a freshly-prepared aqueous solution containing 0.04 per cent. indole, 2.5 per cent. ethyl alcohol, and 2.0 per cent. ammonium carbonate. It has been demonstrated (Mackerras and Mackerras 1944) that alcoholic indole plugs are more attractive to flies than the soiled wet breech, slightly less attractive than the breech touled by scouring, and much less attractive than the struck breech. The addition of ammonium carbonate results in a slight increase in the attractiveness of alcoholic indole and it is believed that the solution used had the same general order of attractiveness as an unstruck but susceptible sheep.

The sheep were then exposed in an insectary to a dense population of the Australian sheep blowfly *Lucilia cuprina*, containing hundreds of gravid females, an intensity of attack far more severe than the sheep would ever normally encounter in the field. After several hours the number of egg batches, each laid by an individual female, was estimated (Mackerras and Mackerras 1944) and the plugs and eggs were removed to prevent strikes from developing. Eggs were generally laid only in the immediate vicinity of the plugs and could be found quite readily. The sheep were allowed to graze in the open except when under test in the insectary, exposure to flies being repeated at intervals until the treatments no longer afforded protection.

A fairly constant density of flies was maintained in the insectary, which was held at 25°C. or above by thermostatically controlled heaters. Except when the temperatures rose in summer above about 32°C., the flies usually laid between 2 and 10 egg batches per control area at each test.

(*c*) *Interpretation of Results*

The degree of protection by an insecticidal treatment was assessed by comparing (*a*) the number of treated and control areas on which eggs were laid and (*b*) the numbers of egg batches laid on those areas. It was considered that breakdown of protection was not as clearly indicated when eggs were deposited on only one of the areas treated in a particular way as when the same number of egg batches was distributed over a greater number of such treated areas. The basis for this assumption was the probability that, when eggs were deposited on only one treated area, oviposition was due to some abnormality in that particular area (e.g. accidental exposure of untreated fleece) but that, when eggs were deposited on two or more areas, this indicated that the insecticide was losing its protective effect. A rating method was used which took both comparisons into account (Table 1), the range from complete protection to no protection

being arbitrarily divided into 5 categories. These are represented graphically (Figs. 1-8) by the width of shading, which diminishes with lessening of the degree of protection.

TABLE 1

BASIS FOR RATING THE PROTECTION GIVEN BY INSECTICIDES AGAINST OVIPOSITION

Protection	Shown on Figures as	$\frac{\text{Egg Batches on Treated Areas}}{\text{Egg Batches on Control Areas}} \times 100$	$\frac{\text{Treated Areas showing Eggs}}{\text{Control Areas showing Eggs}} \times 100$
Complete	Widest shading	0	0
Good	Medium-width shading	1-20 1-10	1-25 1-50
Fair	Thin shading	21-40 11-40	25-50 50-75
Slight	Thin line	41-60	25-100
Poor or none	Dot	61-100	25-100

With all insecticides tested there was some fluctuation in the degree of protection (Figs. 1-8), days with relatively poor protection being followed by days on which greater protection was given. Several factors undoubtedly contributed to this variability. One was the small number of sheep used in the experiments. Another was that, unavoidably, the intensity of fly attack varied considerably from day to day. When the oviposition rate was high the chance of eggs being laid on treated areas was greater than when it was low. A further factor was that within the treated areas untreated wool was at times exposed to oviposition by mechanical disarrangement of the staple, either by the operator when inserting the indole plugs or by the movements of the sheep. In spite of the magnitude of the fluctuations thus caused, the main trend of results was generally quite clear, although it was difficult to assess the exact period for which any particular treatment gave a certain degree of protection. However, a fairly reliable comparison could be made between the treatments applied in a particular experiment.

III. RESULTS

(*a*) *Experiments* 1-4

Experiments 1-4 (Figs. 1-4) indicated the efficacy of DDT treatments. When applied as a solution in kerosene, an increase in DDT concentration*

* All concentrations of DDT refer to the *pp'* isomer.

from 0.5 per cent. through 1 per cent. to 2 per cent. gave a corresponding increase in length of protection (Fig. 1), but a further increase to 4 per

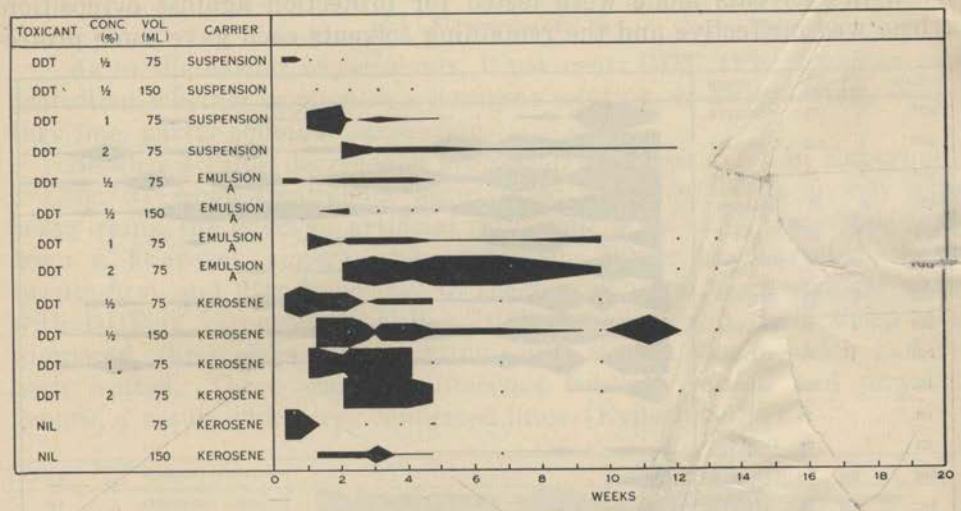

Fig. 1.—Experiment 1: 4 ewes per treatment; treated area 10 in. by 12 in.; treatment applied November 7, 1945.

cent. did not confer any additional protection (Figs. 2 and 3). Increasing the volume of spray applied from 75 ml. to 150 ml. did not affect the results appreciably (Figs. 1 and 2).

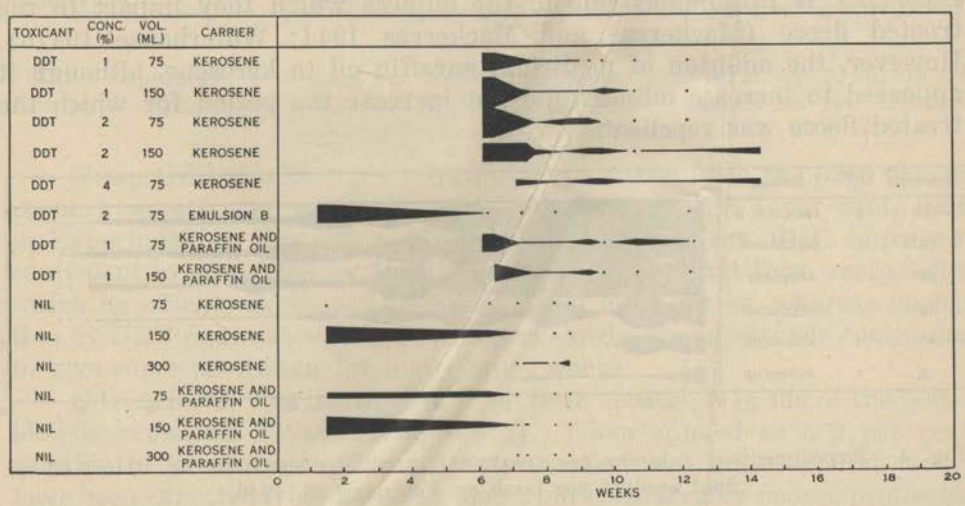

Fig. 2.—Experiment 2: 4 ewes per treatment; treated area 10 in. by 12 in.; treatment applied December 2, 1945.

DDT was tested (*a*) in three different types of emulsion, (*b*) as a very fine, partly colloidal, suspension, (*c*) as a coarse suspension, and (*d*)

as solutions in *Eucalyptus dives* oil, in crude xylene, in kerosene, and in a mixture of 25 per cent. medicinal paraffin and 75 per cent. kerosene. When the solvents alone were tested for protection against oviposition, xylene was ineffective and the remaining solvents each gave some protec-

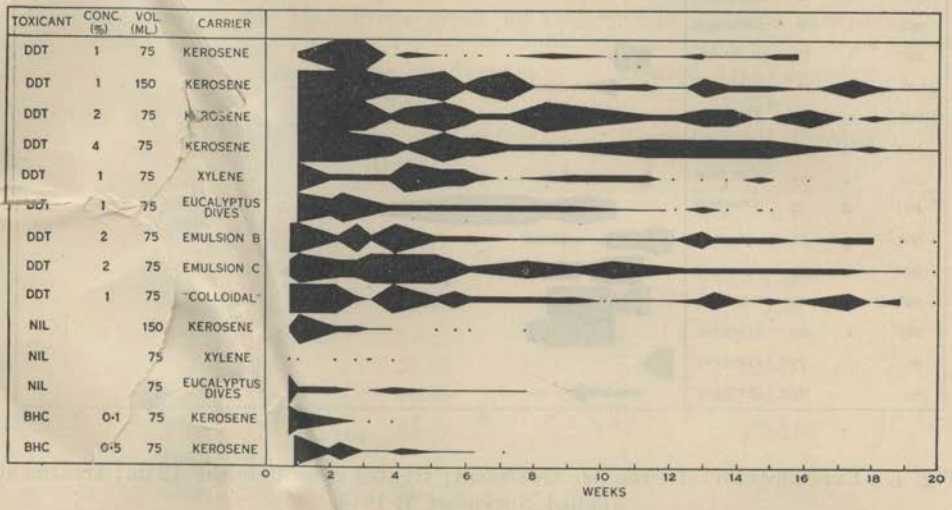

Fig. 3.—Experiment 3: 4 wethers per treatment; treated area 10 in. by 12 in.; treatment applied February 6, 1946.

tion, but of insufficient duration to affect the experiments. This slight repellency is presumably due to the oiliness which they impart to the treated fleece (Mackerras and Mackerras 1944; Waterhouse 1947*b*). However, the addition of medicinal paraffin oil to kerosene, although it appeared to increase oiliness, did not increase the period for which the treated fleece was repellent.

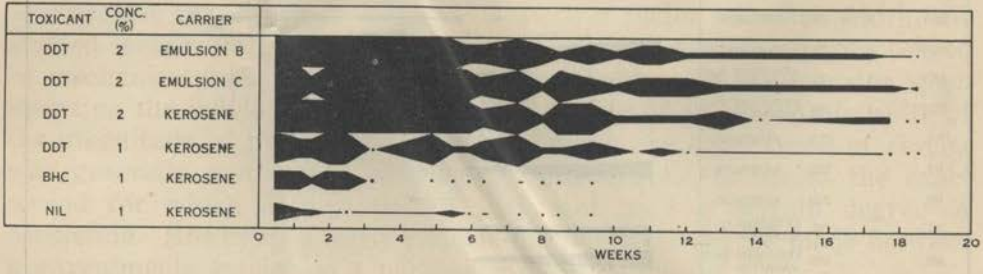

Fig. 4.—Experiment 4: 4 lambs per treatment; treated area 10 in. by 10 in.; 63 ml. fluid applied per lamb on February 26, 1946.

The pure gamma isomer of BHC*, dissolved in kerosene and tested at 0.1 per cent. and 0.5 per cent. concentrations, gave only slight protection and was no more effective than some of the solvents used alone. At 1.0

* All BHC concentrations are expressed in terms of the gamma isomer.

per cent., BHC gave slightly better protection, but was markedly inferior to an equivalent concentration of DDT.

(*b*) *Experiment* 5

As in the earlier experiments, 2 per cent. DDT (Fig. 5) gave good protection whether applied as a kerosene solution, as an emulsion, or as a very fine, partly colloidal suspension.

Because a rapid decrease in protection had been noted in Experiment 2 (Fig. 2) when treatments were tested at seven weeks shortly after heavy rains, the effect of artificial rain (half a gallon of water per sheep from a knapsack spray) was examined. Water was applied on the twenty-first and fifty-third day to sheep which had been sprayed either with DDT in kerosene or with a DDT emulsion, and these sheep were compared, three days after wetting, with similar sheep which had not been wetted. There was no difference between wetted and unwetted groups, a result which was confirmed later (Experiment 8).

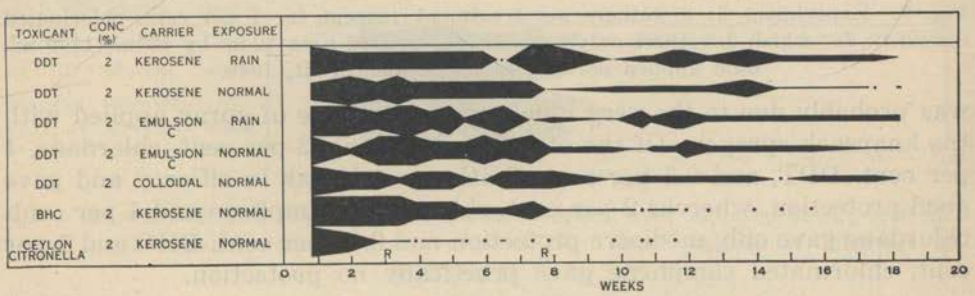

Fig. 5.—Experiment 5: 4 ewes per treatment; treated area 10 in. by 12 in.; 75 ml. fluid applied per ewe on July 12, 1946. 0.5 gal. "rain" applied on two occasions indicated by *R* at bottom of figure.

Sheep treated with 2 per cent. BHC (prepared from the pure gamma isomer) were better protected than those treated with 1 per cent. BHC in Experiment 4. Protection afforded by the 2 per cent. BHC spray was equivalent to protection by 2 per cent. DDT for the first four weeks, after which its efficacy decreased, to disappear at eight weeks, whereas protection by DDT diminished very much more slowly, the insecticide continuing to give some protection for many more weeks.

Citronella oil was included in these tests because it is one of the better blowfly repellents (Waterhouse 1947*b*). When applied as a 2 per cent. solution in kerosene, slightly better protection was afforded than would have been expected from kerosene alone, but considerably poorer protection than from any of the DDT treatments.

(*c*) *Experiment* 6

The four insecticides compared in this experiment (Fig. 6) were emulsified after solution in toluene. For the BHC emulsion the crude

material containing 12.5 per cent. gamma isomer was extracted with toluene, leaving a good deal of insoluble residue.

Whereas 1 per cent. DDT afforded good protection, 2 per cent. DDT was outstanding, giving excellent protection against oviposition for over four months. This is longer protection than obtained in earlier tests and

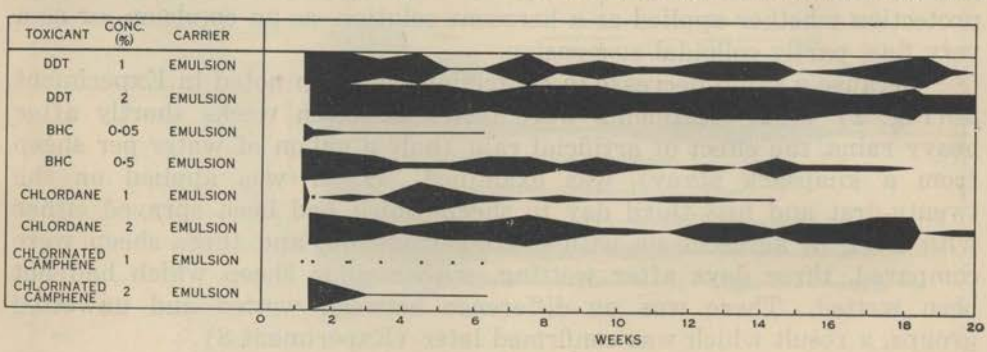

Fig. 6.—Experiment 6: 6 wethers per treatment (except for 1 per cent. chlorinated camphene for which 2 wethers only were used); treated area 12 in. by 12 in.; 1100 ml. fluid applied per wether on September 21, 1948.

was probably due to the very much greater volume of spray applied with the knapsack sprayer. Of the other preparations, 2 per cent. chlordane, 1 per cent. DDT, and 0.5 per cent. BHC were similar in efficacy and gave good protection, whereas 2 per cent. chlorinated camphene and 1 per cent. chlordane gave only mediocre protection and 0.05 per cent. BHC and 1 per cent. chlorinated camphene gave practically no protection.

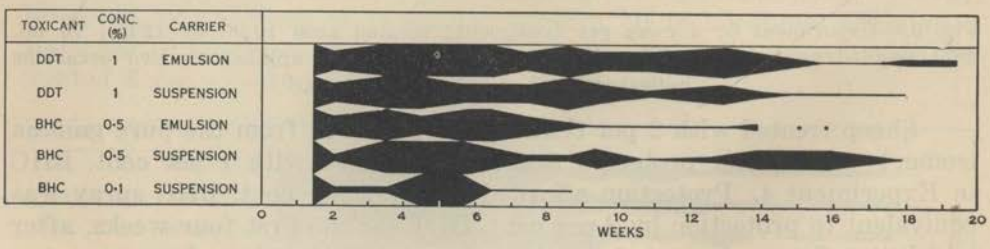

Fig. 7.—Experiment 7: 6 wethers per treatment; treated area 12 in. by 12 in.; 1100 ml. of fluid applied per wether on October 15, 1948.

(*d*) *Experiment* 7

Toluene emulsions of DDT or BHC and proprietary suspensions of these insecticides (Fig. 7) gave similar degrees of protection, although the suspensions were observed to wet the fleece less readily than the emulsions. DDT at 1 per cent. and BHC at 0.5 per cent. each gave a similar degree of protection for the first six or seven weeks. Thereafter, the degree of protection given by BHC diminished more rapidly than that given by DDT. Considerably poorer protection was afforded by 0.1 per cent. BHC than by 0.5 per cent.

(*e*) *Experiment* 8

Experiment 8 (Fig. 8) was carried out to determine whether the period of protection was shortened if sheep sprayed with DDT or BHC were subjected to regular applications of artificial rain. A small pen was fitted with a bank of sprays which delivered approximately one inch of uniformly fine "rain" during a half-hour period. This saturated the tip of the staple, but did not penetrate far into the fleece. The sheep were sprayed with water on the ninth, fourteenth, and sixteenth days after application of the insecticide and first exposed to flies in the insectary on the nineteenth day. Subsequently, bi-weekly sprayings were carried out for three weeks, followed by tri-weekly sprayings for the remainder of the experiment. The sheep were allowed to dry between sprayings and also before being tested in the insectary. This latter procedure was necessary in order to localize the eggs around the indole plugs so that their numbers could be estimated.

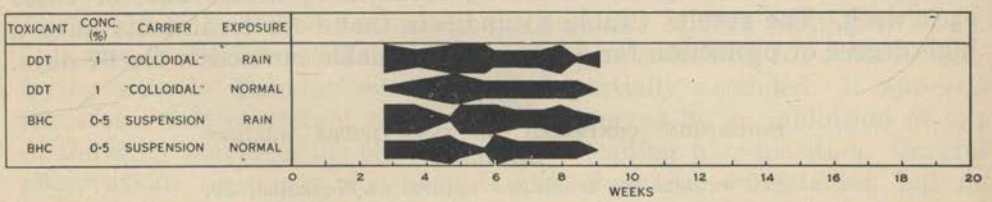

Fig. 8.—Experiment 8: 6 wethers per treatment; treated area 12 in. by 12 in.; 1100 ml. of fluid applied per wether on January 15, 1949. "Rain" applied at intervals as described in text.

The insecticide treatments were 1 per cent. DDT applied as a proprietary colloidal dispersion and 0.5 per cent. BHC applied as a proprietary suspension.

By the end of the experimental period (64 days) there was still no difference in protection between the wetted and unwetted control sheep although a total of 17 inches of rain had been applied. Hence there can have been no significant leaching of the insecticide by water. However, since the sheep were tested only after they had dried out, it provides no information on the protection given by the insecticides to the sheep whilst they are wet, when the flies might be prevented from coming in contact with the insecticide by a film of water and the sheep would probably be more attractive to the flies.

Similar protection was given by 1 per cent. DDT and 0.5 per cent. BHC throughout the nine-week period of this test, a result which agrees with that obtained in Experiment 7.

(*f*) *Experiment* 9

In Experiment 9 an attempt was made to simulate the conditions under which the insecticides would be used in practice. On each sheep a strip

of fleece about 12 inches wide extending from the back of the neck to the base of the tail was sprayed by knapsack sprayer with approximately 1 gallon (4541 ml.) of either 1.0 per cent. DDT (the partly colloidal emulsion) or 0.05 per cent. BHC (a proprietary suspension). All sheep were exposed to one inch of artificial rain each day for several weeks to encourage fleece rot, which developed rapidly. To test the treatments, the sheep were subjected to one inch of "rain" and small cotton-wool plugs, about one inch in diameter, were placed on the skin on the withers and on the rump. These plugs were moistened with 25 ml. of indole solution and the fleece rearranged to hide the plugs completely. The sheep, with their fleeces still moist, were then exposed to fly attack in the insectary. After exposure, the number of egg batches laid in the vicinity of the hidden plugs was roughly estimated and the fleeces moistened again to provide suitable conditions for the eggs to develop. The sheep were subjected to "rain" on subsequent days and any strikes which developed were recorded. This procedure was repeated with exposures to fly attack once or twice each week. The results (Table 2) indicate that both treatments gave a high degree of protection for 54 days and valuable protection for 67 days.

TABLE 2

PROTECTION CONFERRED BY INSECTICIDES AGAINST STRIKE

(12 wethers per treatment applied on November 10, 1949)

Days after Treatment	Per Cent. Struck (Cumulative)	
	DDT	BHC
33	0	8
54	8	25
67	33	42
74	42	50
77	50	92

DDT gave somewhat better protection than BHC, only 50 per cent. of the DDT-treated sheep being struck by the seventy-seventh day compared with 92 per cent. of the BHC-treated sheep. A noticeable feature of these tests was that eggs were deposited on the treated sheep many times, and larvae often hatched and died, before a strike was eventually established. This failure to establish strikes must have been due to the presence of the insecticides in the fleece, because conditions were otherwise very favourable for development of larvae. This type of protection was particularly noticeable among the BHC-treated sheep, which frequently received many more egg batches than those treated with DDT. Indeed, if the criterion were the number of egg batches deposited, the BHC treatment would have been considered ineffective long before protection against strike had in fact ceased.

In this same experiment DDT and BHC were also applied with a commercial fogging machine to other groups of sheep. When these sheep were first exposed in the insectary 19 days after treatment, no protection was afforded against strike, which is a strong indication that, at the concentrations used in these tests, namely 2.0 per cent. BHC and 10 per cent. DDT, fogging is not an efficient method of applying either of these insecticides for the prevention of body strike.

(*g*) *Effect of Treatments on* L. cuprina

The mortality of flies in the insectary was considerably higher in these tests than it normally is in plug experiments with attractants and repellents. As some flies showed DDT tremors, particularly when recently treated sheep were exposed, it was clear that the insecticide treatments were the primary cause of this higher mortality. As noted earlier (Waterhouse 1947*b*), fleece treated with DDT did not appear to be strongly repellent to *L. cuprina*, and the treated and untreated areas could not be distinguished by any difference in the numbers of flies resting on them. However, in DDT-treated fleece there was less searching for suitable egg-laying sites by females with ovipositor partially extended. It appears, therefore, that important protection is conferred by an inhibition of one of the early stages in the chain of reflexes leading to oviposition. Careful observations were not carried out with the other insecticides, but no marked difference in numbers of flies resting on treated and untreated fleece was observed.

(*h*) *Effect of Treatments on the Sheep*

None of the insecticides used in these tests produced any adverse effects on the sheep. The solvents used in some emulsions, however, did cause irritation for about 15 minutes after application. Although kerosene produced no harmful effects when applied as a fine spray in early experiments (1 to 5), its use with coarser sprays in the field caused severe scalding and kerosene solutions were therefore excluded from later experiments.

IV. Effect of Insecticides on Eggs and Young Larvae

Conclusions concerning the relative efficiency of the various insecticides have been based, in the majority of the experiments already described, on the presence or absence of eggs in the treated fleece. It is important to know, however, whether these eggs will be prevented from hatching by the insecticidal treatments and, if hatching occurs, whether the young larvae will be prevented from establishing themselves in a strike wound. The latter effect is suggested by the results of Experiment 9.

(*a*) *Ovicidal Effect*

Eggs laid on treated fleece might be affected in three ways: by direct contact with the dry insecticide, indirectly when a film of water intervenes

between egg and insecticide, and by fumigant action. To test these possibilities 1 ml. of a 1 per cent. solution of the insecticides in acetone was applied to three filter papers 5.5 cm. in diameter which were then dried in an air current for 24 hours. These treated filter papers were used to expose the eggs to the insecticide in three different ways. One treated, but dry, paper was placed in the centre of a petri dish 9 cm. in diameter and encircled by a narrow strip of untreated filter paper, moistened with water. A second treated paper was set up in similar fashion, except that it too was moistened with water. The third treated paper was attached to the undersurface of the lid of a petri dish which carried in the lower half an outer moistened circle of filter paper and an inner dry, untreated 5.5-cm. filter paper. One hundred *L. cuprina* eggs separated in water or in 1 per cent. sodium sulphide were then placed in the centre of the 5.5-cm. filter paper on the bottom of each petri dish and incubated at about 75°F. The moistened filter paper in each petri dish maintained a humidity favourable for development and the percentage hatch was determined after 24 hours. Results obtained with these tests are shown in Table 3.

TABLE 3

AVERAGE PER CENT. HATCH OF EGGS EXPOSED TO INSECTICIDES

	Insecticide Treatment		
	Contact with Dry Insecticide	Contact with Moist Insecticide	Fumigant Action
Control	87	83	86
DDT	88	83	84
BHC	92	77	78
Chlordane	87	74	91
Chlorinated camphene	80	82	78

Three replications, each with 100 eggs, were carried out with the wet and dry insecticides and two replications of tests involving fumigant action. It is clear that none of the insecticides prevents embryonic development. It was observed that all larvae hatched about the same time, so that there was no retardation of development. This agrees with results of previous work which showed that neither DDT (Waterhouse 1947*a*) nor BHC (Duchanois 1947) has any ovicidal effect.

(*b*) *Effect on Young Larvae*

A noticeable feature of the ovicidal tests was that, within a short time after emergence, all the young larvae from the BHC dishes were moribund and some had failed to free themselves completely from the egg shells. This occurred even if the larvae were not in direct contact with BHC. Larvae from other treatments were affected to a lesser extent.

Although respiratory exchanges must have occurred through the egg shell, BHC vapour apparently did not penetrate in lethal doses.

When newly-hatched larvae were placed for three hours on wool which had been dipped in acetone solutions of insecticides and dried for at least 24 hours, the larvicidal effect of BHC was again striking. Whereas all insecticides, if used as 1.0 per cent. or 0.1 per cent. solutions, prevented larvae from developing when transferred to liver after exposure, only BHC prevented all or almost all of the larvae from developing when used at a concentration of 0.01 per cent. Chlorinated camphene appeared to have a slight effect at this concentration but DDT and chlordane had little or no effect. When two-day-old larvae were exposed to wool treated with 0.1 per cent. solutions, BHC prevented further development of most of the larvae whereas the other insecticides had no effect at all.

Similarly, when newly-hatched larvae were placed for two hours in filter paper envelopes treated with insecticides (1 ml. of 1 per cent. solution per 5.5 cm. filter paper) BHC alone completely prevented larvae from developing when all larvae, moribund or alive, were subsequently transferred to liver. Several authors (Duchanois 1947; Loeffler and Hoskins 1946; Shanahan 1946) have also recorded the high toxicity of BHC for fly larvae.

V. Discussion

DDT was the most effective of the insecticides used for preventing oviposition by *L. cuprina*. If an endeavour is made to translate this result into terms of protection against body strike in the field there are two further factors to be considered: any additional protection resulting from the effect of the insecticide on young larvae, and any possible reduction in protection resulting from the treated areas becoming wet or soiled. It is clear from laboratory tests that BHC is a far more effective fumigant for young larvae than any of the other insecticides tested in these experiments. This effect is apparently sufficient to prevent a strike from becoming established after protection against oviposition has partially or completely disappeared. It appears that, for some time after application of BHC, its vapour provides an unbroken barrier of insecticide even if the surface of the fleece is not sufficiently toxic or repellent to prevent oviposition. In general, the protective effect of BHC diminished rapidly once it commenced to decline, whereas DDT continued to exert an appreciable effect for weeks after some oviposition was permitted. These effects were probably due to the considerable differences in the volatility of the two materials.

In the earlier experiments (3-5), in which the pure gamma isomer of BHC was used, poorer protection was obtained than in later experiments with crude BHC, but whether this was due to altered experimental conditions (the rate of application was far higher in the later experiments), to the different types of preparation used, or to some effective

material other than the gamma isomer in crude BHC, was not determined.

The period of time (several months) for which DDT remains on the fleece is striking when compared with the week or 10 days for which it remains on cattle hair, where rapid loss of DDT is mainly due to its removal by licking (Hackman 1947).

The results obtained in these experiments are in general agreement with published records of field tests. In Britain a number of workers (Cragg 1945, 1946, 1947; Harbour and Watt 1945; Hughes, Jenkins, and Jones 1946; Hughes *et al.* 1947; Lyle-Stewart 1946; Stamp, Watt, and Beattie 1948) have obtained valuable protection against strike, for periods up to 6 or 7 weeks, by dipping in emulsions containing 0.5 per cent. DDT. Most of the strikes on treated sheep occurred on the crutch area when this became wet with urine or soiled by faeces and it is clear that under such circumstances DDT does not give effective protection. It was noticed, however, that strikes on DDT-treated sheep were generally restricted in their extent. The number of strikes recorded on control sheep running with treated sheep was lower than expected, although the number of strikes recorded in neighbouring flocks with no treated sheep indicated that conditions for strike were not unusual. It was suggested that this was brought about by flies dying after contact with DDT, thereby reducing the local fly population. Tests with 0.06 per cent. BHC or lower concentrations gave poorer protection than that given by 0.5 per cent. DDT but about the same degree of protection as that given by arsenic.

In South Africa it has been reported that 6 per cent., 5 per cent., and 2.5 per cent. DDT gave good protection against crutch strike for three months (du Toit 1946; du Toit and Goosen 1949). In Australia complete protection against body strike for three weeks was obtained with 0.06 per cent. BHC in one experiment (Shanahan 1948) and for about five weeks with 0.03 to 0.05 per cent. BHC in another (Council for Scientific and Industrial Research 1948), and in a third series of experiments 0.06 per cent. BHC was no more effective than calcium arsenite when used as a jetting mixture to control crutch strike (Shanahan and Morley 1948).

The concentrations found to confer protection in these field experiments are, with one exception, lower than those which would have been selected on the basis of the present tests, namely 1 per cent. or 2 per cent. DDT and 0.5 per cent. or higher BHC. This suggests that conditions under which insectary tests were performed were more severe than encountered in field trials.

Field trials, based on data presented in this paper, are being carried out by colleagues. Results to date are very encouraging, particularly when the incidence of body strike has been high.

In tests in the United States of America young larvae were placed at intervals on areas which had been dressed with various insecticides. Chlorinated camphene prevented artificial strikes from becoming established for 55 to 82 days, chlordane and BHC gave somewhat shorter protec-

tion, and DDT gave comparatively poor protection (Graham and Eddy 1948). Since the relative efficacy of the insecticides differed from that found in the present experiments, it should be pointed out that protection against establishment of larvae is a measure of the contact and stomach toxicity of the insecticides to larvae and differs from protection against oviposition, which was the primary consideration in the present paper. The former is of less importance than the latter when the barrier involved in preventing strike is a layer of treated fleece in which larvae do not feed and in which they do not remain for long to be affected by the insecticide. These differences demonstrate that tests such as the present series cannot entirely replace field trials in which many more factors are involved.

VI. Acknowledgments

The authors wish to acknowledge the assistance of many of their colleagues, and in particular that of Mr. S. H. Dee, in carrying out these experiments.

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The Use of Borax for the Prevention of Fly Breeding in Trap Baits

By D. F. Waterhouse, M.Sc., and Mary E. Fuller, B.Sc.†*

Summary.

Many flies may be produced by untreated baits in blowfly traps, the number bred sometimes exceeding the total number of flies caught by the trap. The flies bred are generally Calliphoras or, less frequently, *Chrysomyia rufifacies*. No *Lucilia cuprina* were produced. Borax (2 per cent. or more by weight), sprinkled over the surface, inhibited larval development, but generally reduced the attractiveness of the bait to all species. *L. cuprina* was sometimes an exception to this statement. The effect of trapping on the fly population is considered and the conclusion is reached that the addition of borax to baits is not warranted.

1. Introduction.

Observations in the field and experience in experimental trapping have shown that larvae from captured flies develop freely in the bait chambers of traps and escape to produce a new generation of flies. M. E. Fuller had early appreciated this fact and had undertaken a number of experiments with borax and other substances to prevent larval development in the baits. Her observations were uncompleted at the time of her death and at a later stage D. F. Waterhouse repeated and extended them, arriving at somewhat different conclusions from those to which the earlier work pointed. In the present paper both sets of work are brought together, but the interpretation of them is solely the responsibility of D. F. Waterhouse.

The work on bait treatment previously reported (Freney, 1932A and B, 1937; Fuller, 1934A) aimed primarily at enhancing attractiveness, prevention of maggot development being a minor consideration. This paper records the results of bait treatment to prevent larval growth and the effect of such treatment on the attractiveness of the bait.

The Western Australian trap (Newman and Clark, 1941) which is the best type available for field use, allows free access of flies to the bait and also unhampered escape of prepupae through the entrance

* An officer of the Division of Economic Entomology.

† The late Miss Fuller was an officer of the Division of Economic Entomology at the time her part of the work described was carried out.

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slits. Many modifications have been tested, but no simple and efficient trap with mechanical means of preventing the flies from ovipositing or the maggots from escaping has yet been devised. Gravid female flies will readily deposit eggs or larvae in a trap, and any device which allows the escape of attractive carrion odours whilst preventing access of the flies to the bait, greatly increases the complexity and therefore the cost of the trap. It will be shown in this paper that the number of maggots escaping from a trap is often large and, under some conditions, may exceed the number of flies captured.

2. Method.

The experiments were carried out at Canberra, A.C.T., in Western Australian traps and in small, standardized, glass traps built on the Western Australian principle; these were used on the Nicholson turntable (Freney, 1937). The results were then checked in Western Australian traps set under normal field conditions. The traps were placed in shallow, sand-filled, maggot-proof containers to retain the maggots escaping from the baits. Only those species which are important to the sheep blowfly problem or cause domestic annoyance were included in the figures considered in this paper. These are the primary* species *Lucilia cuprina* Wied., *L. sericata* Meig., *Calliphora stygia* Fabr., and *C. augur* Fabr., and the secondary fly *Chrysomyia rufifacies* Macq. Particular attention was paid to *L. cuprina* which is responsible for most of the strikes occurring on sheep.

3. Results.

(i) *A comparison of the number of flies caught and the number of flies produced by a bait.*

Many factors have been found to influence the number of maggots escaping from a trap and developing into adults. The more important of these were:—

(*a*) The amount of bait.

(*b*) The type of bait.

(*c*) The competition of the larvae for food, and the weather conditions influencing severity of competition. For instance, of 35 untreated baits in glass traps (Table 1), seven produced no flies owing to the effects of competition, and two dried up before any flies were caught.

(*d*) The presence of predators and parasites which concentrate around traps and greatly reduce the number of flies bred. The beetles, *Saprinus* (Histeridae) and *Creophilus* (Staphylinidae), and also ants seize the maggots as they emerge from the traps, and in experiments of this kind the predators have an unusual advantage in that the maggots, being confined to the outer containers, have little chance to escape them. However, by collecting the maggots daily in some experiments a fairly true estimate was obtained of the number which would probably escape and develop under field conditions. Five, at least, of the above-mentioned 35 baits showed evidence that most of the maggots escaping from the bait pans had been destroyed by predators, and the

* Primary flies are able to initiate strikes on sheep, while the secondaries follow later.

few survivors were attacked by parasites, so that a very small number of flies was produced. The number of flies bred from all the other baits was also considerably reduced by the depredations of parasites or predators, the activity of these being largely influenced by weather conditions.

Summing up, of the 35 untreated baits exposed in glass traps between November and March (Table 1), 7 produced no flies from the bait owing to competition, 2 dried up before any flies were caught, 5 had almost all the maggots or puparia destroyed by predators and parasites, and the remaining 21 produced numbers of flies. Of these 21 traps, 13 produced more flies than they caught, and 1 produced as many. Although 8,096 flies were caught, the total population difference produced by the 35 traps was the destruction of 1,467 flies. A total of 158 *L. cuprina* was caught in the traps (an average of 4·5 per trap), while none were recorded as having bred in the baits.

TABLE 1.—COMPARISON BETWEEN THE NUMBER OF FLIES PRODUCED BY THE BAIT AND THE NUMBER CAUGHT—GLASS TRAPS.

Month Exposed.	Number of Flies.		Effect of Trapping on Fly Population.	Bait.
	Caught.	Bred.	+ Increase — Decrease.	
November to December	310	384	+ 74	Rat
	223	180	− 43	
November to December	443	201	−242	Cat
	280	499	+219	
December	194	194	0	Rat
	109	185	+ 76	
	253	145	−108	
	54	201	+147	
December	125	1	−124	Mouse
	105	0	−105	
	149	0	−149	
	95	0	− 95	
December	259	0	−259	Rat
	625	5	−620	
December	136	0	−136	Mouse
December	337	0	−337	Rat
December	0	0	0	Lambs'
	0	0	0	tails
January to February	60	714	+654	Rat
	217	402	+185	
	105	137	+ 32	
January to February	219	78	−141	Liver
	208	333	+125	
	233	339	+106	
	231	728	+497	
January to February	495	580	+ 85	Liver
	320	668	+348	
January to February ..	122	331	+209	Rat
February	356	0	−356	Liver
	271	29	−242	
February	43	4	− 39	Rat
March	227	86	−141	Rat
	475	19	−456	
March to April	448	103	−345	Rat
	369	83	−286	

The figures for Western Australian traps were somewhat different (Table 2). Of 17 baits, two small animals (less than 300 g. in weight) suffered complete and rapid desiccation and produced no flies, three were flooded by rain and all the maggots drowned, and the remaining twelve bred flies in varying numbers. Two bred more than half the number caught and two bred more than they caught. These latter two were guinea pig carcasses which are relatively unattractive, and hence were not overcrowded with maggots. The 17 baits bred 13,118 flies and caught 102,255. Of this total, 1,941 were *L. cuprina,* an average of 11¼ per trap.

TABLE 2.—COMPARISON BETWEEN THE NUMBER OF FLIES PRODUCED BY THE BAIT AND THE NUMBER CAUGHT—WESTERN AUSTRALIAN TRAPS.

Month Exposed.	Number of Flies.		Effect of Trapping on Fly Population.	Bait.
	Caught.	Bred.	+ Increase − Decrease.	
October to November	10,557	4,450	− 6,107	Cat
November	7,143	850	− 6,293	Cat
November to December	2,833	Larvae	− 2,833	Cat
	1,013	drowned	− 1,013	
December	1,958	1,500	− 458	Cat
December	3,949	370	− 3,579	Cat
December	580	Larvae	− 580	Dog
	313	dessicated	− 313	
December to January	5,327	Larvae drowned	− 5,327	Guinea pig
January to February	186	380	+ 194	Guinea
	803	944	+ 141	pig
January to February	6,335	660	− 5,675	Liver
	27,299	966	−26,333	
January to February	15,433	500	−14,933	Liver
February	1,488	839	− 649	Cat
March to April	13,043	503	−12,540	Liver
	3,995	1,156	− 2,839	

The majority of the maggots recorded as escaping from baits in Tables 1 and 2 were *Calliphora augur* while *C. stygia* was occasionally found. When *Ch. rufifacies* was present in the bait, most of the maggots of this species pupated on the bait or in the bait pan, and the resulting flies were trapped immediately on emergence. Occasionally, however, larvae did escape. No *Lucilia* were recorded at any time from the bait.

(ii) *Prevention of Maggot Development.*

In view of the fact that trap baits frequently produced flies, experiments were set up to determine whether breeding could be prevented without lowering the attractiveness of the bait.

Various preliminary experiments and general field observations had demonstrated that, although arsenicals prevent larval development, they so greatly reduce the attractiveness of baits to all flies, and particularly to primary flies, that their use can readily be excluded on this ground. Experimental work had shown that both borax and boric acid were effective maggot poisons, whether dusted on carcasses

(Fuller, unpublished) or incorporated in dressings for struck sheep (Freney *et al.*, 1936; Lennox, 1940, 1941). There is, however, no published information on the attractiveness of borax-treated baits to flies. One of the great advantages of these boron compounds is their low toxicity to mammals, so that they can be handled with perfect safety. Since borax is cheaper than boric acid, extensive tests were conducted with this compound. During five months' trapping, 44 tests were made of the effect of borax on maggot development in both carcass and liver baits (Table 3). The borax was sprinkled on to the surface of the bait, the amount used varying from 2 to 10 per cent. by weight. Some of these baits were left dry and to others water was added but, in every case, the treatment was completely effective in preventing flies from being produced, and the few larvae which developed to full size died before pupating.

Table 3.—The Effect of Bait Treatments on Fly Catches and Number of Flies Produced by the Bait—Glass Traps.

Number of Tests.	Treatment.	Average Number Flies Bred per Trap.	Average Number Flies Caught per Trap.
44	Borax	0	257
7	Na_2S + borax	0	516
7	Na_2S	123	680
7	Nicotine sulphate	40	118
35	Controls	179	231

In the same series of experiments, tests were made of the effect of adding sodium sulphide, a mixture of borax and sodium sulphide, or nicotine sulphate to the bait. Sodium sulphide (2·5 to 5 per cent.) treatment of baits did not prevent maggot development, but it reduced the number of maggots escaping. This was probably due to the more intense competition for food as the bait was more attractive.

A mixture of sodium sulphide and borax in equal quantities (2·5 per cent. of each) prevented adults being produced in all but one instance, when a few *Helicobia* (Sarcophagidae) were bred. Nicotine sulphate in concentrations from 2 to 8 per cent. often prevented adults being produced, although larvae generally began to develop on the baits. None of the baits produced *L. cuprina*.

(iii) *The Attractiveness of Borax-treated Baits.*

Since borax had proved efficient in preventing larval development it was next tested for its effect on bait attractiveness.

(*a*) *Glass traps.*—Considering first of all the results with 91 baits in glass traps (Table 4), borax-treated baits caught more flies than the controls, but this difference was not significant. The figures for primary and secondary flies, and for *L. cuprina*, were also examined. The presence of borax did not influence the catch of primary flies or of *L. cuprina*, but it resulted in a significantly higher catch of the secondary fly *Ch. rufifacies*.

TABLE 4.—THE EFFECT OF BORAX ON THE ATTRACTIVENESS OF BAITS IN GLASS TRAPS.

Number of Traps in Experiment.	Concentration of Borax in Treated Bait (Percentage by Weight).	Average Number of Flies per Trap.			
		Borax.		Control.	
		Total.	*Lucilia cuprina.*	Total.	*Lucilia cuprina.*
6	2	161	1·3	127	0·3
10	2½	75	6·4	174	21·4
4	3	193	4·5	136	9·0
35	5	467	5·7	240	2·6
12	7	338	11·7	198	6·3
20	8	241	5·8	246	7·4
4	10	313	4·0	403	13·0

Early work by Freney (1932A, B, 1937) and Fuller (1934A) demonstrated that the addition of sodium sulphide usually rendered baits much more attractive than untreated ones. Now sodium sulphide alone has been shown to be an ineffective maggot poison (Table 3), but when borax was added the mixture prevented larval development. Borax was used at 5 per cent. concentration, sulphide at 2½ per cent., and borax + sulphide at 2½ per cent. each. An analysis of seven comparisons which are summarized in Table 5 showed that borax did not have any significant effect upon the attractiveness either of liver or liver plus sodium sulphide baits. In these tests, borax treated baits caught slightly fewer primary flies and about the same number of secondaries as the controls. The presence of sodium sulphide enhanced the attractiveness of the bait whether borax was present or not.

TABLE 5.—THE EFFECT OF BAIT TREATMENT ON BLOWFLY CATCHES—GLASS TRAPS.

—	Borax + Na_2S.	Na_2S.	Borax.	Control.
Catch per trap	516	683	222	266
Percentage of primaries in catch ..	67	69	75	88
L. cuprina per trap	2	6	3	4

(*b*) *Western Australian Traps.*—While borax sprinkled on the baits of Western Australian traps was invariably successful in preventing maggot development, it considerably reduced the total number of flies caught (Table 6), the borax-treated baits catching only about half as many flies as the controls. These experiments were conducted over two seasons and involved more than 300,000 flies, so doubtless they give a truer picture of what occurs under field conditions than the experiments with glass traps. It is important to note, however, in these

experiments there was no difference in the number of *L. cuprina* caught and there was also some indication that the borax-treated bait caught a higher proportion of primary flies than the control bait.

TABLE 6.—THE EFFECT OF BAIT TREATMENT ON THE ATTRACTIVENESS OF BAITS IN WESTERN AUSTRALIAN TRAPS.

Experiment.	Borax.				Control.			
	Number of Traps.	Average Flies per Trap.		Percentage Primaries.	Number of Traps.	Average Flies per Trap.		Percentage Primaries.
		Total.	*Lucilia cuprina.*			Total.	*Lucilia cuprina.*	
W.A. traps in the field	23	3,101	152	55	17	6,051	114	44
W.A. traps on turn tables	11	3,286	200	68	11	7,103	224	54
Average	..	3,194	176	62	..	6,577	169	49

A comparison was also made of the relative effects of borax on wet and on dry baits (Table 7). For the wet baits water was added until about half of the bait was submerged.

TABLE 7.—THE EFFECT ON WESTERN AUSTRALIAN TRAP CATCHES OF THE ADDITION OF WATER TO THE BAIT.

Bait.	Untreated.				Borax.			
	Wet.		Dry.		Wet.		Dry.	
	Total Flies.	*Lucilia cuprina.*	Total Flies.	*Lucilia cuprina.*	Total Flies.	*Lucilia cuprina.*	Total Flies.	*Lucilia cuprina*
Dog	580	35	313	5	629	17	513	12
Guinea pig ..	803	5	186	0	115	2	321	1
Liver	40,342	365	10,330	226	14,056	431	1,414	49
Totals	41,725	405	10,829	231	14,800	450	2,248	62

It is clear from these few experiments that wet baits were more attractive than dry ones and also that the reduction in numbers of flies caught when borax was added was, if anything, less for wet than for dry baits. The enhancing effect of adding water is most noticeable under dry conditions when moistened baits remain attractive longer than dry baits, and therefore catch more flies; under humid conditions there is often little difference between wet and dry baits. Although there was little difference in the number of *L. cuprina* caught by control and treated wet baits, the number caught by the borax-treated dry bait was much smaller than the number caught by the untreated bait.

The effect of addition of sodium sulphide to control and to borax-treated baits was also investigated (Table 8). Although the addition of 2½ per cent. sodium sulphide enhanced the attractiveness of both baits, the improvement was more noticeable for the bait without the borax. In these experiments, the presence of borax resulted in a distinct lowering of the numbers of flies caught. The number of *L. cuprina* caught was also much lower, although the percentages of this species attracted to the various baits did not vary greatly.

TABLE 8.—THE EFFECT OF BAIT TREATMENT ON BLOWFLY CATCHES—WESTERN AUSTRALIAN TRAPS.

—	Borax + Na_2S.	Na_2S.	Borax.	Control.
Catch per trap	725	5,616	544	3,234
Percentage of primaries in catch ..	88	65	88	83
L. cuprina per trap	63	416	54	297

4. Discussion.

Prevention of fly breeding in trap baits may be considered from two points of view, namely, the effect of such prevention on the population of sheep blowflies, and its effect on the blowflies causing annoyance in houses.

The action of borax in slowing down bacterial decomposition might have been expected to favour a high proportion of primary sheep flies in the total catch, since these species are more attracted to fresh baits than other species. While the bulk of the evidence indicates that a higher proportion of primary flies (including *L. cuprina*) was caught by borax-treated baits than by non-borax baits, the actual numbers caught were generally less. However, the figures are too variable to generalize satisfactorily. Of the primary flies, *L. cuprina* is responsible for many more strikes than any other (Mackerras and Fuller, 1937). Although it was present in almost all the catches, and must often have deposited eggs on the untreated baits, no adults were produced. This is not unexpected, for Fuller (1934B) and Waterhouse (1946) have shown that *L. cuprina* is produced infrequently and in small numbers from carrion. Failure of the eggs laid on the baits to survive is principally due to intense competition for food in the larval stage, other species crowding *Lucilia* out. There is, therefore, no advantage in adding borax to the bait, so far as this species is concerned.

The three species *C. stygia, C. augur,* and *Ch. rufifacies,* which are of some importance as sheep blowflies, are also the main blowflies causing domestic annoyance. All three species breed in the baits, but most *Ch. rufifacies* adults are trapped on emergence since the larvae generally pupate in the bait pan

Unpoisoned baits give the greater catches, but this benefit is likely to be offset in the next generation by production of adults from larvae that escape, which will result in a temporary local increase in population density. Further, if trapping is carried out intensively and on

a large scale, the population decrease will result in a general population increase in the next generation caused by the reduced competition among larvae for food (Fuller, 1934B). As an immediate reduction is generally required, there seems to be no object in adding borax to baits. It can also be concluded that the most promising line of future investigation would be directed towards increasing bait attractiveness.

The factors underlying the very great variability in results from trap to trap and day to day during these experiments have not been elucidated, but it is considered that such variation would necessarily occur in the field, so that any results presented in this paper are valid for practical purposes. The reasons for the difference in results in glass traps and in the Western Australian trap also remain undetermined.

5. Acknowledgments.

Thanks are due to Mr G. A. McIntyre for statistical analyses of the results.

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An Account of Experiments Undertaken to Determine the Natural Population Density of the Sheep Blowfly, *Lucilia cuprina* Wied.

By

DARCY GILMOUR, M.Sc.; D. F. WATERHOUSE, M.Sc.;
AND
G. A. McINTYRE, B.Sc., DIP.ED.

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CONTENTS

SUMMARY.

The natural population density of *Lucilia cuprina* was estimated in an area of 50 square miles of grazing country near Canberra.

The method employed was that of releasing a known number of stained flies and of recording the ratio of stained to unstained flies in the catches of 102 traps placed within the area.

The results of four experiments carried out between November 1941 and March 1942 indicated that the natural population varied between 0.3 and 5.7 adult flies per acre.

The distribution of stained flies was found to agree fairly well with a theoretical curve of distribution based on the assumption that the flies moved outwards at random.

The rate of dispersal of the stained flies varied from experiment to experiment, the differences showing some correlation with meteorological factors.

The error involved in the method of estimation of population density was found to be about 20 per cent. This does not include an error, which could not be measured, due to the possibility that the released flies did not constitute an accurate facsimile of the natural population.

The population density was found to be relatively high in spring and autumn and low during summer, which agreed with previously recorded observations on the seasonal variation in activity of *L. cuprina*.

An analysis of the variation between individual trap catches showed that some of the variability was due to local differences in the natural population.

The mean catch per trap was found to vary directly as the population density, indicating that the effect on the catch of fly activity had been unimportant over the range of weather conditions encountered.

The maximum distance from the point of release at which flies were taken was 4.7 miles. This distance was covered in less than 30 hours.

An Account of Experiments Undertaken to Determine the Natural Population Density of the Sheep Blowfly, *Lucilia cuprina* Wied.*

GENERAL INTRODUCTION.

Investigations on the sheep blowfly problem in Australia have been pursued along two main lines of thought. The first of these has been a study of the biology of the blowflies, with the end in view of controlling blowfly strike by reducing fly abundance. The emphasis of the second has been on the host, and the environment provided by the fleece for the development of maggots, the aim being to control blowfly strike by reducing the susceptibility of the sheep to attack. In the study of the biology of the sheep blowfly, the need for information on the density of natural populations has long been felt. For instance, there has been much argument for and against the value of adult trapping in reducing the numbers of blowflies, and thereby decreasing the incidence of strike. This can be settled only by gathering a body of data on the population density of the blowfly, its fluctuation with time, its relation to the incidence of strike, the density of traps needed to effect a sizeable reduction in fly population, and so on. Some information has been available in the past from adult trapping records which have been continued for many years. From these it is possible to obtain some indication of the relative abundance of flies at different times, but, of course, no estimation of absolute population density was possible.

In the investigation reported in this paper we have attempted to make a start in gathering together this fundamental information on blowfly populations. The aim of the experiments was to determine the population density of the primary sheep blowfly, *Lucilia cuprina*, at a number of times throughout the season of fly abundance. Perhaps equally important as the actual figure obtained was the knowledge we gained of the possibilities and limitations of

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the method. The many questions that presented themselves during the course of the work and remained unanswered have indicated the lines of future investigations. In view of the preliminary nature of the data presented, we have avoided discussing the implications of our results upon the general questions of the relationship between blowfly population and the incidence of strike, and the influence of trapping on population. When the work has been extended further, it should be possible to draw some more general conclusions.

In presenting this paper we have found it advisable to divide the work into two main sections, one largely biological and one largely statistical. The purpose of this has been to avoid confusing the discussion of the biological implications of our results with the details of the statistical methods employed. This has necessitated a certain amount of repetition and we have had to state conclusions in the first part while leaving their detailed proof for the second, but we consider that this disadvantage is offset by the convenience of having all the biological discussion grouped in the one section.

PART 1: DESCRIPTION OF THE EXPERIMENTS AND GENERAL DISCUSSION OF RESULTS

By Darcy Gilmour, M.Sc., and D.F. Waterhouse, M.Sc.

I. INTRODUCTION.

In this part we have considered the details of the method employed in the experiments, the results arrived at, and the general biological discussion arising out of these results. For the details of the calculation of the results and the corrections involved, as well as for proof of some of the statements made in this section, reference must be made to the second part.

II. METHODS.

(i) General.

Stated in general terms, the method employed was that of liberating a known number of marked flies and of sampling by means of traps the population in an area surrounding the point of release. The number of flies within the area covered by the traps was then calculated by multiplying the ratio of unstained to stained flies caught in the traps by the number of stained flies in the trapping area. This was the principle employed by Jackson (1933) in determining the population density of tsetse flies. There was, however, a wide difference in actual technique between our experiments and those of Jackson, owing to the markedly different habits and habitats of the two species studied. A marking technique was also used by Eyles and Cox (1943) to determine the density of a mosquito population.

The following requirements were thought to be necessary for a successful experiment of this type:

1. The area to be covered by the traps should be fairly extensive, and a large number of traps should be used in order to reduce errors due to local variations in population density and variability in the efficiency of the traps.

2. The traps should not be so close together as to cause an artificial enhancement of the attractiveness of the trapped area, such as might draw in flies from the surrounding untrapped area. That is to say, there should be no mass effect of the traps; each trap should sample from an area distinct from those of the surrounding traps.

3. The stained flies should be released some time before the commencement of trapping, so that

they might have an opportunity of mixing to a certain extent with the wild population.

4. If a study of the dispersal of the stained flies from the central point is to be made, the period of trapping should be short, since the density of stained flies at any point is constantly changing.

5. During the time of sampling all or nearly all the stained flies should be still within the area covered by the traps.

6. The released flies should constitute, as far as possible, a replica on a small scale of the wild population. This is particularly important in that it was thought that the traps would probably be attractive in different degrees to gravid and non-gravid flies.

In order to meet these requirements it was first proposed in our experiments to use a total of 102 traps disposed at equal intervals within a circle of six miles diameter; to release 40,000 flies at the centre of this circle one day before the commencement of trapping; and to trap for periods of one day, the number of such periods to be determined by the time elapsing before an appreciable number of the flies had left the area.

Since the possibility of meeting all the requirements listed could not be predicted in advance, a trial experiment was undertaken in which only 40 traps were used. Flies were released at 11 a.m. on one day, and trapping was begun on the succeeding day. It was arranged that about half of the female flies released should be gravid. The results of this experiment, which are given in detail in a later section, indicated that the traps attracted gravid and non-gravid females in about equal numbers, and that the proportion of gravid to non-gravid individuals among the released flies did not differ markedly from that in the wild population. The stained flies, however, reached the outer traps rather more quickly than had been expected, and in order that a sufficient number of stained flies should be within the area covered by the traps during the first trapping period, it was decided to increase the diameter of the circle in subsequent experiments to 8 miles. With the same object in view the time of release of the stained flies was delayed until 3 p.m. on the day prior to the commencement of trapping, a modification which was found later to be of doubtful value in that it increased the possibility of error in our results by entailing a less even distribution of stained flies during the first sampling period.

(ii) Breeding of Flies.

Lucilia cuprina was reared on liver in a constant temperature room at about 25°C. One lot of 20,000 was bred to emerge 5 days before liberation, and was fed after emergence on liver slices, sugar, and water. This treatment ensured that about 90 per cent. of the females in this batch would have mature ovaries at the time of liberation. Another 20,000 were bred to emerge 2 days before liberation and were fed on sugar and water only. The females in this batch were non-gravid.

Weighed lots of puparia were counted and the figure for

average pupal weight thus obtained was used in weighing out into cages the required number of puparia. An excess was added to compensate for the expected mortality. Samples were taken and counted for percentage emergence, and this figure was used in computing the actual total emergence.

(iii) Staining

The flies were stained with a saturated alcoholic solution of neutral red, brilliant green, or gentian violet, with shellac added to a concentration of 2 per cent. Aqueous solutions of dyes were tested, but were found to be much less reliable than the alcoholic. The slight narcotic effect of the alcohol was quite transient and the rate of movement appeared subsequently to be normal. Mortality counts extending over 18 days after staining showed that the treatment did not increase the death rate. Staining was carried out several hours before the flies were liberated. Two treatments were given, the first with an electric power sprayer, the second with a hand atomizer. The two treatments were separated by an interval of one hour for recovery. Examination of the flies with a low-power binocular microscope showed that 99 per cent. or more were recognisably marked by this procedure. The stain was most conspicuous on the white squamae and the wings.

(iv) Traps.

These were of the Western Australian type (Newman and Clark, 1941) with some modifications to ensure greater rigidity and uniformity. Parts were made interchangeable. The traps were protected by stakes from interference by animals (Plate 1, Fig.1). A strip of muslin soaked in creosote was tied around the base of each bait pan as a protection against ants. Experience showed that additional protection was needed in some localities. In these cases the traps were set up about 18 inches from the ground on posts, which were liberally treated with creosote (Plate 1, Fig.2). Each trap was baited with 1 kg. of minced sheep's liver and 1 litre of a 1 per cent. solution of sodium sulphide in water. The bait was mixed in large drums at the laboratory and carried out to the traps by truck. The contents of the drums were stirred before each lot of bait was removed, in order to keep the mixture as uniform as possible.

(v) Collection of Catches.

In view of the variation in the activity of flies which occurs during the day, it was necessary that the period of sampling should be the same for all traps. Since it was not possible to empty all the traps simultaneously this meant collecting the catches at a time when the flies were not active. To achieve this, three parties, totalling 13 people and travelling in three trucks, were employed each morning from 5 a.m. till 9 a.m. visiting the traps. The trucks were driven to such traps as were accessible; others were visited on foot. A fairly rigid time schedule was arranged to coordinate the movements of the trucks and the people walking out across country from them. By this means everyone was constantly employed during the four hours either in walking or driving a truck, so that it was possible to visit all the traps in the limited time available. Actually, flies were observed to be active from about 8 a.m. on, but it was not possible to reduce any further the time required for the collecting of catches. Since the order of collecting was the same on every day this slight encroachment on the period of fly activity was not thought to be serious.

On the morning following the day on which the flies were liberated, the traps were baited. On the succeeding mornings the catches were removed from the traps, the baits were stirred, and water was added wherever considerable evaporation had occurred. This treatment of the baits was necessary in order to prevent the formation of a surface scum which reduced their attractiveness.

The trapped flies were killed with cyanide gas. A convenient source of gas for field use was provided by cotton wool pads impregnated with "Cyanogas G", and covere with a muslin wrapper. The procedure at each trap was as follows. The top of the trap was removed and placed in a large calico bag which had been painted to render it reasonably gas-tight (Plate 1, Fig. 2). A Cyanogas pad was pl within the cone of the trap and the mouth of the bag tied up. When the flies were dead, which was usually after about three minutes exposure to the gas, they were removed to a labelled cardboard box and the top was replaced on the trap. Where it was possible to reach the traps by truck the time of waiting at each trap was reduced by carrying a number of spare trap tops, one of which was substituted for the top containing the trapped flies. The catch could then be killed as the truck moved on to the next position.

(vi) Terrain.

The experiments were carried out on a tract of land south-west of Canberra, between the city and the Murrumbidgee river (Plates 2 and 3). The country is undulating, ranging in elevation above sea level from 1,700 to 2,700 feet. The original vegetation is mainly open savannah woodland, but most of the land is cleared to a varying extent and is used for the grazing of sheep. Some fairly extensive areas are practically denuded of trees (Plate 3, Fig. 1). Included within the 8-mile circle was part of a plantation of pine trees mostly 30 to 40 feet high (Plate 3).

III. DETAILS OF THE EXPERIMENTS.

(i) The Preliminary Experiment.

Twenty-seven traps were uniformly spaced at 90-chain intervals within a circle six miles in diameter. The centre of this circle was about one mile west of the centr of the circle shown in the map.* An additional 13 traps were scattered outside the circle, the furthest being 4.7 miles from the centre.

At 11 a.m. on November 1, 1941, 19,700 liver-fed flie stained green, and 19,700 non-liver-fed flies, stained red were released at the centre of the circle. Traps were baited on November 2 and catches collected on November 3, 4, and 7. On the first two days the catches were brought immediately to the laboratory where the *Lucilia cuprina* were sorted out and sexed. The females were dissected and the ovaries examined for the presence or absence of mature eggs. The numbers of flies caught and the results of the dissections are shown in Tables 1 and 2.

* See folder at back of Bulletin.

TABLE 1

Experiment 1. Total Catches of Stained and Unstained Flies

Trapping period	Stained flies				Total unstained flies	Mean natural population density flies per acre *
	Red-stained flies (non-liver-fed)	Green-stained flies (liver-fed)	Total	Stained flies caught as percentage of stained flies in trapped area		
2/11/41	291	259	550	1.8	1122	3.5
3/11/41	127	92	219	0.9	618	3.7
4/11/41 to 6/11/41	68	31	99	-	3024	-

* For method of calculation see pp. 19 and 36-38.

TABLE 2*

Experiment 2. Results of Detailed Examination of the Catches

Trapping period	Classification	Males	Females	Sex ratio $\frac{\text{males}}{\text{females}}$	Gravid females: Per cent. of total females
2/11/41	Red. Non-liver-fed	117	166	0.71	0
	Green. Liver-fed	66	185	0.36	83
	Total stained	183	351	0.52	46
	Unstained	136	876	0.16	39
3/11/41	Red. Non-liver-fed	32	85	0.38	39
	Green. Liver-fed	14	58	0.24	73
	Total stained	46	143	0.32	55
	Unstained	67	393	0.17	43
4/11/41 to 6/11/41	Red. Non-liver-fed	15	53	0.28	
	Green. Liver-fed	8	23	0.35	
	Total stained	23	76	0.30	
	Unstained	696	2380	0.29	

* The total number of flies recorded in this table is somewhat less than in Table 1, since some of the flies were so damaged that their sex was unrecognisable.

The details of the method of calculation of the population density are discussed in later sections. No calculation of population was made from the results of trapping from November 4 to 6, since by that time the stained flies had spread so widely that a large proportion had left the circle. Even on the first day stained flies were caught in the most remote trap, 4.7 miles from the point of release. This evidence of rapid dispersal prompted the modifications in procedure already enumerated.

Fewer green, liver-fed flies were caught than red, non-liver-fed flies, the difference being significant in the second and third trapping periods, but not in the first. The percentage of females with mature eggs in the stained flies, however, did not differ much from that in the unstained flies, indicating that the procedure of allowing half the stained flies to feed on protein resulted in a fairly good simulation of the actual field population, insofar as maturity of the ovaries was concerned. In the first day's trapping 82 per cent. of the green liver-fed females were found to be gravid, whereas none of the red flies were gravid. On the second day, however, 38 per cent. of the red flies had mature or nearly mature eggs in the ovaries, which meant that this proportion of females had been successful in finding animal protein within one day of being released in the field.

On the first and second days the proportion of males to females caught in the traps was significantly higher in the stained flies than in the unstained. This difference disappeared in the third catch (3 to 5 days after release). In addition, on the first day the sex ratio of the red-stained flies caught was significantly greater than that of the green-stained flies caught, a difference which also in subsequent catches became less significant and finally disappeared. In other words, the traps were either relatively more attractive to stained males than to wild males, or less attractive to stained females than to wild females. The difference in sex ratio between the red and green flies, on the other hand, can be associated almost entirely with the males: on the first day the traps caught only 66 green males, as against 117 red males, whereas green females (185) were only slightly in excess of red females (166). That is, the traps were more attractive to two-day-old males not fed on protein than they were to five-day-old males fed on protein. This difference in behaviour might be thought to be the result of the different diets of the two batches of flies, but dissimilarity of diet alone could not explain the difference in sex ratio between stained flies as a whole and the natural population, in view of the fact that the results of the examination of the females had suggested that the opportunity of obtaining a protein feed in the field was about the same as in the laboratory cultures. A more probable explanation would be that the younger flies were simply more active and consequently more successful in finding the traps, and that in the population released by us there was a higher proportion of young flies than in the natural population. This is supported by the observation that the sex ratios approach more nearly to one another as the time from the moment of release increases (see, however, Experiment 2).

This difference in sex ratio emphasizes the fact that we were unable to release anything like a true replica of the natural population, particularly insofar as age distribution and activity were concerned. The sex ratio of

the natural population of *L. cuprina* may not be 1.0, in view particularly of possibly different expectations of life of males and females, but we had no way of calculating from our data what the natural sex ratio might be, and considered that any artificial alteration of the sex ratio in the released flies would not have been justified.

It was decided, therefore, in the main experiments to release a population of stained flies of similar constitution to that used in the preliminary experiment. In future work, when more data on the natural sex ratio and age distribution have been collected, it may be possible to approach more closely to the ideal stained population. The method used by Jackson (1933), of marking flies captured in the field, was not feasible in our experiments in view of the large number of stained flies required.

(ii) Large-scale Experiments.

Further experiments were carried out in December, 1941, and January and March, 1942, the object being to embrace, as far as possible, the seasonal variation in the population of *Lucilia cuprina*. In each experiment all the released flies were stained the same colour. The folder shows the circle enclosing the area in which trapping was undertaken, and the location of the 102 traps, which were placed three-quarters of a mile apart. The results of these experiments are summarized in Table 3.

TABLE 3

Total Catches for the Three Large-scale Experiments

Experiment	Trapping period	Number of stained flies released	Number of stained flies caught	Recovery of stained flies *per cent. of stained flies present in trapping area*	Number of unstained flies caught	Mean natural population density: *flies per acre* *
2	7/12/41	36,300	782	2.2	1005	1.4
	8/12/41		452	1.3	794	1.9
	9/12/41		332	1.0	1839	5.7
3	18/1/42	38,700	1456	3.8	513	0.4
	19/1/42		386	1.2	124	0.3
4	13/3/42	37,950	725	1.9	690	1.1
	14/3/42		432	1.2	643	1.6

* For method of calculation see pp. 19 and 36-38.

The sex of the trapped flies was recorded in the case of Experiment 2. The numbers of males and females caught are shown in Table 4.

TABLE 4

Experiment 2. Sex Ratios in Stained and Unstained Flies Caught

Trapping period	Classification	Males	Females	Sex ratio $\frac{\text{males}}{\text{females}}$
7/12/41	Stained	162	615	0.26
	Unstained	168	804	0.21
8/12/41	Stained	161	303	0.53
	Unstained	163	601	0.27
9/12/41	Stained	117	214	0.55
	Unstained	402	1233	0.33

Here again the sex ratio of the stained flies is significantly higher than that of the unstained flies. But in this case the difference is significant only in the second and third days, and the sex ratio of the stained flies increased with time instead of decreasing, as it did in the first experiment. The increase in the sex ratio of the unstained flies, coincident with a rapid rise in the natural population density (Table 3), might be thought to be an indication of the presence, and increasing importance in the population of young, more active males, but the evidence from the stained flies contradicts that of Experiment 1, and reveals the possibility of the sex ratio of the stained flies in the traps increasing with age. We are unable to suggest any explanation of this contradiction.

IV. DISCUSSION.

(i) Dispersal of the Stained Flies.

Fig. 1 is a graphical representation of the distributions of the stained flies on the two days of the fourth experiment. Each diagram is made by representing the catch of each trap by a number of dots placed in the neighbourhood of the position of the trap. These scatter diagrams indicate that on the day following release the stained flies were largely bunched at the centre of the trapping area, there being a steep gradient in the size of the catch of stained flies from the central traps outward. By the second day, however, the continued dispersal of the stained flies had to a large extent flattened this gradient.

The dispersal illustrated for the fourth experiment was typical of all the experiments. The scatter seemed to have the characteristics of a random diffusion outward from the central point, and in Part 2 of this paper it will be demonstrated that the distribution agrees in

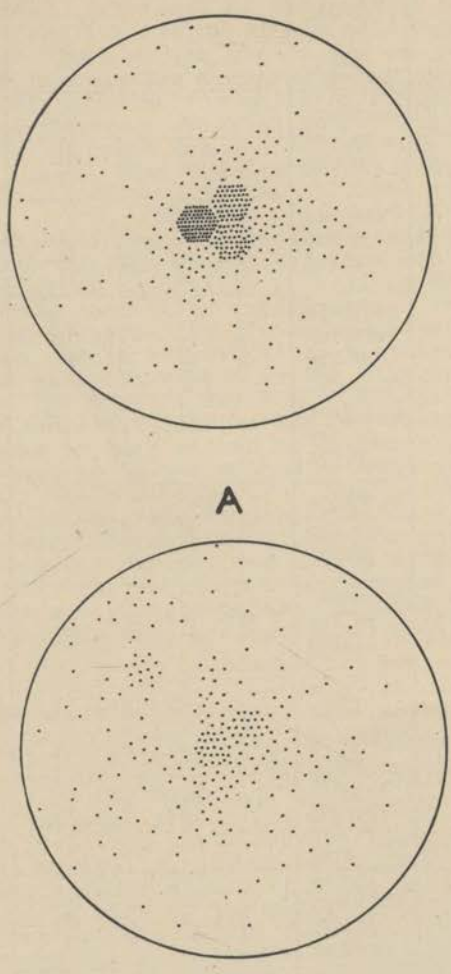


Fig. 1 - Scatter diagrams showing the distribution of stained flies on the two days of the fourth experiment: (A) 13/3/42, (B) 14/3/42. Each dot represents two flies caught in the traps. The number of dots representing the catch of each trap has been distributed evenly throughout a hexagonal figure which represents the theoretical sampling area of the trap.

general with a theoretical distribution based on the assumption of random movement. No evidence was found at any time of a departure of the centre of dispersal from the point of release, that is, of a mass movement of the stained flies, in spite of the fact that for part of one experiment there was a wind of about 20 miles per hour blowing across the area.

Although the general form of the dispersal was the same in all experiments, the actual rate obviously differed. In the first experiment the rate of dispersal was the most rapid, while it was slowest in the second. In Table 5 the rates of dispersal in the four experiments are compared with temperature, humidity, and wind records for the

TABLE 5

A Comparison of the Rates of Dispersal of Stained Flies with Meteorological Data

Experiment	Dispersal index * *miles*	Mean temperature °C.				Mean relative humidity *per cent.*				Wind *miles per 24 hours*			
		Day of release of flies	First day of trapping	Second day of trapping	Third day of trapping	Day of release of flies	First day of trapping	Second day of trapping	Third day of trapping	Day of release of flies	First day of trapping	Second day of trapping	Third day of trapping
1	2.15	20.0	21.4	21.8	-	43	38	43	-	89.5	98.2	143.0	-
2	< 0.43	16.0	17.2	18.8	19.9	51	59	78	70	256.2	208.6	159.3	122.9
3	1.70	23.0	22.4	29.3	-	16	36	28	-	219.5	167.8	132.2	-
4	1.40	22.0	16.3	17.9	-	11	28	37	-	96.8	236.1	113.5	-

* Radius of a circle beyond which half of the released flies had passed at the end of the second day of trapping.

appropriate times. An index of the rate of dispersal is obtained by finding the radius of a circle which encloses half the stained flies at the end of the second day of trapping. The figures for temperature and humidity are averages for the period of fly activity (approximately 8 a.m. to 5 p.m. from our observations). On the day of release they are averages for the period from the time of release until 5 p.m. The anemometer readings are totals for 24 hours.

An examination of this table shows that differences in rates of dispersal can be related to a certain extent to the meteorological conditions prevailing during the early parts of the respective experiments. For instance, the slow rate of dispersal in the second experiment seems to have been the result of cold windy weather, a conclusion which confirmed our observation of the sluggish behaviour of the flies at the time of release. In the fourth experiment similar conditions on the day following release of the flies seem to have slowed up dispersal, though not to the same extent as in the second experiment. Temperature is probably the most important single factor affecting activity, although the rate of dispersal does not vary directly with temperature. The highest temperature (in the third experiment) was not associated with the most rapid rate of dispersal, but in this case the effect of high temperature was perhaps counterbalanced by the lower humidity and stronger winds, as compared with the first experiment.

(ii) The Population Density.

The figures for population density shown in Tables 1 and 3 were obtained by multiplying the ratio of unstained to stained flies by the number of stained flies per acre present in the trapping area. The number of stained flies present was derived from the number released by applying corrections for mortality, number of flies previously caught (if any), and the number of flies which had escaped from the area. Details of the method of calculating these corrections and the actual figures obtained in every experiment are set forth in Part 2. The mortality of the stained flies after their release could only be guessed at, and was set at 2 per cent. per day. This was considered to allow for a reasonable increase in mortality, as a result of natural hazards, over the observed mortality of about 1 per cent. per day of the flies in the cages before release. The error of the estimate of the natural population density, determined by considering all the measurable sources of variability (also discussed in detail in Part 2) was found to be on the average about 20 per cent. There remains the possibility of additional error arising from the fact that the stained flies did not behave exactly as the natural population. In addition, the validity of the method of estimation of the population density rests on the assumption that, activity being uniform, the catch of a trap varies directly with the population density. This may not be so, and we have some evidence (v. Part 2, p. 28) which could be construed to suggest that the catches of stained flies in the central traps, where the population is high, are disproportionately large. At present we have insufficient data to determine the importance of these factors, but we consider that the results we have obtained give a fairly reliable estimate of the true population.

(iii) Seasonal Fluctuations in Population Density.

There is a well-marked variation from week to week

throughout the warmer months of the year in the number of *L. cuprina* caught in traps (cf. Fuller, 1934). This fluctuation is illustrated in Fig. 2, in which the solid line is a plot of the catch of a Western Australian type trap averaged week by week for corresponding periods of the past nine years, the broken line being the weekly catch record for the period at which the population experiments were done. The actual population densities found are also illustrated at the appropriate times for the four experimen

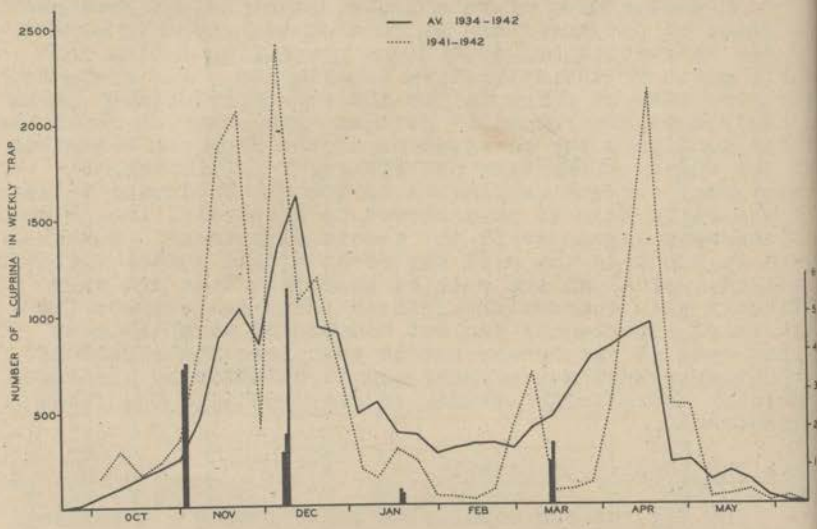


Fig. 2 - Population density compared with the weekly catches of a Western Australian type trap for the season of fly activity 1941-42, and the average weekly catches for corresponding periods of the years 1934 to 1942 inclusive. Population densities, as determined by our experiments, are shown in the four histograms; the continuous graphs are the records of the weekly trap catches. Records for the early part of 1941 have been excluded from the average weekly catch as the results at this time were prejudiced by the escape of flies from an insectary near the trap.

Variation from week to week in the catch of a single trap is obviously the result of differences both in density of population and fly activity. It might be expected, however, that by averaging results over a number of years the effect on fly activity of short term fluctuations in the weather would be largely eliminated, and that the curve for average weekly catch would give a fair representation of relative population densities throughout the fly season. That there is a certain amount of agreement between population density and the curve for average weekly catch can be seen from the figure. In other words, the weekly catch record suggests that *L. cuprina* is relatively more abundant in spring and autumn than during the hotter summer months, and our results have confirmed this. Beyond this the agreement cannot be said to go, and it is obvious that the correlation is even less good between the figure for population density and the catch of a single trap over the period of experimentation. It is apparent that the catch of a single trap cannot be used as a continuous index of population density. This must be due in the first place to the essential unreliability of single trap records, and

secondly to the effect on fly activity of sudden severe changes in weather (rain, excessive cold, etc.).

If we are to consider our results reliable, there must have been a tremendous increase in the natural population during the course of the second experiment. An apparent increase of this sort could have been obtained if there had been a very great mortality in the released flies. The figure of 2 per cent. per day, used in the calculations may have been an underestimate of the mortality under field conditions, but if the population during the second experiment had in fact been uniform, then the mortality of the stained flies must have been greater than 30 per cent. per day. The number of flies caught per trap normally falls off fairly rapidly as the attractiveness of the bait to *L. cuprina* declines. The number of stained flies caught from day to day in the second experiment illustrates a continual decrease which has every appearance of being "normal", that is, there is no evidence of excessive mortality. In the catches of unstained flies, however, there is a large and quite unusual increase on the third day, as compared with the second. This, while not conclusive, constitutes at least presumptive evidence for the occurrence of an increase in the wild population. The evidence seems to suggest, therefore, that the increase in population we have recorded was a real one, although our figures may have exaggerated it slightly.

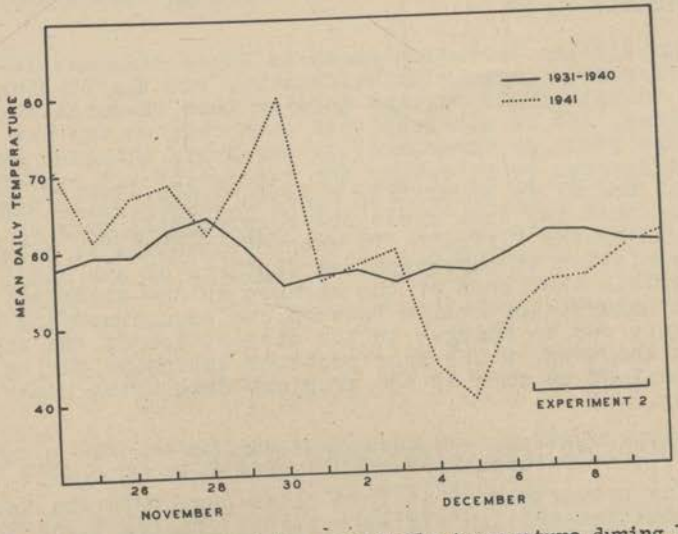


Fig. 3 - Comparison of the mean daily temperature during late late November and early December, 1941, with the average of mean daily temperatures of the corresponding period for the previous ten years. The figure is based on the average of 9 a.m. and 4 p.m. readings.

A probable cause for this sudden rise in population can be found in the weather conditions immediately prior to and during the experiment. Early in December, 1941, a period of unseasonably cold weather was experienced at Canberra. Just before the start of the second population experiment, however, conditions became suddenly much milder. This change is illustrated in Fig. 3, in which the mean daily temperature for the period under consideration is compared with the mean daily temperature averaged over the past 10 years. The fall in temperature would probably have

killed off a large part of the existing adult population and interrupted the development of numerous pupae. The succeeding rise in temperature would cause the more or less coincident emergence of numbers of flies which would normally have emerged during the preceding few days. The combination of these circumstances would produce a sharp rise in the natural population while the experiment was in progress, thereby exaggerating the spring increase in population which normally occurs at about this time.

(iv) Distribution of the Natural Population.

Included in the second part of this paper is a detailed analysis of the variation in trap catches, from which it is concluded that our results demonstrate the existance of local variations in natural population density. In Fig. 4 are shown scatter diagrams which demonstrate this uneven distribution of population. In these diagrams the average catch of unstained flies of a trap for each large-scale experiment (or in Fig. 4-D, for all the experiments) is expressed as being spread uniformly over a hexagonal figure surrounding the trap. Before estimation of the average for any experiment, the number of flies caught in each trap was multiplied by a factor to bring the total of all catches on each day to the same value, that is, the same weight was given to each day's catching. These figures were combined to give the average figure for all experiments (4-D).

Not all the variation shown in these diagrams is due to differences in population density, but the figures do serve to illustrate regions more or less favourable to the flies. It is apparent that such regions are not constant from one experiment to the next, although even in the diagram representing the average of all experiments general trends in population density distribution can be traced, that is, some areas are consistently more or less favoured by the flies. It was not possible to relate this variation to topographical features of any kind. It is possible that some of the changes in the distribution of the natural population between one experiment and the next were due to changes in the disposition of the sheep within the area, which were probably extensive during this period, as many of the graziers were using hand-feeding.

(v) Catch per Trap of Unstained Flies.

The proportion of stained flies caught in the traps was about the same in all experiments (Tables 1 and 3). This indicates that the effect on the mean catch per trap of varying weather conditions has not been very great, providing we accept the validity of the basic assumption that under uniform conditions of fly activity the mean catch per trap is proportional to the population density in the vicinity of the trap.

If variations in fly activity have had so little effect on the total catch of stained flies, then it should be possible to demonstrate a constant relation between the mean catch per trap of unstained flies and population density throughout the four experiments. This has been done in Fig. 5, in which mean catch per trap of unstained flies has been plotted against population density separately for the first and second days of the experiments. From the slope of the straight lines in the two graphs can be determined a figure for the actual daily "trap area", that

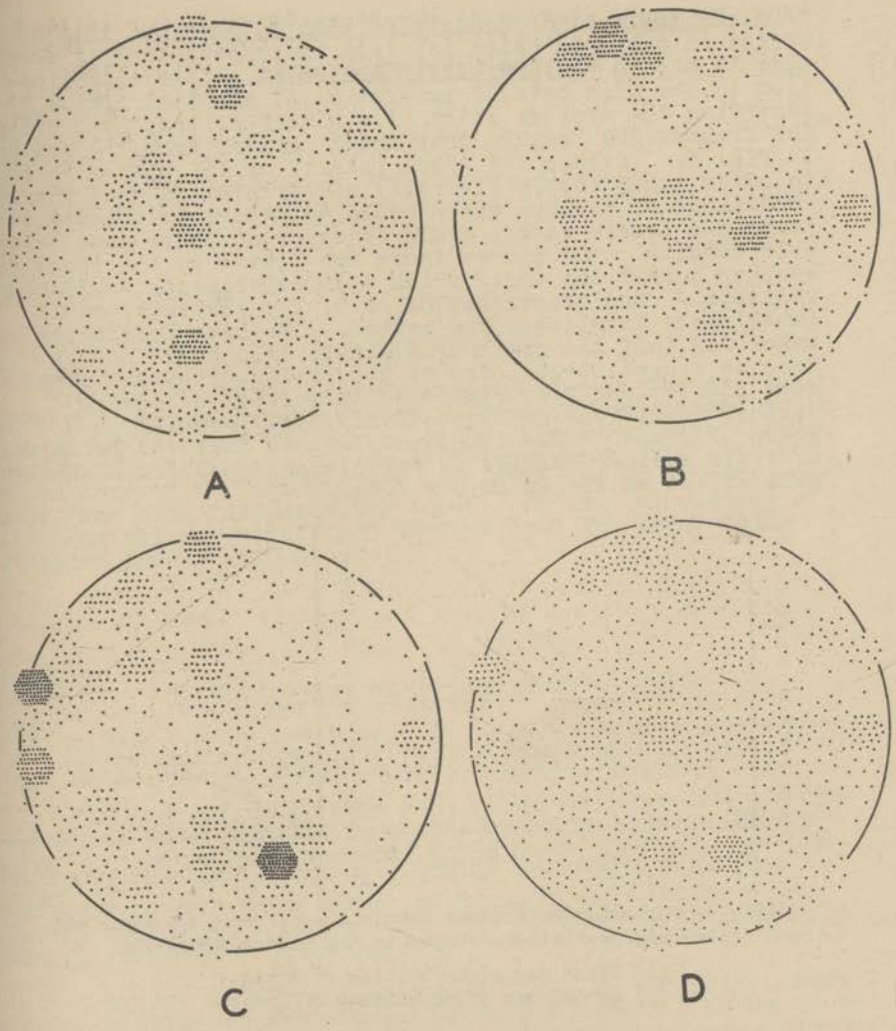


Fig. 4 - Scatter diagrams showing the distribution of unstained flies: (A) Experiment 2 7/12/41 - 9/12/41
(B) " 3 18/1/42 - 19/1/42
(C) " 4 13/3/42 - 14/3/42
(D) Mean of experiments 2, 3, 4.

Each dot represents one fly caught. The number of dots representing the catch of each trap has been distributed evenly throughout a hexagonal figure which represents the theoretical sampling area of the trap.

is, the area from which the mean trap can be considered to remove all the flies in any one day. This area is 9 acres for the first day after exposure of the baits, and 4.5 acres for the second day. That is, the traps are, on the average, half as efficient on the second day as on the first.

The range of weather conditions experienced during the experiments was fairly wide (cf. Table 5), although not extreme. The fact that variations in fly activity arising

from varying weather conditions seem to have had little effect on the mean catch per trap is important. It means that it should be possible to arrive at a fair approximation to the mean natural population at any time simply by recording the average catch of a number of traps and using the figures for "actual trap area" derived from the graphs in Fig. 5. Even a few traps (say 10 or 15) placed so as to cover a reasonable diversity of terrain and used under conditions of weather which could be described broadly as "fine and fairly warm", could be expected to produce reasonable results. Of course there are limits to the range of weather conditions under which such experiments would be reliable. The occurrence of rain, for instance, would immediately invalidate the method. We have previously concluded that the catch of a single trap could not be used as a continuous index of population density. The same limitations with regard to the effect of severe weather conditions on fly activity apply equally to a group of traps. The important fact is that the relatively moderate weather changes we experienced produced no noticeable effect on the catch per trap.

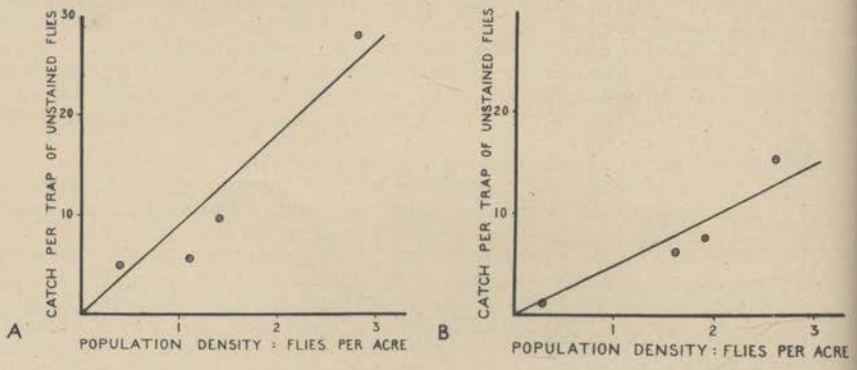


Fig. 5 - The relation between mean catch per trap of unstained flies and population density in the four experiments:

(A) First day after baiting of traps.
(B) Second day after baiting of traps.

(vi) Range of Flight.

Our experiments were not designed to record maximum range of flight. The longest radius of dispersal we did record was 4.7 miles, which was reached within 30 hours of the time of release. It is obvious from the scatter diagrams for stained flies (Fig. 1) that the average rate of dispersal was much less than this. The range of flight of blowflies in Australia had previously been studied by Gurney and Woodhill (1926). They recorded results for *Lucilia sericata*, but since at the time their experiments were done *L. cuprina* was not distinguished from *L. sericata*, and since their work was done in a locality outside the normal range of *L. sericata*, it seems most likely that they were actually working with *L. cuprina*. They recorded a maximum range of flight of four miles (16 days after the time of release).

PART 2: DETAILS OF THE CALCULATIONS AND MATHEMATICAL ANALYSIS OF THE RESULTS

By G.A. McIntyre, B.Sc., Dip Ed., Darcy Gilmour, M.Sc., and D.F. Waterhouse, M.Sc.

I. INTRODUCTION.

This part of the paper is concerned with the derivation of a theoretical curve of distribution of the stained flies and an analysis of the variation in catches of both stained and unstained flies. It forms the mathematical basis on which some of the conclusions stated in the first part were made, and also indicates the details of the calculation of the mean natural population density, and the error to be attached to our estimates.

II. DISTRIBUTION OF THE STAINED FLIES.

(i) Derivation of a Theoretical Curve of Distribution for Flies Moving at Random from a Common Centre.

The aims of this study were first to determine the distribution after a given time of a group of flies released from one centre, and secondly to estimate the mean density at any given point over an interval of time. This last estimate is necessary since the density of released flies in the vicinity of a trap will vary throughout the day as the flies disperse more and more from the point of release.

Consider an individual fly at time, t, at coordinates x, y, relative to the release point O,O, and the probability that such a fly should be at that point at that time. Suppose that the fly moves at a miles an hour on the average, and that the number of random movements per hour is n, so that the mean distance covered per movement is a/n. The projection of each movement on the X axis will on the average be $2a/n\pi$ in a positive or negative sense. In t hours the number of movements will be nt and the probability that a fly will be between $x + \frac{1}{2}dx$ and $x - \frac{1}{2}dx$ miles will be given by the ordinate at x of the normal distribution multiplied by dx.

$$\frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-x^2}{2\sigma^2}} dx$$

Where

$$\sigma^2 = nt\left(\frac{2a}{n\pi}\right)^2$$

$$= \frac{4a^2t}{\pi^2 n}$$

If the number of individuals be N, the number between

$x + \frac{1}{2}dx$ and $x - \frac{1}{2}dx$ will be

$$\frac{N}{\sigma\sqrt{2\pi}} e^{\frac{-x^2}{2\sigma^2}} . dx$$

Similarly the projection of the numbers at all points y, where y lies between $y + \frac{1}{2}dy$ and $y - \frac{1}{2}dy$ on the Y axis will be

$$\frac{N}{\sigma\sqrt{2\pi}} e^{\frac{-y^2}{2\sigma^2}} . dy$$

Evidently, the density function is a function of r, the distance from the point of release, i.e. it is of the form $f(x^2 + y^2)$.

The projection of flies at xy on the X axis will contribute $f(x^2 + y^2)dxdy$ to the number at this point, and the sum of projections with the same x value on the X axis must be equal to

$$\frac{N}{\sigma\sqrt{2\pi}} . e^{\frac{-x^2}{2\sigma^2}} dx$$

$$\therefore \quad dx \int_{-\infty}^{+\infty} f(x^2 + y^2)\, dy = \frac{N}{\sigma\sqrt{2\pi}} e^{\frac{-x^2}{2\sigma^2}} dx$$

$$f(x^2 + y^2) = \frac{N}{\sigma\sqrt{2\pi}} . \frac{1}{\sigma\sqrt{2\pi}} . e^{\frac{-(x^2 + y^2)}{2\sigma^2}}$$

$$= \frac{N}{2\pi\sigma^2} e^{\frac{-(x^2 + y^2)}{2\sigma^2}}$$

The density at point xy is $\frac{N}{2\pi\sigma^2} e^{\frac{-(x^2 + y^2)}{2\sigma^2}}$

$$= \frac{N}{\frac{8a^2 t}{\pi n}} e^{\frac{-(x^2 + y^2)}{\frac{8a^2 t}{\pi^2 n}}}$$

and the mean density from time t_1 to t_2 is

$$\frac{N}{(t_2 - t_1) . \frac{8a^2}{\pi n}} \int_{t_1}^{t_2} \frac{1}{t} . e^{\frac{-r^2}{\frac{8a^2}{\pi^2 n} t}} dt ,$$

where r is the distance from the point of release i.e.

$$r^2 = x^2 + y^2$$

By substituting

$$z = \frac{r^2}{\frac{8a^2}{\pi^2 n} t} \qquad k = \frac{8a^2}{\pi^2 n}$$

this expression becomes

$$\frac{N}{(t_2 - t_1)k\pi} \int_{r^2/kt_2}^{r^2/kt_1} \frac{1}{z} \cdot e^{-z}\, dz$$

If $\int_{r^2/t_2}^{r^2/t_1} \frac{1}{z} \cdot e^{-z}\, dz$ be evaluated for all values of r corresponding to fixed values for t_1 and t_2, and these values are plotted against r on logarithmic paper (log scales on both axes) to form a curve, then the similar curve derived from the above expression for any value of k, N, and the same ratio of t_2 to t_1 can be superimposed exactly on the former curve by horizontal and vertical movements.

The integral $\int \frac{1}{z} \cdot e^{-z}\, dz$ was first evaluated to $z = 3$ by substitution in the oscillating series.

$$\log_e z - z + \frac{z^2}{2\lfloor 2} - \frac{z^3}{3\lfloor 3} \cdots \cdots ;$$

Higher values were obtained by approximate integration.*

Definite integrals for $r = .01$ to $r = 6$, for $t_1 = 1$ and $t_2 = 1.9$, 2.6, 5 and 6 were calculated, and a curve of the definite integral plotted against r on logarithmic paper for each value of t_2/t_1 was prepared.

The form of the curves for different values of t_2/t_1 differ only slightly from one another in the range which it is necessary to consider. This is a fortunate feature in view of the roughness of the approximation in assuming the same level of activity from 8 a.m. to 5 p.m. and on each day.

(ii) Application of the Theoretical Curve to the Experimental Data.

The period of fly activity was assumed on the basis of field observations to be from 8 a.m. to 5 p.m. The values t_1 and t_2 used in the calculation of the density curves in the first experiment, in which flies were released at 11 a.m., were therefore, for the first day, the 6th hour and the 15th hour (ratio $t_2:t_1 = 2.5$) and, for the second day, the 15th and 24th hours (ratio $t_2:t_1 = 1.6$). In the second, third, and fourth experiments, in which the flies were released at 3 p.m., the corresponding periods were the 2nd and the 11th hours (ratio $t_2:t_1 = 5.5$) and the 11th and the 20th hours (ratio $t_2:t_1 = 1.8$).

The data for the marked flies were plotted on transparent logarithmic paper with scales identical with the paper used for the distribution curves. The data were then fitted by eye to the appropriate $t_2:t_1$ distribution curve by vertical and horizontal movements of the transparent

* Subsequently it was found that the exponential integral had been tabulated by J.W.L. Glaisher and by J.R. Airey. The latter table has been reprinted in the British Association for Advancement of Science, Mathematical Tables, Vol.1, London 1931.

sheet superimposed on the plotted curve, the axes of the two graphs being parallel.

For the first day of the second, third, and fourth experiments the ratio $t_2 : t_1$ is 11:2. In view of the sensitivity of this ratio to the assumptions concerning activity, the experimental data were fitted to $t_2 : t_1 = 5$ or 6 according to which gave the best fit. For the second experiment the catches of stained flies in each trap on the second and third days were pooled, the ratio $t_2 : t_1$ appropriate to this sum being 29:11 or 2.6. For the first experiment, curves for $t_2 : t_1$ = 15:6 and 24:15 were interpolated and extrapolated from the curves already prepared.

The form of the curves is independent of the value of k. All were prepared with $k = 1$. The trapping results were pooled in rings of traps equidistant from the centre, and the ratios of stained to unstained flies were used rather than the actual numbers of stained flies, in order to eliminate as much as possible the effect of local favourableness or unfavourableness. These ratios may be converted to theoretical numbers of stained flies caught in the traps, if the conditions were uniformly favourable, by multiplying by the mean number of unstained flies per trap.

Two sets of experimental data and the appropriate theoretical curves are shown in Fig. 6. Generally speaking

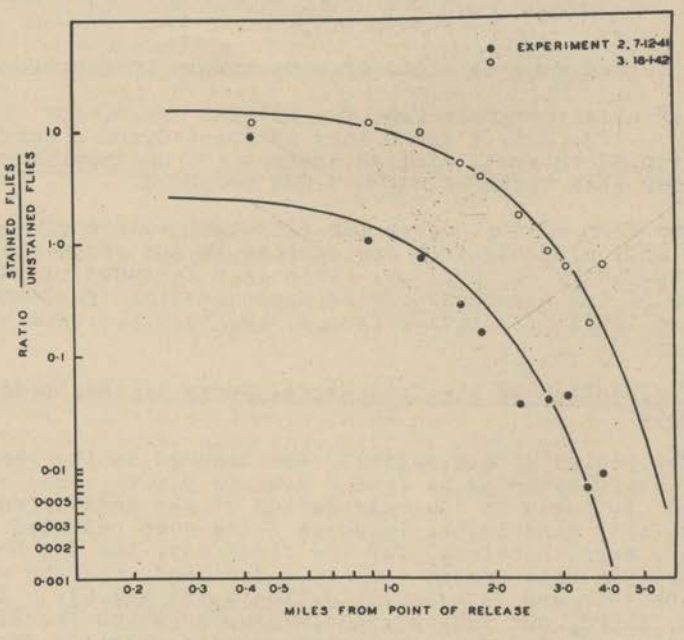


Fig. 6 - Curves of distribution of the mean population densities of stained flies relative to unstained flies.

the experimental data fitted quite well to the theoretical distribution. The two cases illustrated were selected to show examples of a good and a bad fit. The points departing most seriously from the theoretical curve are those relating to the inner circles of traps. In this region the observations are based on the catches of few traps, and the population of marked flies is large and probably changing quite

rapidly so that a high variability is to be expected. The departure in this region does suggest, however, that the catch per trap under uniform conditions may not be directly proportional to the population density. It could also be explained by the occurrence of different activity among the stained flies. The persistence in the centre of any considerable number of individuals more sluggish than the average would cause a departure from the theoretical curve in this region.

(iii) Use of the Distribution Curve in the Estimation of Error.

An attempt was made to arrive at an estimate of the error in the numbers of stained flies caught by using the goodness of fit of the experimental points to the theoretical curve. A series of positions of the distribution curve embracing the limits of reasonable fitting to the experimental data were determined. From these the limits of the number of stained flies that might have been expected to be caught could be determined. When this test was made it was found that only in about half of the separate day's counts did the actual number of stained flies caught fall within the expected limits. In some instances the divergence was very marked, due largely to a bad fit of the experimental points near the centre of the circle. The catch of 7/12/41, for example, was 782, whereas the expected limits were 164 and 447. The estimation of error arrived at by this procedure would obviously be much greater than that determined by any other method which did not presuppose a definite form for the distribution. It was decided, therefore, that the agreement of the experimental data with the theoretical distribution curve was not sufficiently exact to justify the use of the curve in the determination of error.

(iv) Estimation of the Number of Stained Flies Outside the Circle.

Since the flies spread outwards from the centre very rapidly it was necessary in each determination of the population to calculate the mean number of stained flies that were outside the trapping area in the trapping day. The theoretical distribution curves were used to make this estimate.

In the first place it was necessary to convert mean daily numbers per unit area at different points to numbers in concentric rings. To do this, the mean density common to all points at the same radius value was multiplied by $2\pi r \,.dr$ to give the number in the concentric ring of infinitesimal width dr at radius r. These products of density $\times 2\pi r$ were plotted against r and the points joined to give a smooth curve.

The distribution curve corresponding to the value t_2/t_1 for the experimental data was superimposed on the plotted experimental data, as described before, to get the best fit. The r values on the axis of the theoretical curve corresponding to two and four miles on the graph of the experimental data were read off and transferred to the curve relating number of flies in concentric rings to distance from the centre. The area enclosed by this curve between the ordinates corresponding to the two- and four-mile positions was determined and compared with the area outside the ordinate corresponding to the four-mile position. From the ratio of these two areas and the known number of flies caught between two and four miles, the number of flies that would have been caught outside the four-mile point was

determined. Similarly, from the ratio of the number that would have been caught outside the four-mile circle to the number that were caught inside the circle, and the number of stained flies released, the mean number of stained flies outside the circle during the trapping day was determined. Only the part of the curve between two and four miles was used since it was in this region that the experimental data showed the best agreement with the theoretical distribution. In the first experiment, in which only a three-mile circle was trapped systematically, the curve was fitted between the one- and three-mile points. It should be noted, also, that in the first experiment the estimate of the number of flies outside the circle, being based on the results of a much smaller number of traps, is less reliable than in the other experiments. The results of these calculations are shown in Table 6.

TABLE 6

Number of Stained Flies Estimated to be Outside the Trapping Area During the Trapping Periods

Trapping period	No. of stained flies released	No. of stained flies outside circle
2/11/41	39,400	8,540
3/11/41		14,970
7/12/41		0
8/12/41	36,300	0
9/12/41		210
18/1/42	38,700	80
19/1/42		2,250
13/3/42	37,950	0
14/3/42		670

This table illustrates the variation in the rate of dispersal of the stained flies, which is discussed in detail in Part 1 (pp. 17-19). It must be remembered that in the first experiment the circle was six miles in diameter and the flies were released at 11 a.m. on the day prior to the first trapping period, whereas in the other experiments the circle was eight miles in diameter and the flies were released at 3 p.m. on the day prior to the commencement of trapping.

III. ANALYSIS OF THE VARIATION IN THE TRAP CATCHES OF STAINED AND UNSTAINED FLIES.

(i) Components of the Variation.

The variation in the catches of unstained flies in different traps on any one day is very large (maximum range 0 to 106 flies). It seemed possible that some of this variation was due to actual differences in population density from place to place. We have attempted in this section to examine the relative importance of the various factors contributing to the variation in trap catches, in order to

find whether the data we have collected provide any evidence for the existence of local differences in population density within the area studied, and finally, by a consideration of the appropriate sources of variation, to determine the standard error of the estimate of the mean population density in the three major experiments. Both stained and unstained flies have been included in this analysis, the stained flies being compared within rings of traps equidistant from the centre.

The area covered by the 102 traps may be considered to be divided into a series of hexagons of equal size, at the centre of each of which is a trap. In the following discussion these hexagons have been considered to be the unit trap areas, that is, each trap is assumed to sample the population of its appropriate hexagon. Of course the actual sphere of influence of a trap must be much less than this area, and cannot be defined exactly since the attractiveness of a trap decreases with distance, but the concept of a "trap area" is convenient for the purposes of this discussion.

Four possible sources of variation between the catches of different traps were considered. These were (1) purely chance variation such as would occur in the catches of exactly similar traps sampling from a uniform population; (2) variation due to differences in traps and baits; (3) variation due to differences in mean population density in different hexagons; and (4) variation due to differences in the behaviour, for example, the activity, of populations in different hexagons. Factors (3) and (4) cannot be differentiated from one another and will be referred to together as variation due to differences in density-activity. In determining the standard error of the estimate of mean population density, variability due to differences in density-activity within the area should be excluded as far as possible. Another source of error which should be included, however, is that due to the probability that the density of population at the centre of any particular hexagon is not the mean density over the whole hexagon; that is, that the trap is not sampling the mean density within the hexagon. In the following sections an attempt has been made to differentiate the four (or rather, three, since the third and fourth factors are considered together) major sources of variation.

If the variability in successive catches of single traps on successive days of an experiment be compared with the variability in the trap catches on any one day, the former is found to be significantly less than the latter. This means that the variability in the catches of different traps on any day is greater than that to be expected by pure chance. This extra variation can be ascribed to intrinsic differences in trap efficiencies (trap variation) and to differences in density-activity between hexagons. The material collected in the population experiments did not provide us directly with the data necessary to effect a separation of these two factors. To do this it was necessary to consider the results of other experiments. This has been done in the next section.

(ii) Trap Variation.

Information regarding the variation to be expected between traps for different population densities was obtained from a set of data on the standardization of Western Australian traps previously accumulated by one of us (D.F.W.). A number of traps were compared by placing them on a turntable which revolved about once every three minutes, thus exposing each trap to identical conditions. Catches of *Lucilia cuprina*,

L. sericata, *Calliphora stygia*, and *C. augur* were recorded over a number of days. For each day the mean and the standard deviation of the catches for each species were calculated. These were plotted against one another on logarithmic paper. All points for each species fell very closely about a straight line, the equation for which was:

$$\log M = 1.17 \log \sigma ,$$

where M = mean

and σ = standard deviation

So that the variance $= M^{\frac{2}{1.17}}$

$$= M^{1.709}$$

The mean populations for some species were far in excess of the numbers of *L. cuprina* caught in any trapping experiment, so that errors of extrapolation will not occur when transferring this relation to the trapping data.

The variation between the means of *L. cuprina* catches in different traps was greater than would be expected from the variation for the same traps on successive days but not significantly so.

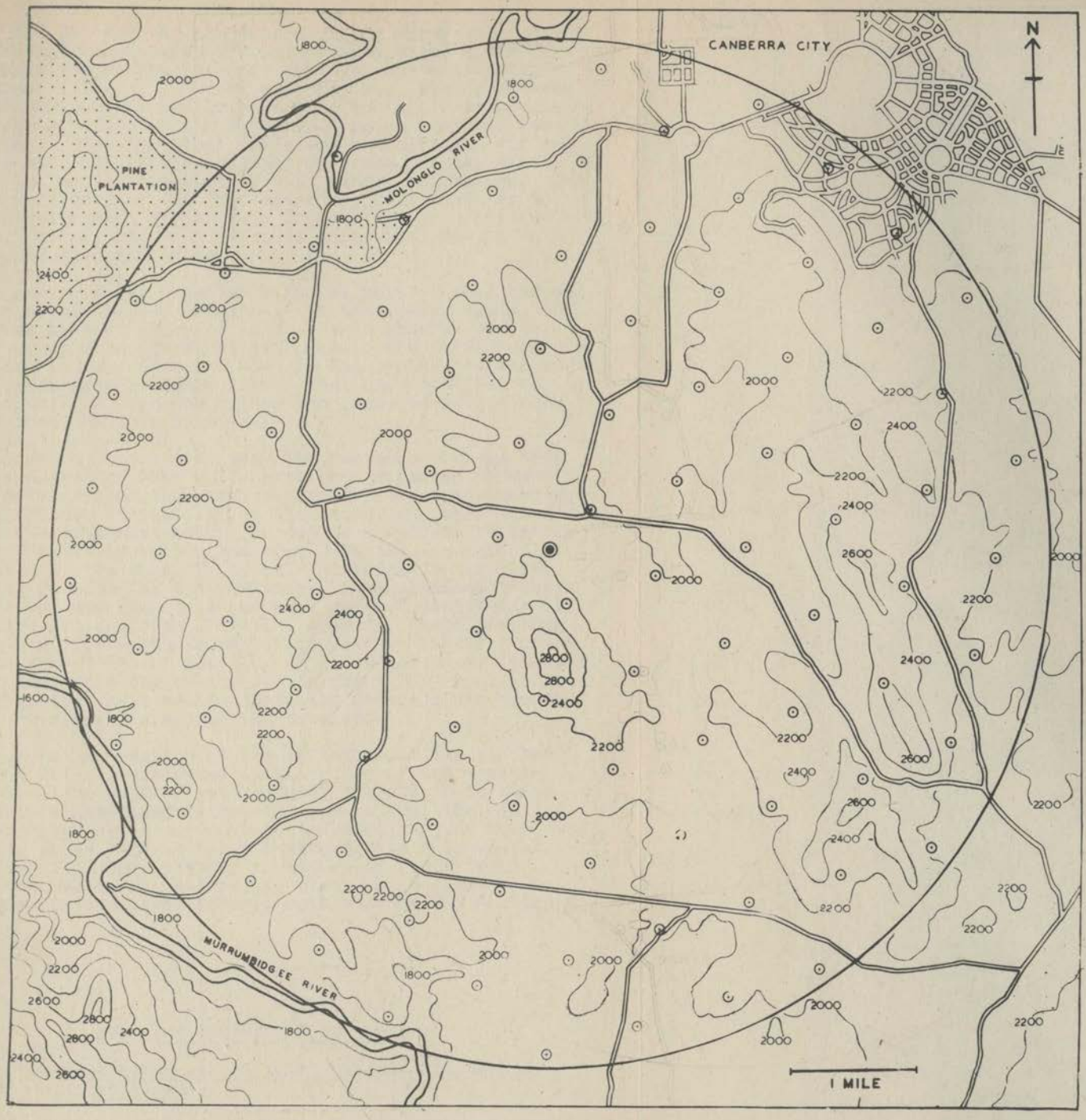
(iii) Variation in Density-Activity between Hexagons.

If the flies were spread uniformly over the whole area, it would be expected that the variance of the trap catches about the mean catch would approximate to the variance of the mean catch as estimated from the standardization data of the previous section. If it did not, and was greater, then the increase in variance could be ascribed to (1) greater variation in the field traps than in the standardization traps; or (2) a real variation in density-activity of the flies. The comparison was made, and it was found that the actual variance was always much greater than the expected variance derived from the standardization data, the actual variance ranging from 2.5 to 5.7 times as great as the expected variance.

Both the traps and the baits were kept as uniform as possible in the field experiments, so that it seems unlikely that there was much more variation in the traps than there had been in the standardization experiment. The increase in the variance in the field experiments was thus thought to constitute quite strong evidence for the existence of differences in density-activity between hexagons.

It must be emphasized also that the comparison of traps in close proximity to one another as in the standardization experiment probably gives an estimate of variability much in excess of what one would expect if the same traps had been compared in a uniform population at some distance from one another, since the flies, attracted to the spot where, in the turntable experiment, the traps were massed, would tend to discriminate and be caught mostly in the trap which was perhaps only slightly more attractive than its neighbours. Further evidence for the existence of variation in density-activity was sought in the population experiments themselves.

The basic unit of area was increased from a single hexagon containing one trap to three contiguous hexagons containing three traps. Grouping the data in this way



Map of the locality in which trapping was carried out.

The circle indicates the area of trapping in the three major experiments. In the preliminary experiment the area of trapping was a circle 6 miles in diameter centred at a point about one mile west of the central point shown above. Contours are at 200 ft. intervals.

⊙ Position of trap. ◉ Point of release of stained flies.

provided a basis for the comparison of the error mean square within the unit areas with the mean square between the unit areas. This comparison was made for each day's trapping of unstained flies. In every case the mean square between groups of three traps was greater than the error mean square within groups, although the difference in no instance was significant. The fact that there was a consistent difference, however, constitutes further evidence for the existence of an irregular distribution of the natural population. The relative smallness of the difference may have been due to the fact that marked differences in density occur between contiguous traps, these differences being included in both of the contrasted variances.

The analysis was repeated with the unit of area reduced to a pair of contiguous hexagons. In this case, again, the mean square between pairs of traps was in general greater than the error mean square within pairs. The difference was significant in two cases and also in the pooled results for all the trappings. It seems likely that even with paired traps the unit of area was rather too large to define some local differences in population.

It appears, therefore, that the variation in the trap catches was greater than would have been expected by mere chance, greater also than what could have been accounted for by the intrinsic variability of the traps, and that this extra variability must have been due to a natural variation in density-activity within the area. We have no direct information on the possibility of differences in activity accounting for some of the variation in populations between hexagons, but approximately the same general conditions of temperature and humidity prevailed over the whole trapping area, and in view of the conclusions reached in Part 1 with regard to the effect of activity on catch per trap, it seems unlikely that this factor was of any great importance. It is probable that most of the variation observed was due to actual differences in population density.

Since an examination of the variation in the catches of stained flies (considered within rings of traps equidistant from the centre) gave results comparable to those obtained from unstained flies, it appears that the stained flies were themselves unevenly distributed, within such concentric zones, and it seems reasonable to suppose that the stained flies had assumed a pattern of distribution similar to that of the natural population, the same areas being more or less attractive to both stained and unstained flies.

IV. THE ESTIMATION OF ERROR.

(i) The Standard Error of the Total Catches of Stained and Unstained Flies.

It is apparent that an estimate of the standard error based on the variation of the trap catches about the mean catch will be an overestimate, since part of the variation of trap catches is due to an uneven distribution of the population within the trapping area. Variation due to differences in population density may be eliminated to a certain extent by considering only the variation within the larger unit areas (two or three hexagons) as a basis for calculation, and ignoring the variation between these areas.

Another approach is to use the information provided by the standardization data. The population density is considered to be variable and the catch of each trap is taken to represent the mean catch that would have been obtained if each trap in turn had sampled the population at that location. The variance corresponding to this mean can be obtained from the standardization curve, and the total variance is then compounded by totalling the variance of the individual trap catches.

In Table 7 are shown the standard errors obtained by each of these three methods, i.e. from the variations with groups of three traps, within pairs of traps, and from the variation as determined by the standardization curve (that is, using single traps).

In the case of the stained flies, as mentioned above, it was necessary to consider the variation within rings of traps equidistant from the point of release in order to eliminate the effect of the uneven distribution of the flies due to their dispersal from the central point. The error derived from a consideration of the variation within groups of three traps was omitted in the case of the stained flies on account of the difficulty of grouping the three trap units in rings around the centre. Data from pairs of traps grouped in rings were included, however.

There is in general a decrease in the estimated error one passes from three-trap to two-trap and from two-trap to single traps as the basis of computation. This decrease must be due to the decreasing importance of local variation in population density within the unit areas. In the case the three-trap units the distance of the traps from their centroid is greater than it is for the two-trap units. When the estimate of the error of the total population is based on single traps, variation due to differences in population density within the unit area is ignored, since the method computation postulates that the catch of each trap is, in a measure of the mean population at that point. In other words, the variation included in this estimate is that due chance and intrinsic trap variability. The correct estimate should include not only this variation but also variation to the density at the trap not coinciding with the mean density within the hexagon. Thebest estimate of standard error must therefore lie somewhere between the values obtained by using two-trap units and single traps. The estimate based on single traps is probably much closer to the true error, however, since it has been shown that even the slight reduction in distance between the individual traps and their centroid produced by changing from three-trap to two-trap units causes on the average a reduction in the estimated error, and the reduction to be expected in changing from trap units to single traps would be considerably greater. chance of the centroid for the mean population in a hexagon being distant from the centre by more than a third of the radius would be very small. The estimate of error based on single traps was therefore used in compounding the total e

The existence of differences in density-activity between hexagons affects indirectly the estimate of standard error based on individual traps. Since the variance is not proportional to the mean, but to the mean raised to the power 1.7, the total variance would be greater in the case of traps sampling a population with varying density than one with the same number of flies distributed uniformly over the same area. The effect of varying density is illustrated by the greatly increased total error in the catches of

TABLE 7

Estimation by Various Methods of the Errors of the Total Catches of Stained and Unstained Flies

	Trapping period	No. of flies caught	Standard error			S.E. X 100/Number		
			Using three-trap units	Using two-trap units	Using standard-ization data	Using three-trap units	Using two-trap units	Using standard-ization data
Stained flies	7/12/41	782		170.5	182.3		21.8	23.3
	8/12/41	452		122.0	95.1		27.0	21.0
	9/12/41	332		81.5	52.5		24.5	15.8
	18/1/42	1456		175.4	187.3		12.0	12.9
	19/1/42	386		45.5	52.4		11.8	13.6
	13/3/42	725		84.1	125.1		11.6	17.3
	14/3/42	432		53.9	58.8		12.5	13.6
Unstained flies	7/12/41	1005	120.4	115.7	95.8	12.0	11.5	9.5
	8/12/41	794	89.8	89.4	76.0	11.3	11.3	9.6
	9/12/41	1839	196.7	214.1	154.3	10.7	11.6	8.4
	18/1/42	513	83.0	53.4	63.5	16.2	10.4	12.4
	19/1/42	124	19.0	19.3	19.0	15.3	15.6	15.3
	13/3/42	690	125.5	119.4	79.3	18.2	17.3	11.5
	14/3/42	643	91.3	81.9	70.8	14.2	12.7	11.0

stained flies as compared with unstained flies where the total catches are about the same. The population of stained flies varies from very high at the centre to almost zero at the circumference of the circle and the high catches in the inner six traps are responsible for a very large part of the total variation.

(ii) The Error of the Estimate of Mean Population Density.

The equation for the determination of the mean natural population is as follows:

$$N_{unst} = N_{st} \times \frac{a}{b}$$

where N_{unst} = the mean population within the trapping area

N_{st} = the number of stained flies within the trapping area

a = the number of unstained flies caught in the traps

b = the number of stained flies caught in the traps

An error can be attached to each of the three factors involved in the estimation of the natural population density.

The method of breeding the flies destined to be released has been detailed in Part 1 (p.10). From this description it can be seen that the estimate of the number of stained flies released is itself subject to three errors. These are (1) the error introduced by the method of estimating the number of pupae (counting of weighed samples), (2) the error of estimate of percentage emergence of adult flies, and (3) the error of the estimate of mortality between emergence and the time of release. The first two errors were calculated from our experimental data. The mortality after emergence was assumed, on the basis of previous experience, to be about 1 per cent. per day (1200 flies in each experiment). This was confirmed within broad limits by an examination of the cages, but not by detailed counts. For this reason a rather high figure for the error (± 500) was assumed for this component. In addition, the number of stained flies actually present during the trapping day is subject to another error arising from the error of estimate of the number of flies which had passed out of the area. This number was relatively quite small, however, and since calculation of the error presented considerable difficulty, this source of error was ignored.

The errors to be attached to the catches of stained and unstained flies have been detailed in the last section.

The numbers of stained and unstained flies caught in traps equidistant from the centre show some slight evidence of positive correlation. Presumably the characteristics of the traps which cause or contribute to the excessive variation between traps will affect both the number of stained and unstained flies caught. However, the inner eighteen traps caught not less than 60 per cent. of the stained flies even on the second day after release, so that only a small proportion of the traps have much effect on the total catch of stained flies. Further it is likely that topographical and weather factors tending to canalize the movement of flies from the point of release will

TABLE 8

The Error of the Estimate of the Mean Natural Population Density, and its Component Factors

Experiment	Trapping period	Number of stained flies present in circle N_{st}	S.E. of estimate of number of stained flies present	Number of unstained flies caught a	Sampling error for unstained flies	Number of stained flies caught b	Sampling error for stained flies	Number of unstained flies present in circle $N_{unst} = \frac{N_{st}\, a}{b}$	S.E. of estimate of number of unstained flies	S.E. X 100 / Number for unstained flies	Number of unstained flies per acre
2	7/12/41	35,690	644	1005	95.8	782	182.3	45,450	11,580	25.5	1.4
	8/12/41	34,190	644	794	76.0	452	95.1	57,060	13,920	24.4	1.9
	9/12/41	32,800	644	1839	154.3	332	52.5	166,200	32,760	19.7	5.7
3	18/1/42	37,970	653	513	63.5	1456	187.3	11,860	2,398	20.2	0.4
	19/1/42	33,570	653	124	19.0	386	52.4	10,200	2,221	21.8	0.3
4	13/3/42	37,290	650	690	79.3	725	125.1	34,910	7,380	21.1	1.1
	14/3/42	35,160	650	643	70.8	432	58.8	49,700	9,208	18.5	1.6

contribute much to the irregularity in stained fly catches near the centre. Lastly, in order that adjustments could be made in the estimate of error of the wild population density, to take account of the fact that the efficiency of each trap was affecting both stained and unstained fly catches, one would have to be able to analyse the variability between traps into two components, the variability inherent in the same trap under identical conditions and the variabili between true trap means under the same conditions. No satisfactory information of this character is available to us

In ignoring the correlation between catches of stained and unstained flies, the estimate of error of the wild population will tend to be slightly over-estimated.

The factors involved in the calculation of the mean popu lation density and the errors to be attached to them are sho in Table 8. After the first day in each experiment, the numb of stained flies originally released is reduced by the number caught during the previous day and by an arbitrary figure for their mortality in the field. This figure was 2 per cent. per day, that is, twice the mortality in the laboratory of flies of this age (v. Part 1, p. 19). The number of flies originally released is also reduced by the number which had moved out of the four-mile circle, this number being calculated from the curve of distribution of marked flies (Table 6).

The compounded standard error averages about 20 per cent. of the estimated number of unstained flies. It must be emphasized again that this estimate does not include certain at present immeasurable, and perhaps important, sources of error arising out of the possibility that the stained population does not behave exactly as the natural population.

ACKNOWLEDGMENTS.

An investigation of the type we have described could only be carried out by the co-operation of a large number of workers. We wish to thank those of our colleagues within the laboratory, and friends outside of it, who by their willing assistance made this work possible. These helpers, too numerous to mention by name, cheerfully undertook the task of collecting the catches in the early morning and of working long hours thereafterwards at sorting and classification. Our thanks are due also to Mr. Edwards and Mr. Morrow of the Department of Interior, who furnished us with maps and gave us much information; and to the Photographic Survey Section, R.A.A.F. Station, Canberra, which provided an aerial photograph. We gratefully acknowledge the criticism and advice of Dr. A.J. Nicholson which was of much assistance in the planning of this investigation and the preparation of the paper. Finally we wish to thank the graziers who were so ready in their permission for us to use their land for this work and who co-operated so willingly while the experiments were in progress.

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PLATE 1.

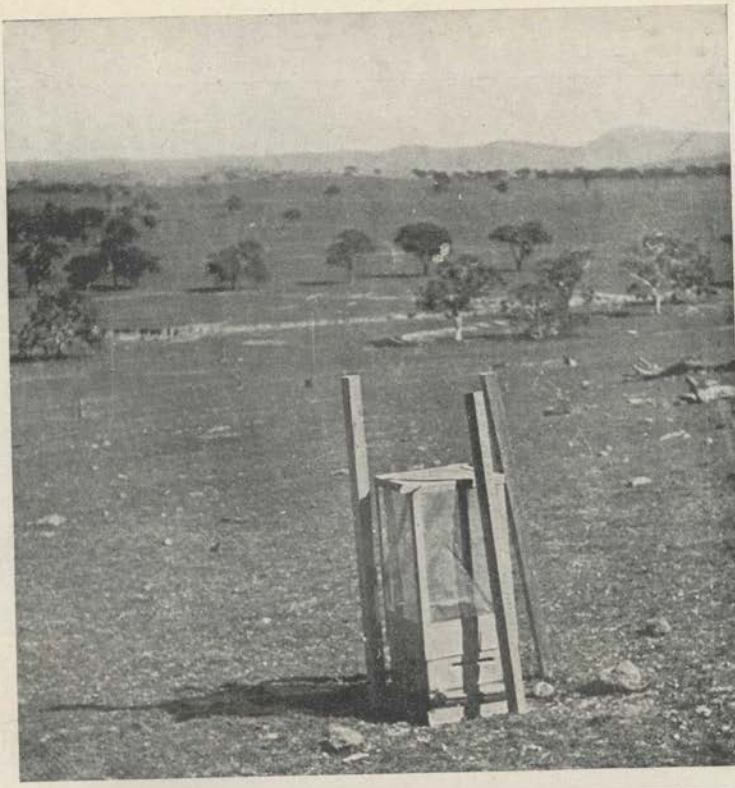

FIG. 1 (above).—One of the Western Australian type blowfly traps used in the experiments. The three stakes are to prevent the trap being overturned by stock. The strip of creosote-treated tape for protection against ants can be seen around the base of the trap.

FIG. 2 (below).—Dismantling the trap for removal of the catch. The trap top, with a cyanide-impregnated pad in the cone, is being placed in the fumigating bag. The cardboard box in which the dead flies are stored is seen in the foreground. Extra protection against ants has been provided for this trap by placing it on the creosote-treated post seen between the three stakes.

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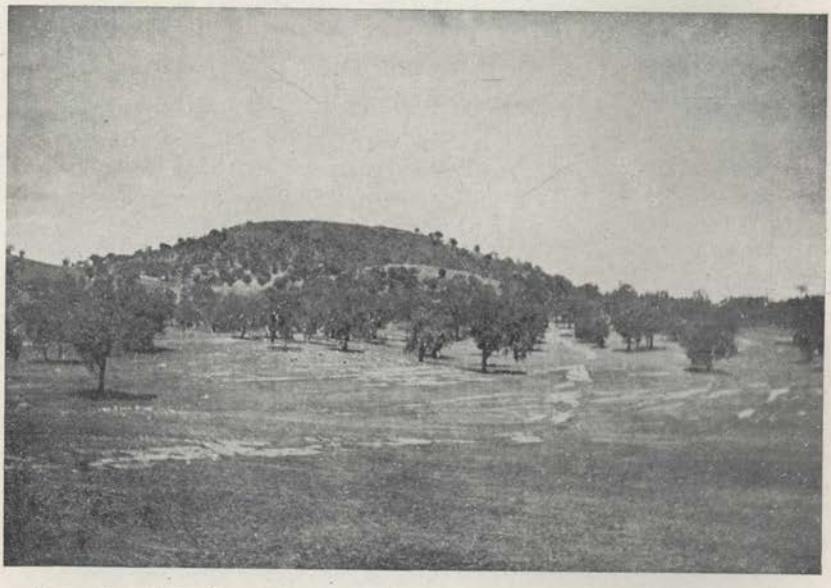

FIGS. 1 and 2.—Partially cleared savannah woodland typical of most of the trapping area. Fig. 1 was taken from the outskirts of Canberra and is a view looking north-west across the width of the experimental site.

PLATE 3.

FIG. 1 (above).—Part of an extensive area of grassland practically denuded of trees. The plantation of pine trees can be seen in the distance.

FIG. 2 (below).—Part of the pine plantation included in the trapping area.

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THE EFFECT OF COLOUR ON THE NUMBERS OF HOUSEFLIES RESTING ON PAINTED SURFACES

By D. F. WATERHOUSE*

[*Accepted for Publication September 22, 1947*]

Summary

A simple technique for determining the preference of houseflies for surfaces of various colours is described. This consists of liberating adults into a Peet-Grady testing chamber fitted with movable coloured corners. The numbers of flies resting on the coloured corners are recorded at intervals to provide adequate data for comparison.

The ascending order of preference for the particular colours tested was white and sky blue (equal), light grey, green, yellow and medium grey (equal), dusky blue, and red. From measurements of the visual reflectances of the various colours it was found that the order of preference could be explained largely by a reaction to the intensity of the light reflected by the coloured surfaces, darker surfaces being preferred to lighter surfaces.

I. INTRODUCTION

Colour perception in insects has been reviewed extensively by Weiss (1943, 1944, 1946), who points out that most of the available information indicates that light intensity is more important than wavelength in producing reactions. However, von Frisch and others have shown that, for bees at least, there is a definite wavelength discrimination (Wigglesworth 1939).

The present tests were initiated when a proposal was made during the war that army kitchens and hospitals should be painted dark blue to repel flies. While it is well known that the common bush fly, *Musca vetustissima*, will not enter darkened rooms, the reaction of the housefly, *M. domestica*, is quite different. Thus there is a general trend in the evidence of Freeborn and Berry (1935) and Atkeson *et al.* (1943), in experiments carried out in dairy barns, that flies (mainly houseflies) preferred darker to lighter colours. However, because of the absence of precise experimental control and the lack of adequate physical data on the amount of light reflected by the various colours, it was considered desirable to carry out tests paying attention to these factors.

II. METHODS

(*a*) *Apparatus*

The tests were carried out in a standard 6 ft. by 6 ft. by 6 ft. white lacquered Peet-Grady testing chamber (Anon. 1943). Into each of the four corners of the chamber was fitted a coloured, movable, plywood corner. Each plywood corner consisted of two 5 ft. 11 in. by 2 ft. 3 in. pieces hinged together so that they

*Division of Economic Entomology, C.S.I.R.

would stand up on their shorter ends at right angles to one another. During a test, these hinged pieces rested on a 2 ft. 4 in. by 2 ft. 4 in. piece of plywood placed on the floor in one corner of the chamber and were roofed by a similar piece of plywood. By this means, from one to four colours could be exposed simultaneously in the four corners of the chamber. Each false corner was separated from its neighbour by an 18 in. strip of white chamber wall.

The paints used were selected from a range of full gloss, lacquer finish colours obtained from a commercial firm. They were applied with a spray gun after appropriate undercoats.

The chamber was illuminated by a light bulb set in a reflector and shining in through a square glass panel in the centre of the roof. Normally a 200-watt frosted bulb was used, but in some experiments the light intensity was varied by inserting other bulbs. Although the light in this position provided equal illumination for each of the four coloured corners, the intensity of illumination varied from place to place on each corner. Thus the roof and upper portion of each corner was in shadow, while the remainder was directly illuminated, but situated at varying distances from the source of light. However, since each corner had an identical distribution of light from the bulb, this variation in light intensity over any one corner should not introduce any irregularities when comparing the relative attractiveness of colours.

The temperature of the chamber was thermostatically controlled, the majority of tests being carried out at 75° ± 1°F.

(*b*) *Flies Used*

The test insects were adult houseflies (*Musca domestica* L.), bred in general accordance with the standard Peet-Grady technique (Anon. 1943). They were fed on a mixture of equal parts of milk and water and were usually tested when three to seven days old. Most batches of flies consisted of approximately equal numbers of males and females. However, in one test, females alone were used.

(*c*) *Test Procedure*

Four hundred to five hundred flies were liberated in the chamber in which corners of the desired colours had been placed. The operator sat on a stool in the centre of the chamber and disturbed all flies from each of the four corners in rotation and then from the glass panel under the light. After an interval of three minutes to allow the flies to settle, the numbers of flies on each coloured corner were counted as rapidly as possible, starting with the corner which had been disturbed first. Following this count, the flies were again disturbed and counted after settling, on this occasion the corner first disturbed being the one adjacent and clockwise to the one first disturbed previously. When each of the four corners had been the first to be disturbed, a cycle of four counts was complete. At least four repetitions of each cycle of four counts were usually carried out with each batch of flies to provide sufficient data for statistical analysis.

The above cyclical procedure was necessary to compensate for the fact that the corner last to be disturbed for any one count always had fewer flies resting on it than would have been expected from the counts when it was not disturbed last. The validity of the cyclical method of counting is discussed later.

In one series of experiments, a cycle of counts taken inside the chamber in the fashion just mentioned was alternated with a cycle taken by an operator through glass panels in opposite sides of the chamber. During the outside cycle of counts the flies were not disturbed but, as before, counts were taken at three-minute intervals. This series of experiments was designed to detect the presence of any "self-preservation" reaction which might cause the flies to react differently to coloured panels when disturbed than when allowed free choice to settle and move without disturbance.

(d) Colours Tested

Table 1 contains information* on the colours tested. Plywood painted with each colour and illuminated by illuminant A (light from a gas-filled tungsten lamp operating at a colour temperature of 2,840°K.) was matched to the nearest shade in the Munsell Book of Colour. From the Munsell notation the ISCC-NBS name was derived (Judd and Kelly 1939), and the I.C.I. trichomatic coefficients for illuminant A (gas-filled light bulb) obtained (Kelly *et al.* 1943). The visual reflectances were measured with illuminant A, incident normally on the sample and viewed at 45° to the normal. This source of light is the standard equivalent of the 200-watt bulb used to illuminate the chamber.

TABLE 1

DESCRIPTION OF THE COLOURS TESTED

Name Used	Munsell Notation	ISCC-NBS Name	I.C.I. Trichromatic Coefficients			Visual Reflectance (=Y)
			x	y	z	
White	10Y8/2	Pale yellow-green	0.460	0.422	0.118	0.68
Sky blue	5BG7/2	Pale blue-green	0.421	0.420	0.159	0.50
Dusky blue	5B3/4	Dusky blue	0.333	0.390	0.277	0.04
Green	10GY6/6	Strong yellowish-green	0.419	0.482	0.099	0.31
Yellow	10YR7/10	Moderate yellowish-orange	0.545	0.431	0.024	0.37
Red	5R4/14	Vivid red	0.641	0.331	0.028	0.16
Light grey	N7/	Light grey	0.446	0.408	0.146	0.45
Medium grey	N5/	Medium grey	0.446	0.407	0.147	0.23

*Supplied by the Division of Physics, C.S.I.R.

Light grey was prepared by mixing one part of black paint with eleven parts of white, while medium grey was a mixture of one part of black and three parts of white.

(*e*) *Analysis of Results*

The relative attractiveness of colours was compared on the basis of the ratio of the numbers of flies on the panels in question, these numbers being summated over the four phases of a cycle. Logarithms of ratios for replicate cycles were used for tests of significance. When a number of cycles was involved the mean value for a colour ratio was calculated as the antilog of the mean log of the ratios of each cycle.

III. Results

A. standardization experiments

(*a*) *Symmetry of Method of Counting*

In order to test for any possible asymmetry in the test chamber or in the method of taking counts, one pair of diagonally opposite corners was set up with white plywood and the other with dusky blue plywood. One hundred and six cycles, each of four counts, were then made, as described earlier, ten different batches of flies being used. Between tests with each batch of flies both the painted corners and the chamber were wiped clean with a moist cloth. The results obtained are shown in Table 2.

Table 2

Counts of Flies Resting on White and Dusky Blue Corners

Corner	1	2	3	4
Colour	Blue	White	Blue	White
No. of flies	24,389	3,797	24,715	3,741

There was no significant difference in the ratios of the numbers of flies resting on the two blue or the two white corners. This indicates that there is no asymmetry of the chamber, and that no asymmetry is introduced by the method of counting. Hence we can conclude that it is unnecessary to have more than one corner of any colour in order to obtain reliable results. Absence of asymmetry due to counting was further substantiated when the ratio of blue to white was compared for the first and second halves of each cycle of four counts. It was found that there was no significant difference, indicating that the ratio could be established satisfactorily by half a cycle when only two colours were compared. It is clear, however, that a complete cycle is necessary when four colours are compared simultaneously.

(*b*) *Effect of Recent Painting*

As a regular precaution, panels were allowed to age for at least five days after painting, before use. However, on one occasion (see Table 3) a test was

carried out to compare the attractiveness of a surface painted the day before with one which had been painted five days previously. These two corners were again compared on three successive days. The more recently painted panel had fewer flies (not significant) resting on it on the first and second days after painting than the other panel. However, by the third day any trend that there may have been had disappeared. The five-day minimum ageing period adopted as a standard was therefore considered ample.

TABLE 3

COMPARISON OF THE NUMBERS OF FLIES RESTING ON PANELS ALLOWED TO AGE FOR DIFFERENT TIMES AFTER PAINTING WITH DUSKY BLUE

Panel Painted	Date Tested			
	22.v.43	23.v.43	24.v.43	25.v.43
17.v.43	954	2,035	1,309	1,047
21.v.43	920	1,795	1,478	1,060

(*c*) *Effect of Light Intensity and Temperature*

Several tests were carried out using 150-and 60-watt globes in place of the 200-watt globe. The results were somewhat conflicting, although the bulk of the evidence indicated that alteration of light intensity over this range did not affect the ratios obtained. Similarly, several tests were carried out at 70°, 80°, and 85°F. instead of at 75°F. In some tests the ratios were lower at a higher temperature that at a lower one, but this trend was not consistent. There was no occasion on which alteration in light intensity or temperature altered the relative order of attractiveness of the colours.

(*d*) *Effect of Sex of Fly*

On one occasion the chamber was populated with female flies only, white and red panels being compared. The white : red ratio from eight cycles was 1 : 6.2 which is very similar to the 1 : 6.9 ratio obtained from different cultures using both males and females. There is no indication, therefore, that the sex of the fly influences its response to colour.

B. RESULTS OF COLOUR COMPARISONS

Unless specifically mentioned, all tests in this section were carried out at 75°F. using a 200-watt light bulb. Table 4 shows the ratios obtained in tests when two colours only were compared. Owing to the variation in ratios from day to day and from culture to culture, it is only satisfactory to use, for an accurate comparison of various colours, the ratios obtained when these colours are directly compared. However, the ratios given in Table 4 do indicate quite well the relative attractiveness of the various colours.

TABLE 4

RATIOS OBTAINED FROM COMPARISON OF TWO COLOURS ONLY

Colour	White : Colour = 1 :	Green : Colour = 1 :	Yellow : Colour = 1 :
Sky blue	1		
Light grey	(1.5) *		
Green	2.0		
Yellow	2.3	1.2	
Medium grey	(2.4) *		
Dusky blue	6.2		
Red	6.9	4.3	5.3

*Derived from comparison when other colours were present.

(*a*) *Sky Blue*

Sky blue was compared only with white, 25 cycles giving total counts of 5,259 flies on white and 5,290 on sky blue, the mean ratio not departing significantly from 1. The white : sky blue ratios for individual cycles varied from 1 : 0.76 to 1 : 1.4

(*b*) *Light Grey*

Light grey was compared with white on a number of occasions, but each time in the presence of other colours. The white : light grey ratios recorded were 1 : 1.5 (12 cycles in the presence of yellow and red), 1 : 1.5 (4 cycles with medium grey and red), and 1 : 2.2 (4 cycles with medium grey and yellow).

The light grey : yellow ratios obtained from these comparisons were both 1 : 1.4 and there is confirmatory evidence from tests at 80°F. that light grey is less attractive than yellow.

No comparisons with green were carried out at 75°F. but, at 80°F., the average light grey : green ratio of 26 cycles in the presence of white and yellow was 1 : 1.8 (and of light grey to yellow, 1 : 2.0). Light grey is, therefore, less attractive than green, but more attractive than white.

(*c*) *Green*

The white : green ratio for 13 single-comparison cycles was 1 : 2.0, while the average ratio from 26 cycles in the presence of yellow and red was 1 : 1.4. The green : yellow ratio from these tests was 1 : 1.4, and the green : red ratio 1 : 4.8. This green : red ratio was of a similar order also in other tests, for instance, 1 : 4.3 for 19 cycles of these two colours alone and 1 : 4.7 for 18 cycles in the presence of yellow.

The green : yellow ratio was further investigated with 23 cycles in which these two colours alone were compared (1 : 1.2) and 18 cycles in which red was also present (1 : 1.3). Green is, therefore, less attractive than yellow.

(*d*) *Yellow*

The white : yellow ratio for 8 single-comparison cycles was 1 : 2.3 Other ratios obtained were 1 : 2.0 (26 cycles with green and red) 1 : 2.0 (12 cycles with light grey and red) and 1 : 3.1 (4 cycles with light and medium grey).

In addition to comparisons already mentioned, yellow has been compared with medium grey and red. The yellow : red ratios obtained were 1 : 5.3 (14 single-comparison cycles), 1 : 3.6 (26 cycles with white and green), 1 : 3.7 (18 cycles with green), and 1 : 5.0 (12 cycles with white and light grey). The yellow : medium grey ratios obtained were 1 : 0.9 (8 cycles with white and green), and 1 : 1.1 (13 cycles with white and light grey). There was no significant difference in the relative attractiveness of these two colours.

(*e*) *Medium Grey*

The white : medium grey ratios obtained were 1 : 2.4 (4 cycles with light grey and red) and 1 : 3.2 (4 cycles with light grey and yellow).

Medium grey has already been shown to be similar in attractiveness to yellow; it is less attractive than red, and more attractive than green (green : medium grey = 1 : 1.25 for 8 cycles with white and yellow).

(*f*) *Dusky Blue*

This paint had been selected by a manufacturer as a fly-repellant blue paint. A total of 72 cycles of comparisons with white was performed, giving an average white : blue ratio of 1 : 6.2; 5,659 flies being counted on white corners and 34,451 on dusky blue corners. These tests were carried out over quite a long period of time and involved many different cultures of flies. It is of interest to note that the lowest ratio recorded for a cycle was 1 : 3.5 and the highest 1 : 15.3. Low or high ratios appeared to be associated with particular batches of flies, but no explanation was found for their varying behaviour.

(*g*) *Red*

The white : red ratio for 25 single-comparison cycles was 1 : 6.9. Values also obtained for this ratio were 1 : 6.2 (26 cycles with green and yellow) and 1 : 9.0 (20 cycles with light grey and yellow). No comparisons were made of dusky blue and red, but it seems reasonable to assume that these colours both have the same order of attractiveness.

It is apparent from the foregoing results that the ratios may vary considerably at different times. Thus, when the attractiveness of two colours is close, e.g. sky blue and white, medium grey and yellow, or even occasionally with green and yellow, either one or other colour may record the greater number of flies. When the attractiveness is quite different the ratios obtained with different fly cultures may vary greatly, e.g. the fourfold variation for white: dusky blue, although the order of attractiveness of the colours remains the same.

A series of tests was set up to determine the quantitative accuracy of ratios obtained for various colour combinations with the same batch of flies. Four cycles of counts were carried out with each of the following pair comparisons: green *v.* red, yellow *v.* red, and yellow *v.* green. The results are shown in Table 5.

TABLE 5

RATIOS OBTAINED FROM COLOUR TESTS ON THE SAME BATCH OF FLIES

Green : Red	= 1 : 5.87
Yellow : Red	= 1 : 6.28
Yellow : Green	= 1 : 1.07

The observed ratios are exactly the same as those obtained by calculation. For example, the observed green : yellow ratio is the same as that calculated from the green : red and yellow : red ratios. This suggests that for a single batch of flies there is a fixed quantitative difference in response to the colours. It is interesting to note that in this test the green corners had more flies on them than the yellow corners.

As a means of testing the stability of ratios in a changing environment, 22 cycles of green, yellow, and red were run, in which the fourth panel was white, and these were interspersed with 18 cycles in which the fourth panel was another red one. The values obtained for the three panels which remained constant throughout are shown in Table 6, there being no significant difference in the two sets of ratios. In other words, the relative attractiveness of the three colours tested was not influenced by the presence of a varying fourth colour.

TABLE 6

RATIOS OBTAINED FROM COLOUR TESTS

Fourth Colour	Ratios
White	Green : Yellow : Red = 1 : 1.4 : 5.1
Red	Green : Yellow : Red = 1 : 1.3 : 4.9

In the counts carried out inside the chamber it was thought that there might be, superimposed on a colour preference, some sort of "self-preservation" response whereby flies which were endeavouring to escape from a disturbing influence would rest preferentially on darker colours. To test this possibility, counts inside the chamber were alternated with counts taken from outside through windows in opposite sides of the chamber. The results are shown in Table 7. There is a significant difference in the relative numbers of flies on the various colours when the observer counts from inside and outside the chamber, the effect of the observer in the chamber, taking white as reference, being given by the following:

White	*Light Grey*	*Green*	*Dark Grey*	*Yellow*
1.000	1.023	1.042	1.119	1.127

Subject to the remote possibility of observer bias (omitting to count all the flies on the darker panels) one can state that the presence of the observer in the chamber tends to make the darker colours relatively more attractive. However, because of the correlation of the effect of the observer with the attractiveness of the colours, the ranking remains the same. This is the only relevant issue,

TABLE 7

COMPARISON OF RATIOS OBTAINED FROM COUNTS OUTSIDE AND INSIDE THE CHAMBER

	White	Light Grey	Green	Dark Grey	Yellow	No. of Cycles
Outside	1	1.2	1.7		2.0	20
Inside	1	1.1	1.9		2.2	28
Inside	1	1.8		2.8	3.2	13
Outside	1	1.6		2.6	2.9	9
Inside	1		2.3	2.8	2.5	8
Outside	1		2.3	2.5	2.3	8

since the general conclusions reached from the data already presented are thus valid under the two sets of circumstances, either of which may be encountered by the fly during the selection of a colour upon which to settle.

IV. DISCUSSION

It has been clearly established that houseflies have a definite order of preference for surfaces of various colours. Such a preference may be either for the colour of the surface, or the intensity of light reflected, or a combination of both factors.

If the ratios obtained when the colours were compared with white (Table 4) are plotted against the visual reflectances of these colours (Table 1), it can be seen (Fig. 1) that there is a good correlation between the amount of light reflected by the painted surface and its attractiveness to flies, the lighter colours being less attractive than the darker colours. In Figure 1 there are several irregularities in the order of the colours, but these are probably due in part to the fact that the ratios were established on different cultures of flies and are, therefore, not strictly comparable. In addition, without knowing the curve for the relative stimulative efficiency of different wavelengths for the housefly retina, it is not legitimate to assume that the figures used for visual reflectance are true values for the relative intensities perceived by the flies.

Indeed there is good evidence that insects are less sensitive to the red end, and more sensitive to the violet end, of the spectrum than the human eye (see Wigglesworth 1939). This might well account for the fact that both yellow and red gave ratios suggesting that they appeared less bright to the flies than one would have expected from their visual reflectances. The fact that reduction in

intensity of illumination did not alter the relative order of attractiveness of colours suggests that the optimum intensity of reflected light must be zero, or close to it. No clear evidence in these tests was obtained of a response to wavelength.

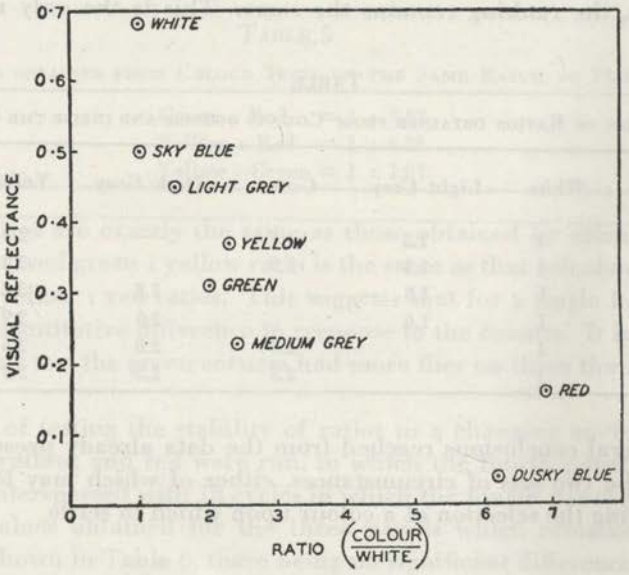

Fig. 1.—Graph showing the relationship between visual reflectance and attractiveness of colours to houseflies.

Considering the use of colours to deter flies from entering rooms, it seems highly improbable from general observations that even the least attractive colours will have an overriding effect on the powerful chemotropic stimuli guiding housefiies to an attractive odour. Once the flies have fed or ceased to respond to the odour it is possible that the colour of their surroundings may affect the numbers which remain or leave. Lighter shades of colours are, therefore, to be preferred to darker shades. However, the effectiveness of residual deposits of poisons, such as DDT, suggests that colour will only be called on to play, at the best, an accessory part in affecting the accumulation of flies in rooms. Dark colours, for instance, might well be used to attract houseflies to surfaces treated with DDT, thereby restricting the areas which it is necessary to treat.

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An Examination of the Peet-Grady Method for the Evaluation of Household Fly Sprays

By
D. F. WATERHOUSE, M.Sc.

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CONTENTS

SUMMARY

The Large Group Peet-Grady method is examined with particular reference to its suitability for the evaluation of household fly sprays. Conclusions are based on tests with pyrethrins, Thanite, and Lethane 384 standard. The future of the Peet-Grady method is discussed in the light, both of the results obtained and of the likelihood that DDT with its high toxicity and slow knockdown properties will be a component of all household fly sprays.

It is pointed out that the standard dosage of 12 ml. per 216 cu. ft. is far higher than normally used in actual practice and that this high dosage results in higher knockdown and mortality than when the spray is used in a room. On the other hand, a dosage of 2ml. per 216 cu. ft. in the Peet-Grady chamber gave results similar to those obtained in room tests.

Reduction in exposure time from the standard 10 minutes to 1 minute produces a slight reduction in knockdown and mortality, but the reduction is not great. If the spray is introduced into the chamber while it is being aerated the reduction is greater, but conditions as severe as this are not likely to occur frequently in actual practice.

A number of factors examined by other workers are brought together and discussed. It is pointed out for instance that, unless the sex ratios of all cages of flies, the results from which are to be compared, are the same, sexing the flies and basing mortality on a 50:50 sex ratio will improve the reliability of results.

Uniformity of flies in various cages of the same batch, uniformity of spraying pressure and spraying procedure, and the validity of using a standard reference insecticide were examined to evaluate their influence on the variability of results obtained by the Peet-Grady method. The variation in activity and distribution of flies in the chamber from test to test was considered to be the most probable explanation for the fact that the amount of insecticide received by different individuals varied greatly. Variation in spray distribution from test to test may also be a contributing factor. Although the variability in results is reduced by reduction in chamber size, this change favours any fumigating effect which the spray may possess, and also renders flight conditions less like those in actual practice.

Difficulties which arise in any method of testing fly sprays are the variation in relative susceptibility of laboratory-reared and wild flies and the difficulty of deciding whether to class moribund flies as dead or alive.

An Examination of the Peet-Grady Method for the Evaluation of Household Fly Sprays*

1. Introduction

The essential features of a laboratory method for contact toxicity studies against flying insects and of a laboratory method to evaluate fly and mosquito sprays for household and field use are not necessarily the same. In the evaluation of sprays for actual use, it is desirable to choose a testing technique which approximates as closely as possible to the average circumstances under which the spray will be used or will give parallel results to service tests. On the other hand, for contact toxicity studies against flying insects it is permissible to select arbitrarily convenient testing conditions which permit easy handling of test insects and rapid replication of tests.

The Large Group Peet-Grady† (P.G.) method (Anon., 1943) has come to be accepted as the standard test for evaluating household fly sprays and, as such, has been used in Australia for several years. In employing the P.G. method in Canberra for the selection and standardization of mosquito and fly sprays for army and civilian use, several serious disadvantages have become apparent and these are discussed below. Suggestions are made for modifying the P.G. procedure if results more nearly applicable to actual conditions of fly spray use are desired, and also for the general improvement of the accuracy of the standard P.G. method. All the data in the present paper relate to the housefly, since the standardization tests carried out with mosquitoes were not as extensive (but see Waterhouse, 1947).

The fact that low concentrations (0·1 per cent.) of DDT in kerosene will kill all or almost all flies which are hit by the spray, although the rate of knockdown is slow, has necessitated a complete re-orientation of pre-war ideas on household sprays. It is now necessary to pay particular attention to knockdown, for 100 per cent. mortality is easily produced with safe and reasonably cheap sprays. Since flies sprayed with a lethal dose of DDT in kerosene are still able to fly around for 5 or 10 minutes, or even longer, it is necessary for household use to include in the spray a material which will knock the flies down in a much shorter time. While examining the usefulness of the P.G. method for the evaluation as opposed to the mere standardization of sprays, attention has been paid to the reliability for household use of the information on knockdown which is provided by the standard tests.

2. The Essential Features of the Peet-Grady Method

Briefly, the Large Group Peet-Grady method consists of liberating approximately 500 adult houseflies (*Musca domestica* L.) of a certain known age and bred under standard conditions into a 6 ft. by 6 ft. by 6 ft. chamber maintained at a constant temperature. Here they are sprayed by means of a standard gun with a standard volume (12 ml.) of the insecticide delivered at a standard constant pressure (12·5 lb. per sq. in.). The flies are exposed to the spray in the closed chamber for 10 minutes. The chamber is then ventilated to remove air-borne

* Typescript received August 26, 1946.

† P.G. is used as an abreviation for Peet-Grady in this paper.

spray particles and the paralysed flies are picked up and transferred to a cage, while the unparalysed flies are counted and removed. Between tests the spray residue is removed by wiping walls and roof with a cleaning mixture and the floor is covered with fresh absorbent paper.

Insecticides are evaluated by comparing the mortality produced by the unknown with that caused by the standard insecticide under the same experimental conditions, the evaluation being based on the average differences in tests with three different cultures of flies. The standard insecticide, known as the Official Test Insecticide, or O.T.I., is a kerosene-extract of pyrethrum flowers and is prepared annually by the National Association of Insecticide and Disinfectant Manufacturers Inc., U.S.A. It contains approximately 0·1 per cent. w/v pyrethrins. The strain of flies used must be such that the O.T.I. will cause between 30 and 55 per cent. mortality. Mortalities from the two O.T.I. tests which are performed with each culture must agree within 10 per cent., or the entire test is discarded.

The standard P.G. method has been found by many laboratories to be suitable for tests in which a comparison of the toxicity of a number of fly sprays is required. The conditions of testing are conveniently and arbitrarily fixed to eliminate many sources of variation in results, and to facilitate routine testing and handling of flies. It has been found that, with adequate precautions, replicated tests with the same spray yield fairly uniform results. These results are in a form which is suitable for treatment by statistical methods. An important feature of the P.G. procedure is that a standard reference insecticide is readily available from a central source. This standard is used to compensate for the variability in resistance to poisons which occurs from batch to batch of flies and to reveal batches of flies of aberrant susceptibility.

3. A Critical Analysis of the Large Group Peet-Grady Method

Some of the disadvantages and possible disadvantages mentioned below would apply to many, but not all, methods of testing sprays. In contrast to the advantages of the method, the disadvantages are discussed in detail for it is only possible in this way to demonstrate their importance. Difficulties which appear to be inherent in all tests with houseflies are discussed later in a separate section. Modifications of the standard method have been suggested where they appear necessary.

(i) *Spray Dosage.*

In the standard P.G. method the dosage of spray used is 12 ml. for the 216 cu. ft. chamber, which is equivalent, approximately, to 1 fl. oz. per 500 cu. ft. This volume of spray produces a dense mist of droplets in the chamber and normally causes a 30–50 per cent. mortality of houseflies when the standard 0·1 per cent. w/v pyrethrins solution (O.T.I.) is used. This range of mortalities was selected for standard reference because it covers that portion of the concentration-mortality curve most sensitive to variation in pyrethrin concentration and is thus most suitable for use when comparing the mortalities produced by unknown sprays. The 12 ml. dosage is, therefore, satisfactory for the laboratory comparison of the relative toxicities of sprays possessing this particular order of toxicity. On the other hand, this dosage is so high as to be impracticable under normal conditions of household use. Using an ordinary, efficient, hand spray gun the dosage delivered in,

say, a 2,000 cu. ft. room by an average individual before the onset of fatigue is certainly no more than 2/3 fl. oz. (approximately 150 strokes with a good gun), and often considerably less*. On the other hand, it may be argued that, in actual practice, the spray is aimed at the insects present, so that they are subjected to a far higher concentration of spray than is the average for the room. Ford (1941) comments on the high P.G. dosage, pointing out that an equivalent amount of spray would only be used in a room if an electric sprayer or a vacuum cleaner with an attachment for spraying was available.

To obtain information on the effect of using a dosage in the P.G. chamber similar to that normally used in a room, a series of tests was carried out in which the standard 12 ml. dosage in the P.G. chamber was compared with a 2 ml. dosage (equivalent to 1 fl. oz. per 3,000 cu. ft.) in the P.G. chamber and a 1 fl. oz. per 3,000 cu. ft. dosage in a 2,000 cu. ft. room. The standard P.G. gun (De Vilbiss No. 5004), operated at 12·5 lb. pressure, was used for tests in the P.G. chamber and a good hand spray gun for the room tests. The hand gun† was of the non-continuous type in which the liquid jet is completely surrounded by the air jet. The metal spray container of the hand gun was replaced with a glass vial to facilitate accurate dosage. The test room was maintained at the same temperature as the P.G. chamber and the door and two windows were closed during each test. Delivery of the spray, which was aimed at the flies as they flew about, took between 1½ and 2 minutes and the exposure period was 10 minutes from the middle of this period, after which the room was aerated and the flies collected and counted as in the P.G. method. Only one test was carried out in the room on any one day. The average number of flies per test was 404. The results from tests with pyrethrins, Thanite‡, and Lethane 384§ are summarized in Table 1.

All results were adjusted for a 50 : 50 sex ratio.

TABLE 1.—THE COMPARATIVE EFFECTIVENESS OF THE STANDARD P.G SPRAY DOSAGE (6 fl. oz. PER 3,000 cu. ft.) IN THE P.G. CHAMBER AND A DOSAGE OF 1 fl. oz. PER 3,000 cu. ft. APPLIED IN THE P.G. CHAMBER AND IN A 2,000 cu. ft. ROOM.

Spray.	Number Tests.	K.D. (10 mins.)			Mortality (24 hrs.)			O.T.I. in P.G. for Same Tests.	
		12 ml. P.G.	2 ml. P.G.	≡2 ml. Room.	12. ml P.G.	2 ml. P.G.	≡2 ml. Room.	K.D.	Mort.
		%	%	%	%	%	%	%	%
0·13 per cent. w/v pyrethrins ..	4	99·5	60	70	40	5	7	99·6	35
3·5 per cent. Thanite	4	99·9	84	84	59	31	25	99·0	37
5·0 per cent. Lethane 384	4	99·9	95	68	63	4	17	98·4	40

* The recommended Australian Army dosage for mosquito spray is 1 fl. oz. per 3,000 cu. ft. or 1/6 of the P.G. dosage.

† Shelltox gun, manufactured by Rega Products Pty. Ltd., Sydney, N.S.W.

‡ Thanite is bornyl thiocyanoacetate.

§ Lethane 384 is β-butoxy-β'-thiocyanodiethyl ether 50 per cent., petroleum distillate 50 per cent.

When the results with the two dosages in the P.G. chamber were compared, it was found that both knockdown and mortality were significantly lower for the 2 ml. dosage than for the 12 ml. dosage, the difference being far greater for mortality than for knockdown.

Room test results showed that there was no significant difference for pyrethrins or Thanite in the mortalities produced by equivalent dosages in the P.G. chamber and the 2,000 cu. ft. room, although Lethane produced a significantly higher kill in the room than in the P.G. chamber. Similarly, there was no significant difference in the knockdown for pyrethrins and Thanite, although Lethane produced a lower knockdown in the room than in the P.G. chamber. The absence of significant difference in knockdowns for pyrethrins was confirmed by an additional three tests in which the knockdowns in the P.G. chamber and the room were compared, resulting in average figures (for the seven tests) of P.G. 59 per cent., room 57 per cent.

It is difficult to explain the lower knockdown and higher mortality with Lethane in room tests than in the P.G. chamber. When two treatments are compared, however, it is not necessarily an anomaly if the higher knockdown results in the lower kill. Faster knockdown will result in the flies being exposed to the spray mist for a shorter time than the slower knockdown and hence the flies of the latter group have the opportunity of collecting more spray on their wings and bodies, which may subsequently result in a higher mortality. Insects motionless on the floor of the chamber collect very much less spray than those allowed to fly about (David and Bracey, 1944A). There is a compensating factor, however (David, 1945), since flies which are not knocked down quickly may, by their cleaning movements, rapidly remove a significant amount of spray from their bodies to the substratum.

One factor which might introduce a differential effect in room versus P.G. tests is the use of different syray guns. However, figures obtained by White (1945) indicated that the distribution of droplet sizes produced by both guns was substantially the same within each toxic material. The sprays tested were 0·1 per cent. pyrethrins, 3·5 per cent. Thanite, and 5·0 per cent. Lethane.

In view, therefore, of the apparent absence of any irregularities in carrying out the tests with Lethane, the conclusion is inescapable that with the same dosage (1 fl. oz. per 3,000 cu. ft.) significantly different results are obtained in P.G. and room tests. Four additional tests with Lethane, which are not included in Table 1 because the O.T.I. mortality was below 30 per cent., confirmed this finding. Thus, whereas pyrethins and Thanite can be satisfactorily evaluated for household use by reducing the P.G. dosage, it would seem that Lethane cannot be. These conclusions, it must be pointed out, are based on a single concentration of each material.

It is apparent from the above results that a 2 ml. dosage in the P.G. chamber provides a far more accurate evaluation of a spray *for actual conditions of usage* than does the standard 12 ml. dose. The room tests on which this conclusion is based were carried out under conditions at least as favourable to the sprays as normal conditions of fly spray usage, where the dosage would often be less and the room frequently not closed for 10 minutes after spraying.

Perhaps the most serious objection to a 2 ml. dose in the P.G. chamber is that it is not as easy to distribute evenly through the eight spraying holes (two in each wall) as the 12 ml. dose. This could, perhaps, be improved by using one hole in the centre of each of the four walls. It is also clear that, with a 2 ml. dosage, O.T.I. would produce such low knockdown and mortality as not to be a satisfactory standard, so that a second more concentrated standard would be required for the modified tests.

Table 2 gives the results of tests in which 2 ml. dosages of 0·3, 0·4, and 0·5 per cent. w/v pyrethrins were compared with a 12 ml. dosage of O.T.I. in the P.G. chamber.

TABLE 2.—AVERAGE FIGURES FROM THREE TESTS IN THE P.G. CHAMBER COMPARING 2 ML. DOSAGES OF CONCENTRATED PYRETHRIN SOLUTIONS WITH THE STANDARD DOSAGE (12 ML.) OF O.T.I. ALL RESULTS CALCULATED ON A 50 : 50 SEX RATIO.

Concentration w/v Pyrethrins.	Dosage.	Knockdown at—		Mortality.
		5 mins.	10 mins.	
%	ml.	%	%	%
0·1 (O.T.I.)	12	84	95	42
0·3	2	52	66	25
0·4	2	56	75	34
0·5	2	65	80	44

Considering mortality first of all, it can be seen that 2 ml. of 0·5 per cent. pyrethrins produces approximately the same kill as 12 ml. O.T.I. On mortality figures, therefore, this concentration would be a satisfactory secondary standard for tests with reduced dosage. The figures in Table 2 are consistent with the assumption that there is a close correlation between mortality and the amount of toxic ingredient introduced into the chamber. Thus a reduction in volume of spray to 1/6 was compensated by a five-fold increase in concentration of toxic ingredient. Further evidence on this point is given by Lindquist *et al.* (1945) who demonstrate the practicability of using small quantities of highly concentrated sprays rather than larger quantities of more dilute ones.

When the figures for knockdown are examined, it can be seen that the knockdown produced by the secondary standard (0·5 per cent. pyrethrins) is lower than it is for O.T.I. This result (lower knockdown with the same mortality) is presumably due to the greater chance with reduced dosage of flies escaping contact with the spray, although a greater number of those hit are killed because of the greater amount of pyrethrins in each droplet of spray.

The question of the suitability of the secondary standard because of its 80 per cent. knockdown in ten minutes leads to an examination of various aspects of knockdown. There is no doubt that it is easier

to perform tests in the P.G. chamber when the proportion of flies knocked down is very high. If there are many flies still able to fly, it is, for example, less easy to collect those which are knocked down without letting the others escape. However, it is necessary to count and sex the flies which are not knocked down, since these figures are required for the calculation of percentage knockdown and mortality.

One method of dealing with the flies which are not knocked down is to spray them again with a material causing quick knockdown, wait for them to fall, and then collect them for counting. In this method these flies are regarded as having survived the spray and are included as such in the 24-hour mortality counts. This procedure, however, is open to severe criticism, for the assumption that flies not knocked down at 10 minutes will survive the spray treatment is valid only for rapidly acting materials such as pyrethrins, Thanite, or Lethane, but invalid for slow acting materials such as DDT.

An alternative means of dealing with the flies not knocked down at 10 minutes is to leave them in the chamber for a longer period (with DDT about 30 minutes) until knockdown is substantially complete. This suffers from the disadvantage that the flies may accumulate an additional dosage during their prolonged period in the chamber (e.g., with DDT), although increased dosage may not be very important with some toxic agents (e.g., pyrethrins), in view of the evidence available on the influence of exposure time on mortality (see next section). The foregoing points are emphasized if we consider, for example, a DDT spray which has been used in just sufficient concentration and dosage to produce 100 per cent. mortality in 24 hours. This spray will produce very much less than 100 per cent. knockdown in 10 minutes; in actual fact, the 10-minute knockdown will probably be less than 50 per cent. If only the flies knocked down at 10 minutes are retained for 24 hours for the mortality count and the remainder counted as alive, then the spray will give a result of 50 per cent. knockdown (10 minutes), 50 per cent. mortality (24 hours), whereas the mortality in 24 hours would actually be 100 per cent. On the other hand, there is great difficulty in collecting without injury for a 24-hour count the 50 per cent. of flies not knocked down at 10 minutes. As already pointed out, if the flies are left in the chamber the possibility of their accumulating an additional toxic dose of DDT from the walls is high.

Thus there is no doubt that there are many advantages, as regards manipulation of flies, in using a dosage and exposure time which will result in a knockdown of 95 per cent. or better, particularly if one is concerned with the final mortality obtained. On the other hand, little information is provided by the standard P.G. tests shown in Table 1 on the relative efficiency as knockdown agents under room conditions of pyrethrins, Thanite, and Lethane, which all rate between 99·5 and 99·9 per cent. knockdown. More information is provided by the reduced dosage experiments (Table 1). It should be pointed out, however, that this table provides no direct comparison between different toxic materials since the materials were not tested on the same batches of flies.

The foregoing discussion, and the conclusions arising from it, can be summarized as follows:—

(1) The standard P.G. dosage gives a greater knockdown than is obtained under conditions of actual usage.

(2) While a knockdown of over 95 per cent. is desirable for mortality ratings, it is difficult to get a reliable comparison of the efficiencies of materials as knockdown agents at this level. To do this satisfactorily, separate tests are required in which conditions are adjusted to provide a knockdown of less than 90 per cent. Mortality counts would only be incidental to such tests.

(3) A P.G. test, using 1/6 standard dosage, would provide a better evaluation of the performance of a spray, and would at the same time give knockdown figures of an order suitable for reliable comparison.

(4) If a reduced-dosage P.G. test was used to establish a mortality-based rating of the household effectiveness of a spray, a secondary standard, comprising a spray containing at least 0·5 per cent. pyrethrins, would be required. Such a standard would have the same limitations as those shown later to be possessed by O.T.I.

(5) The standard P.G. procedure, at normal or reduced dosage, is not suited for the individual evaluation of materials, such as DDT, which produces slow knockdown in 10 minutes and high 24-hour mortality.

(ii) *The 10-minute Exposure Period.*

Under normal conditions of fly spray use, it is not often that all doors and windows of a room are closed before spraying and left closed for 10 minutes afterwards and, indeed, under some conditions such a procedure would not be possible. For practical use it is desirable that a spray should give a high knockdown in 5 minutes or less, although as pointed out earlier, very rapid knockdown may result in a lower kill than less rapid knockdown.

To relate P.G. results to conditions more nearly approximating to actual spray usage, it is desirable to determine what effect reduced exposure time has on knockdown and mortality. Knockdown figures for intervals shorter than 10 minutes have been published by Lederer (1940) who recorded 1, 3, 5, 7, and 10 minute knockdown counts during ten-minute exposure periods. He showed that, for most sprays, the knockdown increased progressively. For example, using the 1940 O.T.I. his figures for the above exposure periods were 11·4, 32·8, 63·2, 91·3, and 94·6 per cent. respectively. Ford (1941) reported the results of cooperative tests carried out by 15 laboratories to determine the effect on 10-minute knockdown and 24-hour mortality of reduction in exposure time from 10 minutes to 5 minutes using both 12 ml. and 6 ml. doses of spray. All sprays gave slightly greater average 10-minute knockdowns for the 10-minute than for the 5-minute exposure. Percentage knockdown at 5 and 10 minutes was determined by six laboratories, but only for the 6 ml. dosage. The average knockdown at 10 minutes was 85·5 per cent. and at 5 minutes it was 83·3 per cent. Reduction in exposure time from 10 to 5 minutes did

not affect the mortality produced by the sprays. Hurst (1943) also observed that over a considerable range in exposure times the mortality remained relatively constant. Thus the kills obtained for exposure periods of 5, 10, 15, 20, 30, 60, and 120 minutes were 35, 36, 32, 34, 36, 43, and 49 per cent., respectively.

Information is not available for the lower end of the exposure scale, and in order to determine the effect of very short exposures, a series of experiments was carried out with pyrethrins, Thanite, and Lethane 384, in which the results from the standard 10 minute exposure period were compared with those from a 1-minute exposure period and from tests in which the spray was atomized with the vents of the P.G. chamber open and the fan on. It is probable that conditions of actual usage would seldom be more severe than spraying with the fan on all the time, and that they would more often approximate to an exposure for 1 minute followed by aeration.

In the 10-minute and 1-minute exposure tests, the time was measured from the commencement of spraying, this operation occupying just under 1 minute. Knockdown counts were taken for all exposures 10 minutes after spraying. To prevent flies being sucked against the fly wire covering the exhaust duct during the shorter exposure periods, a fly-wire cage 12 in. by 19 in. by 4 in. high was attached to the inside of the chamber. This kept the flies at least 4 inches from the duct and, at this distance, the air pressure was insufficient to hold the flies against the wire. The results, adjusted to a 50 : 50 sex ratio, are shown in Table 3.

TABLE 3.—THE EFFECT OF REDUCED EXPOSURE TIME ON 10-MINUTE KNOCKDOWN AND 24-HOUR MORTALITY, USING THE STANDARD PEET-GRADY DOSAGE.

Spray.	Number Tests.	K.D. (10 mins.) Exposure Conditions.			Mortality (24 hrs.) Exposure Conditions.			O.T.I. 10 mins., Same Tests.	
		10 min.	1 min.	Fan on.	10 min.	1 min.	Fan on.	K.D.	Mort.
		%	%	%	%	%	%	%	%
0·13 per cent. w/v pyrethrins ..	3	99·0	96·3	85·0	46	40	32	98·7	37
3·5 per cent. Thanite	3	99·9	99·9	97·3	61	58	56	98·9	37
5·0 per cent. Lethane 384	3	100	99·6	98·5	50	34	21	99·3	38

On examination of the knockdown figures it can be seen that reduction in exposure time from 10 minutes to 1 minute or less resulted in a reduced 10-minute knockdown. The maximum reduction, which was recorded on each occasion for the shorter exposure, was 14 per cent. for pyrethrins, 2·6 per cent. for Thanite, and 1·5 per cent. for Lethane. The 24-hour mortality was also reduced by reduction in exposure time, the maximum reduction being 14 per cent. for pyrethrins, 5 per cent. for Thanite, and 29 per cent. for Lethane and, as for knockdown, the reduction in mortality was greatest when the exhaust fan was left on.

While it might be anticipated that the very great reduction in exposure time would result both in reduced knockdown and mortality, the reasons for the considerable variation in the reduction for the three spray materials are not clear. If one of the principal effects of Lethane (which shows a considerable reduction) were a fumigating action (Hurst, 1943), this would be interfered with by aeration. Tests carried out by White (1945) showed that 3·5 per cent. Thanite and 5·0 per cent. Lethane produce a similar particle size distribution when sprayed at 12·5 lb. pressure through the P.G. gun, while 0·1 per cent. pyrethrins produces somewhat smaller particles. This relationship holds for size counts taken during 1 minute after and 5 minutes after spraying. Particle size counts therefore do not afford any explanation of the differential effect for Thanite and Lethane of reduced exposure time. On the other hand, the relatively smaller pyrethrin droplets would presumably be removed by the exhaust fan more readily than the larger particles of the other sprays, since the rate of fall of the droplets (which is proportional to the square of the radius) would have an effect upon their rate of removal through a duct in the roof.

It is clear that the effect of reduced exposure time is not nearly as marked as that of reduced dosage (Table 1). Assuming that a 1-minute exposure approximates to conditions of actual usage, the degree of reduction in knockdown and mortality which was observed is not considerable for pyrethrins or Thanite, although it is for Lethane for mortality, but not for knockdown (see Table 3). The general conclusion to be drawn is that toxicity is largely dependent upon the initial dosage transmitted to the flies during, and shortly after, spraying.

(iii) *Sexing of Flies.*

Many authors have discussed the importance of sexing all flies used, although this is not at present required by the P.G. test. Miller and Simanton (1938) provide comprehensive data to show that, if the sex ratio is not the same in all batches of flies from a single culture, a variable error in rating is introduced which is equal to about half the percentage difference in sex ratios between the O.T.I. and the unknown spray populations.

The difference in resistance of males and females is very marked, not only with pyrethrins, but also with other toxic materials. Table 4 shows representative figures for differential mortality of sexes obtained from tests with pyrethrins, Thanite, and Lethane.

TABLE 4.—DIFFERENTIAL MORTALITY OF SEXES DEMONSTRATED BY STANDARD P.G. TESTS WITH 3 TOXIC AGENTS.

Spray.	Number Tests.	Average Percentage Mortality.			Difference in Percentage Mortalities.
		Males.	Females.	50 : 50 Sex Ratio.	
O.T.I.	57	81	13	47	68
3·5 per cent. Thanite ..	9	92	25	58	67
5·0 per cent. Lethane ..	7	90	20	55	70

For an average mortality (50 : 50 sex ratio) of about 50 per cent. there is no great difference in the relative susceptibility of the sexes to the three poisons. It should be noted (see Fig. 1) that the divergence in susceptibility of sexes for pyrethrins is greatest at about the 50 per cent. mortality level and diminishes both above and below. Therefore, without the concentration-mortality curves for both sexes for Thanite and Lethane it is not safe to make any generalization about the relative susceptibility of the sexes to the three poisons.

Uniformity in the sex ratio of the flies in a set of cages is directly dependent upon the uniformity of the pupae from which they were derived. Very thorough mixing and extremely careful sampling of pupae for a set of cages are essential if the sex ratios are to be close. As an indication of the results when no particular attention was paid to this point, in a series of 20 tests with sets of 4 cages with approximately 500 flies in each cage the maximum variation in percentage of males was 27 and the average 12. Even the mean maximum variation of 5·63 per cent. which would be expected by pure chance if 1,000 male and 1,000 female pupae were mixed and divided into 4 batches each containing 500 flies, is of some importance, since, as pointed out above, variations in sex ratio are responsible, to the extent of half their numerical value, for an error in rating.

To sum up, while sexing of flies increases the work involved in P.G. tests, it not only improves the accuracy of the rating even if the pupae are adequately sampled, but it also safeguards against accidental inadequate mixing or sampling.

(iv) *Uniformity of Dose per Fly.*

Using 24 ml. of dyed pyrethrin spray per test in the P.G. chamber, Murray (1940) determined colorimetrically the individual dosages received by each fly. The ratio of the greatest dose to the least dose varied in different tests from 2·60 to 6·44 for male flies and 2·58 to 5·15 for females. It is clear that this variation in individual dosage reduces greatly the sensitivity of the P.G. test. Thus in each test the individual doses may be divided into (i) a sub-lethal zone which includes all doses which are too low to kill any flies, (ii) an intermediate zone including doses from which the flies die or recover depending upon their inherent individual susceptibilities, and (iii) a lethal zone comprising doses from which no flies recover. Only flies receiving intermediate doses contribute to the sensitivity of the test. Because of the great variation in dosage error from test to test, real differences in insecticidal power of samples may not be shown without extensive replication of tests.

The dosage error can probably be attributed mainly to variation from test to test in the spraying technique and distribution of spray particles, and to variation in the activity and distribution of flies in the chamber. With regard to spraying technique, although it is probable that the gross characteristics of spray distribution are fairly closely replicated from experiment to experiment, no standard method has been laid down for distributing the spray in the chamber. During spraying, the nozzle of the spray gun may be held still, moved back and forth, or in a circular motion, each method no doubt producing a different spatial distribution of droplets. However, Murray demonstrated large differences from test to test using the same method of spraying in all tests, so that even a standard spraying routine does not eliminate the individual dosage error.

Whether or not the dosage error would be reduced by introducing the flies into the chamber after (David, 1946), instead of before, spraying was not investigated, although this might have the effect of reducing the number of heavy individual dosages received. A spray gun producing only particles of a closely similar size, instead of the P.G. gun with its wide distribution of particle size, should also improve uniformity of dosage, but this would involve a major alteration in the official P.G. method. While the use of particles of a uniform size is unlike conditions at present obtained in actual usage, this modification would not necessarily be a disadvantage if it lowered the variability in the results of laboratory tests.

Turning to the second, and probably more important, factor, a considerable variation in activity and distribution of flies in the P.G. chamber before testing can be observed over a series of tests. While the activity of different cultures of flies bred under apparently identical conditions may vary, the activity of all batches of a single culture should be fairly uniform.

David and Bracey (1944A) have demonstrated for mosquitoes that activity is a major factor in determining dosage. Because pyrethrins stimulate mosquitoes to great activity, they suggest (1944B) the incorporation of a sub-lethal concentration of pyrethrins in all sprays in order to produce uniformly active insects during testing. Murray used a pyrethrin spray in his tests, but the fact that his individual dosages were extremely variable indicates that pyrethrins did not render fly activity and distribution uniform.

Lack of uniformity, even with pyrethrins, is supported by experiments (Hurst, 1943; Kearns and March, 1943) showing that, with decrease in the size of the spray chamber, it is possible to obtain more uniform results because the irregularities of fly distribution and spray distribution on individual fly dosage are decreased. In the smaller chamber it is possible to attain more uniform and higher initial spray concentrations than is practicable with the P.G. chamber. On the other hand, there was a higher rate of knockdown in the smaller chambers, especially where the toxic materials used exerted fumigant activity. Since it is necessary to have information on knockdown as affecting sprays under household conditions, this fumigating effect due to restricted conditions is clearly undesirable.

(v) *Spraying Pressure.*

The importance of spraying pressure has recently been demonstrated by Hurst (1943), who showed that the toxicity of O.T.I. in the P.G. chamber increased markedly with spraying pressure, pressures of 2, 12·5, and 25 lb. per square inch producing mortalities of 15, 36, and 45 per cent., respectively.

Spraying pressure may influence mortality in three ways. These are its influence on (*a*) the rate at which the spray is delivered, (*b*) the velocity of the spray particles, and (*c*) the particle size distribution. It is well established that there is an optimum particle size above and below which a progressively greater concentration of toxicant is required per given volume to produce the same mortality. Too much stress, however, should not be placed on the effect of minor variation in spraying pressure on particle size distribution, since the relationship

is to the cube root of the pressure. Thus White (1945) found that there was no significant difference in particle size distribution when a spray was applied at 12·5 lb. and 25 lb. pressure from a standard P.G. gun. This conclusion was based on four kerosene based sprays containing 0·1 per cent. pyrethrins, 3·5 per cent. Thanite, 5·0 per cent. Lethane 384, and 5·0 per cent. S.A.E. 30 lubricating oil, respectively.

(vi) *Comparison of Sprays with O.T.I.*

Because of the great variation in susceptibility to sprays of flies from different cultures reared either in the same laboratory or, more particularly, in different laboratories, the use of a standard reference insecticide (the official test insecticide or O.T.I.) was adopted. For each culture of flies the mortalities produced by the unknown sprays are compared with the kill produced by the reference insecticide. Insecticides are then evaluated on the basis of ratings which are the plus or minus differences in percentage kill from the standard, this procedure being designed to compensate for the different levels of susceptibility in different cultures.

The use of a standard reference insecticide does not do away with the possibility of error due to the facts that (Murray, 1940; Ford, 1941):—

(i) A given increase in active principle content will produce a different (either greater or less) additional kill with weak flies than with strong flies. Hence there may be appreciable differences in ratings obtained with cultures of flies of different susceptibilities.

(ii) The variation from culture to culture in susceptibility to pyrethrins is different from the variation in susceptibility to poisons, such as rotenone, acting in a different fashion. This means that samples containing pyrethrins as the sole toxic principle can be rated more accurately in comparison with the O.T.I. than samples containing other toxic principles.

It should also be borne in mind that the ratings of sprays are not directly proportional to their toxic content; this is due to the fact that the concentration-mortality relationship is not linear.

For the standard reference insecticide, a kerosene solution, containing approximately 0·1 per cent. w/v total pyrethrins was selected because, under standard P.G. conditions, it produces a 30-55 per cent. mortality of houseflies. As can be seen in Fig. 1, based on Miller and Simanton (1938), for a 50 : 50 sex ratio the 30-55 per cent. mortality range is on the portion of the concentration-mortality curve most sensitive to variation in pyrethrin concentration. Owing, however, to the variation in relative resistance of males and females, this range corresponds to males, 50-83 per cent., and females, 10-27 per cent. mortality. These ranges do not correspond with the portions of greatest slope of the respective curves. The male mortality (with a range of 33 per cent.) has more influence on the sensitivity of the 50 : 50 sex ratio curve over the 30-55 per cent. mortality range than the female mortality (with a range of 17 per cent.). Both male and female flies carry disease and cause annoyance, so that conclusions regarding practical use must

be based on results with both sexes. Unless males and females are to be considered separately and tested over their most sensitive range, and this would approximately double the amount of work required at present, or unless comparisons are based on one sex only, the method of averaging results on a 50 : 50 sex ratio basis appears to be the only one to adopt. It should be clearly realized, however, that the 30-55 per cent. mortality range is somewhat less satisfactory than it appears to be and is really a convenient compromise.

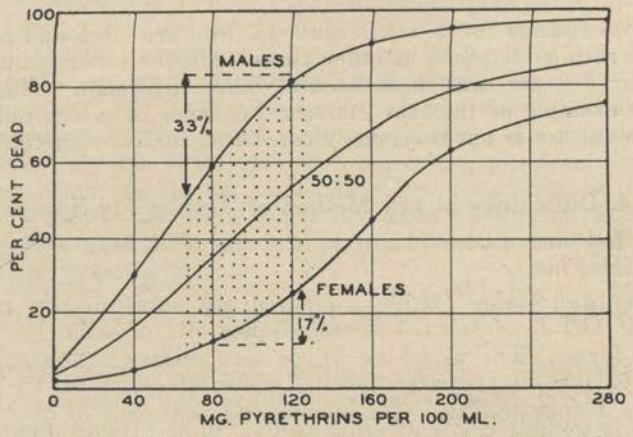

FIG. 1.—Concentration-mortality curves for male and female houseflies (Miller and Simanton, 1938).

In spite of these drawbacks, the use of O.T.I. has undoubtedly improved the uniformity of P.G. tests and has been particularly useful in revealing cultures of flies of aberrant susceptibility to sprays. The variation in susceptibility that may occur between cultures of houseflies reared for the P.G. test, is great and this species is certainly no exception to the general conclusion of Tattersfield and Potter (1943) concerning standard cultures of insects for insecticide tests: "The level of resistance of any one species does not remain constant. The factors influencing the innate susceptibility and variation are not fully understood. Even when insects are reared from the same stock for a number of years under fairly standard conditions and show no sign of disease or parasitism, they may still fluctuate in susceptibility from one set of cultures to the next."

Table 5 shows the ratings for pyrethrins, Thanite, and Lethane obtained from the figures in Tables 1 and 3.

TABLE 5.—RATINGS FOR PYRETHRINS, THANITE, AND LETHANE.

Spray.	Rating.	
	Table 1.	Table 3.
0·13 per cent. w/v pyrethrins	+ 5	+ 9
3·5 per cent. Thanite	+ 22	+ 24
5·0 per cent. Lethane	+ 23	+ 12

The 0·13 per cent. pyrethrins spray was prepared from a 2·4 per cent. concentrate analysed by the Ripert (1934, 1935) method. On the basis of tests carried out at different times with sprays prepared from other concentrates, and of Fig. 1, a rating of about +13 would have been expected. Although too much reliability should not be placed on a direct comparison with Fig. 1, which is based on results from another laboratory, there is some evidence that the biological tests indicate here a lower concentration of toxic ingredients than the chemical analyses.

The two ratings for 3·5 per cent. Thanite are close and also agree well with ratings obtained at other times. On the other hand, the two ratings for 5·0 per cent. Lethane are widely divergent. This may be a further example of the fact that the variation in susceptibility from culture to culture is not necessarily correlated for two different poisons.

4. Difficulties in any Method of Testing Fly Sprays

(i) *The Relative Susceptibility to Poisons of Laboratory-reared and Wild Flies.*

Murray and Caler (1938) compared the susceptibility to 14 ml. dosages of O.T.I. of laboratory-reared flies and of wild flies collected from two farms. The wild flies, which were similar in size and general activity to laboratory-reared adults, were offered food for at least two hours before testing. The mortalities for over 10,000 wild flies were, males 63 per cent., females 57 per cent., average 59 per cent.; and for nearly 12,000 laboratory flies, males 63 per cent., females 7 per cent., average 35 per cent. Thus wild females in these tests were far more susceptible to pyrethrins than laboratory-reared females; there was no difference in the susceptibility of males. If these results are typical for all wild flies, care must be taken when using tests with laboratory-reared flies as a basis for dosages for practical conditions. On the other hand, it is known that a source of great variation in results would be introduced if wild flies were used instead of flies reared under carefully standardized conditions.

(ii) *Moribund Flies.*

When a 24-hour mortality count is made, it is found that some flies are dead, some are quite normal, and, with certain sprays, that some are alive but abnormal in their reactions. These latter flies are termed moribund. There is no unanimity in favour of the standard practice of classing these moribund flies as alive. The main advantage of this procedure is that this separation is easy. For sprays containing pyrethrins as the sole toxic agent the problem is not an important one, since moribund flies seldom form more than 1 per cent. of the total. However, with many other spray ingredients, including rotenone, large numbers of flies may be moribund at 24 hours. It is almost certain that recovery conditions in the P.G. test are more favourable than in actual practice, so that many of the flies classed as moribund would either die, or at least never become sufficiently active to cause annoyance. Many of the moribund flies may die between 24 and 48 hours after spraying, but it is not convenient in routine testing to standardize on a 48-hour recovery period. Except for the difficulty in deciding where to draw the line between moribund and alive, it would

often be more satisfactory, for practical purposes, to regard moribund flies as dead, although it should be clearly realized that a spray which kills flies outright is more satisfactory than one which achieves the same result by killing some and producing a long delayed action on others. For uniformity, moribund flies have been counted as alive in the tests quoted in the present paper as laid down in the standard P.G. method.

Whitmire (1939) suggests two standard methods for separating moribund and normal flies. In the first, flies placed on a piece of paper 18 inches square at the 24-hour count and able to crawl off are regarded as normal, the remainder as moribund (if able to move) or dead; in the second, the flies are allowed to recover in standard cages with an opening in the top, and flies sufficiently unaffected by sprays to escape by the end of 24 hours into a larger container are regarded as normal. One disadvantage of the second method is that some flies which escape may subsequently die before the 24-hour count, although there is no reason why these should not be included with the dead in the inner container. Unless this were done, the exact time after spraying at which the flies were placed in the inner container would influence the result with slow-acting poisons.

5. Discussion

A critical examination of the Large Group Peet-Grady method reveals it to be subject to certain errors, or liability to error, to which variation in spray distribution and variation in distribution and activity of the test insects appear to be the most important contributory factors. Some of the errors are unavoidable in any practicable standardized testing technique using houseflies. In the case of others, alteration of conditions or technique so as to minimize them would introduce new error, or at any rate some undesirable feature.

While it is not difficult to find fault with the method, and indeed many of the points mentioned in this paper have been established by earlier workers, it is extremely difficult to suggest an alternative method of testing household sprays that would be an all-round improvement on the Peet-Grady technique. One thing about the P.G. test, of course, offsets to a large extent the weaknesses that are inherent to it: this is the fact that it has been, and is the only method to have been, widely accepted and used.

A major criticism levelled against the P.G. test in this paper, a criticism which arose as a result of the necessity of obtaining results from which a spray could be formulated for use by the Services, is that the knockdown and kill given by a spray in the P.G. chamber, using the standard dosage, does not provide a true indication of the actual, as opposed to relative, performance under conditions of ordinary usage. For the evaluation of actual performance, a secondary test was suggested, using 1/6 standard dosage, which is equivalent to the dosage one could reasonably expect to be dispersed with a hand spray-gun in a room of normal size. While the results of such tests might be valuable and illuminating to the prospective user of a spray, one is forced to recognize, at the outset, that the procedure is unlikely to find favour with manufacturers; or, more correctly, it would not have been welcomed by manufacturers, say, five years ago. In this connexion it is unnecessary to do more than point out that in such a test (see

Table 1) a spray containing 0·13 per cent. pyrethrins, which would be considered a fair average quality spray, or better, in the 1930's, gave only a 60 per cent. 10-minute knockdown and a 5 per cent. kill.

The incorporation of DDT in household sprays, which is likely to become standard practice, if it is not so already, has created an entirely new situation; and it is of interest to consider what role the P.G. chamber is likely to fulfil usefully in the future.

The development of the P.G. method was related to the particular properties and mode of action of the pyrethrins, which are not easily, nor even satisfactorily, assayed chemically; and the standard P.G. technique is only really suitable for insecticides with a mode of action generally similar to that of pyrethrum. As has already been indicated, the P.G. test is not particularly suitable for the rating of sprays containing DDT. Apart from the complication that the 24-hour mortality normally may be higher than the 10-minute knockdown, it is anticipated that the great majority of sprays marketed will be formulated to contain more than enough DDT to give 100 per cent. kill with the standard P.G. dosage (or probably even with 1/6 standard dosage). Furthermore, it would be difficult, if not impracticable, to remove all traces of DDT from the walls of the chamber after each test, and thus there would be a real danger of a residual toxicity effect providing an ever-present complication in any chamber subject to regular use.

The P.G. method, particularly if the refinements suggested in previous sections are incorporated, will remain a most valuable technique for the laboratory study and assay of certain types of insecticide. However, one is forced to the conclusion that the P.G. test, as at present standardized, can no longer serve its primary function, which is to provide a satisfactory comparative rating or evaluation of proprietary household sprays. This does not mean that the test could not be modified to provide a rating that would be of great practical value as an indication of the performance of a spray.

Until an insecticide is developed which has a toxicity comparable with that of DDT, and a much greater rapidity of action, household fly sprays will have to depend on one component for kill, and another for knockdown. Although it is unlikely that this will hold for long, at the present time no satisfactory substitute for DDT can be recommended for the former purpose. The incorporation of a certain minimum quantity of DDT will ensure adequate killing power; and the killing power of a spray would be determined more conveniently by a chemical assay of the DDT than by a biological test. For the knockdown component of a spray, pyrethrum, or a material with similar action, would have to be used; and it would be more convenient to assess the knockdown properties of a spray by a biological test. It is considered that a modified P.G. test would do this admirably.

An indication of the means by which knockdown could be evaluated most accurately in the P.G. chamber has been given in a previous section. In order to reduce percentage knockdown to a figure falling within the range providing the optimum basis of comparison (i.e., say from 30 to 70 per cent.), a reduction of the standard P.G. dosage would be necessary. It is suggested that 1/6 standard dosage, and a 5-minute rather than a 10-minute count might be adopted. This

should give, at any rate for sprays of a desirable standard, percentage knockdown figures within the required range; and would have the additional advantage of approximating reasonably closely to conditions of normal usage.

For the new P.G. rating, even though it would be based entirely on knockdown, a notation similar to the present one, and derived in the same manner, could be adopted. The complication due to residual toxicity of DDT on the walls of the test chamber would be of no consequence, as even if a significant deposit remained, the effect on the flies would not be noticeable within 5, or even 10 minutes.

Of the domestic pests against which household insect sprays are used in Australia, the most important are houseflies, blowflies, mosquitoes, and silverfish. Their relative prevalence varies with locality and latitude; so that it is difficult to say against which type of pest sprays would, in the aggregate, be applied most. One can say, however, that in certain areas sprays would be used as much against mosquitoes as against houseflies, if not more, and that more spray is used against blowflies, mosquitoes, and silverfish together than against houseflies. When it is realized that the different species of domestic pests differ markedly in their susceptibility to the insecticides used in household sprays, these considerations will be seen to have an important bearing on the value of the P.G. test.

Houseflies are less susceptible to pyrethrum than are the other three types of insects mentioned above, particularly mosquitoes* and silverfish. On the other hand, mosquitoes and, as far as is known, silverfish are markedly less susceptible to DDT than are houseflies and blowflies.

With the type of spray that is likely to be most common for some time to come, i.e., one containing pyrethrum for knockdown and DDT for kill,† the modified P.G. test, giving a rating based on housefly knockdown, would at the same time provide a reliable indication of the all-round efficacy of the spray against mosquitoes and silverfish. This, however, would not necessarily apply when another insecticide is substituted for pyrethrum as the "knockdown component." It is possible to suggest one material at least which, in a concentration sufficient to give as good a housefly knockdown as 0·1 per cent. pyrethrins, would almost certainly give a less satisfactory knockdown and kill of mosquitoes, even in combination with 0·5 per cent. DDT, than 0·1 per cent. pyrethrins alone. Other insecticides, which may be developed in the future, might have an even more marked differential toxicity. It is clear, therefore, that P.G. tests using houseflies, even if modified to suit present day conditions, cannot in themselves be accepted as giving a reliable indication of the performance of household sprays. Data on the toxicity of the finished spray or of its components to the other important domestic insect pests are also required.

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* The results of experiments with various sprays against mosquitoes and against silverfish are being prepared for publication.

† The C.S.I.R. Division of Economic Entomology in May, 1945, recommended a minimum of 0·1 per cent. total pyrethrins and 0·5 per cent. DDT (para para isomer) w/v, although it now appears that for houseflies the concentration of DDT could be reduced to 0·1 per cent.

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The author desires to acknowledge the helpful assistance of Mr. F. N. Ratcliffe during the preparation of this paper. He is also indebted to Mr. G. L. White, of Munitions Supply Laboratories, Maribyrnong, for particle size data, to Mr. G. A. McIntyre for the statistical analysis of results, and to Mr. R. F. Powning for pyrethrum analyses.

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BULLETIN No. 219

Spray Tests against Adult Mosquitoes

1. Laboratory Spray Tests with Culicine (*Culex fatigans*) Adults

By
D. F. WATERHOUSE, M.Sc.

2. Spray Tests with Anopheline (*Anopheles punctulatus farauti*) Adults

By
D. F. WATERHOUSE, M Sc.
and
D. O. ATHERTON, M.Sc.AGR.

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FOREWORD

The investigations described in this Bulletin were carried out in 1942 and 1943 to provide a basis upon which to formulate specifications for a spray for use against the mosquito vectors of malaria and dengue fever.

When this work was commenced, most of the existing information on toxic ingredients for sprays was derived from tests against house flies. It was soon shown that these results could not be used to formulate anti-mosquito sprays.

The first paper by D. F. Waterhouse deals with the initial exploratory tests against Culicine mosquitoes under standardized laboratory conditions in Canberra. There was sufficient evidence from these tests to show that the species used, *Culex fatigans*, was more resistant to toxic materials than *Aedes aegypti*, the principal vector of dengue fever. However, there was no information upon the relative susceptibility of *Anopheles punctulatus*, the principal vector of malaria in New Guinea and neighbouring islands. Accordingly, Captain D. F. Waterhouse carried out tests with this species in New Guinea with the cooperation of Major D. O. Atherton. The results obtained are described in the second paper.

These investigations were undertaken when pyrethrum was in such short supply that its use had to be restricted to the control of Anopheline mosquitoes and it was essential that the minimum concentration consistent with efficiency and a reasonable margin of safety should be determined as quickly as possible. On the basis of the tests a standard for mosquito sprays was decided upon and this was adopted by the Australian Army and used consistently until the advent of DDT, in the last year of the war, in sufficient quantity to reinforce the spray and convert it into an all-purpose one.

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2. Spray Tests with Anopheline *(Anopheles punctulatus farauti)* Adults

SUMMARY

1. Laboratory Spray Tests with Culicine (*Culex fatigans*) Adults

Spray tests were carried out with *C. fatigans* Wied. using the standard Peet-Grady chamber and procedure, with the exceptions that the spray dosage was reduced from 12 ml. to 2 ml. (to approximate actual usage conditions) and both knocked-down and not-knocked-down mosquitoes were collected after the 10-minute exposure period to assess mortality.

Concentration-mortality and concentration-knockdown curves for both sexes for pyrethrin concentrations from 0·01 per cent. w/v to 0·14 per cent. w/v are given. For females 0·01 per cent. pyrethrins gave 79·2 per cent. knockdown and 74·5 per cent. mortality, 0·1 per cent. pyrethrins gave 94·4 per cent. knockdown and 97·5 per cent. mortality, and 0·14 per cent. pyrethrins gave 99·3 per cent. knockdown and mortality. Figures for 10-minute knockdown and 24-hour mortality were generally close.

The mortality produced by pyrethrin sprays appeared to be dependent more on the amount of pyrethrins atomized than on the volume of spray used.

Kerosene, which was used as the base for most sprays, produced very low mortality. The addition of creosote did not result in a satisfactory spray.

Undiluted oils of *Zieria smithii* or *Dacrydium franklinii* (Huon pine), or 50 per cent. *Backhousia myrtifolia* oil in kerosene, gave high mortalities. No increase in mortality was observed when these oils were added to pyrethrin sprays. Similarly, no increase in mortality was observed on the addition of eucalyptus oils, sesame oil, or eudesmin to pyrethrin sprays.

Sprays containing up to 10 per cent. Lethane special were not highly effective, although 15 per cent. produced a high mortality. The addition of Lethane to a pyrethrin spray did not increase the mortality.

A spray containing 3·5 per cent. Thanite produced 98 per cent. knockdown and 99 per cent. mortality of females, giving a higher mortality than a 0·1 per cent. pyrethrin spray tested at the same time. 3·0 per cent. Thanite caused 95 per cent. mortality and 2·5 per cent. caused 93 per cent. mortality. However, the addition of 2·5 per cent. Thanite to a 0·01 per cent. pyrethrins spray resulted in a lowering of knockdown and mortality.

No additional mortality of females resulted when 5 per cent D.H.S. activator was added to a 0·01 per cent. pyrethrins spray, although the knockdown was higher.

The addition of 0·34 per cent. N-isobutyl undecylenamide to 0·01 per cent. pyrethrins caused no significant increase in mortality. Two higher concentrations (1·0 and 2·0 per cent.) of N-isobutyl undecylenamide depressed knockdown very considerably and mortality slightly.

A spray containing 1·0 per cent. DDT gave poor (55 per cent.) knockdown, but high (94 per cent.) mortality of females, while a 0·1 per cent. solution of DDT gave 6·5 knockdown and 50 per cent. mortality. The addition of 0·1 per cent. DDT to a 0·01 per cent. pyrethrins spray caused quite a large increase (16 per cent.) in mortality.

Undiluted dimethyl phthalate gave 63 per cent. knockdown and 47 per cent. mortality.

A Freon bomb containing pyrethrins and sesame oil gave excellent knockdown and mortality at the recommended dosage of four seconds spraying per 1,000 cu. ft.

From an examination of figures for differential mortality of males and females to a number of sprays, it can be seen that males are slightly more susceptible than females. The one exception was Lethane.

A difference in susceptibility of different species was noted. *C. fatigans* was more resistant than *Aedes aegypti, A. notoscriptus,* or *A. alboannulatus.*

2. Spray Tests against Anopheline (*Anopheles punctulatus farauti*) Adults

Spray tests were carried out in New Guniea with caged wild females of *Anopheles punctulatus farauti* Lav. (=*moluccensis* Sw. & Sw. de Graaf) in tents and in an airy native hut. Dosages of 1 fl. oz. per 2,100 cu. ft. and 1 fl. oz. per 3,000 cu. ft. were atomized, both by pressure sprayer and a hand spray gun. The effect of 10-minute and very short exposure to the spray was recorded as percentage knockdown in 10 minutes and percentage mortality after 18 hours.

The carrier for the sprays, lighting kerosene, was shown to have no effect on the mosquitoes under the conditions of the test. The lowest concentration of pyrethrins found to give both good knockdown (99·5 per cent.) and kill (99 per cent.) was 0·07 per cent. w/v. Data for two higher and three lower concentrations of pyrethrins are given. A 0·5 per cent. DDT spray gave good mortality (97 per cent.), but poor knockdown, while lower concentrations of DDT gave poor mortality also. The knockdown of the 0·5 per cent. DDT spray was rendered satisfactory by the addition of 0·03 per cent. w/v. pyrethrins or 3·5 per cent. Thanite. A spray containing 0·25 per cent. DDT and 0·05 per cent. w/v pyrethrins also gave good results.

The following sprays gave inadequate (less than 85 per cent.) mortality: 5 and 10 per cent. Lethane 384 standard, 3·5 and 5 per cent. Thanite, dimethyl phthalate. The comparative ineffectiveness of Thanite in these experiments is possibly due to the absence of conditions favouring a fumigating action.

Dimethyl phthalate, dibutyl phthalate, and Crystox had no activating effect under the conditions of test.

Little difference was noted with pyrethrins, DDT, or a mixture of the two when 10-minute and 1-minute exposures to the spray were compared. With Thanite, both knockdown and mortality were lower for the short exposure.

Little difference in results was noted for pyrethrins, DDT, or a mixture of the two when spray tests were carried out in a closed tent and in an open native hut.

An examination of figures for a number of sprays for 10-minute knockdown, and 1, 5 and 18-hour mortality indicated that it was necessary to make an 18-hour count to obtain reliable information of the effectiveness of sprays, particularly of those containing DDT.

Spray Tests against Adult Mosquitoes*

1. Laboratory Spray Tests with Culicine (*Culex fatigans*) Adults

By D. F. Waterhouse, M.Sc.

I. INTRODUCTION

In the early days of the Pacific War, when the advice of the C.S.I.R. was sought by the Army on specifications for fly and mosquito sprays, it soon became clear that almost all of the existing information on toxic ingredients for sprays was derived from tests against flies. Data were urgently required on the effectiveness of these materials when used against the mosquito vectors of malaria and dengue fever.

Because of various wartime factors, pyrethrum flowers, the most commonly used basis of effective fly sprays, were in extremely short supply. The present tests were designed to provide information on the minimum effective concentration of pyrethrins in mosquito sprays, and also to evaluate the effectiveness of a number of synthetic materials, such as Lethane, Thanite, and DDT (pp'dichlordiphenyl trichlorethane).

Owing to the necessity for providing as rapidly as possible one or more formulae for effective mosquito sprays, the information acquired on many aspects of the investigation, which did not appear after the first few tests to be promising, was not extensive. An effective spray was primarily required for use against the species of Anopheline mosquitoes transmitting malaria in New Guinea and neighbouring islands. When these investigations were carried out (July 1942 to July 1943) there was no possibility of culturing in the laboratory, or obtaining from local breeding grounds adequate supplies of Anophelines. It was therefore decided to carry out rapid preliminary tests with the more readily available Culicine mosquitoes and to check the main results later in New Guinea using *Anopheles punctulatus*. After several preliminary tests with various species of Culicines, it was decided to use *Culex fatigans* Wied. as the test species. Although this mosquito is not easy to breed in the laboratory, plentiful field supplies are available for seven or eight months of the year. *Aedes aegypti* L., whilst easy to culture, was so much more susceptible to sprays that it was regarded as a probably less desirable test insect, although, as the principal vector of dengue fever, it was one of the species against which the sprays were to be used. In the second paper in this bulletin, it is shown that the resistance to toxic agents of adult *An. punctulatus farauti* Lav. (= *moluccensis* Sw. and Sw. de Graaf) is of the same general order as that of *C. fatigans*.

II. METHODS

Eggs or larvae of *C fatigans* were collected in the field and reared to the adult stage in a laboratory conditioned to 80° ± 1° F. and 70 ± 2 per cent relative humidity. The adults were kept in mosquito net cages and were fed on slices of apple and banana suspended from the top of the cage. Adults were most frequently used from four to six days after emergence. They were not fed on blood.

* Typescript received 30th September, 1946.

Tests were carried out in the Peet-Grady Chamber (Anon., 1943) using the standard spray gun and a 2 ml. dosage of spray, 1 ml. being applied from each of two diagonal corners. This dosage is 1/6th of the standard Peet-Grady dose for flies and is equivalent to 1 fl. oz. per 3,000 cu. ft. Care must be taken to distribute this small quantity of spray uniformly around the chamber. This dosage was selected because it is not practicable with a hand spray gun to deliver more than 1 fl. oz. per 3,000 cu. ft. under most conditions of usage (Waterhouse, 1947), and it was therefore considered most undesirable to use a higher dosage for the standardization of the sprays.

Mosquitoes were liberated into the chamber, spray applied using 12·5 lb. air pressure, and the mosquitoes exposed to the spray mist for 10 minutes. All mosquitoes, both knocked down and still flying, were then collected. This provided a figure for percentage knockdown. The mosquitoes were placed in a clean cage, supplied with a fresh slice of apple, and returned to the conditioned room for 24 hours, after which a count was taken to provide percentage mortality. Only those adults able to fly were classified as alive. There were sometimes a number of individuals able to move their legs, but unable to fly. Since these would have no opportunity of biting, they were classed as dead. The mosquitoes were sexed in all counts. Between tests, the chamber was aerated, the walls and roof wiped down with rubbing alcohol containing 10 per cent. acetone, and fresh paper placed on the floor.

III. RESULTS

The following results were based on approximately 350 tests involving over 30,000 mosquitoes.

1. Concentration-Mortality and Concentration-Knockdown Data for Pyrethrins

Table 1 shows percentage mortality obtained with pyrethrin sprays of various concentrations. Concentrations of 0·1 per cent. w/v and below were prepared from the Official Test Insecticide* containing 0·1 per cent. w/v pyrethrins. Above 0·1 per cent. the pyrethrin content was determined by a slightly modified Ripert method (1934, 1935).

TABLE 1.—CONCENTRATION-MORTALITY AND CONCENTRATION-KNOCKDOWN DATA FOR PYRETHRINS.

Spray (Percentage w/v Pyrethrins).	Number of Tests.	Average of Mortalities.		Adjusted Mortality.		Highest Mortality.		Lowest Mortality.		Adjusted Knockdown.	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
		%	%	%	%	%	%	%	%	%	%
0·14	4	98·8	99·3	98·7	100	100	100	95·0	97·0	98·8	99·3
0·10	56	99·1	97·5	99·1	97·5	100	100	86·0	88·0	97·2	94·4
0·09	1	100	93·0	99·1	93·5	..	..	..	..	92·4	95·7
0·08	1	100	96·8	99·1	97·3	..	..	..	..	97·2	97·6
0·07	1	92·3	94·5	91·5	95·0	..	..	..	..	93·4	93·7
0·06	1	100	90·5	99·1	91·0	..	..	..	..	97·2	89·8
0·05	11	97·9	92·0	97·7	90·1	100	99·0	92·0	81·0	94·5	88·1
0·04	3	100	98·1	99·1	96·6	100	100	100	96·0	84·2	90·3
0·03	3	97·0	93·3	96·1	91·9	100	100	90·0	88·9	84·8	88·8
0·025	5	88·4	91·4	88·9	92·0	100	96·0	79·0	85·0	89·3	88·8
0·02	3	91·6	90·7	90·8	89·3	100	97·4	84·8	81·0	83·0	85·4
0·01	33	80·4	74·5	85·4	73·3	100	97	23·0	13·0	77·9	79·2

* Prepared annually by the National Association of Insecticide and Disinfectant Manufacturers Inc., New York, U.S.A.

These tests were carried out at various times and, with few exceptions, not primarily to obtain data for a concentration-mortality curve. In order to obtain some common basis of comparison, and as a first approximation only, the average of the percentage mortalities for 0·1 per cent. w/v pyrethrins over the same series of tests as the concentrations in question was determined and a factor obtained to adjust the figures so that they were comparable with the average result for the 56 tests with 0·1 per cent. w/v pyrethrins.

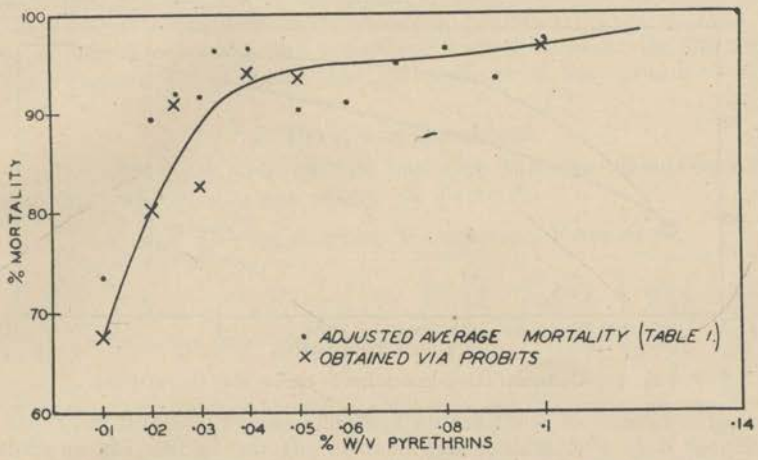

FIG. 1.—Concentration-mortality curve for female *C. fatigans*.

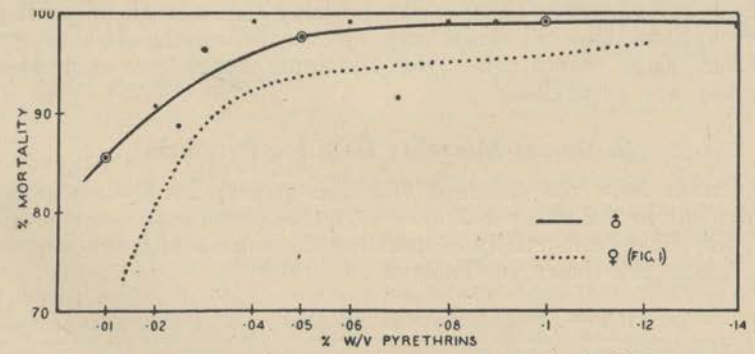

FIG. 2.—Concentration-mortality curve for male *C. fatigans*.

Fig. 1 shows the concentration-mortality data for female *C. fatigans*. The dots represent the adjusted average percentage mortalities just mentioned. More reliable data for the construction of a curve was calculated from a number of test series in which two or more concentrations were involved, the majority of these tests including 0·1 per cent. pyrethrins. Best estimates for probits corresponding to the various dosages were determined by least squares. These estimates were then converted to percentage kills and have been plotted as crosses. It is clear that the mortality decreases rapidly from about 0·05 per cent. downwards. Above this value the curve is fairly flat, although it seems reasonable to infer that 0·14 per cent. w/v will produce very nearly

100 per cent. mortality. The curve for males (Fig. 2), drawn through the three points for which more than ten observations were available, follows a similar trend to that for females, but a higher mortality is recorded for each concentration.

Fig. 3 shows the percentage knockdown at 10 minutes. Like mortality, knockdown commences to fall off rapidly below 0·05 per cent. pyrethrins. It may also be noted that at about 0·01 per cent. the curves intersect, so that knockdown for males is lower than that for females.

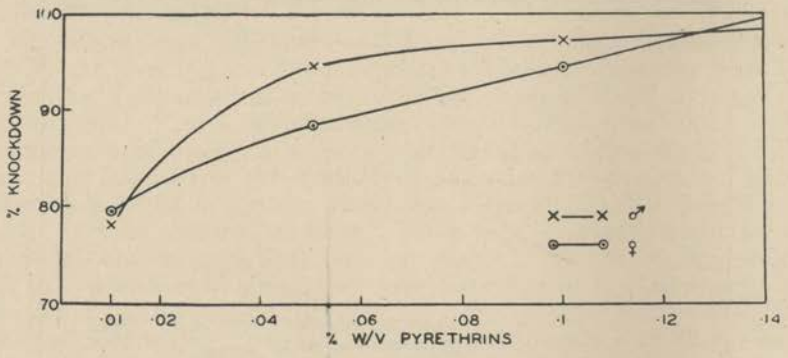

Fig. 3.—Concentration-knockdown curve for *C. fatigans*.

This is the reverse of what occurs at higher concentrations, but there are insufficient data to establish significance. If the figures for knockdown are compared with those for mortality, it will be seen that some of the mosquitoes which are still able to fly 10 minutes after spraying may die in the next 24 hours. There is also evidence that a small proportion of the mosquitoes knocked down may recover before the 24-hour count. However, as a general rule, the 10-minute knockdown and 24-hour mortality are very close.

2. Dosage-Mortality Data for Pyrethrins

In most tests, the standard (for this paper) 2 ml. dose of spray (equivalent to 1 fl. oz. per 2,000 cu. ft.) was used. In a series of five tests, the effect on mortality of doubling the volume of spray was noted. The results are shown in Table 2.

Table 2.—Dosage-Mortality Data for Pyrethrins.

Spray (Percentage Pyrethrins).	Dosage.	Average of Percentage Mortalities.		
		♂	♀	Average.
	ml.			
0·1	2	98·6	98·2	98·4
0·05	4	100	100	100
0·05	2	97·0	90·8	93·9
0·025	4	95·4	90·8	93·1
0·025	2	88·4	91·4	89·9

On the basis of the average figures (column 5), it would appear that mortality is dependent upon the amount of pyrethrins present in the chamber, although the increased volume of spray used may have been responsible for increasing the mortality from 4 ml. of a 0·05 per cent. spray above that from 2 ml. of a 0·1 per cent. spray. This interpretation holds good when the figures for each sex are examined, with the exception of the high figure for females for 2 ml. of the 0·025 per cent. spray. This figure for females (91·4 per cent.) appears to be aberrant since from Fig. 1 a mortality of about 80 per cent. would be expected. These results are in agreement with those of Lindquist *et al.* (1945), who showed that knockdown and mortality were dependent on the amount of toxic ingredients atomized and independent of the volume of spray.

3. Pyrethrin Emulsions

Preliminary tests were carried out with three emulsions containing pyrethrins. The results are shown in Table 3.

TABLE 3.—TESTS WITH WATER-BASE EMULSIONS.

Spray Number.	Spray (Active Ingredients).	Number Tests.	Average of Mortalities.		Average of Mortalities with O.T.I. in Same Tests.		Adjusted Mortality for Spray.		Expected Mortality for Equivalent Pyrethrins.	
			♂	♀	♂	♀	♂	♀	♂	♀
			%	%	%	%	%	%	%	%
1	0·043 per cent. pyrethrins 0·051 per cent. rotenone	4	96	82	99	95	96	84	97	88
2	0·1 per cent. pyrethrins, 0·1 per cent. rotenone ..	1	75	50	100	92	74	53	99	97
3	0·1 per cent. pyrethrins ..	1	100	67	100	100	99	65	99	97

Spray No. 1 contained a sulphonated alcohol emulsifier. The concentrate was diluted with 34 parts of water before use. Spray No. 2 contained a casein emulsifying agent. This spray produced a definite white deposit which would render the spray unsuitable for many indoor uses. Spray No. 3 contained the sodium salt of an alkyl naphthalene sulphonic acid plus pine oil, a proprietary emulsifier which is stable under a wide range of conditions, including hard water.

While it is not possible to draw definite conclusions from these few tests, it is interesting to observe a consistent trend. If sprays of equivalent pyrethrin content are compared, in five out of six instances (three for each sex) the mortality caused by water-base sprays is lower than that caused by equivalent kerosene-base sprays. This trend would be even more marked if it were possible to make an allowance for the contributing toxicity of the rotenone present in sprays 1 and 2. Since no data were obtained on the toxicity of rotenone under these conditions, it is safer to assume here that it has no effect.

For all sprays the observed mortality was adjusted on the same basis as the mortalities in Fig. 1 and 2 and, where necessary, this adjusted mortality compared with that obtained for the same concentration of pyrethrins from these curves.

4. Kerosene and Creosote

Fly-spray kerosene (Table 4) caused negligible mortality under the conditions of test. Knockdown was only slightly higher than mortality. Low mortality is interesting in view of the evidence that pyrethrins are more toxic in kerosene than in emulsion form. It is likely that better atomization is produced with a kerosene spray, but it is also probable that the kerosene carrier assists the penetration of pyrethrins through the cuticle. This is in accord with recent work on cuticular penetration (see Hurst, 1943).

TABLE 4.—TOXICITY OF KEROSENE AND CREOSOTE TO *C. fatigans*.

Spray.	Dosage.	Number Tests.	Average Mortality.	
			♂	♀
	ml.		%	%
Fly-spray kerosene	2	4	4·3	1·8
Fly-spray kerosene	12	1	91·0	0
0·1 per cent. pyrethrins in fly-spray kerosene	2	3	100	95·3
0·1 per cent. pyrethrins in lighting kerosene	2	3	100	100
25 per cent. creosote in fly-spray kerosene	2	3	45	37
50 per cent. creosote in fly-spray kerosene	2	3	60	42

Owing to wartime difficulties, it became impossible to import deodorized fly-spray kerosene for use in sprays. Tests were therefore carried out comparing fly-spray kerosene with lighting kerosene to determine what effect the carrier had on the toxicity of pyrethrins. The lighting kerosene possessed a higher content of aromatics, such as naphthalene, than pre-war lighting kerosene, but did not give rise to any objectionable odour when sprayed into a room. As can be seen, the lighting kerosene appeared to be toxicologically a slightly better base than fly-spray kerosene.

Creosote 259* in 25 or 50 per cent. concentration in kerosene produces only fair mortality and was very irritant to the eyes and nose. A spray involving creosote as the only toxic ingredient would not be very effective and would be unpleasant to use.

5. Essential Oils as Substitutes or Synergists for Pyrethrins

Undiluted oil of Huon pine (*Dacrydium franklinii*) was toxic to mosquitoes, producing a 90 per cent. mortality of females (Table 5). There was no evidence, however, that addition of this oil to a pyrethrin spray increased the mortality. Five such mixtures were used, and four times for each sex the mortality was below that of a straight spray containing the same amount of pyrethrins. Since the basis of comparison has been derived in three instances from the concentration-mortality curves (Figs. 1 and 2), care must be taken in drawing

* Timbrol Ltd , Sydney, N.S.W.

conclusions. It is clear, however, that there was certainly no advantage gained by the addition of Huon pine oil to a pyrethrin spray, and there is a suggestion that further tests would reveal an antagonistic effect. The knockdown of a pyrethrin spray was decreased by the addition of Huon pine oil in the two sprays for which a direct comparison can be made (Table 6). The absence of increased mortality is directly in contrast with Peet-Grady tests with houseflies in which a +17 rating* was obtained for 0·1 per cent. w/v pyrethrins + 2 per cent. Huon pine oil (Kerr, 194–).

TABLE 5.—TOXICITY OF ESSENTIAL OILS AND ESSENTIAL OILS PLUS PYRETHRINS TO *C. fatigans*.

Spray.	Number Tests.	Average of Mortalities with Spray.		Mortality with Same Concentration of Pyrethrins Alone.				Average of Mortalities with 0·1 Percentage Pyrethrins in Same Tests.	
				Observed in Same Tests.		Calculated (Figs. 1 and 2).			
		♂	♀	♂	♀	♂	♀	♂	♀
		%	%	%	%	%	%	%	%
Huon pine oil	2	97·5	90·0	..	..	..	..	97·5	96·3
0·1 per cent. pyrethrins + 2 per cent. H.P. oil	7	97·6	97·1	99·0	95·9	99·1	97·5	99·0	95·9
0·09 per cent. pyrethrins + 2 per cent. H.P. oil	2	98·0	88·5	..	..	99·1	96·7	100	96·0
0·07 per cent. pyrethrins + 2 per cent. H.P. oil	2	97·0	93·5	..	..	99·1	94·5	100	97·0
0·01 per cent. pyrethrins + 2 per cent. H.P. oil	3	53·9	28·8	59·0	36·3	85·4	73·3	98·5	95·5
0·05 per cent. pyrethrins + 1 per cent. H.P. oil	1	100	92·0	..	..	97·7	90·1	100	100
Zieria oil	1	95·5	81·5	..	..	..	..	100	100
10 per cent. *Zieria* oil ..	1	43·5	48·6	..	..	..	..	100	100
0·1 per cent. pyrethrins + 10 per cent. *Zieria* oil	1	100	100	100	100	99·1	97·5	100	100
0·01 per cent. pyrethrins + 10 per cent. *Zieria* oil	2	89·5	68·1	54·6	39·4	85·4	73·3	100	100
0·01 per cent. pyrethrins + 2 per cent. *Zieria* oil	3	56·2	37·2	59·0	36·3	85·4	73·3	98·5	95·5
50 per cent. *Backhousia* oil ..	4	98·6	92·1	..	..	..	..	..	..
2 per cent. *Backhousia* oil ..	3	57·2	55·8	..	..	..	..	..	..
0·01 per cent. pyrethrins + 2 per cent. *Backhousia* oil ..	10	91·3	79·5	93·3	80·9	85·4	73·3	..	..
0·005 per cent. pyrethrins + 2 per cent. *Backhousia* oil ..	1	83·4	61·5	95·5	91·6	85·4	73·3	..	..
0·01 per cent. pyrethrins + 10 per cent. *E. citriodora* oil ..	5	80·1	63·7	78·0	65·4	85·4	73·3	..	..
0·06 per cent. pyrethrins + Eucalyptus oils A	3	98·0	94·0	..	..	98·9	92·9	98·7	94·3
Eucalyptus oils B	2	23·0	7·5	..	..	..	..	99·0	95·5
0·01 per cent. pyrethrins + 5 per cent. sesame oil ..	3	85·7	69·1	90·4	72·8	85·4	73·3	..	..
0·01 per cent. pyrethrins + 0·2 per cent. eudesmin	3	94·0	78·0	89·0	76·0	85·4	73·3	..	..

Eucalyptus oils A
- Phellandrene .. .. 0·46 per cent.
- *E. dives* .. .. 0·46 per cent.
- Piperitone residues .. 0·38 per cent.

Eucalyptus oils B
- Phellandrene .. .. 5·2 per cent.
- *E. dives* .. .. 5·2 per cent.
- Piperitone residues .. 0·38 per cent.

* This means that, in the same series of tests, the spray containing Huon pine oil caused an average mortality 17 per cent. higher than one containing 0·1 per cent. pyrethrins alone.

Undiluted oil of *Zieria smithii* was toxic to mosquitoes (81·5 per cent. mortality of females), although a 10 per cent. concentration in fly-spray kerosene caused less than 50 per cent. mortality (Table 5). As with Huon pine oil, no advantage was gained by the addition of *Zieria* oil to a pyrethrin spray. Once again, this is in contrast with Peet-Grady tests with houseflies in which a +20 rating was obtained for 0·1 per cent. w/v pyrethrins + 2 per cent. *Zieria* oil (Kerr, 194–). The percentage knockdown of mosquitoes was sometimes increased and sometimes decreased by the addition of *Zieria* oil to a pyrethrins spray (Table 6).

TABLE 6.—KNOCKDOWN PRODUCED BY ESSENTIAL OILS AND ESSENTIAL OILS PLUS PYRETHRINS.

Spray.	Number Tests.	Average of Knockdowns with Spray.		Knockdown with Same Concentration of Pyrethrins Alone.				Average of Knockdowns with 0·1 Percentage Pyrethrins in Same Tests.	
				Same Tests.		Calculated (Fig. 3).			
		♂	♀	♂	♀	♂	♀	♂	♀
		%	%	%	%	%	%	%	%
Huon pine oil	2	97·6	83·2	..	..	..	..	100	99
0·1 per cent. pyrethrins + 2 per cent. H.P. oil	7	95·4	94·5	96·4	96·4	..	..	96·4	96·4
0·09 per cent pyrethrins + 2 per cent. H.P. oil	2	95·0	91·0	..	..	97·6	93·0	97·9	95·8
0·07 per cent. pyrethrins + 2 per cent. H.P. oil	2	96·0	92·0	..	..	96·0	90·6	97·9	95·9
0·01 per cent. pyethrins + 2 per cent. H.P. oil	3	57·8	47·8	69·6	69·3	..	..	90·2	91·1
0·05 per cent. pyrethrins + 1 per cent. H.P. oil	1	100	94·6	..	..	94·5	88·1	91·0	100
Zieria oil	1	86·4	59·2	..	..	..	..	91·1	93·3
10 per cent. *Zieria* oil	1	26·1	14·7	..	..	..	..	100	100
0·1 per cent. pyrethrins + 10 per cent. *Zieria* oil	1	100	100	91·1	93·3	..	..	91·1	93·3
0·01 per cent. pyrethrins + 10 per cent. *Zieria* oil	2	72·4	54·8	53·2	55·0	..	..	..	..
0·01 per cent. pyrethrins + 2 per cent. *Zieria* oil	2	47·8	36·2	69·6	69·3	..	..	..	..
								0·01 per cent. pyrethrins	
50 per cent. *Backhousia* oil ..	4	91·0	83·1	..	..	..	..	86·0	76·1
2 per cent. *Backhousia* oil ..	3	21·7	37·1	..	..	..	..	88·8	76·8
0·01 per cent. pyrethrins + 2 per cent. *Backhousia* oil ..	10	78·2	67·7	81·9	75·7	..	..	..	..
0·005 per cent. pyrethrins + 2 per cent. *Backhousia* oil ..	1	66·6	61·6	..	..	72·0	77·0	95·4	79·2
0·01 per cent. pyrethrins + 10 per cent. *E. citriodora* oil ..	5	55·1	56·1	73·1	65·1	..	..	..	..
								0·1 per cent. pyrethrins	
0·06 per cent. pyrethrins + Eucalyptus oils A*	3	69·5	80·1	..	..	95·3	89·5	95·3	92·7
Eucalyptus oils B	2	36·2	18·7	..	..	..	..	95·5	94·0
0·01 per cent. pyrethrins + 5 per cent. sesame oil	3	34·8	31·1	74·2	67·3	..	..	..	..
0·01 per cent. pyrethrins + 0.2 per cent. eudesmin	3	72·1	67·0	74·2	67·3	..	..	..	..

* See explanation at foot of Table 5.

A spray containing 50 per cent. oil of *Backhousia myrtifolia* in lighting kerosene gave good results (Table 5) showing up as slightly more toxic (92·1 per cent. mortality of females) than either undiluted Huon pine oil or undiluted *Zieria* oil. The knockdown of females produced was the same as for the Huon pine oil spray and higher than for the *Zieria* spray. Two per cent. *Backhousia* oil in kerosene killed 56 per cent. of mosquitoes and was slightly more effective than 10 per cent. *Zieria* oil. In ten tests, the addition of 2 per cent. *Backhousia* oil to 0·01 per cent. pyrethrins did not influence the kill produced by the pyrethrins alone, although it lowered slightly the percentage knockdown at 10 minutes (Table 6). Against houseflies, 0·1 per cent. pyrethrins + 2 per cent. *Backhousia* oil gave a +34 rating (Kerr, 194–).

The results presented above for Huon pine oil, *Zieria* oil, and *Backhousia* oil, provide striking evidence of the lack of effectiveness against mosquitoes of certain materials which are extremely effective synergists for pyrethrins when tested against houseflies. These three oils were tested because they were the most effective mosquito repellents of a series of essential oils (McCulloch and Waterhouse, 1947). In laboratory repellent tests, it had been noted that mortality of *A. aegypti* frequently occurred following exposure to these repellents, but not to various less effective repellents. The principal constituent of Huon pine oil is methyl eugenol, and of *Zieria* and *Backhousia,* elemicin (see (McCulloch and Waterhouse, 1947). A spray containing 10 per cent. *Eucalyptus citriodora* oil and 0·01 per cent. pyrethrins, gave approximately the same mortality as 0·01 per cent. pyrethrins alone, although the knockdown was lower. A mixture of eucalyptus oils in kerosene, but without pyrethrins (Eucalyptus oils B), gave a low knockdown and mortality. These oils did not affect the mortality when added to 0·06 per cent. pyrethrins.

Three tests with 5 per cent. sesame oil + 0·01 per cent. pyrethrins gave no indication of pyrethrin activation, although the knockdown was considerably depressed. However, tests against houseflies with sprays prepared from the same sample of oil also failed to reveal any activation, and it is probable therefore that the sesame oil was an inactive sample.

In three tests 0·2 per cent. eudesmin + 0·01 per cent. pyrethrins gave a slightly (though not significantly) higher mortality than 0·01 per cent. pyrethrins alone, and a slightly lower knockdown. Eudesmin is closely related to sesamin from sesame oil and occurs naturally in the resinous exudate of certain Australian eucalypts.

6. Synthetic Compounds as Substitutes or Synergists for Pyrethrins

(i) *Lethane.**

Two lethanes were tested, Lethane 384 standard (50 per cent. β-butoxy-β′-thiocyanodiethyl ether + 50 per cent. petroleum distillate) and Lethane 384 special (12·5 per cent. β-butoxy-β′-thiocyanodiethyl ether, 37·5 per cent. β-thiocyanoethyl laurate, 50 per cent. refined kerosene). The results are shown in Table 7.

* Rohm and Haas Co., U.S.A.

TABLE 7.—MORTALITY AND KNOCKDOWN PRODUCED BY LETHANE SPRAYS.

Spray.	Number Tests.	Percentage Mortality.						Percentage Knockdown.					
		Spray.		Same Concentration Pyrethrins, Same Tests.		0·1 Per Cent. Pyrethrins, Same Tests.		Spray.		Same Concentration Pyrethrins, Same Tests.		0·1 Per Cent. Pyrethrins, Same Tests.	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Lethane Special 5 per cent.	1	61·9	36·5	..	..	98	88	61·9	27·3	..	..	98·0	96·0
Lethane Special 10 per cent.	4	85·0	85·0	..	..	98·7	96·5	58·4	70·2	..	..	96·9	97·8
Lethane Standard 10 per cent. ..	1	61·3	89·5	..	..	100	98	64·5	73·7	..	..	100	98·0
Lethane Special 15 per cent.	1	93·8	100	..	..	96·6	100	75·0	88·4	..	..	89·7	97·1
Lethane Special 5 per cent. + 0·1 per cent. pyrethrins	1	96·3	94·5	100	100	100	100	88·9	90·7	100	100	100	100
Lethane Special 10 per cent. + 0·1 per cent. pyrethrins	1	96·8	77·3	100	98	100	98	96·7	86·4	100	98·0	100	98·0
Lethane Special 15 per cent. + 0·1 per cent. pyrethrins	1	100	100	96·6	100	96·6	100	78·4	88·4	89·7	97·1	89·7	97·1
Lethane Special 10 per cent. + 0·025 per cent. pyrethrins	1	91·5	87·2	78·6	91·7	100	97·9	80·8	87·2	83·4	83·4	98·5	92·8
Lethane Special 10 per cent. + 0·01 per cent. pyrethrins	3	76·1	78	81·2	76·4	..	..	86·0	87·7	86·0	76·1	..	..

It can be seen that sprays containing 5 or 10 per cent. Lethane were considerably less effective than 0·1 per cent. pyrethrins, although 15 per cent. Lethane special was almost as effective. The addition of 5 or 10 per cent. Lethane special to a 0·1 per cent. pyrethrins spray depressed the mortality of both sexes. The addition of 10 per cent. Lethane special to 0·025 per cent. pyrethrins depressed the female mortality and increased the male mortality, while it slightly increased the mortality of females produced by 0·01 per cent. pyrethrins, but depressed the male mortality. None of these changes is significant, but they indicate that Lethane is not an effective activator for pyrethrins.

The figures for knockdown follow a very similar trend to those for mortality, the lower concentrations producing relatively poor knockdown. The highest concentration, namely 15 per cent. Lethane special, produced 88 per cent. knockdown of females which is approximately equivalent to that given by a 0·04 per cent. pyrethrins spray. The addition of Lethane to a pyrethrins spray depressed the knockdown of both sexes, except for one spray (10 per cent. Lethane + 0·01 per cent. pyrethrins) when the knockdown of females was increased from 76 to 88 per cent.

While the number of tests is small, the general conclusion can be drawn that Lethane special is neither highly toxic to mosquitoes nor does it serve any useful purpose when added to a pyrethrum spray.

In sprays containing more than 5 per cent., both Lethane and Lethane special have a rather unpleasant smell. A 5·0 per cent. Lethane standard spray gave 99·9 to 100 per cent. knockdown, and a +12 to +23 rating against houseflies (Waterhouse, 1947). Murphy and Vandenberg (1936) state that the speed of action of Lethane standard in knocking down flies is greater than that of pyrethrum. This is certainly not so for *C. fatigans*.

(ii) *Thanite.**

The results of tests with Thanite (bornyl thiocyanoacetate) are shown in Table 8. A spray containing 3·5 per cent. Thanite caused an average female mortality of 99·2 per cent. in five tests and thus appears to be about equivalent in effectiveness to a 0·14 per cent. pyrethrins spray. It gave a lower knockdown, but a higher mortality, than a 0·1 per cent. pyrethrins spray tested at the same time. Reduction in Thanite concentration through 3·0 to 2·5 per cent. caused a progressive dimunition in both mortality and knockdown. In ten tests 2·5 per cent. Thanite gave the same knockdown as 0·01 per cent. pyrethrins, but a considerably higher mortality (93 compared with 82 per cent.).

The addition of 2·5 per cent. Thanite to a 0·01 per cent. pyrethrins spray resulted in a lowering of mortality and knockdown of both sexes. On this evidence (three tests only) there is no advantage to be gained by adding Thanite to a dilute pyrethrins spray.

Thanite also gave promising results against the housefly, a 3·5 per cent. solution recording a 99·9 per cent. knockdown and a +22 to +24 rating (Waterhouse, 1947). Sprays containing 3·5 per cent. or higher concentrations of Thanite tend to cause nasal irritation.

* Hercules Powder Co., U.S.A.

TABLE 8.—RESULTS OF TESTS WITH SPRAYS CONTAINING THANITE.

Spray.	Number Tests.	Percentage Mortality.						Percentage Knockdown.					
		Spray.		0·1 Per Cent. Pyrethrins, Same Tests.		0·01 Per Cent. Pyrethrins, Same Tests.		Spray.		0·1 Per Cent. Pyrethrins, Same Tests.		0·01 Per Cent. Pyrethrins, Same Tests.	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
3·5 per cent. Thanite	5	98·5	99·2	96·6	98·7	..	..	95·6	97·9	100	99·1	..	..
3·0 per cent. Thanite	4	93·9	95·3	99·5	97·8	..	..	87·4	76·6	100	98·9	..	..
2·5 per cent. Thanite	10	94·4	93·2	..	..	82·1	82·4	75·1	72·5	..	..	67·1	75·4
2·0 per cent. Thanite	1	93·8	94·0	100	98·5	..	..	87·5	94·0	100	100	..	..
2·5 per cent. Thanite + 0·01 per cent. pyrethrins..	3	89·6	79·1	94·5	99	93·8	91·6	68·2	70·3	100	100	84·1	82·8

(iii) *D.H.S. Activator.**

D.H.S. Activator (ethylene glycol ether of pinene) was tested in 5 per cent. concentration in a 0·01 per cent. pyrethrins spray (Table 9).

TABLE 9.—RESULTS OF TESTS WITH D.H.S. ACTIVATOR AND PYRETHRINS.

Spray.	Average Mortality.		Average Knockdown.	
	♂	♀	♂	♀
	%	%	%	%
5·0 per cent. D.H.S. Activator + 0·01 per cent. pyrethrins	80·7	74·7	68·4	85·5
0·01 per cent. pyrethrins	76·2	74·4	59·2	73·4

In the ten tests carried out, the spray containing D.H.S. Activator caused a slight increase (4·5 per cent.) in male mortality, but no change in female mortality. The knockdown was significantly increased for both males and females. In view, however, of the absence of increase in female mortality, it was concluded that no really useful purpose would be served by the addition of D.H.S. Activator to a pyrethrin spray. Pierpont (1941) demonstrated the value of adding D.H.S. Activator to a pyrethrin and Lethane spray for use against houseflies, but there is no published information on its effectiveness against mosquitoes.

(iv) *N-Isobutyl Undecylenamide*† *(IN930).*

This material has been shown to be an effective synergist for pyrethrins against houseflies. The 24 tests carried out with various concentrations of isobutyl undecylenamide (IN930) and pyrethrins are shown in Table 10.

TABLE 10.—RESULTS OF TESTS WITH ISOBUTYL UNDECYLENAMIDE (IN930) AND PYRETHRINS.

Spray.	Number Tests.	Percentage Mortality.				Percentage Knockdown.			
		Spray.		Same Concentration Pyrethrins, Same Tests.		Spray.		Same Concentration Pyrethrins, Same Tests.	
		♂	♀	♂	♀	♂	♀	♂	♀
0·01 per cent. pyrethrins + 0·1 per cent. IN930 ..	2	84·6	67·3	94·1	76·2	66·1	64·0	54·1	71·1
0·01 per cent. pyrethrins + 0·2 per cent. IN930 ..	1	88·9	85·9	100	85·7	81·5	75·0	61·2	82·2
0·01 per cent. pyrethrins + 0·34 per cent. IN930 ..	11	82·5	76·9	80·1	75·5	69·6	74·0	77·6	77·4
0·01 per cent. pyrethrins + 1·0 per cent. IN930 ..	2	81·3	77·7	84·5	81·6	57·2	57·4	67·8	62·9
0·01 per cent. pyrethrins + 2·0 per cent. IN930 ..	2	86·9	70·6	84·5	81·6	45·0	28·0	67·8	62·9
0·05 per cent. pyrethrins + 0·34 per cent. IN930 ..	4	97·8	97·9	99·2	94·7	82·5	90·8	97·5	88·9
0·075 per cent. pyrethrins + 0·34 per cent. IN930 ..	2	100	100	(99·0‡)	(95·0‡)	96·2	96.7	(96·0§)	(91·0§)

* Hercules Powder Co., U.S.A. † Du Pont de Nemours and Co. Inc., U.S.A.
‡ Taken from Figs. 1 and 2. § Taken from Fig. 3.

The addition of 0·1 or 0·2 per cent. IN930 to a 0·1 per cent. pyrethrins spray did not increase the mortality. The addition of 0·34 per cent. IN930, however, did cause a slight, but not significant, rise in both male and female mortality. This is the only spray for which adequate data are available. Ten of the eleven tests were carried out at the same time as those in which D.H.S. Activator was used (Table 9). The IN930 spray gave a slightly higher mortality of both sexes than the D.H.S. spray. Increasing either the concentration of IN930, or of pyrethrins, did not give any consistent indication that the addition of IN930 to a pyrethrum spray had any value.

With few exceptions, the presence of IN930 in a pyrethrum spray depressed the knockdown, the greatest depression being observed with the highest concentration of IN930. It is interesting to note that, although there was a very considerable depression in knockdown of 0·01 per cent. pyrethrins to which 2·0 per cent. IN930 had been added, the mortality was not correspondingly lowered; actually, it was higher for males although somewhat lower for females.

(v) *pp'-Dichlordiphenyl Trichlorethane (DDT).*

The sample of DDT used contained about 95 per cent. of the pp'-isomer. The results of tests, which are shown in Table 11, show that DDT possesses marked toxicity, although it is clearly not as effective as pyrethrins. The knockdown is poor, and this is in agreement with the slow knockdown produced when DDT alone is used against houseflies.

TABLE 11.—RESULTS OF TESTS WITH DDT.

Spray.	Number Tests.	Average Percentage Mortality.				Average Percentage Knockdown.			
		Spray.		0·1 Per Cent. Pyrethrins, Same Tests.		Spray.		0·1 Per Cent. Pyrethrins, Same Tests.	
		♂	♀	♂	♀	♂	♀	♂	♀
1·0 per cent. DDT ..	4	96·0	94·0	100	100	64·1	55·3	100	99·7
0·3 per cent. DDT ..	2	94·0	80·0	100	100	11·9	18·2	100	100
0·1 per cent. DDT ..	2	60·0	50·0	100	100	10·0	6·5	100	99·0
				0·01 per cent. pyrethrins				0·01 per cent. pyrethrins	
0·1 per cent. DDT + 0·01 per cent. pyrethrins ..	6	84·9	80·1	61·9	63·7	71·2	51·8	61·5	64·0

A mortality of 100 per cent. was produced with houseflies using 0·1 per cent. DDT in kerosene. DDT is the only material tested which caused a large increase in mortality when added to a pyrethrum spray (0·1 per cent. DDT + 0·01 per cent. pyrethrins).

(vi) *Dimethyl Phthalate.*

Two tests were carried out with undiluted dimethyl phthalate (Table 12) which was tested because of its effectiveness as a mosquito repellent (McCulloch and Waterhouse, 1947).

The knockdown obtained was not outstanding, and a mortality of about 50 per cent. in both sexes was produced. The spray mist was irritant to the nose and throat.

TABLE 12.—RESULTS OF TESTS WITH DIMETHYL PHTHALATE.

Spray.	Number Tests.	Average Mortality.		Average Knockdown.	
		♂	♀	♂	♀
		%	%	%	%
Dimethyl phthalate ..	2	54·5	47·0	76·7	62·5
0·1 per cent. pyrethrins ..	2	97·5	96·3	100	99·3

7. The Freon Bomb

Several tests were made on a Westinghouse Freon-aerosol insecticide bomb which was stated to contain 1 per cent. pyrethrins and 2 per cent. sesame oil in Freon 12 (dichlorodifluoromethane). The dosage recommended was 4 seconds spraying per 1,000 cubic feet, which, from data supplied with the bomb, results in the expulsion of 0·0216 grams pyrethrins per 1,000 cubic feet. A 1-second dosage in the 216 cubic feet Peet-Grady chamber is slightly greater than that recommended. The results obtained with 1, 2, and 3-second dosages are shown in Table 13.

TABLE 13.—FREON BOMB TESTS IN THE PEET-GRADY CHAMBER.

Freon Bomb Dosage.	Number Tests.	Average Mortality.				Average Knockdown.			
		Bomb.		0·1 Per Cent. Pyrethrins.		Bomb.		0·1 Per Cent. Pyrethrins.	
		♂	♀	♂	♀	♂	♀	♂	♀
		%	%	%	%	%	%	%	%
One second	3	99·0	99·0	100	100	97·9	98·2	100	100
Two seconds	4	100	100	100	97	100	98·6	100	99·5
Three seconds ..	3	100	100	100	96	100	100	100	99·3

In these tests, a 1-second dosage corresponded approximately in effectiveness with a 2-ml. dosage of 0·1 per cent. pyrethrins producing very high (99 per cent.) knockdown and kill.

One test was carried out using an 8-second exposure in a 2,000 cubic feet room. Five cages of mosquitoes were placed respectively at floor level and 1½, 2½, 4, and 8 feet above the floor. One hundred per cent. mortality was recorded for both sexes in all cages except that at the 1½-ft. level, for which the mortalities were males 86 per cent., females 89 per cent.

8. Sex Differences in Resistance to Sprays

Table 14 shows the percentage mortality for both sexes of *C. fatigans* for most of the sprays dealt with in earlier sections. The figures are based on the total mosquitoes surviving in tests where 100 per cent. mortality of both sexes was not obtained. In most cases when different concentrations of the same spray were used, the results were combined for the purpose of distinguishing differential sex mortality. Inspection

of the results for the various concentrations indicated that this should not introduce any important errors, and was adopted as the only convenient method of gathering together results of the tests.

TABLE 14.—PERCENTAGE MORTALITY OF MALE AND FEMALE *C. fatigans*.

Spray.	Number Tests.	Percentage Mortality.	
		♂	♀
0·1 per cent. pyrethrins	35	98·7	96·2
0·05 per cent. pyrethrins	11	96·7	92·7
0·01 per cent. pyrethrins	33	85·0	75·4
Other straight pyrethrins	23	94·3	92·4
Pyrethrins + Huon pine oil	13	90·3	84·2
Pyrethrins + *Zieria* oil	5	71·3	53·0
Pyrethrins + *E. citriodora* oil	5	66·6	52·0
Pyrethrins + *Backhousia* oil	10	90·2	76·1
Pyrethrins + Eudesmin	1	100·0	95·5
Pyrethrins + Rotenone (emulsion) ..	1	75·0	50 0
Pyrethrins + Sesame oil	3	91·3	69 2
Pyrethrins + DDT	7	82·5	62·0
Pyrethrins + D.H.S. Activator	10	82·1	79 0
Pyrethrins + isobutyl undecylenamide ..	21	85·1	81·9
Pyrethrum emulsion	1	100·0	66·7
Pyrethrum + Rotenone emulsion ..	4	94·4	75·3
Huon pine oil	2	97·6	92 3
Zieria oil	2	68·7	57·9
Backhousia oil	7	81·4	77·6
Dimethyl phthalate	2	52·9	50·0
DDT	5	94 9	86·6
Creosote + kerosene	2	61·0	43·2
Kerosene (fly spray)	3	5·0	0·1
Thanite	17	94·3	94·6
Thanite + pyrethrins	3	88·5	78·9
Lethane special	7	77·9	86·9
Lethane special + pyrethrins	7	89·4	86·1

For sprays containing only pyrethrins, 92·8 per cent. of males and 89·3 per cent. of females were killed. This difference is significant as is also that for 0·1 and 0·01 per cent. pyrethrins. The data available for other sprays are not extensive enough for satisfactory analysis. It is clear, however, that the recorded mortality of males is higher than that of females except for two sprays, namely Lethane special and Thanite, although the difference is extremely small and certainly not significant for Thanite. The addition of pyrethrins to these two materials resulted in a higher mortality of males than females.

In view of the comparatively small overall difference in mortality for the two sexes and the presence of males in all batches of test insects, figures for males as well as for females have been shown in all tables. Male mortalities may be used to lend added weight to the results obtained with females, particularly when the number of tests is small, although it is only against the disease-carrying and annoyance-causing females that the sprays are used. The differential mortality of the sexes of *C. fatigans* is not nearly as great as that for the housefly *Musca domestica* against which 0·1 per cent. pyrethrins produced 81 per cent. mortality of males, and 13 per cent. of females (Waterhouse, 1947). A similar difference for flies was also noted for Lethane 384 and Thanite.

9. Species Differences in Resistance to Sprays

Larvae of *C. fatigans* and *Aedes alboannulatus* Macq. were collected from the same pool, and larvae of *A. notoscriptus* Skuse from a nearby tank. They were kept under identical laboratory conditions and tested simultaneously in the Peet-Grady chamber. The results of twelve tests with pyrethrin sprays of various concentrations are pooled in Table 15.

TABLE 15.—TWENTY-FOUR-HOUR MORTALITY OBTAINED IN SPRAY TESTS AGAINST MIXED MOSQUITOES.

Species.	Percentage Mortality.	
	♂	♀
C. fatigans	71	53
A. notoscriptus	86	81
A. alboannulatus	100	89

While the numbers used were not great, there is good evidence that *C. fatigans* is most resistant, *A. alboannulatus* least resistant, and *A. notoscriptus* occupying an intermediate position. For each species the mortality of males is higher than that for females. Untreated controls were not run with these experiments, since control mortality was not high over the experimental period. However, the impression was gained that *A. alboannulatus* died off more rapidly in the stock cages than the other species.

In a further series of tests with *A. notoscriptus,* the following results were obtained.

TABLE 16.—MORTALITY OF *A. notoscriptus* 24 HOURS AFTER SPRAYING.

Spray.	Number Tests.	Percentage Mortality.	
		♂	♀
0·1 per cent. pyrethrins	3	100	100
0·04 per cent. pyrethrins	2	100	100
0·03 per cent. pyrethrins	3	100	97·5
0·02 per cent. pyrethrins	3	96·7	96·1
0·01 per cent. pyrethrins	3	97·9	98·4
0·01 per cent. pyrethrins + 0·01 per cent. DDT	4	100	100

If these figures are compared with those for *C. fatigans* (Tables 1 and 11), it can be seen that *A. notoscriptus* was consistently more susceptible than *C. fatigans* to the sprays.

Ten tests using a 0·01 per cent. pyrethrins spray were carried out to compare the relative susceptibility of laboratory reared *A. aegypti* with field collected *C. fatigans*. The results, which are shown in Table 17, indicate quite clearly that *A. aegypti* is considerably more susceptible to pyrethrins than is *C. fatigans*.

TABLE 17.—THE RELATIVE RESISTANCE OF *C. fatigans* AND *A. aegypti* TO A PYRETHRUM SPRAY.

Species.	Percentage Mortality.	
	♂	♀
C. fatigans	79	73
A. aegypti	100	100

In addition, in three tests with a 0·005 per cent. pyrethrins spray a 100 per cent. mortality of *A. aegypti* was obtained. Simultaneous tests with *C. fatigans* were not carried out but, on the basis of the data shown in Fig. 1, the mortality of this species would not have been higher than 70 per cent. Several single tests with toxic materials other than pyrethrins (Thanite, Lethane, DDT) served to confirm that *A. aegypti* was more readily killed by toxic sprays than *C. fatigans*. This difference may have been partly due to the greater activity of *A. aegypti* in the Peet-Grady chamber resulting in a heavier dosage of spray being picked up (David and Bracey, 1944). Preliminary counts of the numbers of adults in flight both before and shortly after spraying indicated that, under Peet-Grady conditions, the general level of activity of *A. aegypti* was higher than that of *C. fatigans*, but this aspect of the work was not followed up. Higher activity of males than of females could explain the difference in mortality noted in the previous section. Only the impaction of sprays on motionless insects or the application of individual doses could provide absolute measurements, although these would not be of as much practical value as the data already obtained.

IV. DISCUSSION

A very noticeable feature of these tests of fly spray ingredients against mosquitoes is the fact that the results obtained with mosquitoes are quite different from those recorded against flies. Thus, pyrethrins are particularly effective against mosquitoes, both in producing high mortality and high knockdown; against houseflies, they produce high knockdown, but relatively low mortality. Thanite and DDT (both effective against flies) were the only other materials tested which showed distinct promise against *C. fatigans*. The rate of knockdown is poor with DDT against both mosquitoes and flies. Lethane special and a number of materials which are effective synergists for pyrethrins when used against houseflies had little or no effect on pyrethrum sprays used against *C. fatigans* under the conditions of test.

David and Bracey (1944), have reported results which differ in some respects from those described here, notably in that they found that IN930, and some other substances of low vapour pressure, acted as

synergists for pyrethrins against *A. aegypti*, increasing mortality by depressing knockdown and increasing the persistence of spray droplets. However, their technique differed in several respects from the normal Peet-Grady procedure, probably the most important being that, in their tests, the mosquitoes were introduced into the chamber four minutes after spraying which would result in the insects being exposed to a different environment, both as regards droplet size and concentration of spray in the air than if they were present during spraying. Under these conditions, any materials delaying volatilization of the droplets would show up to best advantage. In addition, the Aerograph M.P. spray gun used by David and Bracey may produce a different droplet spectrum from the Peet-Grady gun used in the present tests.

Unfortunately, the present tests were concluded some time before the paper by David and Bracey appeared, so it was not possible to carry out parallel tests to theirs to establish whether the difference in technique was, in fact, the cause of the differing results.

The main conclusions drawn from the present tests were

(1) that a pyrethrum concentration greater than 0·1 per cent. w/v would probably be necessary in the field, and

(2) that none of the synergists or substitutes, except possibly Thanite, was likely to be of value. While DDT was very promising it was not, at that time, available in commercial quantities.

V. ACKNOWLEDGMENTS

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2. Spray Tests with Anopheline (*Anopheles punctulatus farauti*) Adults

By D. F. Waterhouse, M.Sc., and D. O. Atherton, M.Sc.Agr.

I. INTRODUCTION

The present tests were an extension of the laboratory experiments reported in the previous paper. It was necessary to confirm the earlier findings with tests against the principal vector of malaria in New Guinea, *Anopheles punctulatus,* before mosquito spray formulae could be recommended with confidence for routine use by the Services. In particular, information on the minimum effective concentration of pyrethrins was required, and also on the usefulness of DDT (pp'dichlordiphenyl trichlorethane) and Thanite in sprays for use against malaria mosquitoes.

The tests were carried out in the native village of Lalapipi at the mouth of the Lakekamu River, New Guinea, where, at the time of the experiments, September 1943, there was an abundance of adult *A. punctulatus farauti* Lav.

Information on the composition and source of many of the materials used is given in the previous paper and is therefore not included again here.

II. METHODS

It was found at an early stage that a method such as the liberation of a known number of adult mosquitoes into a tent, the floor of which was covered with white material, followed by spraying and observing results was not satisfactory. There were several reasons, namely that the number of tests which could be done each day would often be limited to one per tent, that it was difficult to prevent adults from escaping from the tent and, finally, that ants would carry away mosquitoes temporarily or permanently affected by the spray thereby affecting final counts of survivors and dead. The following method was therefore adopted. Approximately equal numbers of adults were introduced into 18 in. by 9 in. by 9 in. high cages with painted wooden bases, wire frames, and interchangeable mosquito net covers provided with sleeves. These cages were suspended during testing in the centre of a tent, and about 2 feet below the ridge pole. The tents measurd 12 ft. by 14 ft. and, as slung, enclosed 750 cubic feet (Plate 1).

In most tests, 10 ml. of spray was atomized in a standard manner into the tent, this giving a dosage of approximately 1 fluid oz. per 2,100 cubic feet. A number of tests was also carried out with 7·1 ml. equivalent to 1 fluid oz. per 3,000 cubic feet. This dosage was selected as being the maximum practicable volume for field use (Waterhouse, 1947, and the previous paper). A Rega* Model L hand-operated, compressed air, spray gun (Plate 2) with a 0·018 in. diameter spray nozzle and a 0·032 in. diameter air nozzle, was operated at 30 to 35 lb. pressure (read by pressure gauge) and under these conditions a finely atomized mist of spray was thrown for about 6 feet. Tests were also carried out

* Rega Products Pty. Ltd., Sydney, N.S.W.

using an ordinary, non-continuous hand-spray gun. With the hand gun, it was extremely difficult to deliver accurately a given volume of spray owing to the great variation in output from stroke to stroke. However, by giving a standard number of strokes, it was possible to obtain a fairly uniform dosage. Although the flaps of the tent were closed during and after spraying, a certain amount of spray leakage occurred through these, also through two net-covered 7 in. by 5 in. windows in the roof.

A native hut, having a volume of approximately 1,300 cubic feet, was used in a number of confirmatory experiments.

In most tests, an exposure of 10 minutes was allowed after spraying, but tests were also carried out in which the cages were removed immediately the dose had been atomized. After the 10-minute exposure period had elapsed and before further use, the tent was allowed to air for 20 minutes with flaps thrown back. Sufficient mosquito net covers for cages were available to allow them to be aired for two days between tests. Where necessary, the covers were decontaminated by soaking in kerosene and boiling in soap and water.

Percentage knockdown was noted after the 10-minute exposure period, and the number of adults which were capable and incapable of flying was recorded at 1, 5, and 18 to 20 hours after testing. An unsprayed cage was left in each series to determine control mortality. In all cases where control mortality occurred, this was allowed for in assessing results by Abbot's formula:

$$\text{Percentage mortality due to spray} = \frac{M - C}{100 - C} \times 100, \text{ where}$$

M = percentage mortality observed in test, and C = percentage control mortality.

Adults were at first obtained for experiments by catching them in small 3 in. by 1 in. glass tubes in the jungle on the day of the experiment, but during the greater part of the experimental period, the village children provided about 700 per day caught while biting the night before. In this way a high proportion of engorged females was always used. The adults were caught in tubes and transferred immediately to mosquito net cages in which they remained until the following morning.

For preliminary experiments adults had been collected at night by sweeping with a butterfly net around the hut; it was possible to obtain large numbers in this manner—up to 250 in half an hour. Such treatment, however, was too rough, and hardly any adults in unsprayed cages survived the period of test. Catching individuals by means of tubes was, therefore, adopted as the standard procedure. Under these conditions, the control mortality varies from 0 to 41 per cent., with an average over 24 tests of 19 per cent. This applies to females only. The percentage of males in the catches varied from 0, in those caught biting in the village, to about 50 on rare occasions in those caught resting in the jungle. Males very rarely survived the 18-hour test period and appeared to be far less viable than females. Males were not included in any of the calculations.

A total of 10,866 female *farauti* was used in 236 experiments in which 32 sprays were tested. This gives an average of 46 adults per test.

Both temperature and humidity were recorded during the tests, the former varied between 82° and 90°F. and the latter between 68 and 92 per cent. R.H.

Sprays containing pyrethrins were made up every day or every two days from a fairly concentrated stock solution (0·7 per cent. w/v pyrethrins) which was kept in a screw-top tin. Other sprays were made up in bulk as required.

In all tests ants were prevented from attacking the mosquitoes in the experimental cages by keeping these on a hanging shelf, the suspending wires of which passed through tins containing kerosene.

III. RESULTS

1. Spray Tests with a Compressed-air Spray Gun

The Rega Model L gun was used in all tests in this section; the dosage was 1 fluid oz. per 2,100 cubic feet.

(i) *Kerosene.*

In all tests, lighting kerosene was used as the base for the sprays. Three tests were carried out using kerosene alone. There was an average 10-minute knockdown of 5 per cent. and an excess mortality of 2 per cent. over the control mortality. It is clear that the carrier, by itself, exerts no toxic action.

(ii) *Pyrethrins.*

The results of tests with pyrethrins alone are shown in Table 1. Above 0·07 per cent. w/v pyrethrins, knockdown and kill were complete, the higher concentrations merely knocking the mosquitoes down more rapidly than the lower. Below 0·05 per cent. the mortality fell away rapidly, far more so than the knockdown. It is clear from the table that quite a number of mosquitoes which are knocked down may recover completely. As a knockdown component of an otherwise satisfactory spray 0·03 per cent. or a higher concentration of pyrethrins should be used. For a spray containing pyrethrins alone, it would not be desirable to use solutions weaker than 0·07 per cent. Owing to the possibility of

TABLE 1.—KNOCKDOWN AND MORTALITY WITH PYRETHRUM SPRAYS.

w/v Pyrethrins.	Number Tests.	Knockdown (10 minutes).	Mortality (18 hours).
%		%	%
0·14	1	100	100
0·1	1	100	100
0·07	4	99·5	99
0·05	10	99	95
0·03	12	98	88
0·01	15	75	56

rapid deterioration of pyrethrins in unsealed tins in warm climates, initial concentrations lower than 0·07 per cent. might become too low to give complete kill if stored for any length of time.

(iii) *pp'-Dichlordiphenyl Trichlorethane (DDT).*

The results with DDT sprays are shown in Table 2. The DDT used contained approximately 96 per cent. pp'-isomer.

TABLE 2.—KNOCKDOWN AND MORTALITY WITH DDT SPRAYS.

DDT.	Number Tests.	Knockdown (10 minutes).	Mortality (18 hours).
%		%	%
0·5	15	33	97
0·25	16	15	91
0·1	5	0	39

A 0·5 per cent. DDT spray gave good kill (97 per cent.), but very poor knockdown (33 per cent.). Many of the adults which were not down at 10 minutes appeared normal, though they gradually became affected by the spray and eventually died. One hour after spraying, an average of 71 per cent. was down, and unfed females which were alive would still bite freely, although most of them would die in the next few hours.

A 0·25 per cent. DDT solution gave quite high mortality (91 per cent.), but exceedingly poor knockdown, while a 0·1 per cent. solution was unsatisfactory in both respects. Lindquist *et al.* (1945) record a 9 per cent. knockdown and 97 per cent. mortality from the use of a 1 per cent. DDT spray against *Anopheles quadrimaculatus.* In their tests, there was a 1 minute interval between spraying and exposure of insects, which may explain the somewhat lower effectiveness than would have been expected from the present tests. Rice *et al.* (1945) have recorded that 1 per cent. DDT produces poor knockdown, but 100 per cent. mortality, against *An. quadrimaculatus,* under test conditions which were considerably different from those in the present paper.

DDT sprays were not unpleasant to use.

(iv) *DDT + Pyrethrins.*

Table 3 shows the results of tests with DDT + pyrethrins sprays. With 0·5 per cent. DDT, 100 per cent. mortality was obtained with the addition of 0·03, 0·02, or 0·01 per cent. pyrethrins. The knockdown, however, diminished progressively, being 98, 88, and 55 per cent. respectively. In sprays containing 0·25 per cent. DDT, 0·05 per cent. pyrethrins gave complete knockdown and kill, and 0·03 per cent. pyrethrins 99 per cent. kill and fairly satisfactory knockdown (89 per cent.). A spray containing 0·01 per cent. pyrethrins and 0·1 per cent. DDT gave poor knockdown and kill.

TABLE 3.—DDT + PYRETHRUM SPRAYS.

Spray.	Number Tests.	Knock-down (10 mins.).	Mortality (18 hours).	In Same Tests Individual Components Gave—			
				Pyrethrins.		DDT.	
				K.D.	Mor-tality.	K.D.	Mor-tality.
		%	%	%	%	%	%
0·5 per cent. DDT + 0·03 per cent. pyrethrins ..	9	98	100	99	86	22	95
0·5 per cent. DDT + 0·02 per cent. pyrethrins ..	5	88	100	..	..	22	94
0·5 per cent. DDT + 0·01 per cent. pyrethrins ..	5	55	100	73	39	22	94
0·25 per cent. DDT + 0·05 per cent. pyrethrins ..	2	100	100	98	98	9	98
0·25 per cent. DDT + 0·03 per cent. pyrethrins ..	10	89	99	97	89	29	80
0·1 per cent. DDT + 0·01 per cent. pyrethrins ..	5	49	70	73	39	0	39

For all but one concentration in these tests, the knockdown produced by straight pyrethrum sprays was depressed slightly by the presence of DDT.

(v) *Thanite and Thanite + DDT.*

The results with Thanite and Thanite + DDT sprays are shown in Table 4.

TABLE 4.—THANITE AND THANITE + DDT SPRAYS.

Spray.	Number Tests.	Knockdown (10 mins.).	Mortality (18 hours).	In Same Tests 0·5 Percentage DDT Gave—	
				K.D.	Mortality.
		%	%	%	%
3·5 per cent. Thanite ..	3	99	76	..	..
5·0 per cent. Thanite ..	9	99	82	..	..
0·5 per cent. DDT + 3·5 per cent. Thanite ..	3	95	96	50	99
0·5 per cent. DDT + 1·0 per cent. Thanite ..	3	77	96	50	99

In these tests, 3·5 per cent. Thanite gave 76 per cent. mortality and 5·0 per cent. Thanite 82 per cent. mortality, while both sprays produced 99 per cent. knockdown. At 3·5 per cent. concentration and the dosage used, Thanite is slightly irritating to the nostrils and produced uncomfortable sensations in some people when used in a tent, although this was not nearly as noticeable in an open hut. 5·0 per cent. Thanite is about the upper limit of concentration which can be used with any

degree of comfort, even in an open hut. This concentration does not give a sufficiently high kill to warrant using a spray containing only Thanite. Thanite as the knockdown component of a DDT spray was therefore tested. The incorporation in a 0·5 per cent. DDT solution of 1·0 per cent. Thanite improved the knockdown considerably without affecting the mortality. A 3·5 per cent. Thanite + 0·5 per cent. DDT mixture produced a spray incorporating the good knockdown of Thanite (95 per cent.) and the high mortality due to DDT (96 per cent.). This spray, like the straight 3·5 per cent. Thanite spray, is just sufficiently unpleasant in closed tents under tropical conditions to make its use justified only if pyrethrins were not available for use as the knockdown component. Rice *et al.* (1945), using *An. quadrimaculatus,* have also shown that the addition of Thanite to a DDT spray renders the knockdown satisfactory.

(vi) *Lethane 384 Standard.*

The results are shown in Table 5.

TABLE 5.—LETHANE SPRAYS.

Spray.	Number Tests.	Knockdown (10 mins.).	Mortality (18 hours).
		%	%
5 per cent. Lethane ..	4	37	46
10 per cent. Lethane ..	4	73	71

Two concentrations were used, 5 and 10 per cent. Both gave poor knockdown and kill, and 10 per cent. Lethane in a closed tent was far too unpleasant to permit its use.

(vii) *Other Synthetic Compounds as Substitutes or Synergists for Pyrethrins.*

Tests were performed with five further substances to determine whether they would act as pyrethrin activators and the results are shown in Table 6.

(*a*) *Dimethyl phthalate.*—Undiluted dimethyl phthalate, when used as a spray, was very irritant to the nose and throat. It gave poor knockdown and even poorer kill, some adults recovering after being apparently dead. Its limit of solubility in kerosene lies between 1 and 2 per cent. Both 1 per cent. and saturated solutions of dimethyl phthalate were tested as the activating component of a 0·01 per cent. pyrethrins spray. Although the saturated solution appeared to increase the kill somewhat, the spray containing 1 per cent. dimethyl phthalate and 0·01 per cent. pyrethrins was slightly inferior to a 0·01 per cent. pyrethrins spray used in the same tests.

(*b*) *Dibutyl phthalate.*—The results obtained with dibutyl phthalate are very similar to those with dimethyl phthalate, except that the undiluted material produced 98 per cent. mortality. It was very irritant. 0·01 per cent. pyrethrins + 1 per cent. dibutyl phthalate gave slightly lower knockdown and kill than 0·01 per cent. pyrethrins alone. Dibutyl phthalate is far more soluble in kerosene than dimethyl phthalate and

TABLE 6.—SYNTHETIC COMPOUNDS AS SUBSTITUTES OR SYNERGISTS FOR PYRETHRINS.

Spray.	Number Tests.	Knockdown (10 mins.).	Mortality (18 hours).	0·01 Per Cent. Pyrethrins in Same Tests.	
				Knockdown.	Mortality.
		%	%	%	%
Dimethyl phthalate ..	2	67	45	..	..
Dibutyl phthalate ..	2	63	98	..	..
0·01 per cent. pyrethrins + 1 per cent. Me_2 phthalate	3	73	54	77	63
0·01 per cent. pyrethrins + satd. Me_2 phthalate ..	2	85	77	72	57
0·01 per cent. pyrethrins + 1 per cent. Bu_2 phthalate	3	60	50	77	63
0·01 per cent. pyrethrins + 5 per cent. Bu_2 phthalate	2	93	77	72	57
0·01 per cent. pyrethrins + 5 per cent. Crystox ..	3	87	79	85	74
0·01 per cent. pyrethrins + 5 per cent. D.H.S. activator	1	90	91	94	80
0·01 per cent. pyrethrins + 0·34 per cent. IN. 930 ..	1	86	87	94	80

a spray containing 0·01 per cent. pyrethrins + 5 per cent. dibutyl phthalate was therefore also tested. This gave improved knockdown and mortality, but proved somewhat irritating to the nose and throat.

(*c*) *Crystox* (Shell Co., London).—The addition of 5 per cent. Crystox to a 0·01 per cent. pyrethrins solution did not cause any significant change in the knockdown or mortality. There is, therefore, no advantage to be gained by its use, under these conditions. Crystox is stated to be a product of the condensation of two molecules of isophorone.

(*d*) *D.H.S. Activator and N-isobutyl undecylenamide (IN930).*—One test only was done with each of these activators. Both gave some indication of increased kill and decreased knockdown, though not of very great magnitude. An extensive series of tests with *C. fatigans* had demonstrated that no advantage was gained by the addition of these compounds to a pyrethrum spray (see previous paper). Since at this time these materials were reported to be unavailable for use by the Australian forces, no further tests were carried out.

(viii) *The Freon Bomb.*

A 4-second dose in the 750 cubic feet tent from the bomb used in the tests described in the previous paper gave 100 per cent. mortality and 100 per cent. knockdown in three tests. The fine mist produced was slightly irritant to the nose and throat.

2. Comparison of Sprays Applied at the Rate of 1 fl. oz. per 3,000 cu. ft. and 1 fl. oz. per 2,100 cu. ft.

In these tests, both the Model L and a hand gun were used. A comparison of the effectiveness of three sprays applied with the Model L gun at the two dosages reveals (Table 7) that the dosages were almost equally effective. When a hand gun was used to deliver

the dose, the mortality recorded for a 1 fluid oz. per 3,000 cubic feet dose was about the same as when this dosage was applied with the Model L gun.

Although the number of comparisons is small the results with the two dosages are sufficiently close to render valid for the lower dosage the main conclusions reached from tests with the higher dosage.

TABLE 7.—COMPARISON OF SPRAYS APPLIED AT THE RATE OF 1 FLUID OZ./3,000 CUBIC FEET AND 1 FLUID OZ./2,100 CUBIC FEET USING BOTH HAND GUN AND MODEL L.

Spray.	Hand Gun.			Model L.					
	Dose 1 fl. oz./3000 cu. ft.						1 fl. oz./2100 cu. ft.		
	Number Tests.	K.D.	Mortality.	Number Tests.	K.D.	Mortality.	Number Tests.	K.D.	Mortality.
		%	%		%	%		%	%
0·07 per cent. pyrethrins	2	100	99	2	100	100	4	99·5	99
0·05 per cent. pyrethrins	2	100	88	..	..	..	10	99	95
0·03 per cent. pyrethrins + 0·5 per cent. DDT	2	100	99	2	98	98	9	98	100
0·5 per cent. DDT ..	2	60	100	1	78	100	15	33	97
5 per cent. Thanite ..	2	97	74	..	..	..	9	99	82

3. Comparison of 10-Minute and Short Exposures to Sprays

To obtain information on the effect of sprays on adults flying through them, and then out into the open, a comparison was made between leaving the test cage in the treated hut for the standard 10-minute exposure period and removing it immediately the tent had been sprayed. From commencement of spraying to removal of the cage occupied less than 1 minute. The results are shown in Table 8.

TABLE 8.—COMPARISON OF 10-MINUTE AND SHORT EXPOSURES TO SPRAYS.

Spray.	Number Tests.	10 Minutes.		Short Exposure.	
		K.D.	Mortality.	K.D.	Mortality.
		%	%	%	%
0·07 per cent. pyrethrins ..	2	100	99	100	98
0·05 per cent. pyrethrins ..	2	99·5	94	95	92
0·03 per cent. pyrethrins + 0·5 per cent. DDT ..	2	96	100	83	100
0·5 per cent. DDT ..	2	50	99	88	100
5·0 per cent. Thanite ..	5	100	88	81	75

With pyrethrins, DDT, or a mixture of these two, both knockdown and mortality caused by long and short exposures were approximately the same, although on three out of five occasions, both knockdown and mortality were slightly higher for the longer exposure. The conclusion to be drawn from these observations is that the initial contact with the spray particles is all important in determining eventual mortality and that the fumigating effect of these sprays is unimportant. Adults flying through a mist of spray should die whether or not they remain in the sprayed tent or hut. The comparatively small effect of reducing exposure time is in accordance with the results obtained with flies by Waterhouse (1947).

Quite different results were obtained when 5 per cent. Thanite was used. Over a series of five tests, both knockdown and mortality were lower in short than in 10-minute exposures. This suggests that Thanite has a fumigating effect as well as contact action and it may explain why 3·5 per cent. Thanite gave 100 per cent. mortality of *C. fatigans* in the sealed Peet-Grady chamber tests (see previous paper) but only 76 per cent. mortality in the present tent tests.

4. Relative Effectiveness of Sprays in a Tent and a Native Hut

To determine whether the results obtained in the tent at a dosage of 1 fl. oz./3,000 cu. ft. were applicable to native quarters, a typical hut was chosen. This had ill-fitting plank flooring raised about 4 feet from the ground, and roof and incomplete sides both made of Beeri palm leaves. A slight breeze was blowing freely through the hut during the tests.

The results with both Model L and hand gun are shown in Table 9.

TABLE 9.—COMPARISON OF EFFECTIVENESS OF SPRAYS IN AN OPEN NATIVE HUT AND IN A TENT. ALL DOSAGES AT RATE OF 1 FL. OZ./3,000 CU. FT.

Spray.	Native Hut.						Tent.					
	Model L.			Hand Gun.			Model L.			Hand Gun.		
	Number Tests.	K.D.	Mortality.	Number Tests.	K.D.	Mortality.	Number Tests.	K.D.	Mortality.	Number Tests.	K.D.	Mortality.
		%	%		%	%		%	%		%	%
0·07 per cent. pyrethrins	3	99	98	3	98	96	2	100	100	2	100	99
0·03 per cent. pyrethrins + 0.5 per cent. DDT	3	89	98	3	90	99	2	98	98	2	100	99
0·5 per cent. DDT ..	3	33	97	..	..	..	1	78	100	2	60	100

The mortalities observed, both in the tent and in the hut, are very close indeed, although in three out of five comparisons, the mortality was slightly lower in the hut. In all five comparisons, the percentage knockdown at 10 minutes was lower in the hut than in the tent.

However, there is good reason to believe that the results obtained in the extensive series of tent tests would be generally applicable to open huts.

Both 0·07 per cent. pyrethrins and 0·03 per cent. pyrethrins + 0·5 per cent. DDT gave satisfactory knockdown and kill under all conditions of testing.

5. Comparison of Knockdown, 1, 5 and 18-20 Hour Mortality as Criteria of Spray Efficiency

In many of the experiments, records were kept of the number of mosquitoes not able to fly at 1 and at 5 hours after spraying. The figures are summarized in Table 10.

TABLE 10.—KNOCKDOWN AND MORTALITY OF VARIOUS SPRAYS.

Spray.	Percentage Knockdown.	Percentage Inactivated.			Number Tests.
		1 Hour.	5 Hours.	18–20 hours.	
0·07 per cent. pyrethrins..	99	100	100	99	4
0·05 per cent. pyrethrins..	98	99	98	96	8
0·03 per cent. pyrethrins..	99	92	87	91	6
0·01 per cent. pyrethrins..	72	51	52	62	11
0·5 per cent. DDT ..	28	71	94	98	11
0·25 per cent. DDT ..	18	45	68	90	8
0·1 per cent. DDT ..	1	19	40	53	4
0·5 per cent. DDT + 0·03 per cent. pyrethrins ..	95	98	100	100	9
0·5 per cent. DDT + 0·02 per cent. pyrethrins ..	88	83	100	100	5
0·5 per cent. DDT + 0·01 per cent. pyrethrins ..	55	94	99	100	5
0·25 per cent. DDT + 0·30 per cent. pyrethrins ..	74	97	97	98	4
0·5 per cent. DDT + 3·5 per cent. Thanite ..	98	92	85	96	1
0·5 per cent. DDT + 1·0 per cent. Thanite ..	77	77	79	95	1
3·5 per cent. Thanite ..	100	92	96	86	2
5·0 per cent. Thanite ..	90	70	77	82	2
0·01 per cent. pyrethrins + 1 per cent. Me_2 phthalate	88	87	87	81	3
0·01 per cent. pyrethrins + 1 per cent. Bu_2 phthalate	86	87	89	76	3

With pyrethrins the percentage knockdown was always greater than the mortality, except of course where there was 100 per cent. mortality. With high concentrations a slightly greater number of mosquitoes was inactivated at 1 hour than at 10 minutes. With lower concentrations there was a gradual recovery from 10 minutes to 5 hours, at which period mortality was lowest, and then some apparently normal adults became inactivated again.

With DDT there was an increase in mortality up to the 18-hour count. The greatest increase generally occurred between the 10-minute and 1-hour readings.

The addition of pyrethrins to DDT resulted in a high knockdown although mosquitoes not knocked down at 10 minutes were dead 18 hours later. With the Thanite + DDT spray, there was a slight recovery after initial high knockdown, followed by eventual death. With Thanite alone after initial high knockdown, recovery was gradual up to 18 hours. Pyrethrins plus phthalates showed slight recovery after initial knockdown.

Since significant changes may occur after either the 10-minute, 1-hour, or 5-hour periods, particularly with sprays containing DDT, 1 and 5-hour mortality figures are not sufficiently satisfactory to enable tests to be stopped at either of these stages.

IV. DISCUSSION

The results reported in the previous paper and those obtained with *An. punctulatus farauti*, agree well for all toxic ingredients with the exception of Thanite. The reason for this discrepancy probably lies in the greater fumigating effect in the Peet-Grady chamber than in field tests. This is an important point to remember when using the Peet-Grady chamber to assess sprays for field use.

It appears that *farauti* is slightly more susceptible to pyrethrum sprays than *fatigans*, although it is not possible to draw a direct comparison since the two sets of tests were carried out under different conditions and at different temperatures. Male *farauti* were so susceptible to the conditions of handling during tests that it was not possible to obtain any idea of their susceptibility to sprays. One of the unavoidable disadvantages of the present series of tests was that it was necessary to use caged mosquitoes. The presence of mosquito netting may restrict the impaction of spray droplets on the mosquitoes, and the cages, although fairly large, restrict the flight of the insects to a certain extent.

No difficulty was found in atomizing 1 fl. oz. per 3,000 cu. ft. in a tent using either a Model L or a continuous hand spray gun. With an ordinary hand spray gun, selected for its good atomization, 60 to 80 strokes provided the 7 ml. dosage required. This is about the limit to which the energy of a normal individual would run under tropical conditions, so that any enclosure larger than 750 cu. ft. would probably receive less than the recommended dose in any one application. If the dosage is lower, a proportionately higher concentration of toxic ingredients in the spray is required. The difficulty in operation and the variation in amount delivered per stroke makes a hand gun of the type used unsatisfactory for a series of comparative tests.

Under conditions of great anopheline abundance, as at Lalapipi, the effect of spraying at night in native huts could be clearly observed. With clouds of anophelines continually seeking to bite at night, spraying a hut with the recommended dose of a satisfactory spray (0·07 per cent. pyrethrins, 0·5 per cent. DDT plus 0·03 per cent. pyrethrins, 5·0 per cent. Thanite) gave freedom from attack for about five minutes. After this period, mosquitoes became troublesome again.

There was no indication that atomization of 5 to 7 times the standard dose of spray each day for 24 days into two tents affected the number of engorged and unengorged anophelines found resting therein each morning. Nor did these adults, when collected and caged, die off more

rapidly than usual. With good atomization, the amount of spray deposited on the roof and walls is apparently not sufficient to have a persistent lethal effect, even although DDT and pyrethrins were generally each used several times each day. On the other hand, following treatment of one of these tents with DDT at the rate of 50 mg. per sq. ft., no anophelines were found resting during the observation period of several weeks (Waterhouse, unpublished).

Because of the above evidence, and especially the fact that the average soldier was most unlikely to spray at the full recommended dosage, the Army decided not to vary the 0·14 per cent. pyrethrins spray, adopted provisionally as a result of the tests reported in the previous paper, and not to include a synergist.

V. ACKNOWLEDGMENTS

The authors are indebted to Lt.-Col. I. M. Mackerras for arranging facilities for these tests and for his interest and encouragement.

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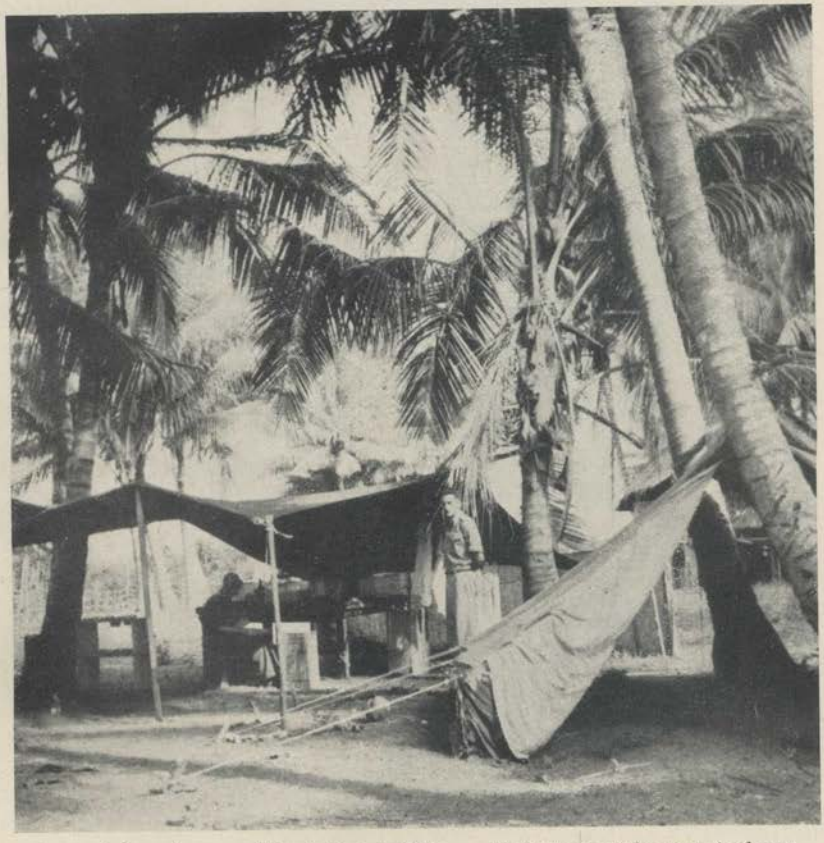
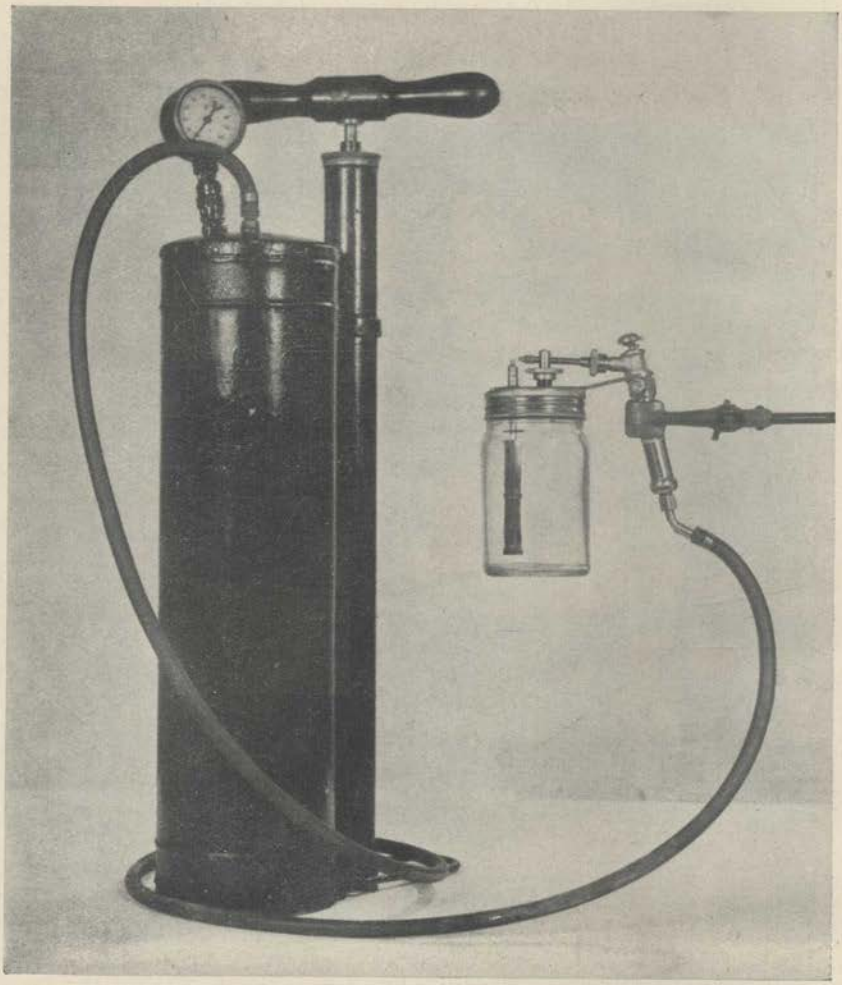
PLATE 1



General view of some of the testing facilities. Half of one of the tents is shown, end on, at the right. A working bench can be seen in the background.

PLATE 2



The Rega Model L spray gun. For small dosages the glass jar is replaced by a tube.

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Laboratory and Field Tests of Mosquito Repellents

By

R. N. McCulloch, B.Sc., B.Sc.Agr.,
and
D. F. Waterhouse, M.Sc.

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FOREWORD.

When the Australian Forces commenced operations in the highly malarious islands of the south-west Pacific theatre, one of the most urgent requirements was a really effective mosquito repellent. The standard Army citronella cream had proved unsatisfactory: its repellent properties were inadequate, and it was unpleasant to use under tropical conditions.

Accordingly, the investigations described in this paper by Major R. N. McCulloch and Captain D. F. Waterhouse were intitiated. The work, in the form of field experiments, was pioneered by Major McCulloch; but it soon became evident that a more detailed and intensive study was required. The cooperation of the Council for Scientific and Industrial Research was therefore sought; and the facilities of its Division of Economic Entomology at Canberra, and the services of Mr. Waterhouse and his associates, were promptly and generously made available to the Army. Laboratory investigations were pushed ahead; and Mr. Waterhouse was brought on to the Reserve of Officers, and called up for full-time duty when required. He was thus able to continue his work in Canberra as an officer of the C.S.I.R., and pursue it into the field as an officer of the A.A.M.C., at first in collaboration with Major McCulloch, and later in association with other Army entomologists.

As a result of the A.A.M.C.-C.S.I.R. repellent investigations, and information received from the U.S.A. (where experiments, developed on a much larger scale than the Australian, were carried out under the aegis of the Department of Agriculture) the local manufacture of dimethyl phthalate was organized, and this very effective repellent was made available to the Australian Army, Navy, and Air Force well before the end of 1943, some special forces being supplied with it as early as March, 1943.

It would be profitless to attempt to assess the relative importance of the work of Major McCulloch and Captain Waterhouse on the one hand, and the reports of experimental results received from overseas on the other, in determining the selection of dimethyl phthalate for use by the Australian armed forces. When the final decision to adopt dimethyl phthalate was made, we were armed with a considerable amount of valuable data furnished by the U.S. authorities (including the results of toxicity tests with mammals, which indicated that the substance would be safe to apply to the human skin), in addition to the Australian results. The latter carried special weight through the work having dealt with the mosquito responsible for most of the malaria transmission in the south-west Pacific area. Perhaps the question can best be summed up by saying that either the results of the Australian work, or the reports of the American, would have led to the selection of dimethyl phthalate; but in the light of the mutual confirmation of the two independent investigations the decision was made with a much greater degree of confidence.

S. R. BURSTON, Major-General.
Director-General of Medical Services,
Australian Military Forces.

CONTENTS.

SUMMARY.

Laboratory and field tests of more than 125 substances for use as repellents against both Anopheline and Culicine mosquitoes are reported. The main species used were the two important vectors of disease, *Anopheles punctulatus farauti* and *Aedes aegypti*, and the common pest mosquito of coastal New South Wales, *Aedes vigilax*. The time to the first bite on treated skin was taken as the first indication of efficiency, but, in distinguishing between better repellents, much attention was paid to the speed with which subsequent biting occurred. Cage tests were found to be most convenient for selecting the more effective repellents for field testing, but tended to indicate, for better repellents, longer protection than was obtained in field tests.

The most effective repellents, giving nearly complete protection for 45 to 60 minutes under the most severe conditions, were:

Dimethyl phthalate
612 (2-ethylhexane-1, 3-diol)
Staway
Oils of: *Dacrydium franklinii* (Huon pine)
Melaleuca bracteata
Zieria smithii
Backhousia myrtifolia.

Under similar conditions, Ceylon citronella oil gave protection for no more than twenty minutes.

Oils of Huon pine and *M. bracteata* owe their repellency to their high content of methyl eugenol, but are unsuitable for use because of their somewhat irritant effect on the skin, and because they cause some individuals to become nauseated when the oil is applied to the face. *Backhousia* and *Zieria*, which both contain a high proportion of elemicin, are also somewhat irritant to the skin, and have a pungent smell which renders them less desirable to use than dimethyl phthalate. Staway was excluded from serious consideration when American work indicated that it might be unsafe for human use. 612 has a distinct smell, and in most tests gave results equally as good as dimethyl phthalate. Dimethyl phthalate is almost odourless, is non-irritant, and was comparatively pleasant to use under service conditions.

Dilutions of dimethyl phthalate with ethyl alcohol were slightly to considerably less effective than the pure repellent. Methyl ethyl phthalate was almost as effective as dimethyl phthalate and far more effective than either diethyl or dibutyl phthalates. When all factors—effective protection, absence of objectionable features, availability, &c.—were considered, dimethyl phthalate was by far the most satisfactory repellent tested.

A number of other synthetic and naturally occurring substances were tested with indifferent results. Of a large number of Australian essential oils, other than those mentioned above (tea-tree oils, eucalyptus oils, &c.), the most promising were two sassafras oils which were too irritant to use. Many essential oils were quite ineffective. Pyrethrum preparations gave consistently poor protection, although a toxic effect on mosquitoes biting through them was observed. The four effective essential oil repellents listed above also exerted a toxic effect. Indalone gave very variable results, but even the best repellent times were not comparable with those obtained with dimethyl phthalate.

The treatment of clothing with dimethyl phthalate prevented mosquitoes biting through it for two or three days.

Laboratory and Field Tests of Mosquito Repellents.*

I. INTRODUCTION.

The present study was a cooperative one between the C.S.I.R. and the Army, its purpose being to discover a more satisfactory repellent than the citronella cream which had repeatedly been shown to confer inadequate protection against the severity of biting often encountered in the field. Since the repellent was required urgently for use by the armed forces, it had to be available in large quantities, preferably from Australian sources. Throughout the experiments, the object was to select the best repellent as quickly as possible. No time was devoted to putting in precise order the various materials tried and found less effective.

Before 1938, work with mosquito repellents dealt mainly with the study of essential oils and their constituents, and with attempts to prolong their protection by various means, such as by adding to them non-volatile oils or greases to act as fixatives. Ceylon citronella oil and citronella creams were probably the most popular repellents available at this time. In 1939, MacNay† described a mixture based on pyrethrum extract and claimed repellency greatly superior to that of any earlier preparation. In 1942, when the present investigations commenced, the most important published work on mosquito repellents was that of Granett (1940)‡ in U.S.A., who demonstrated that Staway§, of which the effective ingredients were synthetic organic compounds, was far superior to any of the other mixtures he tested, which included both pyrethrum and citronella oil preparations.

The first comprehensive list of substances to be examined in the present series of tests included, in addition to many Australian essential oils, pure dimethyl and diethyl phthalates since these were stated to be the active constituents respectively of a proprietary repellent cream and repellent lotion submitted for test by the Vacuum Oil Company Pty. Ltd. on behalf of their manufacturers, Stanco (Australia) Pty. Ltd. These materials received their first tests in the laboratory in Canberra in August, 1942, and first field tests shortly afterwards in New South Wales. Dimethyl phthalate, 612, and Indalone, were described by United States Department of Agriculture entomologists at Orlando, Florida, as outstanding repellents in confidential reports made available towards the end of September, 1942, to Australian military authorities visiting America. When this information reached us dimethyl phthalate had already come to be regarded as the best material available. Indalone and 612 received their first Australian tests some time later.

Since Culicine mosquitoes in New South Wales occur in far greater abundance in the field than Anophelines, and are far more easily dealt with in the laboratory, it was decided that the initial sorting of

* Typescript received May 6, 1946.

† MacNay, C. G. (1939).—Studies on repellents for biting flies. *Canad. Ent.* 71: 38-44.

‡ Granett, P. (1940).—Studies of mosquito repellents. I. Test procedure and method of evaluating test data. *J. Econ. Ent.* 33: 563-5. II. Relative performance of certain chemicals and commercially available mixtures as mosquito repellents. Ibid. 33: 566-72.

§ For formulae, see Appendix.

repellents should be carried out in parallel laboratory studies (by D.F.W.) and field tests (by R.N.McC.), both employing Culicines. The laboratory promised continuity of work uninterrupted by adverse weather, though it was realized that final evaluation of a repellent must take place in the field. When a comparison of the two sets of results had shown the general reliability of laboratory tests for Culicines, the more promising repellents were taken by both authors to Cairns in December, 1942, for laboratory tests against *Anopheles punctulatus farauti* Lav. (= *moluccensis* Swell. and Swell. de Graef), the principal vector of malaria in Australia and New Guinea, of which populations sufficiently dense for field tests were not available in Australia. Subsequently, confirmatory field tests against *A. punctulatus farauti* were carried out in New Guinea (D.F.W.).

II. METHODS.

1. Laboratory Tests.

(i) *Tent Tests.*

In the first method used for testing in the laboratory, suitable biting conditions were produced by the liberation of adult mosquitoes into a mosquito-proof tent 8 ft. by 8 ft. by 7 ft. high, with a 4 ft. by 4 ft. zippered baffle chamber at one end. The walls were mainly of organdie, roof and floor of calico. Fully grown larvae and pupae of *Aedes notoscriptus* Skuse and A. *alboannulatus* Macq. were collected from local breeding grounds, and the resulting adults allowed to emerge, either into cages or directly into the tent. A population of 1,000-2,000 females was generally maintained, of which a hundred or so might attempt to bite during any one experiment. As an alternative food to blood, slices of apple and banana were available. Temperature, humidity, and light intensity in the room containing the tent could be adjusted within certain fairly wide limits.

It was thought that tent tests would reproduce field conditions far more closely than cage tests, since legs, arms, and face of one or more persons could be exposed to attack at one and the same time. To a certain extent this was true, although it was found that most mosquitoes spent their time around the ankles and feet, and attacked the face relatively infrequently. Similar behaviour, although not so marked, has often been observed in the field where the greatest intensity of attack is near the ground. In the tent, more even attack of exposed surfaces was produced if the subject sat on the floor. One of the main objections to the tent method was the build-up of repellent vapours which occurred during testing. The most spectacular occasion was when a 6·6 per cent. solution of pyrethrins was tested one afternoon: next morning, at least half of the mosquitoes in the tent, many of which had not fed, were dead or dying. To avoid interference of one repellent with another, it was necessary to test only one or two repellents each day.

(ii) *Cage Tests.*

Two types of cage were used, single units measuring 18 in. by 9 in. by 9 in. high, with wooden bases, wire frames, and mosquito net covers containing a sleeve at one end, and double units twice the width and possessing two sleeves in one end.

Some 100 or more female *Aedes aegypti* L., with accompanying males, were maintained in each cage. These were fed on human blood and on slices of apple and banana. Eggs were laid on cones of moistened filter paper dipping down into beakers of water. Larvae were reared on powdered yeast and fish meal, or on Farex, a proprietary baby food. Feeding and testing of *aegypti* was carried out at 80°F. and 65 per cent. R.H. For *An. punctulatus farauti* tests, larvae were collected in the field and reared to the adult stage in enamel trays in the laboratory.

The cage with two sleeves was used when it was desired to test two repellents simultaneously in order to compare their repellency under identical conditions. Tests with *aegypti* were carried out using a shaded light. With *farauti* they were done at night, the number of bites being recorded by the intermittent use of a flashlight.

Cage tests have the advantage over the tent method that, by concentrating the mosquitoes in the vicinity of hands and arms, tests can be carried out with small numbers of mosquitoes—down to 50 females per cage, although usually more than this were used. With Anophelines in particular, it may be argued that cages allow the maintenance of a concentration of blood-hungry females rarely encountered in the field. In addition, cage tests can be carried out with adults bred from larvae collected in the field, and this avoids risk of malaria infection.

A number of Culicine species was tested for suitability for cage tests at Canberra—*Culex fatigans* Wied., *Aedes alboannulatus*, *A. notoscriptus*, *A. concolor* Tayl., *A. vigilax* Skuse, and *A. aegypti*. Finally, *A. aegypti* was selected for routine tests because it could be reared continuously and without difficulty, because it bit fiercely under test conditions, and because it was being used for laboratory repellent tests in other parts of the world. Difficulty arose in cage tests from the fact that vigorous biting usually faded after one to two hours' testing in each cage. This was due, in some cases, to the elimination by feeding of blood-hungry females, but for the better repellents, in which few bites were received, there is good reason to believe that the toxicity of the material under test played a part. Even when willing to bite, therefore, each cage was seldom used for more than one or two tests each day, and two or more cages were sometimes used to test the same application of repellent.

2. Field Tests

Field tests in New South Wales were carried out in coastal tea-tree or mangrove scrubs, where the common pest mosquito of coastal bush, *Aedes vigilax* (with a few *A. vittiger* Skuse and *A. alternans* Westw.), provided a biting frequency of 25 to 50 per minute on an untreated limb. Such conditions occurred near Newcastle in April, October, and November, 1942, providing suitable biting frequencies from about 6 a.m. to 6 p.m. In mid-summer (January and February), good test conditions prevailed only for about an hour at dusk and dawn. When the mosquitoes were active throughout the day, it was noted that the biting fell off sharply as the shade temperature fell below 65° F. or rose above 88° F. At the lower temperature saturation was approached, while at the higher, the atmosphere was often very dry (35 per cent. R.H. or lower). Intense biting was recorded with a temperature of 82° F. and a relative humidity of 35 per cent.

In Cairns, in mid-summer, *A. similis* Theo. was the predominant species in field tests. The tests in New Guinea were carried out at Lalapipi, Lakekamu River, against a mixed population of *An. punctulatus farauti* and Culicines.

In general, maximum biting activity occurred only in the scrub. It was possible, therefore, for test personnel to apply, or to have applied, treatments while in open country where biting was not unduly severe, then to walk a short distance to previously selected sites of high biting frequency, and, when a test was finished, to return to the open, wash and dry the skin, and apply material for a new trial.

During exposure, experimental personnel sat in clouds of mosquitoes with faces, ears, neck, hands and sometimes legs treated and continuously uncovered. The men killed mosquitoes that had bitten, calling the bites and their situations to the time keeper. At intervals of fifteen minutes an untreated limb was exposed and the bites occurring noted. This was usually done after a move from place to place. These moves of only 10 yards or so at a time were dictated by a phenomenon noted also by American workers. It appeared as though in the dense mosquito population at any one place, there were a few individuals relatively unaffected by repellents, and when they were fed, or seriously affected by contact with the repellent, a period of immunity followed. A move of only a few yards would often be followed by some bites, so that the policy was adopted of giving the mosquito population every opportunity to bite.

Since, for some reason not understood (it seemed not to be connected with hairiness), several better mixtures gave longer repellent times on the legs than on face and hands, and because it is for face and hands that a repellent is required in practice, final decision between nearly equal mixtures was arrived at by comparing them simultaneously on right and left arms and hands of each of a group of test personnel. While this was being done, dimethyl phthalate was often used on the head, so that many records were made of its performance on the faces of men exposed to exceptionally severe conditions.

3. General

(i) *Repellent Time.*

Granett (loc. cit.) adopted the time to the first bite as his "repellent time" by which he compared the effectiveness of different repellents. In the present tests it was found that an occasional bite might be received even with the best repellents a comparatively short time after application, although very considerable protection would be afforded for an hour or more. As a result, attention was paid to the rate of loss of protective power after the first bite. The routine was adopted of stopping an individual test when, for weak repellents, biting reached one bite per minute on a treated limb and, for better mixtures, when about five or six bites had occurred, or, alternatively, at the end of 60 or 80 minutes.

(ii) *Dosage.*

Repellents were applied to some, or all, of the face, arms, hands, and legs in field and tent tests, and to the arms and hands for cage tests. The volume of repellent required to provide a liberal uniform film over the surface to be protected was recorded in each instance. This volume varied somewhat under different conditions of testing, and also from

person to person, according to the area of skin to be covered, and the amount of hair, but was held constant for the same individual within the same series of tests. In tent tests, 1·5 ml. sufficed for the face, one arm, and one hand; in cage tests, the dosage for various individuals varied from 0·5 ml. to 0·8 ml. for a hand and arm to the elbow. In field tests, the standard dosage was 3 ml. per leg from the boot top to high on the thigh, 1 ml. to the hand and arm to just above the elbow, and 1 ml. to the head (face, ears, and neck). Treatment extended under clothing edges, so that no doubts existed about marginal biting. During tests of head treatments hats were worn.

(iii) *Control Biting Rate.*

In all tests, an untreated limb was exposed at about fifteen-minute intervals to provide a control biting rate. Biting frequencies of more than 25 per minute came to be regarded as essential for comparisons of the best repellents, but lower frequencies were used in the elimination of weaker mixtures.

III. RESULTS

1. Reaction of Mosquitoes to Repellents

It was soon found that skin to be protected by repellent had to be covered completely; mosquitoes seemed to find quickly even a small untreated area. The typical reaction of mosquitoes to repellents is seen most clearly in field tests. A man, fully-clothed and with hands and face treated, would, on entering the scrub, be surrounded by clouds of mosquitoes, hundreds resting on, or probing at, his hat, shirt and especially gaiters and boots. The reaction of mosquitoes to treated skin appeared to vary with the repellent, as it did also in cage tests. Some materials, although they had poor lasting effect (e.g., citronella), appeared to keep the mosquitoes at a distance—several inches—for almost the duration of their period of protection. Others (e.g., dimethyl phthalate) did not prevent the mosquitoes from approaching the treated skin, although actual contact with the surface did not occur for some time, then a few individuals would touch and fly off again. Then probing would occur, the mosquito leaving without stinging or sucking blood. Finally, the first bite would be noticed in which the sting might be distinct, although in the early stages the mosquito might not stay to feed. The hairless parts of the body were usually attacked earlier and far more severely than the hairy parts. This was not dependent simply on the presence of hair, since shaving part of a leg did not make it as attractive as a hairless area.

A feature often noticed in tests in which two or more people took part was their difference in attractiveness, some individuals receiving far more bites than others, both on treated and on untreated surfaces. In addition, the relative order of attractiveness of the same individuals varied from day to day. Great care was therefore taken when comparing results obtained by different people or by the same person on different days. In these experiments it was very difficult to obtain satisfactory replication of numerical results for time to the first bite, and this was no doubt partly due to the variation just described. Also, there may be daily differences in factors which influence the activity and avidity for blood of mosquitoes, and this is reflected in different biting frequencies. The discrepancy in repellent times was greater with the good repellents.

Poor repellents invariably broke down rapidly if the control biting rate was high. On the other hand, good repellents occasionally broke down at a shorter time than would normally be expected.

2. Repellent Results

The results of repellent tests are summarized in rating form in Table 1, the ratings being broadly based on the shortest period for which the repellent gave effective protection. As explained earlier in this paper, this is not necessarily the shortest time ever recorded to the first bite, as for instance when the first bite was not followed by further biting for some time. In addition, it was borne in mind at all times that the principal aim was to select the best repellent rather than to evaluate accurately all substances on the test list. On being clearly surpassed, mixtures were discarded. Subjected to the same intensity of testing as was dimethyl phthalate, many of them would undoubtedly have received a more adverse report than the table indicates. A low biting frequency was sometimes used both in the laboratory and in the field to eliminate weak mixtures, though it was useless for comparing the better substances. The ratings shown, therefore, are not all strictly comparable, but they are the most convenient method of summarizing the results.

As can be seen in Table 1, cage tests gave, for most materials, repellent times of the same order as those obtained in field tests. The

TABLE 1.—RESULTS OF REPELLENT TESTS.

— indicates effective protection for 0–20 minutes
\+ " " " " 20–40 "
++ " " " " 40–60 "
+++ " " " " over 60 "
0 indicates mosquitoes were not given the opportunity to bite earlier.

For details concerning the composition of mixtures tested, see Appendix.

The materials are listed alphabetically in subdivision (*a*) and (*b*), except where a series of similar materials, for instance the phthalates, are grouped together.

Substance.	Cage Tests.		Tent Tests.	Field Tests.		
	A. aegypti.	Anopheline s.		N.S.W.	New Guinea.	Queensland.
(*a*) SYNTHETICS AND PURE CHEMICALS.						
Amyl alcohol ester of coconut fatty acids	—		—	—		
Benzyl benzoate	—			—		
Coumarin (satd. soln. in alcohol) ..	++		—			
Creosote 259* 25 per cent. in lighting kerosene	—					
DDT (pp′-dichlordiphenyltrichlorethane). Deposited from satd. acetone soln.	—					
612 (2-Ethylhexane-1, 3-diol) ..	+++				+	
Furfural	—			—		—
Indalone (*aa′*-dimethyl-*a*-carbo-butoxydihydro-*γ*-pyrone)	++	—		+	—	++

* Timbrols Ltd., New South Wales.

TABLE 1—*continued.*

Substance.	Cage Tests.		Tent Tests.	Field Tests.		
	A. aegypti.	Anophelines.		N.S.W.	New Guinea.	Queensland.
Magnesium sulphate (Epsom salts) (satd. aq. soln.)	−			−		
Methyl stearate	−					
Phthalates—						
Dimethyl phthalate (Me_2p) ..	+++	+++	+++	+++	++	
75 per cent. Me_2p + 25 per cent. Bu_2p by vol.	+++					
50 per cent. Me_2p + 50 per cent. Bu_2p by vol.				++		+++
50 per cent. Me_2p + 50 per cent. Et_2p by vol.				++		+++
75 per cent. Me_2p + 25 per cent. EtOH by vol.	+++			++		
67 per cent. Me_2p + 33 per cent. EtOH by vol.				++		
60 per cent. Me_2p + 40 per cent. EtOH by vol.	+++					
50 per cent. Me_2p + 50 per cent. EtOH by vol.	+++					
40 per cent. Me_2p + 60 per cent. EtOH by vol.	++					
50 per cent. Me_2p + 50 per cent. *Backhousia* by vol.						+
50 per cent. Me_2p + 20 per cent. Et_2p by vol. + 20 per cent. *Backhousia* by vol. + 10 per cent. Huon pine oil by vol. ..						+
Me_2p containing 2 per cent. bentonite by weight				+++		
Me_2p containing 2 per cent. talc by weight				+++		
Me_2p containing 0·1 per cent pyrethrins by weight ..						+++
Me_2p applied after petroleum jelly				+++		
Methyl ethyl phthalate ..	+++				+	
Diethyl phthalate (Et_2p) (see footnote, p. 23)	+	+	−	+		++
Dibutyl phthalate (Bu_2p) ..	+	++	−	−		++
Diamyl phthalate	−					
Dicapryl phthalate	−					
Salicylates—						
Methyl salicylate	+		+	−		−
Butyl salicylate						+
Amyl salicylate	+		−			+
Staway	++	++	+++	++		
Butyl carbitol acetate	++			++		
(*b*) PLANT PRODUCTS.						
Anyme oil (New Caledonia) ..	−		−	−		
Backhousia myrtifolia	+++	+++	+++	++		
B. myrtifolia in rosin soap ..			+++			
B. myrtifolia + Huon pine oil ..			+++			
Callitris glauca (leaf oil) ..	−		−	−		
Callitris glauca (wood oil) ..	+		−	+		
Castor oil 50 per cent. + kerosene 50 per cent.	−			−		
Cineol	−					

TABLE 1—*continued.*

Substance.	Cage Tests.		Tent Tests.	Field Tests.		
	A. aegypti.	Anopheline.		N.S.W.	New Guinea.	Queensland.
Citronella preparations—						
Citronella (Ceylon)	−	−	++0	+	−	+
Citronella (Ceylon) 2nd fraction	+		+++0	−		
Citronella (Java)	−					
Citronella SN3071				+		
Citronella SN3073	+			+		
Citronella SN3073 new ..	+	−	+	−		--
Citronella cream (20 parts Ceylon citronella, 1 part Sp. camphor, 10 parts Ol. cedarwood, 30 parts paraffinum durum, 50 parts paraffinum molle) ..	−	−	−	−		--
Citronellal	−			−		
Citronellol	−			−		
Eremophila mitchelli	−			−		
E. mitchelli, Zieria smithii, Castor oil, equal parts				−		
Eucalyptus oils—						
Eucalyptus dives type	−					
Eucalyptus dives type + 10 per cent. fixative	−		−	−		
Eucalyptus dives var. C. ..	−			−		
Eucalyptus dumosa	+					
Eucalyptus oil (75 per cent. terpineol)	−					
Eucalyptus oil rich in terpineol ..	+			−		
Eucalyptus phellandra	−			−		
Eucalyptus polybractea ..	−					
Eucalyptus oil residues (4 samples)				+		
Eucalyptus alcohols				−		
Evodionol (sat. soln. in EtOH) ..	−					
Geraniol				+		
Huon pine oil (*Dacrydium franklinii*	+++	+++	+++	+++		
75 per cent. Huon pine oil + 25 per cent. beeswax				+++		
Huon pine oil, 1st distillate				+++		
Huon pine oil, 2nd distillate ..				+++		
Huon pine oil, 5 per cent. residues				−		
Huon pine oil, 10 per cent. residues				+++		
Huon pine oil, 20 per cent. residues				+++		
Methyl eugenol	+++			+++		
Mixture L1—Dr. Lahey (see Appendix)						−
Mixture L2						−
Mixture L3						−
Mixture L4						−
Mixture P1						−
Mixture P2						−
Mixture S1						−
Mixture S2						−
Nerolin (synthetic) (dissolved in *M. linariifolia* oil)	−		−			
Nerolin (synthetic) (in lanoline) ..	−		−			
Phellandrene	−			−		
Pyrethrum preparations—						
6·6 per cent. w/v			−			
6·0 per cent. in gum tragacanth	−			−		

TABLE 1—*continued.*

Substance.	Cage Tests.		Tent Tests.	Field Tests.		
	A. aegypti.	Anopheline.		N.S.W.	New Guinea.	Queensland.
0·1 per cent. in paraffin cream ..	–					
British cream (Mark 1) ..	–				–	
MacNay's formula	–			–		
Sandalwood oils—						
Sandalwood oil (*Eucarya spicata*)	–			–		
Sandalwood resin oil	–			–		
Thymol terpenes	–					
Thymol terpenes 75 per cent., Sandalwood oil 25 per cent. ..	–					
Sassafras oils—						
Atherosperma moschatum ..	++					
Doryphora sassafras	+++		++			
Ol. sassafras	+		–	–		
Oil of spike lavender ..	–			–		
Tagetes glandulifera oil	–			–		
Tea-tree oils—						
Melaleuca alternifolia	–			–		
Melaleuca bracteata	+++			++		
Melaleuca ericifolia	–			–		
Melaleuca leucadendron (Q'land)	–	–	+	–		
Melaleuca leucadendron (N.S.W.)	–			–		
Melaleuca linariifolia	+			+		
Melaleuca uncinata	–			–		
M. linariifolia, Pine tar oil, and Castor oil, equal parts ..				–		
Zieria smithii	+++	+++	+++	++		
Zieria smithii, 2nd fraction ..	–		–	–		
Zieria smithii, 5th fraction ..	+++		+++	++		
(c) PROPRIETARY MIXTURES (See Appendix)—						
No. 1 Yarmor pine oil + pyrethrum	++					
No. 2 $MgSO_4$ + gelatin ..	–					
No. 3 to 6 Naphthalene mixtures ..	–					
No. 7 Pyrethrum mixture ..	–					
No. 8 Pyrethrum mixture ..	–					
No. 9 Dimethyl phthalate lotion ..	++	–	+++0	+		
No. 10 Diethyl phthalate lotion ..	–		–	–		
No. 11 Dimethyl phthalate cream				–		
No. 12 Methyl salicylate + essential oils	–					
No. 13 Methyl eugenol 1 per cent.	–					+
No. 14 Indalone + rotenone ..						
No. 15 $CaCl_2$ and $Mg\ Cl_2$ compound of synthetic alcohol. Satd. soln. in alcohol				–		
No. 16 Lauryl thiocyanate (commercial)				–		

most important discrepancy was the more favourable repellent times recorded in cage tests for the better repellents. This has also been noted by overseas workers. As suggested earlier, the longer protection may have been partly due to the adverse effects on the mosquitoes of the repellent vapours in the confined space of a cage. In addition, in field

tests among the generally far larger number attracted, there was a greater chance of individuals comparatively resistant to repellents being present. Cage tests therefore were not regarded as more than a convenient method of selecting the most effective repellents for testing under field conditions.

3. Discussion of Results

Results are discussed under the following headings:—

(i) Dimethyl phthalate repellents—

(*a*) Pure dimethyl phthalate.
(*b*) Mixtures containing dimethyl phthalate.

(ii) Mixtures receiving special attention because of effectiveness or favourable reports by earlier workers—

Methyl ethyl phthalate.
Staway.
612.
Indalone.
Pyrethrum mixtures.
Oil of *Dacrydium franklinii* (Huon pine).
Oil of *Melaleuca bracteata.*
Oil of *Zieria smithii.*
Oil of *Backhousia myrtifolia.*
Oil of citronella and citronella preparations.

(iii) Inferior repellents.

(*a*) Synthetics and pure chemicals.
(*b*) Plant products.
(*c*) Proprietary mixtures.

(i) *Dimethyl Phthalate Repellents.*

(*a*) *Pure dimethyl phthalate.*—In cage tests with Anophelines or Culicines, the first bite with pure dimethyl phthalate was seldom received before one hour (often two hours) and very material protection was afforded long after the first few bites. In almost 100 field tests against Culicines under conditions of very high biting frequency—more than 25 bites per minute per untreated limb—immunity for an hour or more was recorded on many occasions. The least favourable results obtained are shown in Table 2.

TABLE 2.—SOME REPELLENT RESULTS WITH DIMETHYL PHTHALATE OBTAINED IN FIELD TESTS IN NEW SOUTH WALES.

Head.			Arm.		
Dosage.	First Bite.	Total Bites During Test.	Dosage.	First Bite.	Total Bites During Test.
ml.	minutes.	minutes.	ml.	minutes.	minutes.
1·0	13	5 to 36			
1·0	26	2 „ 47	1·0	49	1 to 60, 9 to 80
1·0	26	10 „ 60, 14 to 80 ..	1·0	56	1 „ 60, 3 „ 80
1·0	32	7 „ 60	1·2	34	3 „ 50
1·0	36	8 „ 60	1·2	44	2 „ 60, 2 „ 80
1·0	38	2 „ 60, 5 „ 80 ..	1·2	50	1 „ 60, 3 „ 80
1·0	43	1 „ 60	1·2	56	1 „ 60, 2 „ 80
1·0	48	13 „ 60	1·2	58	1 „ 60, 3 „ 80
1·1	37	4 „ 60, 6 „ 80 ..	1·2	64	– „ 60, 1 „ 80
1·1	54	3 „ 60, 7 „ 80 ..	1·2	64	– „ 60, 3 „ 80
1·2	40	8 „ 60, 14 „ 80 ..	1·2	71	– „ 60, 3 „ 80
1·5	43	8 „ 60, 16 „ 80 ..	1·2	74	– „ 60, 3 „ 80
1·5	45	5 „ 60, 10 „ 80 ..			
1·5	46	2 „ 60, 8 „ 80 ..			
1·5	54	1 „ 60, 5 „ 80 ..			

Under very severe conditions in New Guinea—such that up to 250 *farauti*—not to mention Culicines—could be caught in half an hour by sweeping around oneself with a butterfly net, the first bite might be received about fifteen to twenty minutes after application, but this was for routine use of the repellent during other duties and no particular care was taken to prevent its removal by contact with the clothing or, for instance, when wiping perspiration from the forehead. After the first bite, valuable protection was given, at least up to 45 minutes, and generally much longer. Occasional bites were received, but exposure of an untreated surface rapidly demonstrated that the high degree of protection still persisted. Resmearing, without further addition of repellent, as suggested by U.S.D.A. workers, often conferred fresh protection for a time. Under less severe conditions, protection for two or three hours or more, has been observed.

Dimethyl phthalate is colourless, almost odourless and not particularly unpleasant to use, even under hot and humid conditions which result in excessive perspiration. Immediately after application it causes a moderate, but short-lived, stinging of the skin of the face in some people. It is definitely unpleasant to taste, and if accidentally introduced into the eyes causes severe pain. But after many months of its continued employment (twice-nightly applications) by tens of thousands of men, no reports of dermatitis and few complaints of irritation resulting from it, had been received. This confirms the opinion expressed as a result of skin tests in January, 1943, by Col. A. Dawson, Consultant Dermatologist, A.A.M.C.

(*b*) *Mixtures containing dimethyl phthalate.*—Owing to the restricted availability of the raw materials required for the manufacture of dimethyl phthalate, and the necessity for conserving supplies, various mixtures containing 75 per cent. or less were tested, both in the laboratory and in the field. Table 3 shows the average time to the first bite obtained in a series of simultaneous tests of dimethyl phthalate and various mixtures containing it using two-sleeved cages and *A. aegypti.*

TABLE 3.—RESULTS OF SIMULTANEOUS TESTS IN TWO-SLEEVED CAGES USING *A. aegypti.*

—	Average Time to First Bite.
	minutes.
Me_2 phthalate	162
75 per cent. Me_2 phthalate + 25 per cent. Bu_2 phthalate	166
Me_2 phthalate	153
75 per cent. Me_2 phthalate + 25 per cent. EtOH	121
Me_2 Phthalate	97
60 per cent. Me_2 phthalate + 40 per cent. EtOH	85
Me_2 phthalate	125
50 per cent. Me_2 phthalate + 50 per cent. EtOH	105
Me_2 phthalate	100
40 per cent. Me_2 phthalate + 60 per cent. EtOH	58

There was no difference in these tests between pure dimethyl phthalate and 75 per cent. diemthyl phthalate + 25 per cent. dibutyl phthalate, but this mixture would not have eased the supply position with regard to phthalic anhydride. Mixtures containing dimethyl phthalate and 25, 40, 50, and 60 per cent. alcohol were all inferior to undiluted dimethyl phthalate. Field tests with dimethyl phthalate containing 25 and 33 per cent. alcohol confirmed these results. It was considered unlikely that any appreciable economy in ingredients without loss of protective power could be achieved by dilution, so this line of investigation was not pursued. Other mixtures tested and found to be less repellent, if only slightly so, than the pure material were—

Dimethyl phthalate 50 per cent. + diethyl phthalate 50 per cent.
Dimethyl phthalate 50 per cent. + dibutyl phthalate 50 per cent.
Dimethyl phthalate 50 per cent. + oil of *Backhousia myrtifolia* 50 per cent.
Dimethyl phthalate 50 per cent. + 20 per cent. diethyl phthalate + 20 per cent. oil of *Backhousia* + 10 per cent. Huon pine oil.

Mixtures found no more repellent than dimethyl phthalate were—
Dimethyl phthalate + bentonite (2·0 per cent. by weight).
Dimethyl phthalate + talc (2·0 per cent. by weight).
Dimethyl phthalate + 0·1 per cent. pyrethrins.
Dimethyl phthalate applied to the skin after a coating of petroleum jelly.

(ii) *Mixtures Receiving Special Attention.*

(*a*) *Methyl ethyl phthalate.*—Except for dimethyl phthalate, this is the only phthalate tested which gave promising results. Against *A. aegypti* it gave almost as good protection as dimethyl phthalate, although in New Guinea it was shown to be somewhat less effective.

(*b*) *Staway lotion.*—Staway gave good protection in both cage and field tests, although it was not quite as effective as dimethyl phthalate. In particular, it did not appear to give as long a period of partial protection after the first bite as dimethyl phthalate. The least favourable results from about 25 tests in the field in New South Wales are shown in Table 4.

Staway is pleasant to apply to the skin, but it was eliminated from later tests because American reports (unpublished) indicated that its regular use over extended periods might not be free from health hazards.

TABLE 4.—RESULTS WITH STAWAY: 1·0 ML. APPLIED FOR ALL TESTS.

Head.		Arm.	
First Bite.	Total Bites During Test.	First Bite.	Total Bites During Test.
min.	min.	min.	min.
20	5 to 60	26	12 to 60
20	4 to 40	32	19 to 45
27	4 to 47		

Butyl carbitol acetate, the main ingredient of Staway and present in 50 per cent. concentration, gave a protection time very similar to that of the lotion.

(*c*) 612* (*2-ethylhexane*-1, 3-*diol.*).—612 gave excellent protection in laboratory tests against *A. aegypti,* providing the same average repellent time as dimethyl phthalate when both were tested simultaneously. In field tests in New Guinea, the impression was gained that 612 was slighly inferior, but extensive tests were not carried out because it was understood that this material could not be produced in Australia. 612 has a fairly pungent, although not unpleasant, smell, and slight stinging was recorded after application to sunburnt or tender skin. However, no other signs of irritation were observed.

(*d*) *Indalone.*—Indalone, and various mixtures containing it, gave varying results, but conferred protection inferior to that of dimethyl phthalate. Against Anophelines it gave poor protection in New Guinea, and allowed important biting at twenty minutes in cage tests at Cairns. Indalone caused transient stinging when applied to tender skin.

(*e*) *Pyrethrum preparations***.—Pyrethrum preparations gave very poor protection under all conditions of test. This is in agreement with American results (unpublished), but contrary to evidence obtained by British workers (unpublished).

Preparations tested were: 6·6 per cent. w/v pyrethrins†, 1·0 per cent. w/v pyrethrins in gum tragacanth, 0·1 per cent. w/v pyrethrins in a paraffin paste with and without added citronella, British pyrethrum cream‡, and MacNay's formula§.

They were rarely exposed to severe conditions in the field, but, under biting frequencies of 10 per arm per minute or less, they consistently failed to give protection comparable to that of oil of citronella. The tragacanth mixture on one occasion allowed thirteen bites on face and hands within ten minutes of application. In New Guinea, not only did the British pyrethrum cream give a very much shorter time to the first bite than dimethyl phthalate, but its period of even partial protection had ended by the time the first bite was received on dimethyl phthalate.

It was characteristic of pyrethrin preparations that some mosquitoes would pierce the skin and then leave without sucking blood. Many mosquitoes were visibly affected in cage and tent tests, not only after biting, but even without making contact. While these observations indicate that pyrethrin preparations are toxic, an infected mosquito would transmit infection before the toxic effect became operative, since it has been definitely proved that malaria can be transmitted by simple probing without the mosquito actually sucking blood.

(*f*) *Oil of Dacrydium franklinii* (Huon pine).—Huon pine oil gave the best protection of all the essential oils tested, and was considered to be as effective as dimethyl phthalate. It suffers, however, from the serious drawbacks that, for many people, it is irritant to the skin of the face, and that, when applied to the face, it causes nausea in a high

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* National Carbon Co. Inc., U.S.A.

** The pyrethrin content of these preparations was checked by analyses after they had been used.

† Stafford Allen's M225.

‡ Imperial Chemical Industries' Mark I. 1 May, 1943.

§ See Appendix, page 28.

proportion of test personnel. Vomiting within half an hour after application was recorded more than once. The oil is light-yellow and has, for people who have never used it, a pleasant smell. The nausea and good repellent properties are due to its high content (95 per cent.) of methyl eugenol, since pure methyl eugenol has exactly these same properties.

Much of the later work with Huon pine oil was devoted to attempts to avoid its nauseating effects. These attempts were either unsuccessful, or its efficiency as a repellent was greatly reduced. The tests were carried out with five fractions obtained by distillation, and with various mixtures of the whole oil with beeswax and with oils of *Eremophila, Melaleuca, Zieria, Backhousia,* citronella, and certain residues of *Eucalyptus* oil distillation.

Caged mosquitoes which had been used for tests with Huon pine oil were often affected after exposure of treated limbs. Spray tests (Waterhouse, unpublished) also showed that the oil was toxic to mosquitoes. Huon pine oil is produced in commercial quantities from the sawdust of Tasmanian mills handling Huon pine timber. Large quantities are, therefore, potentially available.

(*g*) *Oil of Melaleuca bracteata**.—Of the oils of six species of tea-tree (*Melaleuca*) tested, only one, *M. bracteata,* showed great promise, while the others gave poor protection. The oil of *M. bracteata* differs from that of other Melaleucas tested in that it is the only one to contain methyl eugenol, which is present to the extent of about 80 per cent. Although this oil has quite a different odour from that of Huon pine oil, it also causes nausea, and is somewhat irritant to apply.

(*h*) *Oil of Zieria smithii**.—*Zieria* oil has given good results under all conditions of test, protection of 45 minutes or more often being obtained. Although it has a strong smell and is slightly irritant to a tender skin, it does not cause nausea. Its principal constituent is elemicin, although it contains a small amount of methyl eugenol. Fractional distillation produced an ineffective 2nd fraction consisting of pinene and carene epoxide and a very effective 5th fraction which was found to be mainly methyl eugenol.

Test mosquitoes were effected by prolonged exposure to *Zieria* oil which proved toxic to them in spray tests.

(*i*) *Oil of Backhousia myrtifolia**.—*Backhousia* oil provided very good protection under many conditions of test, and probably owes its effectiveness to its high content of elemicin (80 per cent.). It did not produce nausea and was only slightly irritant. Like the three other essential oils shown to be good repellents, it affected the mosquitoes during tests and proved toxic when used in the form of a spray.

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* These oils are not produced commercially although some hundreds of gallons could be distilled annually. *Zieria* and *Backhousia* occur more commonly than *M. bracteata,* but none of these species grows naturally in stands sufficiently dense or extensive to permit large-scale exploitation. *M. bracteata* (Myrtaceae) is a tall shrub which grows on the north coast and on the north-western slopes in New South Wales. *B. myrtifolia* (Myrtaceae) is a small tree, widely distributed along creeks in brush forest in northern New South Wales and southern Queensland. *Zieria smithii* (Rutaceae) is a shrub which is widely distributed in small stands on the east coast of Australia.

(*j*) *Oil of citronella and citronella preparations.*—After early experiments, in most of which biting occurred in less than 30 minutes under poor biting conditions, oil of Ceylon citronella was not tested very extensively. Citronella oil from Java was about as effective as oil from Ceylon, as was also a fraction obtained by distilling the Ceylon oil. Two "semi-natural" citronella oils, which were produced by a commercial firm by blending appropriate locally available constituents obtained mainly by distillation of *Eucalyptus* oils, gave protection equal to that of Ceylon citronella, but caused rather more irritation to the skin. In our tests, the citronella cream was never exposed to very severe conditions, but even so, gave very little protection, the first bite in the field tests often occurring in less than five minutes, and nuisance biting in less than fifteen minutes.

Similarly, the addition of castor oil, cedarwood oil, or petroleum jelly in various other proportions to citronella oil did not provide any evidence that the repellent properties of essential oils were prolonged by the addition of non-volatile substances. The impression gained was that the presumed fixatives reduced protection by diluting the repellent ingredients.

(iii) *Inferior Repellents.*

Details of results may be obtained from Table 1. These materials were shown to be slightly to markedly inferior to the better repellents already discussed.

(*a*) *Synthetics and pure chemicals.*

Diethyl phthalate*.
Dibutyl phthalate.
Diamyl phthalate.
Dicapryl phthalate.
Methyl salicylate.
Butyl salicylate.
Amyl salicylate.
Methyl stearate.
Benzyl benzoate.
DDT (pp'-dichlordiphenyltrichlorethane).
Coumarin.
Furfural.
25 per cent. creosote 259† in lighting kerosene.
Amyl alcohol ester of coconut fatty acids.
Magnesium sulphate (Epsom salts).

The fact that methyl stearate gave no protection disposes of a suggestion that effectiveness of some good repellents might be due to their hydrolysis in contact with moist skin resulting in the slow liberation of methyl alcohol. Methyl stearate would break down in this fashion far more readily than the repellents in question.

(*b*) *Plant products.—Tea-tree oils* (*Melaleuca sp.*).—With the exception of *M. bracteata,* all tea-tree oils tested were ineffective.

* After the tests recorded in Table 1 had been completed it was discovered that the sample of diethyl phthalate used was highly impure. At this stage it was only possible to test a pure sample against *A. aegypti.* Protection for over 60 minutes was obtained in cage tests.

† Timbrol's Ltd., New South Wales.

Eucalyptus oils.—Eight *Eucalyptus* oils, four residues from *Eucalyptus* oil distillation, a fraction rich in alcohols, and the following individual constituents:—cineol, citronellal, citronellol, geraniol, and phellandrene, were ineffective.

Sassafras oils.—*Ol. sassafras* and the oils from *Doryphora sassafras* and *Atherosperma moschatum,* both known as sassafras in Australia, were tested. The two Australian oils gave fair protection but were irritant to the skin.

Oil of spike lavender gave poor protection. In a field test under moderate conditions five bites were received on an arm during a three-minute exposure from twelve to fifteen minutes after application.

Other ineffective oils tested included: *Anyme, Callitris glauca* (wood and leaf), *Eremophila mitchelli* ("Buddah"), sandalwood, *Tagetes,* and various mixtures shown in Table 1. Evodionol, Nerolin dissolved in *M. linariifolia* oil, and castor oil 50 per cent. + kerosene 50 per cent., gave very poor protection.

A series of repellents prepared by Dr. F. N. Lahey was also found to be ineffective. The main purpose of the series was to determine the repellent properties of lauryl alcohol and pseudo-ionone. In addition to 30 per cent. solutions of these in alcohol, more complex mixtures containing a "bitter principle"—quinine hydrochloride or *Alstonia* extract—were used. In two preparations, colophony resin softened with castor oil was included in the hope that this might produce a fairly permanent film of the repellent. Finally (S1 and S2) preparations of citronella and soap and of piperitone, sandalwood oil, sassafras oil, and soap, were included in the series. None of these mixtures showed any promise in field tests in Queensland.

(*c*) *Proprietary Mixtures.*—The proprietary mixtures tested are listed in Table 1, and information concerning their composition can be found in the Appendix. All allowed early biting, with the exception of the moderate protection given by the Yarmor pine oil mixture, and the lotion containing dimethyl phthalate.

IV. THE USE OF MOSQUITO REPELLENT IN THE FIELD

Dimethyl phthalate has been in general use in New Guinea since March 1943 and undoubtedly performed a great service in the field, but a number of factors have tended to prevent the realization of full benefits:—

(i) In highly malarious regions, frequently no obvious mosquito nuisance existed. Biting was often imperceptible so that the use of repellent depended on discipline unassisted by the need for protection against nuisance biting.

(ii) The dosage applied was often lighter than that required for full protection. Men frequently used not more than 2 fluid ounces per month, which is equivalent to about 1 ml. per application to head and hands. This was possibly because on sweat-moistened skin, a feeling of complete cover was afforded by the lighter dosage. The applications recommended were at sundown and two hours later. Only troops with a pre-dawn stand-to applied repellent then.

(iii) Protection was sometimes doubted, because mosquitoes were seen to hover over, alight on, or bite, treated skin. Doubt sometimes resulted in failure to apply the repellent, since men did not realize that, for every bite occurring in spite of its use, great numbers were prevented.

(iv) A minor disadvantage of dimethyl phthalate is its ability to dissolve many plastics, such as unbreakable watch "glasses", fountain pens, and spectacle rims.

Virtues in which it overwhelmingly excels essential oils are—

(*a*) it is almost completely odourless, and

(*b*) it has very little or no irritant effect on the skin of the face and hands, and is not unpleasant to use even under very humid conditions.

V. TREATMENT OF CLOTHING WITH MOSQUITO REPELLENT

In February 1943, information was received from the United States that one application of dimethyl phthalate would prevent mosquitoes biting through treated shirts for several days.

In dense populations of *Aedes vigilax,* mosquitoes make almost unbearable attacks through the heaviest army shirts and trousers. Under such conditions, it was found that 8 ml. of dimethyl phthalate distributed over the shirt, and 12 ml. on the trousers (in each case confined to the area where the body would be covered by a single thickness of material) allowed negligible biting at 48 hours, and extremely limited biting at 72 hours after application.

Method of Application.

The method preferred was by shaker-bottle prepared in the following manner:—The metal screw-on top of a bottle, small enough to be held in one hand, was punctured by about 25 holes approximately 0·01 inches in diameter. Dimethyl phthalate, and several times its volume of water, were shaken together to give a temporary emulsion, which was maintained by the shaking required to expel the liquid. Distribution of the liquid from this type of shaker was good, and dosage could be accurately controlled.

Methods considered unsatisfactory for applying the repellent were the household atomizer and the bottle sprayer, as used by barbers, which along with other disadvantages, deliver the liquid respectively too slowly and too fast for the dosage required.

DDT as a Repellent on Clothing.

Observations by one of us (D.F.W.) in New Guinea, more recently confirmed by Roberts (unpublished) though contrary to British and American experience, showed that the mosquitoes encountered preferred not to rest on surfaces treated with DDT where untreated surfaces were available to them. The repellent effect was not important enough to prevent considerable biting through DDT-treated mosquito nets spread over untreated skin. It was noted also that such feeding was not followed by a high mortality among the mosquitoes exposed.

VI. OBSERVATIONS ON THE USE OF REPELLENTS AGAINST INSECTS OTHER THAN MOSQUITOES

In the course of repellent experiments, it was found that dimethyl phthalate and Huon pine oil were both effective repellents for Tabanids (March flies), and for sand flies, both *Culicoides* sp. (Chironomidae) and *Austrosimulium bancrofti* Tayl. (Simuliidae).

None of the effective mosquito repellents, however, gave any protection against the non-biting bush fly, *Musca vetustissima* Walk., which is exceedingly annoying in many parts of Australia during the warmer months of the year. Large numbers of bush flies are attracted to the face, where they seek moisture from the lips, nose, and eyes, in a most persistent and irritating fashion.

It is interesting to note that oils of Huon pine and *Zieria* are at least as promising as Ceylon citronella as deterrants to oviposition for the sheep blowfly *Lucilia cuprina* (Waterhouse, unpublished). Among other oils, which were also used in the present tests, *M. ericifolia, M. linariifolia, E. mitchelli,* and various *Eucalyptus* oils, were not effective against *L. cuprina.*

VII. ACKNOWLEDGMENTS

In the course of these studies, help was asked of, and most freely given by, a great many people. We are particularly indebted to Mr. A. R. Penfold, Professor J. C. Earl, Dr. A. Albert, and Dr. F. N. Lahey for their advice and preparation of materials for test; and to W. Hermon Slade and Co. Ltd., Stanco (Aust.) Pty. Ltd., and Cox Findlayson and Co. We also wish to thank the many volunteers who assisted in carrying out the repellent tests, often with much personal discomfort. Our thanks are, finally, due to the Director-General of Medical Services, Major-General S. R. Burston, for permission to publish the work.

APPENDIX

Information on the Composition of Substances Tested

A. Essential Oils.

Percentage composition approximate only. Data supplied by Mr. A. R. Penfold, Director, and Mr. F. R. Morrison, Chief Chemist, Technological Museum, Sydney.

Anyme Oil (New Caledonia).
Unidentified sesquiterpene alcohols.

Backhousia myrtifolia Hook. and Harv.
elemicin, 75–80 per cent.
pinene.
sesquiterpenes.
unidentified alcoholic and phenolic bodies.

Callitris glauca R. Br. (leaf oil).
borneol, 5–10 per cent.
bornyl acetate, 16–20 per cent.
pinene, limonene, and dipentene, 70 per cent.

Callitris glauca (wood oil).
guaiol, 50 per cent.
d-citronellic acid (Callitrol), 50 per cent.

Ceylon Citronella.
citronellal, 12 per cent.
geraniol, 30 per cent.
terpenes, 12–15 per cent.

Ceylon Citronella 2nd Fraction.
citronellal, about 25 per cent.
geraniol, about 75 per cent.

Java Citronella Oil.
citronellal, 35–50 per cent.
geraniol, 35–45 per cent.
methyl eugenol, about 1 per cent.
citral } trace.
sesquiterpene }

Eremophila mitchelli Benth.
eremophilone.
2-hydroxyeremophilone.
2-hydroxy-2-dihydroeremophilone.

Eucalyptus Oils—

E. dives Schav. type.
piperitone, 40–50 per cent.
commercial phellandrene, 40 per cent.
alcohol esters, terpineol, sesquiterpene.

E. dives type + 10 per cent. fixative.
fixative was eudesmol (from *M. uncinata*).

E. dives var. C.
cineol, 75 per cent.
citral, 3–5 per cent.
terpineol.

E. dumosa A. Cunn.
cineol, 50–60 per cent.
pinene, 20–30 per cent.
aromatic aldehydes—
cuminal,
cryptal,
phellandral, &c.

E. oil rich in terpineol.
a residual oil.
terpineol, over 70 per cent.
unidentified alcohol.

E. phellandra R. T. Bak. and H. G. Smith.
cineol, 30–40 per cent.
commercial phellandrene, 40 per cent.
terpineol.

E. polybractea R. T. Bak.
cineol, 80–90 per cent.
pinene
sesquiterpenes—
aromadendrene.
eudesmene.
aromatic aldehydes, 2–3 per cent. (see above).
australol, 1 per cent.

Huon Pine Oil (*Dacrydium franklinii* Hook.)
methyl eugenol, 90–95 per cent.
eugenol, 1 per cent.
pinene and terpenes.

Mixtures (*Dr. Lahey*)—

L1	lauryl alcohol	30·0
	alcohol to	100·0
L2	lauryl alcohol	30·0
	quinine hydroch.	10·0
	alcohol to	100·0
L3	lauryl alcohol	30·0
	Alstonia ext.	5·0
	dilute HCl to dissolve	
	castor oil	5·0
	colophony	3·0
	alcohol to	100·0
L4	lauryl alcohol	30·0
	Alstonia ext.	10·0
	dilute HCl to dissolve	
	alcohol to	100·0
P1	Pseudo-ionone	30·0
	alcohol to	100·0
P2	Pseudo-ionone	10·0
	Alstonia ext.	5·0
	dilute HCl to dissolve	
	castor oil	5·0
	colophony	3·0
	alcohol to	100·0
S1	citronellal	30·0
	alcoholic soap solution to	100·0
S2	piperitone	10·0
	Australian sandalwood oil	10·0
	sassafras oil	10·0
	alcoholic soap solution to	100·0

Alcoholic soap solution.

Sapo alcoholicus A.P.F. using an alcoholic solution of *Alstonia* extract.

Pyrethrum preparation—

MacNay's Formula—

oil of thyme ..	½ fl. oz.
extract pyrethrum (about 3·5 per cent. w/v) ..	1 fl. oz.
castor oil ..	2–3 fl. oz.

Sandalwood Oil (W.A.) (Eucarya spicata (R.Br.) Sprague and Summ.)

santalol, up to 70 per cent.
unidentified sesquiterpene alcohols.

Sandalwood Resin Oil.

unidentified sesquiterpene alcohols + sandalwood resins.

Sassafras Oils—

Atherosperma moschatum Labill.
pinene, 15–20 per cent.
camphor, 15–20 per cent.
methyl eugenol, 50–60 per cent.
safrol, 5–10 per cent.

Doryphora sassafras Endl. (Monga).
safrol, 60–65 per cent.
camphor, 10–15 per cent.
△'α–pinene, 10 per cent.
sesquiterpenes, 10 per cent.
eugenol, 1 per cent.

Ol. sassafras (U.S.P. *Sassafras officinale*).
safrol, 90 per cent.

Spike Lavender.
borneol.
cineol.
camphor.
camphene.
linalool.

Tagetes glandulifera Schrank.
tagetone.

Tea-tree Oils—

Melaleuca alternifolia Cheel.
α–, and γ–terpinene, cymene, 50–60 per cent.
cineol, 6–8 per cent.
△'–terpinenol–4, 25 per cent.
sesquiterpenes (cadinene) and sesquiterpene alcohols.

M. bracteata F. v. M.
methyl eugenol, over 80 per cent.

M. ericifolia Sm.
linalool, 25–40 per cent.
cineol, 10–20 per cent.
pinene.
sesquiterpene.

M. leucadendron L., Queensland (*?viridiflora*).
cineol, 50 per cent.
terpineol, 25–30 per cent.

M. leucadendron, New South Wales (Terrigal).
nerolidol, 80 per cent.

M. linariifolia Sm.
α– and γ–terpinene, cymene, pinene, 50–60 per cent.
cineol, 16–20 per cent.
terpinenol–4, 20 per cent.
sesquiterpenes.

M. uncinata R.Br.
eudesmol, 20–25 per cent.
cineol, 50 per cent.
pinene, 20 per cent.

"Thymol Terpenes."
thymol, sesquiterpenes.

Zieria smithii Andr.
carene epoxide, 15 per cent.
safrol, methyl eugenol, elemicin, 70 per cent.
pinene and sesquiterpenes.
eugenol, 1 per cent.

Zieria 2nd Fraction.
pinene and linalool, 50 per cent.
carene epoxide, 50 per cent.

Zieria 5th Fraction.
methyl eugenol, 85 per cent.

B. Proprietary Repellents.

Stuxay.
diethylene glycol monobutyl ether acetate (butyl carbitol acetate), 50 per cent.
diethylene glycol monoethyl ether (carbitol), 15 per cent.
corn oil, 7 per cent.
ethyl alcohol, 28 per cent.

No. 1, Yarmor pine oil, (Ex U.S.A.), 40 parts.
pyrethrum extract, 1 part.

No. 2, $MgSO_4$, 95 per cent., gelatin, 5 per cent. (1 oz. of mixture in ½ cup of water and apply to skin.)

Nos. 3–6.
All contain at least 10 per cent. naphthalene. No. 3 was in a paraffin (vaseline) base; the others in various cold and vanishing creams.

No. 7, Pyrethrum, derris, ortho-dichlorbenzene, kerosene, turpentine, methyl salicylate.

No. 8, Alcohol in place of turpentine.

No. 9, 25 per cent. dimethyl phthalate.

No. 10, 25–30 per cent. diethyl phthalate.

No. 11, 30–35 per cent. dimethyl phthalate.

No. 12, Ol. lavender, 2 lb.
methyl salicylate, 1 lb.
formaldehyde, 8 oz.
camphor, 1 lb.
methylated spirits, 1 qrt.
E. citriodora, 1 lb.
citronella, 1 lb.
In a base of—
stearic acid, 5 lb.
paraffin wax, 2 lb.
triethanolamine, 8 oz.
gum tragacanth, 12 oz.
kerosene, 1 gal.
water, 1 gal.

No. 13, methyl eugenol, 1 per cent.
aqueous suspension of alkaline earth silicates, 99 per cent.

J. J. Gourley, Government Printer, Melbourne.

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NOTES ON THE TERRESTRIAL ECOLOGY OF THE FIVE ISLANDS. I.

By Consett Davis, M.Sc., Linnean Macleay Fellow of the Society in Zoology; M. F. Day, B.Sc.; and D. F. Waterhouse, B.Sc.
(From the Zoology Department, Sydney University.)

(Plates xv–xix; eight Text-figures.)

[Read 26th October, 1938.]

Introduction.

The absence of any synecological study of the plant and animal complex (biome) of a terrestrial community in Australia has made impossible the practical illustration in this country of certain well-known ideas, which form the basic principles of animal ecology. In particular, this lack has handicapped the teaching of animal ecology, and has prevented the subject receiving the attention which it merits. With this in mind, the present survey was undertaken rather with a view to illustrate general principles with local examples than to develop any special theory.

The Five Islands, near Port Kembla, N.S.W., were chosen as the location for this survey, because certain factors peculiar to islands appeared to be intrinsically interesting, and also because it was hoped that the terrestrial life of these islands represented a more or less self-contained unit. Belief that we were dealing with a microcosm was soon shattered, and in this paper are set out the numerous ways in which the chosen area fails to represent a 'closed' community. It is certain, however, that these interactions with extraneous communities are far less numerous than would be the case if a mainland area had been selected.

In this paper are discussed the geological and physiographical history of the islands; the climate; the plant ecology; and the various ways in which the islands fail to represent 'closed' communities. The departure from the ideal closed community is due to products of littoral and pelagic communities, and of terrestrial communities from the mainland, becoming involved in the food-chains of the terrestrial life of the islands. Habitats for terrestrial animals, other than those embraced by communities of vascular plants, are also listed. Subsequent papers will comprise lists of all animals collected, with notes on habitat, numbers, food and enemies; from these lists it is hoped that food-chains may be deduced. Later it may be possible to compare these islands with other coastal islands.

General Description of the Five Islands.

Figure 1 shows the general disposition and size of the islands, which we have designated I–V, referring to each island subsequently by the number alone. Certain features are named on Figure 2, the names being bestowed by us for convenience. I, known locally as Big Island or Rabbit Island, is 30 acres in extent, and reaches

a height of 71 feet;[1] it is separated from the mainland at Red Point by only 500 yards, with a reef in intermediate position. Communities of vascular plants[2] occupy 57% of I. II lies to the east of I, and is connected thereto by a low isthmus, wave-swept at high tide during rough weather. II is 18 acres in extent, 49 feet at the highest point, with 45% covered by vascular plant communities. On some maps it is marked as Perkins Island. III, which lies less than 150 yards south-east of II, is 6 acres in extent, 53 feet at the highest point, with only 14% of its area occupied by communities of vascular plants, which are confined to the higher parts of its western half. IV, about 2 miles north of I, and 1¾ miles from the nearest point of the mainland, is 7 acres in extent, 43 feet at the highest point, with 15% of its area occupied by vascular plant communities. It is the island most sheltered

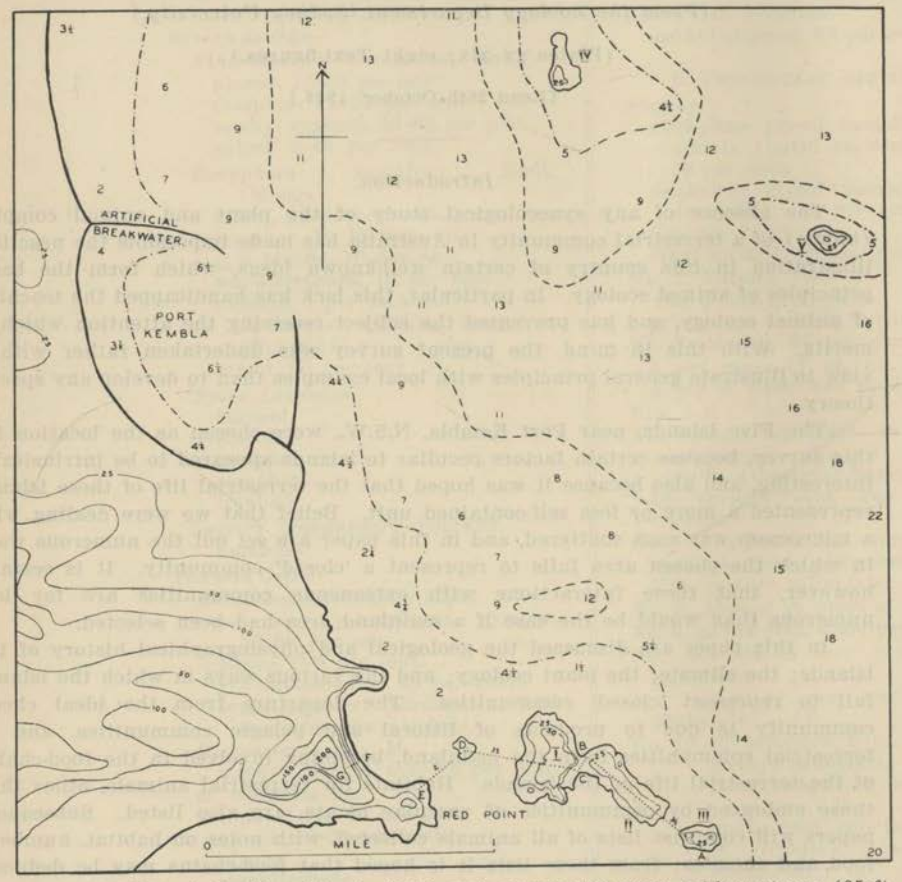


Fig. 1.—Map of the Five Islands (I-V) and surrounding parts, with contours (25-ft., 50-ft., thence at 50-ft. intervals), and ocean soundings (fathoms, low tide). A-B-C-D-E, F-G, line of section for Fig. 6. D represents Midway Reef. —·—·—·—·—, Approximate position of 5-fathom isobath. — — — — — — —, Approximate position of 10-fathom isobath.

[1] Heights are reckoned from mean tide level, areas as those exposed at mean tide level.

[2] All vascular plant communities, including the sparse *Scirpus nodosus* community, with low percentage cover, are included in this figure.

from southerly storms, which produce the heaviest seas to which the islands are subjected. V, the most exposed island, which lies 1 mile to the south-east of IV and 2 miles from the nearest part of the mainland, is 5½ acres in extent, 52 feet at the highest point, with about 3% covered by vascular plant communities. IV and V are referred to on most charts as the Tom Thumb Islands.

In Figure 1 are also indicated 25- and 50-feet contours, and soundings near the islands, with 5- and 10-fathom isobaths. Figures 2–4 give detailed (10-ft.) contours; those on I–IV are fairly accurate, the heights having been surveyed with pole and level and plotted in the field on vertical aerial photographs (scale 10·56 inches to the mile). The contours on V are to be regarded as form-lines, constructed, with the aid of aerial photographs, from a knowledge of the maximum height of the island (obtained trigonometrically), and its profile from several different aspects. Only a few short visits have been made to V, which is difficult of access.

Vertical aerial photographs of the islands are given in Plate xv (from 5,000 ft.), and, for I–III and the coast adjacent, in Plate xvi (from 12,000 ft.). The photographs comprising Plate xv were taken with the sun in approximately north-eastern position, while in Plate xvi the sun was approximately north-west. Shadows indicate various topographic features.

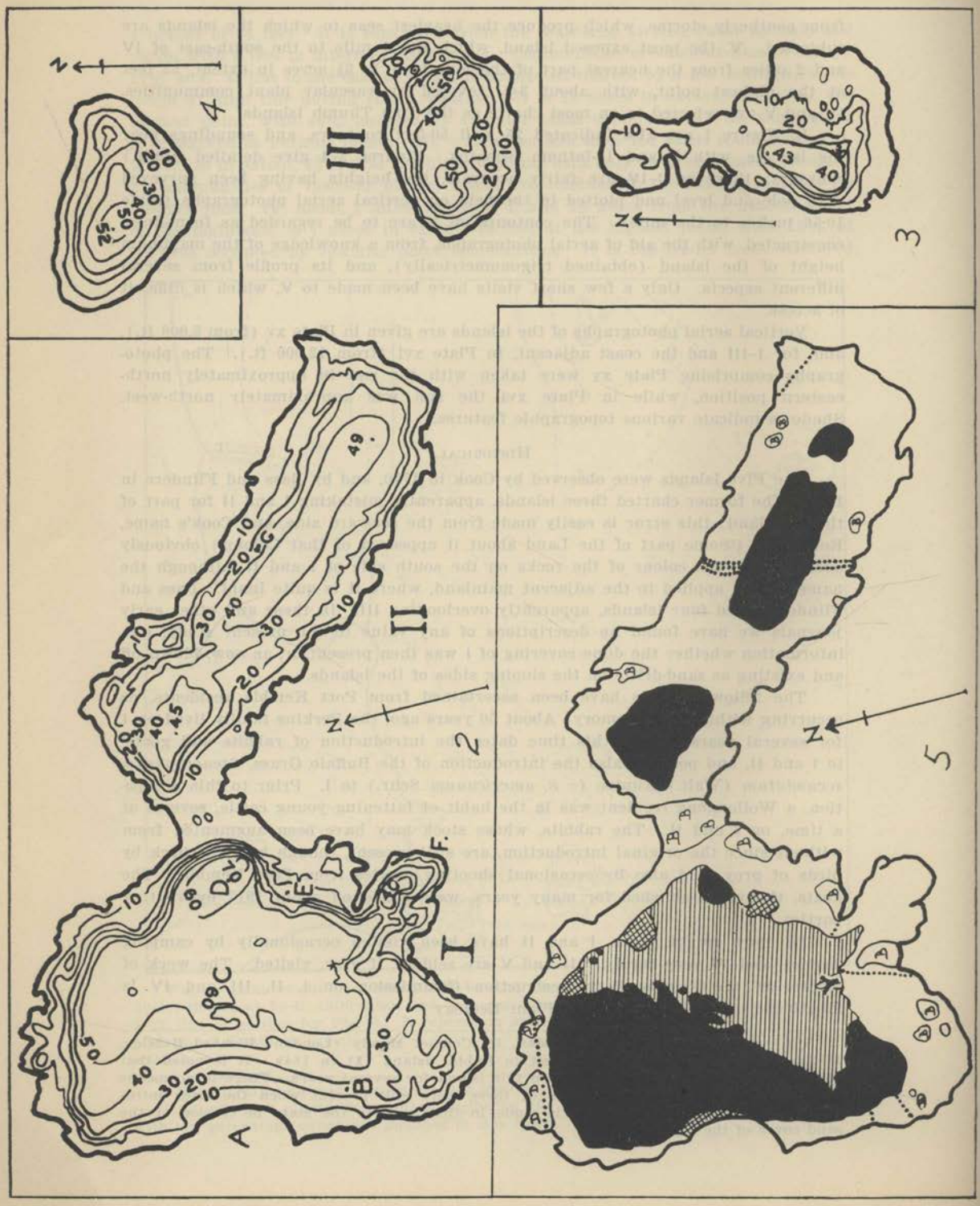
Historical.

The Five Islands were observed by Cook in 1770, and by Bass and Flinders in 1793. The former charted three islands, apparently mistaking I and II for part of the mainland; this error is easily made from the seaward side, and Cook's name, Red Point ('Some part of the Land about it appeared of that Colour') obviously derives from the colour of the rocks on the south side of I and II, although the name is now applied to the adjacent mainland, where it is quite inapt. Bass and Flinders noted four islands, apparently overlooking III. In these and other early journals we have found no descriptions of any value to the present study, e.g., information whether the dune covering of I was then present or, as now, blown off and existing as sand-drifts on the sloping sides of the islands.

The following facts have been ascertained from Port Kembla residents, as occurring within their memory: About 50 years ago, the Perkins family lived on I for several years. From this time dates the introduction of rabbits and goats[3] to I and II, and possibly also the introduction of the Buffalo Grass, *Stenotaphrum secundatum* (Walt.) Kuntze (= *S. americanum* Schr.) to I. Prior to this occupation, a Wollongong resident was in the habit of fattening young cattle, several at a time, on I and II. The rabbits, whose stock may have been augmented from without since the original introduction, are still present, though kept in check by birds of prey, and also by occasional shooting parties from Port Kembla. The goats, though established for many years, were killed off about 1917 by visiting parties.

In more recent years, I and II have been visited occasionally by camping parties, and IV very rarely. III and V are seldom, if ever, visited. The work of officers of the Prickly Pear Destruction Commission on I, II, III and IV is mentioned under the heading of Plant Ecology.

[3] In an early work, 'Our Antipodes', by Colonel Mundy (London: Richard Bentley, 1852), there is a reference to a visit to Rabbit Island (I) in 1849. It is noted that rabbits and goats had been present on this island for several years. There is no means of finding out whether descendants of these were still present when the later introductions occurred. No reference is made in this book to the state of erosion of the sand cover of the island.


N
4
III
3
II
2
5
I
A
B
C
D
E
F.

Reports of Port Kembla residents indicate that no trees have been growing on any of the islands, and that the dune covering of I has been more or less in its present state during the last 50 years. It is probable that the islands never supported trees at any time.

Geology.

Islands I, II, III and V are composed of Dapto-Saddleback Dolerite, which Harper (1915) considers to be a submarine flow of Upper Permian age. The evidence for this is that the dolerite, which lies above sedimentary rocks of the Upper Marine series, is overlaid in the Albion Park district by rocks of similar age containing marine fossils. The dolerite forms an extensive flow on the mainland, the islands being almost certainly remnants of this continuous flow. The lower level of the dolerite—that is, its junction with Upper Marine sedimentary rocks—is of irregular horizon; thus, although the dip of the Upper Marine and all other strata in this district is to the north and west, the lower limit of the dolerite is concealed below sea-level on I, II, III and V, but is more than 50 feet above sea-level at Red Pt., immediately to the east of I. Midway Reef, between I and Red Pt., is composed of Upper Marine argillaceous sandstone, reaching about eight feet above mean tide level; the dolerite has here been removed by erosion, but its lower level must have been above this height. These levels are indicated on Figure 6. They may be explained by faulting, or (as in Fig. 6) by an irregular surface for the Upper Marine at the time of extrusion of the dolerite. The latter sequence is more probable, although, if we follow Harper, this irregularity could only have been due to submarine erosion. The fact that the lower level of the dolerite is below sea-level on the mainland less than a mile north of Red Pt., i.e. lower than the dip of the underlying strata would account for, supports the view that the dolerite was poured out on an irregular surface. An alternative possibility is that the dolerite is not a flow, but an irregular sill. The possibility of small faults between Midway Reef and I, and between III and V, cannot be ruled out entirely, but defies proof, as the critical points are submerged.

The distribution of the different forms of dolerite on I and II is illustrated in Figure 5. The normal rock corresponds to Type A of Browne and White (1929, Fig. 1),[4] whilst variants corresponding to Types B and D of these authors also occur on I and II. These variants seem to weather more readily than the normal type. The isthmus between I and II is composed of Type B, which possibly also occurs (submerged) between II and III. Wherever the variants occur elsewhere they are associated with ravines, inlets or large rock-pools. Tertiary basic dykes are also present on I and II, and appear to be zones of more rapid weathering.

[4] In this paper the nature of the so-called Dapto or Saddleback Dolerite is discussed; it should be termed rather a trachy-basalt.

Fig. 2.—Map of Islands **I-III**, with 10-ft. contours. Scale 10 inches to the mile. Place-names given for reference purposes: A, The Beach; B, Freshwater Springs; C, Camp Hummocks; D, High Hummock; E, Phragmites Soak; F, Periwinkle Point; G, Triangle Pool.

Fig. 3.—Map of Island **IV**, with 10-ft. contours. Scale 10 inches to the mile.

Fig. 4.—Map of Island **V**, with approximate 10-ft. contours. Note: Heights are reckoned from mean tide level in Figs. 2, 3 and 4.

Fig. 5.—Geological map of Islands **I** and **II**. Scale 10 inches to the mile. Plain white, normal type (A) of Dapto dolerite; white with 'B' and 'D', dolerite, Types B and D respectively (see text); dotted lines, dykes; black, dune sand fixed by vegetation; cross-hatched, loose sand; horizontal shading, recent sandstone; 'Y', clay soil below sandstone, exposed by weathering of latter.

Triangle Pool, a large pool just under 30 feet above mean tide level, is closely associated with the dykes diagonally crossing the eastern part of II, and the ravine on I north-east of Periwinkle Pt. is due to the weathering of a dyke in this position.

The dolerite of III and V is entirely of the normal type (A). In all parts the dolerite, in addition to the effects of differential weathering of its local forms, is of very irregular surface, being criss-crossed with large cracks, due to weathering along joint-planes (Pl. xv, xvi and xix, D, F and H). The ravines of III, and at least portion of that on the north side of II near the western end, are associated neither with any variation from the normal type of dolerite, nor with dykes.

There is little evidence on I, II, III and V of raised rock-platforms, such as are found in shales on adjacent parts of the coast. This may possibly be due to the resistant nature of the normal dolerite. In most places the dolerite slopes moderately steeply and smoothly into the sea, save for irregularities due to natural fracture, or differential weathering of the altered forms (B and D, *supra*).

Overlying the dolerite on the top of I and II is a cover of dune sand, thick on I, thin on II. This is in the form of residual hummocks, especially on I, suggesting partial removal, as detailed in the next section. The lower areas between hummocks on I are composed of a very soft recent dune-sandstone, somewhat weathered, below which a heavy clay soil is exposed in a few places. In our opinion, the original dune cover of I and II could not have formed with the sea-level in its present relation to the doleritic rocks of the islands.

IV is composed of trachy-andesite, apparently the only remnant of a local flow. The northern half of the island and the eastern parts of its southern half form a low rock-platform, mostly less than 10 feet high. The western part of the southern half of the island forms a plateau reaching more than 40 feet in height, with steep sides, except to the south-east, where the rock slopes more gently to the platform.[5] In the sides of the plateau, especially near the north-eastern corner, caves are present up to about 25 feet above mean tide level; these are probably due to differential weathering of variants from the normal rock type, rather than to wave action at a period of higher sea-level.

Physiographic Sequence.

The physiographic sequence leading to the separation of the islands may be followed from the time of the completion of the Kosciusko Uplift, probably of the order of one million years ago. At this period faulting possibly occurred between Midway Reef and I, and between III and V. From the time of the uplift, the rocks of this district have been subjected to continuous rapid erosion, resulting in the formation of a coastal plain, at present some 7 miles wide in this district (Davis, 1936*b*, and papers quoted therein). On this plain, the more resistant igneous rocks which now form the islands would, at a period of low sea-level, have been isolated elevations, comparable with the two hillocks of dolerite now existing on the plain just behind Port Kembla (represented by 25-ft. contours on Fig. 1). Neglecting earlier fluctuations in sea-level, and considering only those of the most recent cycle, we find that some 25,000 years ago the sea-level was universally about 250 feet lower than at present, and that, up to about 3,000 years ago, a gradual eustatic rise occurred to a height some 15 feet above the present level.

[5] For the profile afforded from sea-level looking south-east, see Davis, 1936*a*, Pl. xii, 1.

Within the last 3,000 years the level has fallen some 15 feet (Daly, 1934). The application of these changes to the New South Wales coast is generally accepted.[6]

During the process of drowning, then, some 8,000–10,000 years ago, IV and V, at that time hillocks on the coastal plain, were severed from the mainland. The times of severance of I, II and III are more difficult to fix accurately, as moderately rapid erosion is still no doubt proceeding on the isthmus between I and II, and in the shallow water between II and III and between I and Red Pt. It is probable that drowning separated these islands about 4,000 years ago, and that during the 15-ft. fall in the last 3,000 years marine erosion has maintained the depth of intervening water, compensating for the falling level.

This sequence indicates that the islands have been continuously habitable for terrestrial communities such as occupy them at present, since the times of their separation from the mainland.[7] With sea-level 10–15 feet higher than at present (as at 3,000 years ago), V, in its present form, would be barely habitable, while the habitable areas of the other islands would be decreased. The probable decrease in the extent of the rocks forming the islands since the time of maximum sea-level would, however, allow V clearance above sea-level at all times sufficient to support terrestrial vegetation. This is supported by a consideration of the fauna of V, including lizards, Myriapods, etc. IV, though lower than V, would not be rendered uninhabitable by a rise in sea-level of 15 feet, even in relation to its present size, since it is more sheltered from heavy seas.

There is, therefore, no need to postulate that the native plants and wingless animals of the islands are other than relict from the time of severance, although it is possible that some are not lineal descendants of the population at that time, but are more recent colonists.

Prior to the drowning of the land around I–III, undulating sand-dunes, directly continuous with those now present on and to the south of Red Pt., probably covered this area. With the onset of drowning, most of this sand was washed away, leaving a thick cap on I and a thin covering on II (as indicated in Fig. 6, including the contour of the dotted lines), and cutting away the smooth slope of the dune on Red Pt., to give the present form. Below the level of the old dunes, usually at a depth of six feet or more, the sand was bound together to form a very soft sandstone. The binding substances appear to be hydrated oxides of iron, with little or no calcium carbonate. This recent sandstone may be regarded as a compacted illuvial horizon, the great porosity of the sand placing this horizon at a lower level than that normally found in other soils of this climatic zone, where podsols are the prevailing type. The recent sandstone is exposed in some places on the slopes of Red Pt., but is covered in most places by the loose sand talus.

In comparatively recent times, part of the sand covering of I and II has been removed by wind action, as indicated in Figure 6. Some of the sand so

[6] See also Cotton (1926).

[7] This probably applies to all the coastal islands of South-Eastern Australia. The claim that Lady Julia Percy Island, off the Victorian coast, is 'a pure volcanic mass uprisen in the sea, and not . . . a part of the old continental mass sundered from the mainland' (McCoy Society Report, 1937, p. 329) does not seem to be supported by any sound positive evidence. This island is separated from the mainland by about 4 miles of water, up to about 20 fathoms deep. To support the above claim, one must postulate a large and very recent uplift for this sector of the coast, or an improbably recent time for the vulcanicity responsible for the island rocks, with great subsequent reduction in the extent of the flows in a very short period.

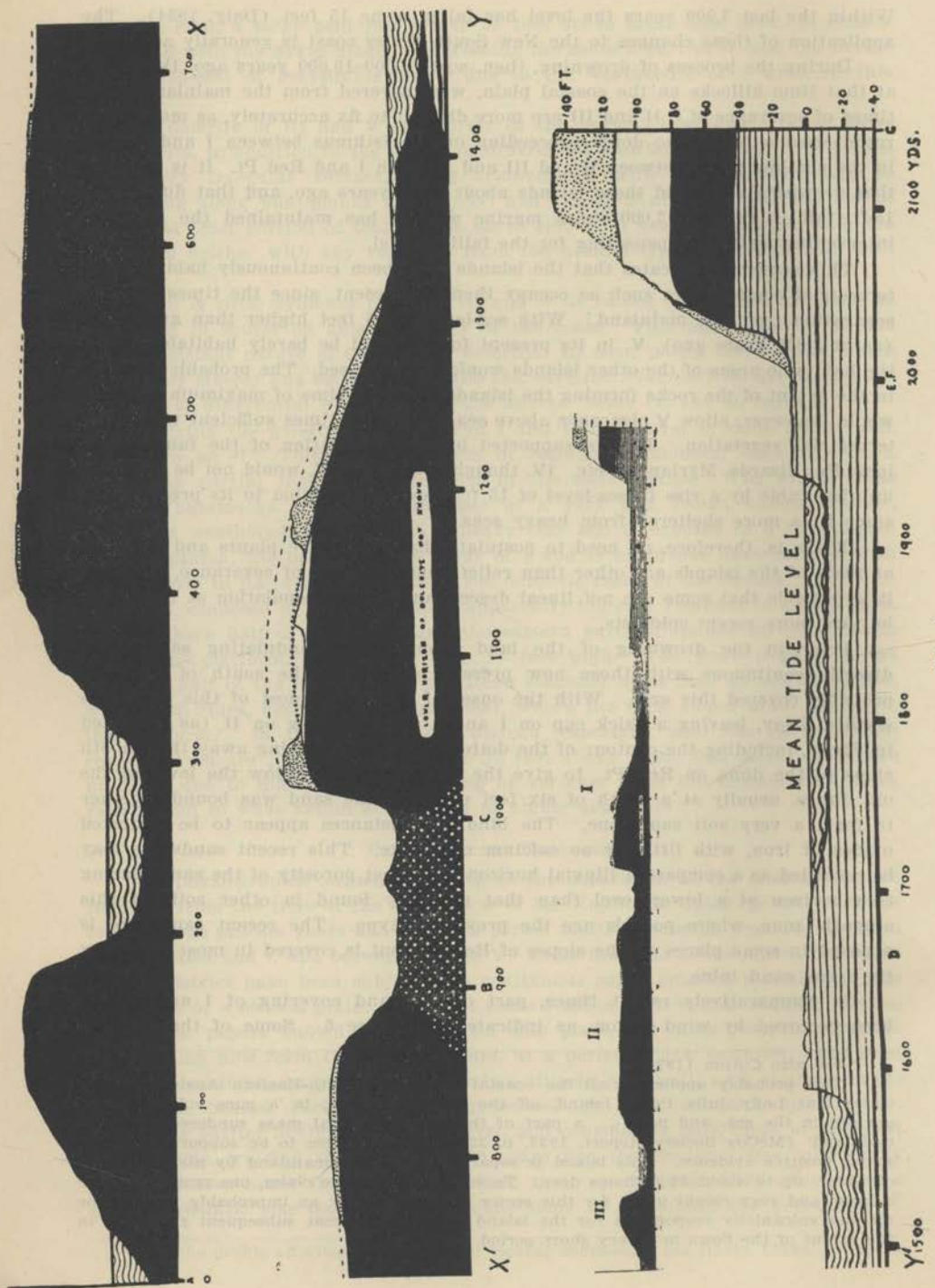


Fig. 6.

removed is now present as bare drifts on the northern slopes of I (Pl. xvii, C), and as a drift, partly stabilized by plants, on its south-eastern slopes. The removal of this sand from II, where its former thickness was not great enough to lead to the formation of underlying sandstone, has laid bare the dolerite; on I the recent sandstone has been exposed. This has weathered to some extent, leaving an irregular surface with local elevations (Fig. 6; Pl. xvii, D). Erosion on the southern side of the exposed sandstone area of I has produced small ravines (Pl. xvii, E, F), revealing in a few cases an underlying heavy clay soil. This was probably derived from the dolerite, pre-dating the covering of this district by dunes. Its texture suggests that it is to be regarded as a 'Y' horizon,[8] the illuvial horizon of a pre-existing soil; the eluvial ('X') horizon has partly been removed by erosion, together with the overlying sandstone, partly become identified with the latter. This old soil is not represented in Figure 6, the sandstone probably resting direct on the dolerite on the section line. It seems to occur only under the southern part of the sandstone tract, where the pre-existing soil had evidently accumulated in a concavity in the surface of the dolerite. More of this clay horizon is continually being exposed by erosion following rain, small ravines cutting back into the 'blow-out' area due to the softer nature of the clay and consequent under-cutting of the sandstone. Both the clay of this 'Y' horizon and also the recent sandstone above it are unsuitable for plant growth, being hard and practically devoid of humus.

The cause and date of the 'blow-out' of the sand covering of I and II are somewhat problematical. The erosion of the underlying elements on I gives some evidence of the date, suggesting possibly several hundred years ago. The hummocks of sand on I are held in place by the introduced Buffalo Grass, and the sand-drifts on the slopes appear to be unstable and in the process of disappearance; but the tempting hypothesis that the 'blow-out' was caused, after the colonization of the islands by Buffalo Grass, by clearance of the stabilizing vegetation by rabbits, must be abandoned, because of the extent of erosion of the underlying formations and the fairly definite information of Port Kembla residents. We must assume that the 'blow-out' occurred before the advent of Europeans, and introduced plants and animals; that the hummocks of sand at the old level were held, at the time of 'blowing-out' of the surrounding sand, by indigenous vegetation (possibly *Sporobolus virginicus* and *Lomandra longifolia*); that this vegetation has now been largely replaced by Buffalo Grass, which has been able to cross the barren areas of exposed sandstone; and that the sand-drifts on the slopes are more stable than first inspection would suggest. The original 'blow-out' may have been caused by wind acting on sand whose vegetational cover had been disturbed by burrowing

[8] For the use of the terms 'X' and 'Y' horizon, see Macdonald Holmes, 1937.

Fig. 6.—Ideal section along line A-B-C-D-E, F-G of Fig. 1, V/H = 3/1. Camp Hummocks projected on section plane between C and D; section carried from E to a point of similar elevation and formation at F, to show formations behind F, which have been denuded behind E and would be omitted by a section continuing from D past E in a straight line.

The section is given in three pieces, X adjoining with X', Y with Y'. A small-scale reproduction of the whole section is given.

Horizontal shading, Upper Marine sedimentary rock; black, normal type of Dapto dolerite; white stippling on black, Dapto dolerite, Type B (see text); plain white, recent sandstone; black stippling on white, dune sand; undulating lines, ocean water; — — — — —, contour of old surface of dune, before 'blow-out'; + + + + +, contour of former upper horizon of recent sandstone, before weathering following 'blow-out' of dune.

marine birds; this process seems to be going on at present on parts of I where the sand cover is still present, and is detailed in the section on plant ecology.

III, IV and V show no indication of former sand cover. The soil of V is shallow and restricted to hollows in the rock on the higher parts of the island; that of III is fairly shallow, and confined to the upper portion of the western end. Both are derived from the dolerite, the latter with a slight admixture of sand particles, probably wind-borne. The plateau of IV possesses a good soil of fine texture, derived from the underlying trachy-andesite. It is at least two feet deep at the centre of the plateau, with definite podsolization, and becomes shallower towards the edge. Considering its size, the vegetation of IV is the richest of the group. This is doubtless attributable to better soil properties, the soil not being shallow and scarce, as on III and V, nor masked by poor dune soil, as on I and II. The shallow sand covering of II is probably in part mixed with soil originating from decomposition of the dolerite.

Climate.

Temperature, humidity and rainfall data for Wollongong have been given by Davis (1936*b*). The averages are: Maximum daily temperature 71·4° F.; minimum daily temperature 54·6° F.; relative humidity 73%; annual rainfall 45·65 inches. The rainfall at the islands is probably somewhat less than at Wollongong. Our observations indicate that the islands miss many showers which strike Wollongong and other points on the coast, and this is supported by theoretical considerations. The mountains behind Wollongong, and behind the coastal plain in general, precipitate the rain, and proceeding east from these mountains to Wollongong there is an observed drop in rainfall, which is probably continued seawards from the coast.

The temperatures detailed are shade temperatures. The terrestrial life of the islands is subjected to much greater extremes. Our observations indicate that the daily range in exposed situations such as the more open parts of the *Scirpus nodosus* community on I may be very considerable.

All the islands are very exposed to winds, V most and IV least so. The question whether this justifies the recognition of shrub communities as a climatic climax for the islands is dealt with in a subsequent section. In rough weather, salt spray is driven over all portions of the islands. We have observed fine spray drifting across the highest point of the islands (High Hummock, I, 71 ft.) in moderately rough weather; at this time, heavy spray was being driven over the whole of II and III, while V was subjected throughout to very heavy spray. Relatively high chloride contents were observed for all soils, particularly poorly-drained soils with little leaching. All plants on the islands must be considered halophiles to a greater or less degree; a large number are common to the islands and to the saline swamp vegetation of the Sydney district described by Hamilton (1919). The effects of spray on all pool communities on the islands is discussed later.

Plant Ecology.

In Table 1 are listed the various species of vascular plants observed, with notes on frequency on each island, life-form, and usual habitat. For purposes of classification we have recognized the following communities: (1) The *Correa-Westringia* Community; (2) The *Stenotaphrum* Community; (3) The *Sporobolus* Community; (4) The *Scirpus nodosus* Community; (5) The *Mesembryanthemum* Community; (6) The *Lomandra* Community; (7) The *Salicornia* Community; (8) The *Spergularia–Claytonia–Portulaca* Community; (9) The *Scirpus cernuus* Community.

This classification includes under the term 'Community' widespread aggregates such as the *Stenotaphrum* or *Correa-Westringia* communities, and small local groups such as the *Scirpus cernuus* community. Communities (6) to (9) might better be termed 'societies'. We have purposely omitted terms such as 'consociation' and 'consocies', with their successional implications; the shrub community (*Correa–Westringia*) is the highest on the islands, but even it is to be regarded as a subclimax. Certain communities (e.g., *Mesembryanthemum aequilaterale* on almost bare rock) can be regarded as seral (stages in a lithosere of which placoid lichens are the initiators); but, as, on the whole, the vegetation is in a retrograde state, due to the gradual marine erosion of the islands, etc., we do not consider that upgrade succession is proceeding to any marked extent. Apart from retrogression by marine erosion, the vegetation is in a highly unstable state, chiefly due to disturbance by man, introduced animals and plants, and burrowing sea-birds.

Plate xv*a* shows the distribution of the communities, with the exception of the *Scirpus cernuus* community, which occupies a small area on I and II, fringing the lower limit of other communities in some parts, especially along the southern side. The habitat factors of the various communities are considered as each is dealt with, but the relations of *Stenotaphrum*, *Sporobolus* and *Scirpus nodosus* may be discussed here. Plate xv*a* shows the presence of *Scirpus nodosus* community on the central part of I and a small portion of II, in those parts where the sand cover has been removed, exposing recent sandstone, hard clay, or (on II) dolerite, as detailed earlier. In the areas of sand remaining, the chief stabilizing agents are the grasses *Stenotaphrum* and *Sporobolus*. The former is introduced, the latter indigenous. The transparency (Pl. xv*a*) suggests that *Stenotaphrum*, probably introduced to the inner (western) side of I, the usual place of landing and residence by visiting parties, has displaced *Sporobolus* on I (except for a remnant on Periwinkle Pt.), and over the inner end of II. Whether this displacement is still proceeding at the ecotone cannot yet be stated with certainty; *Stenotaphrum*, however, became more prominent on II, between its eastern limit as marked on Plate xv*a*, and Triangle Pool, between August, 1937 (when the transparency was prepared), and April, 1938, and in July, 1938, appeared to be further spreading in this region at the expense of the *Sporobolus* community.

Vegetation (*Stenotaphrum* community) reaches a point slightly below the 10-ft. contour behind the beach, on I (Pl. xvii, J), the spot most sheltered from rough seas on any of the islands. Elsewhere, the lower limit of vascular plants is much higher, on I and II between 10 and 20 feet, on the western extremity of III 20 feet, and elsewhere on III 30 feet. Only a few therophytes occur below 20 feet on IV, the entire northern rock-platform being devoid of vascular plants except a few specimens of *Sonchus oleraceus*. On V, most of the vascular plants occur above the 40-ft. contour, 30 feet being the lower limit, except on the north-west corner, where more efficient shelter from south-easterly seas allows them to reach a slightly lower level.

The Correa–Westringia Community: A shrub community 3–4 feet high is developed over extensive areas of II, at the north and south ends of the plateau of IV (a few bushes also on the eastern slopes, IV), for a limited area on the slopes of the western end of III, and, as a few isolated bushes, on the north side of I. The shrubs occur only on well-drained soils, usually not less than six inches in depth. They do not seem to be limited in any way to situations sheltered from the wind. The properties of three typical soil-samples from this community are given in Table 2. The absence of shrubs from the central part of the plateau of IV cannot

be explained satisfactorily. The salinity (as indicated by chloride content) is higher in the central part of the plateau, due to the run-off of water from the slightly higher parts at the north and south ends; the humus content is lower, and the pH higher. The humus content of soils in the shrub community, however, is a direct result of the conditions below the shrubs, where many fallen leaves accumulate. Similar conditions on the margins of the shrub community, where there is cover by branches but absence of rooted plants, should allow the community to spread if humus content alone were the limiting factor in shrub distribution. Neither high salinity nor high pH (a result of high salinity and low humus content) can be limiting, as the figures for a shrub community from II are higher than for soil from the centre of the plateau. No young shrubs have been present at the margins of the community on the plateau at any time since our observations commenced; indeed, some of the older bushes were wholly or partly dead (March, 1938), possibly from severe wind action, possibly from infestation by scale-insects. The factors limiting shrub distribution are not known. The burrowing activities of penguins and mutton birds adversely affect the stability of certain communities on I and II, but, in general, these birds burrow in the more open communities rather than amongst shrubs. However, some of the marginal shrubs bordering the *Mesembryanthemum* community on IV were partly undermined by penguin burrows in July, 1938, and this may possibly have an adverse effect on the roots, promoting the limitation and destruction of the shrubs at and near the centre of the plateau.

Correa alba is dominant in most parts of the shrub community of II, *Westringia rosmariniformis** seldom becoming dominant or co-dominant (Pl. xvii, G). On the plateau of IV, *Westringia* and *Correa* are co-dominant, or each attains local dominance in some areas (Pl. xvii, H), while *Myoporum ellipticum* occurs occasionally, attaining dominance in a small area near the western edge of the plateau (Pl. xvii, I). In the shrub community of III, *Correa* and *Myoporum* are co-dominant, *Westringia* absent.

On I, in the few places where shrubs develop, *Westringia* is the dominant.

The shrub communities of I and III were probably of greater extent before the advent of Europeans and the introduction of exotic plants. *Opuntia inermis*, when unchecked, grows strongly amongst the shrubs on III and parts of II. The fluctuations of *Opuntia* are discussed more fully under the *Stenotaphrum* community.

In its undisturbed state, the *Correa–Westringia* community is practically devoid of other plants. Neighbouring communities, such as *Sporobolus* on II and *Mesembryanthemum* on IV, may enter the shrub community marginally as a ground layer, and occupy the areas between the scattered bushes. In most cases, however, the shrubs grow close together, and the ground below them is devoid of any other vegetation, probably because of lack of light and the presence of an A_0 horizon of fallen leaves, an obstacle to seedling germination. On IV the climber *Kennedya rubicunda* occurs scrambling over the shrubs.

The Stenotaphrum Community, with variant facies: The introduced Buffalo Grass (*Stenotaphrum secundatum*) is dominant on sandy areas over a large proportion of I and the western end of II; it also occurs on V in shallow soil in rock crevices, not reaching the status of a community. It has recently (since 1934) colonized IV, being well established on the eastern slopes and plateau in March, 1938.

* Since the completion of this paper, Mr. E. Cheel has recorded *Westringia fruticosa* Druce as the correct name for the species cited in this paper as *W. rosmariniformis* Sm. (Abstract, Proc. Linn. Soc. N.S.W., 31 Aug., 1938.)

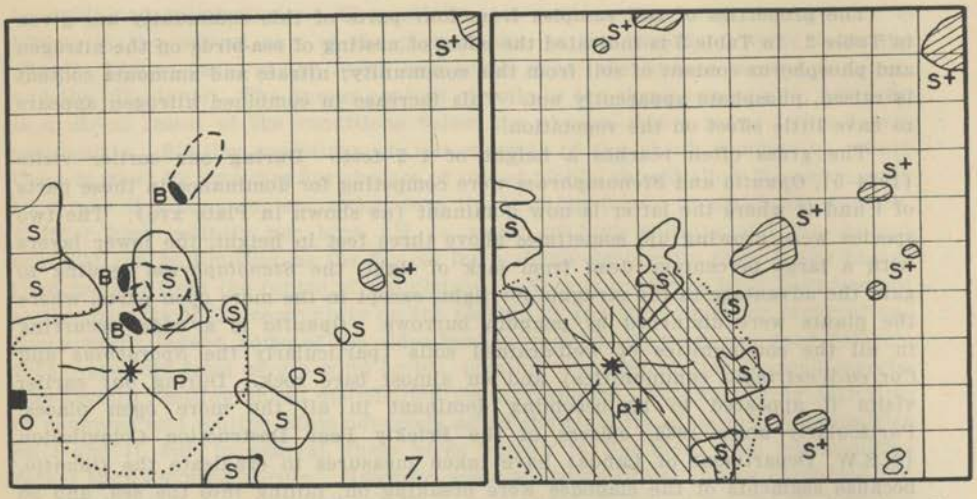
The properties of soil samples from four parts of this community are given in Table 2. In Table 3 is indicated the effect of nesting of sea-birds on the nitrogen and phosphorus content of soil from this community; nitrate and ammonia content is raised, phosphate apparently not. This increase in combined nitrogen appears to have little effect on the vegetation.

The grass often reaches a height of 1–2 feet. During our earlier visits (1934–5), *Opuntia* and *Stenotaphrum* were competing for dominance in those parts of I and II where the latter is now dominant (as shown in Plate xv*a*). The two species were growing up, sometimes above three feet in height, the lower layers with a large percentage dead from lack of light, the *Stenotaphrum* tending to gain the advantage in the struggle for light, except in the more open parts, where the plants were disturbed by penguin burrows. *Opuntia* is a wide, occurring in all the communities of well-drained soils (particularly the *Sporobolus* and *Correa-Westringia* communities) and on almost bare rock. During our earlier visits it appeared to be becoming dominant in all the more open places. Particularly since 1935, officers of the Prickly Pear Destruction Commission (N.S.W. Department of Lands) have taken measures to eradicate the *Opuntia*, because segments of the cladodes were breaking off, falling into the sea, and so being carried down the coast to infect farms on the mainland to the south of the islands. This colonizing ability indicates how *Opuntia* originally reached the islands.

Opuntia has been attacked by the introduction of the eggs of the moth *Cactoblastis cactorum* Berg.; by poisoning with arsenic; and by fire. It has never been exterminated completely, as the attempted eradication measures have been somewhat sporadic, and from time to time it becomes fairly abundant. It is to be regarded as a widespread facies in most communities (especially *Correa-Westringia*, *Stenotaphrum*, *Sporobolus*, *Scirpus nodosus* and *Mesembryanthemum* communities), varying sporadically on account of the biotic factor of human interference (Pl. xviii, C, D, E).

The *Stenotaphrum* community has two other notable facies, those given by *Phytolacca octandra* and *Tetragonia expansa*. The former occupies large areas of the *Stenotaphrum* community to the north and west of I, and is a seasonal facies, *Phytolacca* being an annual. The *Tetragonia* facies is represented by a moderate area behind the beach on I, and a small area on the southern side of the west end of II. *Tetragonia* grows as a perennial on the islands, and in places occurs as an almost pure stand, with very little *Stenotaphrum* admixed. The humus content appears to be higher in the *Tetragonia* facies than elsewhere in the *Stenotaphrum* community.

Other elements of the *Stenotaphrum* community are of rarer occurrence. We have considered as a separate community *Lomandra longifolia*, which may occur alone or with *Stenotaphrum*, as at Camp Hummocks, I (Pl. xvii, A). *Phragmites communis* grows amongst *Stenotaphrum* in the southern portion of I, especially near Phragmites Soak (Pl. xix, G), in most cases in soil more efficiently drained than in its mainland habitats. *Imperata cylindrica* var. *Koenigii* and *Setaria glauca* occur in the *Stenotaphrum* community behind the beach, I, the latter as a seasonal facies. Other elements can be found by reference to the last column of Table 1, in most cases being straggling plants (e.g. *Hibbertia volubilis*, *Commelina cyanea*, *Kennedya rubicunda*), or small herbs, often annual (e.g. *Anagallis arvensis*, *Solanum nigrum*, and many Composites). The bracken (*Pteridium aquilinum*) occurs occasionally in this community.



Figs. 7 and 8.—Permanent quadrat in *Stenotaphrum* community between beach and Camp Hummocks, I. Area disturbed by burrowing activities of penguins and mutton-birds. Fig. 7, 18/12/37; Fig. 8, 24/4/38. Side of quadrat 10 ft. (see also Pl. xviii, A and B). Orientation: North side to the left.

(S, *Stenotaphrum secundatum*, living; S+, do., dead; P, *Phytolacca octandra*, living; P+, do., dead; O, *Opuntia inermis*; B, burrow. The shading indicates dead plants; *Phytolacca* is represented by an asterisk for the main stem, and a dotted line for the limit of branches. Mouths of burrows represented by black ovals, the limits of the underground portions by broken lines.)

Figures 7 and 8 represent a permanent quadrat in portion of the *Stenotaphrum* community behind the beach, I, where nesting sea-birds have disturbed the soil with their burrows. Figure 7 represents the quadrat on 18.12.37, Figure 8 on 24.4.38. Photographs of the quadrat on these dates are given in Plate xviii, A and B. In these four months, disturbance by burrowing allowed the wind to remove a thickness of two inches from the surface of the sand. This has undermined the *Stenotaphrum*, in parts killing it and thereby further hastening the process of wind erosion. In Plate xviii, B and C, the seasonal change of the *Phytolacca* facies is also visible.

The Sporobolus Community: This community, dominated by *Sporobolus virginicus*, occupies most of the eastern end of II wherever shrubs are not developed, a great proportion of the area of III occupied by vascular plants, and the higher parts of Periwinkle Pt. on I. It occurs in dry soil, or in water-logged soil lying in rock hollows; in sandy soil, or soil derived from igneous rock. The dominant has a high salt-toleration. Properties of soils from four parts of this community are listed in Table 2. In the wetter parts of this community, elements of the *Scirpus cernuus* community (detailed later) sometimes occur; in its drier parts, in addition to *Opuntia*, several small herbaceous species, which may be found by reference to the last column of Table 1, are occasionally present. *Zoisia macrantha* occurs in most parts of this community, and is difficult to distinguish from *Sporobolus* in the absence of inflorescences. Identification on characters of the leaf anatomy has shown that *Sporobolus* is always dominant, as is also indicated by a study of the inflorescences when available. The community is shown in Plates xviii, F, and xix, C.

The Scirpus nodosus Community: This community occupies those areas of I and II from which the sand cover has been removed. The plants grow in small accumulations of sand, 1–2 inches deep, collected round the tussock bases. The properties of soil samples from two localities are given in Table 2. In some places the plants are very widely spaced (Pl. xvii, B and D); but in the neighbourhood of shifting sand which can readily collect round the tussocks, as around High Hummock, a dense stand is formed (Pl. xviii, G). Few other elements enter this community, which occupies a very unfavourable habitat: a few plants of *Inula graveolens*, *Mesembryanthemum aequilaterale*, *Enchylaena tomentosa*, *Salsola Kali*, and very rarely *Phytolacca octandra* on I, and of *Tillaea Sieberiana*, *Dichondra repens* and *Cyperus polystachyus* on II.

Scirpus nodosus occurs occasionally on small accumulations of soil on rock on I and II, apart from the 'blow-out' areas, and in the *Mesembryanthemum* community on the plateau of IV.

The Mesembryanthemum Community: Small patches of *Mesembryanthemum aequilaterale* occur on all the islands on rock surfaces almost devoid of soil. Forward succession in time, by the colonization of rock surfaces by this species, has not been observed; it may possibly be going on in isolated cases, since equilibrium does not necessarily obtain at present at the lower limit of vegetation. On III this species forms a definite community in shallow soil on the upper part of the island, surrounded by the *Sporobolus* community. The community reaches its greatest development on those parts of the plateau, IV, on which no shrubs occur; in this situation the mat is mingled with other species, and does not form a pure stand as elsewhere.

As recently as March, 1936, the centre of the plateau of IV was clothed with a mixed community, in which *Stephania hernandifolia* was the commonest species, with *Plectranthus parviflorus*, *Kennedya rubicunda*, *Dichondra repens*, *Senecio lautus*, and several other species admixed (Pl. xviii, I). By March, 1938, all of this area was covered by a mat of *Mesembryanthemum aequilaterale*, with other species (*Stephania*, *Plectranthus*, etc., and *Lepidium hyssopifolium*) occupying a very subsidiary rôle (Pl. xviii, J). We cannot satisfactorily explain this replacement of one community of endemic plants by another of equal or lower integration; however, it was noted in July, 1938, that the mixed community showed some evidence of becoming re-established in a few parts of the centre of the plateau, where the *Mesembryanthemum* mat had been disturbed by burrowing penguins.

Properties of soil samples from two parts of the *Mesembryanthemum* community are given in Table 2, one for the community of the centre of the plateau, IV, the other for shallow soil collected around *Mesembryanthemum* on otherwise bare rock, IV. The latter has a high organic content, suggestive of a stage of a lithosere. In all cases, the situations where this community occurs are well drained.

The Lomandra Community: *Lomandra longifolia* occurs on dune sand, as isolated small hummocks holding the sand at the old level in the 'blow-out' area, on the north side of I (Pl. xvii, B), and, with or without *Stenotaphrum*, on the larger hummocks (Camp Hummocks) in the centre of I (Pl. xvii, A). Properties of soil from the latter situation are given in Table 2 (fourth sample under *Stenotaphrum*). Except for the occasional admixture of *Stenotaphrum* at Camp Hummocks, *Lomandra* forms a pure stand, no other species occurring in this community. On II, *Lomandra* is rare, not forming a definite community.

The Salicornia Community: This community is confined to a hollow on the south side of III, in shallow poorly-drained soil with high salt-content. *Salicornia australis* forms a pure stand, bordered by the *Sporobolus* community on the upper side, where drainage conditions are better (Pl. xix, A). The properties of two soil samples from this community are given in Table 2.

The Spergularia–Claytonia–Portulaca Community: This term has been applied to the vegetation occurring on shallow poorly-drained soils in rock basins on the eastern slopes of IV; similar aggregations occur on the higher parts of V. *Portulaca oleracea* (Pl. xix, B) is the commonest species, but is an annual. Other elements (perennial) are *Claytonia Pickeringi*, *Spergularia rubra*, *Cotula coronopifolia* and *Mesembryanthemum australe*. The species may occur singly or in various combinations. Properties of soil from a typical example of this community are listed in Table 2.

The Scirpus cernuus Community: This term is applied to those aggregates occurring in poorly-drained situations in the lower parts of I and II (Pl. xix, H). *S. cernuus* is often associated with *Samolus repens*, *Apium prostratum* and *Lobelia anceps*, any of which may also occur separately in similar situations. *Samolus* and *Apium* also occur in the moister parts of the *Sporobolus* community.

Soil properties for two parts of this community are listed in Table 2. In some respects, this community corresponds to the *Spergularia–Claytonia–Portulaca* community of IV and V, and the *Salicornia* community of III, but the salt-content appears to be much lower, the highest content being found in soils of the *Salicornia* community. These three communities, however, stand apart from the other communities recognized, in being communities of poorly-drained soils. *Sporobolus* stands in an intermediate position between these three and the remainder in that it tolerates a variety of drainage conditions.

Life-Form Spectra: Table 4 gives life-form spectra (Raunkiaer, 1934) for indigenous and total species for the Five Islands, as a whole and for individual islands. I and II are considered as a single unit. The spectra for Brush Island and for an area of the *Eucalyptus pilularis* association are given for comparison, as well as the 'normal' spectrum. Brush Id. is situated close to the mainland some 75 miles south of the Five Islands, and is composed of augite–olivine–monzonite with a complete dune cover, similar to that which must have been present on I before the 'blow-out' occurred. Its greater area and height, and deeper soil, offer more favourable conditions for plant growth than any on the Five Islands. It possesses 66 species of vascular plants, of which 38 are common to the Five Islands. The *Eucalyptus pilularis* association is given as a typical forest climax on good soil in the same climatic zone as the Five Islands.

It must be remembered that life-form spectra were originally derived for comparing climax vegetations of different climatic zones, although later applied by their originator to local communities other than the climax within one climatic unit. In the present case the spectra are adduced to compare the characteristics of the flora of the islands *inter se* and with other communities, to show correlation with soil formation, shelter, etc. No allowance is made for specific frequency, each species (whether rare or dominant) counting one point; this is the usual procedure, but is not by any means ideal. Again, it is somewhat difficult to apply a terminology based on perennating buds in a climate where most species (except, of course, the therophytes) continue growth at all seasons.

The Five Islands are devoid of phanerophytes other than nanophanerophytes, even these being poorly represented, and more by herbaceous and climbing types than woody shrubs. V, with little soil, and very exposed, has no woody nano-

phanerophytes. The chamaephyte figure is high in all cases; hemicryptophytes are average, geophytes very rare. Therophytes are well represented, and the effect of the introduced element in raising the therophyte percentage is at once apparent. This is due partly to coincidence; many of the introduced species are Composites, which have wind-borne fruit suitable for the colonization of islands, and, as an entirely independent character, happen to be therophytes. In general, introduced species established on the mainland are largely therophytic, being plants able to avail themselves of rapidly-changing areas of disturbance. The only stem-succulent on the islands is also introduced.

We regard the shrub communities of the Five Islands as a subclimax, not as a local climax under the special climatic conditions of the islands. Brush Island, with almost identical climatic conditions (wind exposure, etc.), develops a low forest of *Banksia integrifolia* Linn. f. and *Casuarina glauca* Sieb. The lack of trees on the Five Islands is therefore attributed to lack of suitable soil conditions rather than to an unfavourable climate; climatically, the shrub communities are a subclimax, the climax not forming because of unfavourable edaphic conditions. In addition to microphanerophytes, Brush Island possesses one epiphytic species (*Platycerium bifurcatum* (Cav.) C. Chr.).

The vegetation of the Five Islands and Brush Island, and especially the facts given by the life-form spectra, may be compared with the data given by Osborn (1922, 1923, 1925) for islands off the coast of South Australia.

Introduced Plants: In Table 5 are given the numbers of introduced species recorded from the Five Islands (individually and collectively) and from Brush Island. In addition, the proportion of introduced to total species is given as a percentage. Both figures must be considered for comparative purposes, the percentage reflecting the effect of the habitable area of each island, and the consequent probability that seeds will reach land, and germinate once they have done so. The low figures for Brush Island are due chiefly to the relatively undisturbed nature of the adjacent mainland, and the rarity of visits by man. Brush Island is in a position relative to the nearest point of the mainland comparable to that occupied by I. The number of introduced species of plants growing on the mainland in the settled areas near Port Kembla is considerable.

The only introduced species at the Five Islands which are not therophytes are *Opuntia inermis* (stem-succulent) and *Stenotaphrum secundatum* (chamaephyte).

Crossing from the mainland to the islands was accomplished in half the species (9 out of 18) by wind-borne fruit, while two species (*Phytolacca octandra*, *Lycium ferocissimum*) have fleshy fruits. Ground-larks (*Anthus australis*), part of whose diet consists of seeds, are found commonly on I, II and III, and have frequently been observed flying between II and III, and occasionally between I and the mainland. These birds have not up to the present been observed on IV and V, but probably fly there on some occasions.

The observed ability of *Opuntia* to recolonize mainland areas by its cladodes, floating from the islands in the open sea, explains its original invasion of I, II, III and IV, without the necessity of invoking its fleshy fruit as a means of entry; however, officers of the Prickly Pear Destruction Commission have observed crows (*Corvus coronoides*) feeding on *Opuntia* fruits at the islands.

Of the remaining introduced species, *Stenotaphrum* was probably introduced to I, and possibly to II, by man, but its occurrence on IV and V cannot be so explained. On I, its stolons frequently hang down over rocks in long festoons (Pl. xix, J), and the colonization of IV and V is probably due to the carriage of these vegetative

parts by sea;[9] carriage of the seeds by birds is less probable. *Atriplex patulum*, which was observed near the beach on I in 1934, but now seems to have died out, was probably introduced by man. *Rumex crispus*, a recent migrant to IV, has a swollen, buoyant anthocarp, which may have floated from the mainland. *Datura Stramonium*, with a large, buoyant capsule, is a recent migrant to I, growing just behind the beach, whither its fruits were probably carried by sea. *Anagallis arvensis* (on I, II, and also on Brush Island) may have been introduced as seed by birds, or may have reached I and II with the introduced mammals. *Malva parviflora*, an introduced species on V, possesses a thin mericarp, which might possibly have floated to the island or have been carried by westerly winds.

Other species which may be indigenous, or may have reached the islands by recent colonization, are *Solanum nigrum*, with a fleshy fruit (on I, II, IV, V, and on Brush Island, where *S. laciniatum* Ait. falls within the same category); *Rumex Brownii* (near the beach, I), with a burr fruit, such as would be carried on human clothing or the hair of introduced mammals; and *Echinochloa crus-galli*, the fruit of which might be eaten by birds, and so carried to V. *Lepidium hyssopifolium*, indigenous in New South Wales, has become increasingly numerous on IV during the last few years, suggesting that it may also be a recent migrant to the islands, possibly as seeds eaten by birds.

Pumpkin, tomato and potato plants, introduced by man, have been observed on I near Camp Hummocks. They have never become properly established, only the first-named showing any evidence of doing so. They have been omitted from Table 1, and from the calculations by which Tables 4 and 5 were derived.

Interaction with Extraneous Communities.

Interaction with Mainland Communities: Under this heading comes the disturbance of the ecological equilibrium effected by introduced plants. Their means of entry and behaviour on becoming established have already been discussed. The process is probably still continuing. The remaining interactions may be listed as follows: (A) Introduced animals which have become established on the islands, and now form an integral part of the island life; (B) Animals coming to the islands as temporary but regular visitors, and definitely entering the island food-chains; (C) Animals reaching the islands by chance, of irregular and transient occurrence. (B) and (C) are concerned chiefly with species indigenous in this region.

(A).—1. The introduced fly *Lucilia sericata* Meig. is common on I; the journey from the mainland is well within its normal flight range.

2. The introduced scale-insects *Saissetia oleae* Bern. and *Ceroplastes destructor* Newst. are established on the islands, the former on I and II, the latter on IV. This invasion is possibly to be attributed to the minute first-instar larvae being carried by strong westerly winds.

3. As noted earlier, goats and rabbits were introduced by man many years ago. The latter are established as a definite part of the biome; the former became extinct only by interference from without.

4. The moth *Cactoblastis cactorum* Berg. has been introduced to I by officers of the Prickly Pear Destruction Commission. It has become well established, and has spread to IV, apparently by its own powers of flight.

[9] Stolons of this type, lacking roots, were immersed in sea-water for 18 hours, exposed without soil or water for 14 days, and then placed in moist sand. Within a further 14 days adventitious roots and new leaves were formed.

(B).—1. Human beings disturb the balance of the biome by killing members of the animal population, and add to the islands additional habitats in excreta and discarded food. The human factor pre-dates the advent of Europeans, as is indicated by an aboriginal midden, with discarded flints and large shells, on I (Pl. xvii, D). This may pre-date severance of the island from the mainland, but not necessarily so, as in Cook's journal there is a reference to canoes used by natives in this locality.

2. Ground-larks are probably the only land-birds nesting on the islands. Birds of prey, such as the Nankeen Kestrel (*Cerchneis cenchroides*), derive much of their food supply from the islands, but are not continuous residents. The Boobook Owl (*Spiloglaux novae-seelandiae*) has been observed on I and II, where it rests by day in holes in steep rock-faces, and hunts at night, flying also to IV. It is reasonably certain that it does not nest at the islands. Swallows (*Hirundo neoxena*) are frequently seen hawking over I and II, but have not been observed to breed there; they have been seen flying between I and the mainland. Quail (*Ypsilophorus ypsilophorus*) occur rarely on II, probably seasonally, as on Brush Island. The Black-and-White Fantail (*Leucocirca volitans*) and Silver-Eye (*Zosterops lateralis*) occur, the former having been observed frequently on I, II and III, the latter rarely on I. These birds may possibly breed at the islands, but this has not been observed. In all the above instances, and in the case of the ground-larks breeding at the islands, interaction occurs between the islands themselves, in addition to interaction with the mainland.

3. Odonata (Anisoptera and Zygoptera) have been observed on III, where no breeding habitat exists; these individuals probably developed in Triangle Pool, II. Anisoptera have been observed on IV, where there does not appear to be any breeding habitat; these individuals were probably from the mainland. In fact, it is possible that all the islands are visited by Odonata from the mainland.

4. Although there are no nests on the islands, bees (*Apis mellifera* L.) have been observed on several occasions on IV, gathering nectar from flowers (particularly of *Plectranthus parviflorus*) on the plateau. This is probably a regular occurrence. This example is included here, rather than under (A), although *Apis* is introduced.

(C).—The butterfly *Tisiphone abeona* Don. has been observed on IV, although its food-plant (*Gahnia*) does not occur on the Five Islands. Cicadas (e.g., *Thopha saccata* Fabr.) have also been observed to fly from Red Pt. to I, and have been observed on III following westerly winds. These and other winged insects are irregular visitors to the islands, possibly entering the food-chains, although not in the regular manner comparable with the instances under (B).

Interaction with Marine Communities:

(A).—The following marine birds breed at the Five Islands: The Little Penguin (*Eudyptula minor*), very common on I and II, occasional on III, and IV and V; the Wedge-Tailed Shearwater or Mutton-Bird (*Thyellodroma pacifica*), very common on I, occasional on II and III; the Silver Gull (*Bruchigavia novae-hollandiae*), common on III and probably on V; the White-Faced Storm Petrel (*Pelagodroma marina*), fairly common on II; the Black Oyster-Catcher (*Haematopus ostralegus*), rare on IV; and the Red-Capped Dotterel (*Leucopolius ruficapillus*), rare on I. The last species nests in the more open parts of the *Scirpus nodosus* community on the 'blow-out' area of I, where it may possibly derive some of its food from terrestrial sources. The Crested Tern (*Thalasseus bergii*) is common near the islands, in some years nesting on III, and possibly on V (although we have not been able to land on this island during the breeding season).

The effect of these birds in raising the combined nitrogen in the soil is shown in Table 3. Little effect of the increased nitrogen content on the vegetation can be noticed; *Stenotaphrum* is somewhat more robust than in most mainland situations, but the burrowing activities of *Eudyptula* and *Thyellodroma* more than counteract any benefit the vegetation might receive from the sea-birds. On Brush Island, the creeper *Kennedya rubicunda* develops leaves several times the size of those of plants from other localities. This may be a result of the manuring of the soil by birds. The excreta of birds also raise the phosphate and nitrate content of pool waters, favouring protophyta, the base of some pool food-chains.

The sea-birds, especially those forming burrows, provide a habitat for nest-parasites (Siphonaptera, etc.), whilst lizards (*Lygosoma* spp.) frequently use empty nests as retreats. The ticks *Ornithodorus* and *Ixodes* are true ectoparasites when the birds are present on the islands, at other times resting on and under rocks (IV, V); at this stage they are occasionally eaten by lizards. The sea-birds also appear to form part of the diet of birds of prey; on Brush Island we observed several crows (*Corvus coronoides*) feeding on a freshly-killed Little Penguin, and on Lady Julia Percy Island the Swamp Harrier (*Circus juxta*) is recorded as feeding on mutton-birds during the breeding season (McCoy Society Report, l.c., p. 429). Both *Corvus coronoides* and *Circus juxta* occur frequently on the Five Islands (I–III), but we have not observed this feeding-habit there. However, many sea-birds have been seen dead on the higher parts of I, II and III, probably from this cause. Dead sea-birds also enter terrestrial food-chains as the habitat of carrion insects (Muscoid flies, Histeridae, Staphylinidae, Dermestidae, etc.).

(B).—Two species, the Marine Caddis-Fly (*Philanisus plebejus* Walk.) and the Marine Tipulid (*Limonia* (*Dicranomyia*) *marina* Sk.) occur on the Five Islands, the larvae feeding on algae in the littoral zone, the adults being terrestrial. Adults of *Philanisus* have been taken as far from the shore as Camp Hummocks, in the centre of I. Both species almost certainly enter terrestrial food-chains.

(C).—Insects, particularly Muscoid flies, both larvae and adults, are found on carrion of marine origin about the high-tide mark, especially on the beach, I. Muscoid larvae have been recorded living in dead pieces of the Ascidian *Cynthia* on the beach, I, well below the high-tide mark. The adults of these species certainly enter terrestrial food-chains; lizards (*Lygosoma* (*Hinulia*) *quoyi* Dum. et Bibr.) frequent the zone behind the beach, I, feeding on adult flies, and the Black-and-White Fantail has been observed feeding on flies between tide-marks on the beach.

(D).—Insects, particularly Coleoptera such as Staphylinidae, and Diptera (Phycodromiidae, Anthomyiidae, etc.), have their habitat in aggregations of dead kelp (*Ecklonia*) washed up at the extreme limit of waves, especially on the isthmus between I and II and on the northern rock-platform of IV. These insects form a separate community, which is linked with typically terrestrial communities by spiders which at times frequent it. The habitat of Machilids (*Allomachilis froggatti* Silv.) on IV contains vegetable detritus of terrestrial and marine origin (dead leaves of *Kennedya*, etc.; dead coralline sea-weed, etc.), and either or both may form the food of the Machilids.

(E).—The common littoral crab *Leptograpsus variegatus* (Fabr.) is frequently found in pools above the reach of waves, including those of low salinity (e.g., Triangle Pool). Specimens of this crab have been collected in pools 45 feet above mean tide level on III, with insect remains (larvae and pupae of *Aedes* (*Pseudoskusea*) *concolor* Tayl., and larval Chironomidae) in the crop.

(F).—The periwinkle *Nodilittorina tuberculata* (Menke) occurs on rocks 40 feet and more above mean tide level, both on dry rock-faces and in pools, particularly on Periwinkle Pt., I. *Melarhaphe unifasciata* (Gray) occurs at slightly lower elevations. Marked specimens of *Nodilittorina* remained at about 40 feet above mean tide level on I from August, 1937, to July, 1938, only a small percentage showing any tendency to migrate seawards. This species was observed copulating on rocks 25 feet above mean tide level on IV (March, 1938). It probably derives its food from the zone where it occurs most frequently. The rocks there are covered with placoid lichens, and the periwinkles may possibly feed on these, or on detritus in rock pools. Periwinkles do not appear to act as food (as carrion or otherwise) to any extent for terrestrial animals, so that, even if they derive food from terrestrial sources, the food-chain is a 'blind' one.

Animal Habitats.

From the point of view of animal ecology, each plant community, or for phytophagous species each plant species, may be taken as the habitat unit, and this can be further subdivided. Thus *Correa alba* offers at least five different habitats: (1) The leaves, accommodating leaf-eating beetles, leaf-miners, etc.; (2) the flowers, the habitat for thrips, etc., as well as for nectar-feeders paying occasional visits; (3) the stems, the habitat of Coccidae; (4) the accumulation of dead leaves (A_o horizon) on the soil surface, the habitat of Blattidae, Embioptera, etc.; and (5) the underlying soil, where earth-worms, etc., may occur. Carnivorous species, however, especially birds and winged insects, cannot be assigned to any such community, but are wides ranging through many plant communities, and elsewhere.

It would be superfluous to enumerate all the situations, comprised in the communities of vascular plants, which form habitats for animal communities on the islands; below are enumerated those habitats of terrestrial animals (as opposed to marine) which are not included in vascular plant communities.

(1) *Pool habitats*:

(*a*) *Triangle Pool* (Pl. xix, D): This is the largest pool (other than strictly littoral pools) on the islands; it is some 10 yards long, of varying width, and up to about three feet deep. Its position is marked on Figure 2 (G). The elevation above mean tide level (29 ft.), and the overflow system, maintain the pool at a low salinity, ranging in our experience from 3·5 to 6·0‰ (sea-water 35‰). A dense population of protophyta maintains a high oxygen-content at most times. The phosphate and nitrate supply is good, on account of the presence of sea-birds nesting above the pool. At a time when protophyte production was extremely high (the water being a vivid green), the phosphate content was 90 mgm./cu.m., the nitrate low (c. 10 mgm./cu.m.); these figures are not representative, as much of the soluble nitrate and phosphate was obviously contained in the organic life present. The protophyta are chiefly Desmids, *Scenedesmus* being very abundant.

Triangle Pool supports a varied animal population, notably the larvae of Odonata, Chironomidae, and the mosquito *Aedes concolor*; adults and larvae of Corixids, Notonectids, Dytiscids and Gyrinids; and a surface population of water-skaters (Gerridae).

(*b*) *Freshwater Springs* (Pl. xix, E): These two pools are situated about 28 feet above mean tide level, behind and to the south of the beach, I (Fig. 2, B). They are man-made ponds dug in the *Stenotaphrum* community to a depth of about five feet, the water being retained by a clayey soil, probably the 'Y' horizon referred to earlier. Their salinity is low (within our experience, usually 0·45–0·70‰, rarely

as high as 1·08‰), due both to the shelter of this area from spray, and to continual drainage. The animal population appears to be restricted to larvae of Chironomidae and of *Aedes concolor*.

(*c*) *Phragmites Soak*: Water seeping through a sand-drift on the south side of I, and flowing at all times except in very dry seasons, accumulates in hollows in the dolerite about 25 feet above mean tide level. Almost continual flow keeps the salinity low (normally about 0·50‰ for the highest pool, and 0·95‰ for a pool some 5 feet lower down (Pl. xix, H)), in spite of low elevation. The upper pool (Pl. xix, G) occasionally dries up, passing through stages of high salinity. The population of these pools consists chiefly of larvae of *Aedes concolor*, with Corixids, Notonectids, and a few Amphipods.

(*d*) *Pools of High Salinity*: Many rock-pools above the reach of the tide, but filled frequently with spray and with a salinity approximating to that of sea water, form the habitat of larvae of *Aedes concolor*, but appear to be too saline for other insects. A pool on Periwinkle Pt., I (Pl. xix, F), although 40 feet above mean tide level, falls in this category, its salinity ranging from 18·9 to 50·8‰ in our experience; it seldom overflows, on account of the contour of the rock, and in effect acts as a concentrating-pan for the large amounts of spray which are driven across Periwinkle Pt. by southerly winds. In addition to the larvae of *Aedes concolor*, periwinkles (*Nodilittorina tuberculata*) inhabit this pool.

Pools of this fourth type are common about the 10–20 ft. contours on I and II, and on III occur up to about 45 feet. Some of the pools on the upper parts of III have a fairly low salinity (down to 4·25‰ on some occasions), and Corixids and Chironomid larvae occasionally occur in them; but the other types which are found in Triangle Pool, with a comparable salinity, are not present in the pools of III. On the platform and eastern slopes of IV, pools of the fourth category occur, some with salinity low enough to accommodate Corixids and Notonectids. On the higher parts of V, small rock-pools occur, with larvae of *Aedes concolor*, and species of Cladocera and Ostracoda. The only sample which we have been able to secure from V for salinity determination, from a pool on the top of the island, gave a value of 14·2‰, at a dry period when pools on the other islands were showing unusually high salinities.

(2) *Rock habitats*:

(*a*) *Lichens on rock surfaces*: In nearly all places above the 10-ft. contour where igneous rock is exposed, placoid lichens develop in abundance. The frequent occurrence of *Nodilittorina tuberculata* in this situation suggests that it may feed on lichens. The moth *Halone sinuata* Walk. occurs in this zone, its larvae apparently feeding on lichens.

(*b*) *Boulders*: Accumulations of rounded boulders immediately below the lower limit of vascular plants afford an animal habitat on the south-western part of I and the eastern slopes of IV. In the latter place, Machilids are particularly abundant, and lizards, spiders and ants sometimes occur. Few species occupy this habitat on I. Boulders in the more open parts of the *Scirpus nodosus* community in the 'blow-out' area of I offer shelter to large numbers of Dermaptera, and occasionally to other types.

(*c*) *Mosses and Liverworts*: On I, mosses and the liverwort *Marchantia* occur in moist situations on rocks or soil, and on the eastern slopes of IV mosses grow under similar conditions. The area of this habitat is very restricted, and it is doubtful whether it is occupied by any characteristic animal types.

(3) *Kelp*: This community, which has been mentioned earlier, scarcely constitutes a typically terrestrial habitat, falling rather in the littoral zone.

(4) *Bare sand*: Drifts of bare sand occur on the slopes on the north and south-east of I, and similar situations occur within vascular plant communities, as in the disturbed parts of the *Stenotaphrum* community. Bembecid wasps are the main frequenters of this barren habitat, and they derive their food from other areas.

Variety of Animal Types.

A diverse animal population is present on the Five Islands, including mammals (introduced rabbits), land birds (about a dozen species), reptiles (at least four species of lizards), land Mollusca (one species), insects (16 orders, with more than 50 families represented), numerous species of Arachnids, Myriapods (Chilopoda, Diplopoda), Crustacea (terrestrial Isopods and Amphipods; Cladocera and Ostracoda in pool habitats), and one terrestrial species of Oligochaeta. The animal populations will be dealt with specifically in subsequent papers.

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(In addition to works quoted in the text, the above list includes the sources of most of the taxonomic names used in the paper. The names used for birds are in all cases those given by Mathews, and authors of species have therefore been omitted.)

TABLE 1.

Explanation: For Life-Form symbols, see explanation to Table 4.
Introduced species marked with an asterisk.
Abbreviations: vr, very rare; r, rare; o, occasional; c, common; c1, common (locally dominant), c2, common (locally co-dominant); lc, locally common; o1, occasional (locally common); o2, occasional (locally co-dominant).

	I.	II.	III.	IV.	V.	Life-Form.	Remarks.
Dilleniaceae.							
Hibbertia volubilis Andr.	r	—	—	—	—	N'.	Among *Stenotaphrum* on north-west portion of **I.**
Menispermaceae.							
Stephania hernandifolia (W. et Arn.) Walp.	—	—	—	c	—	Ch.	Epharmonic variation with relatively small leaves, growing as a low straggling creeper on plateau, **IV**; formerly the commonest plant on central part of plateau, now partly displaced by *Mesembryanthemum aequilaterale.*
Cruciferae.							
Lepidium hyssopifolium Desv.	—	—	—	c	—	Th.	Moderately common on plateau, **IV**; has increased since 1936, when it was very rare.
Rutaceae.							
Correa alba Andr... ..	r	c1	o2	c2	—	N.	Dominant or co-dominant in nearly all areas carrying shrubs.
Oxalidaceae.							
Oxalis corniculata Linn. ..	r	r	—	r	—	H.	On bare sandy soil, or in grassland (**I, II**); in centre of plateau (**IV**).
Malvaceae.							
Malva parviflora Linn.*	—	—	—	—	vr	Th.	In shallow soil on higher parts of island.
Portulacaceae.							
Claytonia Pickeringi (A. Gray) F. v. M.	—	—	—	c	—	Ch.	In shallow moist saline soil in rock basins, eastern slopes of **IV.**
Portulaca oleracea Linn.	—	—	o	c	c	Th.	Similar situations to the preceding species, on **III, IV** and **V.**
Caryophyllaceae.							
Spergularia rubra (Linn.) J. et C. Presl.	—	—	vr	o	r	Ch.	As for *Portulaca.*
Chenopodiaceae.							
Atriplex cinereum Poir. ..	—	—	—	r	o	N'.	On **IV**, rare on slopes and plateau; on **V**, in shallow soil in rock crevices, etc.; appears to be increasing in numbers on V (July, 1938).
A. patulum Linn.* ..	vr	—	—	—	—	Th.	In *Stenotaphrum* community near beach, **I**; only seen on earlier visits (1934–5).
Enchylaena tomentosa R.Br.	r	r	o	c	—	Ch.	Usually on dry sandy soil, or in rock crevices with little soil.
Rhagodia baccata (Labill.) Moq.	—	vr	—	—	—	Ch.	In *Stenotaphrum* community.
R. hastata R.Br. ..	o	o	c	o	vr	Ch.	In *Stenotaphrum* community on **I** and **II**; in *Sporobolus* community on higher parts of **III**; on **IV**, both on plateau and eastern slopes.
R. nutans R.Br.	—	—	—	r	—	Ch.	In rocky situations.
Salicornia australis Banks et Sol.	—	—	c1	—	—	Ch.	In wet saline soil in cuppings of the underlying rock.
Salsola Kali Linn. ..	r	—	—	—	—	Th.	In dry sandy situations.
Aizoaceae.							
Mesembryanthemum aequilaterale Haw.	o	o	c	c	o	Ch.	Locally common on rocks, with little soil; also on those parts of the plateau, **IV**, lacking shrubs, where it has become dominant since March, 1936.

TABLE 1.—*Continued.*

	I.	II.	III.	IV.	V.	Life-Form.	Remarks.
M. australe Sol.	—	—	—	vr	—	Ch.	As for *Claytonia*.
Tetragonia expansa Murr.	lc	lc	o	o	o	Ch.	On **I** and **II** forms a distinct local facies of the *Stenotaphrum* community; elsewhere in shallow soil on rocks, or rarely on plateau of **IV**. Listed as an annual by Moore and Betche, but perennial in these localities according to our observations.
Polygonaceae.							
Rumex Brownii Campd. ..	vr	—	—	—	—	N'.	A few plants behind the beach in the *Stenotaphrum* community, **I**; observed only on recent visits (since August, 1937); possibly a recent migrant to the island, though indigenous in this part of New South Wales.
R. crispus Linn.*	—	—	—	vr	—	Th.	On eastern slopes, **IV**; first observed in March, 1938, and probably a recent migrant. Apparently therophytic in this locality.
Phytolaccaceae.							
Phytolacca octandra Linn.*	c	o	—	vr	—	Th.	Usually on sandy soil; forms a distinct local (seasonal) facies of the *Stenotaphrum* community on **I**.
Leguminosae.							
Desmodium varians (Labill.) Endl.	—	—	—	vr	—	Ch.	Eastern slopes of **IV**.
Glycine clandestina Wendl.	—	—	—	r	—	Ch.	On plateau. **IV**.
Kennedya rubicunda Vent.	r	o	vr	o	—	N'.	Climbing over other plants, e.g. *Stenotaphrum* on **I** and **II**, and *Westringia* on plateau of **IV**. Classed as a nanophanerophyte, but more of a chamaephyte on **I** and **II** on considerations of height.
Crassulaceae.							
Tillaea Sieberiana Schultes	r	r	—	o	—	Ch.	In dry rocky situations.
Umbelliferae.							
Apium prostratum Labill.	o	o	o	—	—	Ch.	In moist soil amongst rocks, usually at a low elevation.
Cactaceae.							
Opuntia inermis P.DC.*	c	c	c	c	—	S.	On dry soil or almost bare rocks, often becoming dominant, or co-dominant with *Stenotaphrum*, etc., but subjected from time to time to attempts at eradication.
Compositae.							
Centipeda minima (Linn.) A.Br. et Aschers	—	—	—	o	—	Th.	On plateau, **IV**.
Cirsium lanceolatum (Linn.) Scop.*	r	r	—	—	—	Th.	In more open parts of *Stenotaphrum* community.
Cotula australis (Less.) Hook f.	—	o	—	vr	—	Th.	In *Sporobolus* community, **II**; on eastern slopes. **IV**.
C. coronopifolia Linn. ..	—	—	—	o	—	Ch.	As for *Claytonia*.
Erechthites arguta (A. Rich.) DC.	—	—	—	o	—	Th.	On plateau, **IV**.
Gnaphalium luteo-album Linn.	r	r	—	—	—	Th.	In *Stenotaphrum* and *Sporobolus* communities.
Hypochoeris glabra Linn.*	—	—	—	r	—	Th.	On plateau. Only seen on recent visits (March, 1938), probably a recent migrant.
Inula graveolens (Linn.) Desf.*	o	—	—	—	—	Th.	On bare soil, as in the more open parts of the *Scirpus nodosus* community; very rare on our earlier visits, has increased markedly in numbers (1938).
Onopordon Acanthium Linn.*	r	r	—	—	—	Th.	In more open parts of *Stenotaphrum* community.

TABLE 1.—*Continued.*

	I.	II.	III.	IV.	V.	Life-Form.	Remarks.
Senecio lautus Sol. ..	r	r	c	o	—	Ch.	In *Stenotaphrum* community on **I** and **II**; in *Sporobolus* community on **III**; on plateau, **IV**.
S. mikanioides Otto.* ..	o	o1	—	o	—	Th.	On **I** and **II**, scrambling over *Stenotaphrum* and dead remains of *Opuntia*; on plateau, **IV**.
Sonchus asper Hill* ..	r	r	—	—	—	Th.	On bare soil.
S. maritimus Linn.* ..	r	r	—	r	—	Th.	As *S. asper*.
S. oleraceus Linn.* ..	r	r	r	o	r	Th.	As *S. asper*, sometimes on almost bare rocks; also in more open parts of *Stenotaphrum* community on **I** and **II**.
Taraxacum officinale Weber*	—	—	vr	r	—	Th.	As *Sonchus asper*.
Campanulaceae.							
Lobelia anceps Thunb. ..	o	o	—	c	—	Ch.	In same situations as *Apium*, occasionally in drier situations.
Wahlenbergia gracilis (Forst. f.) A.DC.	vr	vr	—	—	—	Th.	In grassland.
Goodeniaceae.							
Scaevola calendulacea (Andr.) Druce.	vr	—	—	—	—	Ch.	In *Stenotaphrum* community, north-west portion of **I**.
Gentianaceae.							
Erythraea australis R.Br...	r	r	—	—	—	Th.	In grassland.
Plantaginaceae.							
Plantago varia R.Br. ..	r	—	—	c	—	Ch.	Usually in shallow soil in rock crevices.
Primulaceae.							
Anagallis arvensis Linn.*	r	r	—	—	—	Th.	In more open parts of *Stenotaphrum* community.
Samolus repens (Forst.) Pers.	o	o	vr	r	—	Ch.	In similar situations to *Apium*.
Asclepiadaceae.							
Marsdenia rostrata R.Br.	r	—	—	—	—	N'.	Scrambling over *Stenotaphrum*, north-west corner of **I**.
Convolvulaceae.							
Dichondra repens Forst. et f.	r	r	o	c	—	H.	On **I**, **II** and **III**, in dry sandy soil or more open parts of grass communities; on **IV**, on plateau and ledges of westerly rock face.
Solanaceae.							
Datura Stramonium Linn.*	vr	—	—	—	—	Th.	A few plants near the beach, **I**. First observed in 1938, and probably a very recent migrant.
Lycium ferocissimum Miers*	—	—	—	—	vr	Th.	In shallow soil between rocks; apparently therophytic in this locality.
Solanum nigrum Linn. ..	vr	vr	—	vr	vr	Th.	In grassland (**I**, **II**); on plateau (**IV**); in shallow soil on top of **V**. Doubtfully native.
Labiatae.							
Plectranthus parviflorus Henck.	r	r	—	c	—	N'.	Usually in dry shallow soil in rock crevices; also in *Stenotaphrum* community (**I**, **II**), and on plateau (**IV**). Barely falls within the nanophanerophyte class.
Westringia rosmariniformis Sm.	r	o2	—	c2	—	N.	Co-dominant with *Correa* in shrub communities of **IV** and parts of **II**; on **IV** attains local dominance in some places. A few bushes on the north side of **I**.
Myoporaceae.							
Myoporum ellipticum R.Br.	—	—	o2	c	—	N.	Forms a local facies of the shrub community on **IV**; co-dominant on **III** where shrubs are developed.
Liliaceae.							
Lomandra longifolia Labill.	c	vr	—	—	—	H.	As tussocks holding the sandy soil at the former level on **I**; on **II**, rare in the *Stenotaphrum* community.

TABLE 1.—*Continued.*

	I.	II.	III.	IV.	V.	Life-Form.	Remarks.
Commelinaceae.							
Commelina cyanea R.Br.	c	o	r	c	c	Ch.	On **I**, **II** and **III**, in grass communities; on **IV**, on plateau and ledges of westerly rock face; in shallow soil in rock crevices on **V**.
Centrolepidaceae.							
Centrolepis fascicularis Labill.	—	o	—	—	—	Th.	In *Sporobolus* community, locally fairly common.
Cyperaceae.							
Cyperus (*Pycreus*) *polystachyus* (Rottb.)	o	o	—	—	—	H.	In dry shallow soil.
Scirpus cernuus Vahl. ..	o	o	—	vr	—	Ch.	In similar situations to *Lobelia*, *Samolus* and *Apium*.
S. nodosus Rottb... ..	cl	o	r	o	—	H.	In shallow accumulations of sand, or on almost bare rock; also in central part of plateau, **IV**.
Gramineae.							
Agrostis avenacea Gmel. (=*Calamagrostis filiformis* (Forst.) Pilger).	r	—	r	o	—	Th.	Among *Stenotaphrum* or *Sporobolus* (**I**, **III**); in shallow soil amongst rocks, eastern slopes of **IV**.
Calamagrostis quadriseta (Labill.) Spreng.	vr	r	—	—	—	H.	In *Stenotaphrum* community.
Cynodon dactylon Rich. ..	r	r	—	—	—	H.	In *Stenotaphrum* community.
Digitaria marginata Link.	vr	—	—	c	c	Th.	In *Stenotaphrum* community (**I**); in shallow soil amongst rocks (**IV**, **V**).
Eleusine indica Gaertn. ..	—	—	—	—	vr	Ch.	In shallow soil on higher parts of island.
Echinochloa crus-galli (Linn.) Beauv.	—	—	—	—	vr	Th.	In similar situations to the preceding species. Possibly introduced, but allowed as indigenous by Moore and Betche.
Entolasia marginata (R.Br.) Hughes.	—	—	—	r	—	H.	On ledges of western rock face, **IV**.
Imperata cylindrica (Linn.) Beauv. var. *Koenigii* D. and S.	o	r	—	—	—	H.	In *Stenotaphrum* community.
Paspalum distichum Linn.	—	—	—	vr	r	H.	In shallow soil in rock crevices.
Phragmites communis Trin.	lc	—	—	—	—	H.	In *Stenotaphrum* community at low elevations, south side of **I**.
Setaria glauca (Linn.) Beauv.	o	vr	—	—	—	Th.	In *Stenotaphrum* and *Sporobolus* communities.
Sporobolus virginicus (Linn.) Kunth.	o	cl	cl	—	—	H.	Dominant over most of the western part of **III**, and on the eastern part of **II** wherever shrubs are not developed. On **I**, dominant only on Periwinkle Pt., apparently having been displaced elsewhere on **I**, and on the western end of **II**, by *Stenotaphrum*. On dry sandy soil, and on ill-drained soil amongst rocks.
Stenotaphrum secundatum (Walt.) Kuntze*	cl	cl	—	o	o	Ch.	Dominant over much of **I** and **II**, on sand. On **V**, in soil in rock crevices. Since 1934, has colonized **IV**, being now well established on eastern slopes and plateau. Grows as a chamaephyte in these localities, though hemicryptophytic in some localities.
Themeda australis (R.Br.) Stapf.	—	o	—	—	—	H.	In dry shallow soil on rocks, and in *Stenotaphrum* and *Sporobolus* communities.
Zoisia macrantha Desv. ..	r	o	r	—	—	H.	With *Sporobolus*.
Polypodiaceae.							
Pteridium aquilinum (Linn.) Kuhn.	o	o	—	—	—	G.	In *Stenotaphrum* community.

TABLE 2.

Properties of surface soils (0–2 inches) from various communities.

Community and Location.	Height above Mean Tide Level.	pH.	Loss on ignition (%).	W.R.C. (%).	Chloride (%).
	Feet.				
Correa-Westringia community :					
Correa, Centre of **II** : Soil 1 ft. deep, derived from dune sand contaminated with doleritic soil. Well drained*	45	6·0–6·1	11·2	58	0·026
Correa-Westringia, Centre of **II**, south side. Depth, origin and drainage as last	35	5·9–6·0	16·5–17·9	63	0·101–0·112
Correa-Westringia, **IV**, plateau, north end : Soil 1 ft. deep, well drained, derived from trachy-andesite	40	5·2–5·3	45·0–50·2	125	0·034
Stenotaphrum community :					
High Hummock, **I** : Dune sand, 8 ft. deep, very well drained	70	5·3–5·4	1·6–4·5	28–37	0·013
II, west end : Soil 2 ft. 6 in. deep, derived from dune sand, well drained	40	5·3–5·4	10·8	43	0·052
I, between beach and Camp Hummocks : Deep dune sand, well drained. (*Tetragonia* on sample with 11% humus)	25	5·9	8·0–11·0	38–54	0·023
I, Camp Hummocks : Deep dune sand, well drained. With *Lomandra longifolia*	50	5·8	1·2	28	0·014–0·015
Sporobolus community :					
I, Periwinkle Pt. : Soil 8–15 inches deep, derived chiefly from dune sand, with slight contamination from dolerite ; not very well drained	40	6·1–6·2	13·3	51–56	0·032
II, centre, south side : Soil 1 ft. deep, fairly well drained, derived from dune sand contaminated with doleritic soil	35	6·6–6·7	12·5	70	0·064
II, south-east corner : Soil 1 ft. deep, derived chiefly from underlying dolerite ; poorly drained because of cupping of underlying rock*	35	6·5	15·0	104	0·056
III, top of island, west end : Soil about 1 ft. deep, well drained, derived from dolerite, possibly with slight admixture of wind-borne sand	50	5·5	25·6	86–102	0·093
III, near ecotone with *Salicornia* community : Soil 6 in. deep, derived from dolerite, poorly drained	35	7·2–7·4	18·1–21·1	112	0·443–0·653
Scirpus nodosus community :					
I, 'blow-out' area : Recent sandstone, with 1–2 inches of wind-blown sand collected around *Scirpus* tussocks ; very dry and well drained..	50	5·4	3·0	29	0·036
II, 'blow-out' area, near east end : Soil similar to last, but resting on dolerite, not sandstone	45	6·2	3·4	33	0·032–0·033
Mesembryanthemum community :					
IV, Centre of plateau : Soil 2 ft. deep, well drained, podsolized, derived from trachy-andesite	40	5·4–5·5	26·5	101	0·092
IV, edge of plateau : Soil 1–2 inches deep, restricted to vicinity of plant, rock elsewhere bare ; soil well drained, derived from trachy-andesite	35	6·0–6·1	44·9	162	0·203

* Water content (20/8/37) 14% and 84%, respectively, of the dry weight.

TABLE 2.

Properties of surface soils (0–2 inches) from various communities.

Community and Location.	Height above Mean Tide Level.	pH.	Loss on ignition (%).	W.R.C. (%).	Chloride (%).
	Feet.				
Salicornia community:					
III: Soil 3 inches deep, derived from dolerite, very badly drained	30	7·4–7·5	11·4	97	0·848
III: Soil 6 inches deep, as above, but with slightly better drainage; near ecotone with *Sporobolus* community	35	6·9–7·0	30·4	148	0·460
Spergularia-Claytonia-Portulaca community:					
IV, eastern slopes: Soil 1–3 inches deep, derived from trachy-andesite; poorly drained, in rock basin	25	5·7–5·8	29·4–32·3	116	0·117
Scirpus cernuus community:					
I, below High Hummock: Dune sand 3 inches deep, lodged in rock crevices; poorly drained	25	6·8	8·2	48	0·062
I, below *Phragmites* Soak: Soil 1 inch deep, dune sand accumulated in shallow rock-pool, filled from slowly-running soak; salinity of pool when soil collected, Cl 0·76‰	20	6·6–6·7	3·5	33	0·029
Lomandra community:					
See Sample 4 of *Stenotaphrum community.*					

The pH values were measured by the quinhydrone method (gold electrode) as soon as practicable after collection of the sample, usually within three days. Little drift was observed in the pH when the soils were stood with water for varying times, very probably because of natural buffering.

For the other estimations all soils were previously passed through a 1 mm. sieve. The percentage loss on ignition of oven-dry soil gives a fairly reliable measure of the organic matter (humified and unhumified), little clay and practically no calcium carbonate being present. Water-retaining capacity was calculated by weighing saturated soils, contained in squat metal cylinders with gauze bottoms lined with filter-paper, and weighing again after drying in an oven at 90°–100° C. The W.R.C. is expressed as a percentage of the dry soil, allowance being made for the water held by the apparatus at the first weighing. The figures are comparable *inter se*, but higher than would be obtained by most methods. The chloride content (expressed as percentage Cl-ion per unit dry weight of soil) was obtained by lixiviating known weights of oven-dry soil with hot distilled water, and estimating the filtrate with standard silver nitrate. The salinity of the soil solution, involving water-content of the soil, was judged too variable from season to season to merit calculation.

The pH values are controlled by (1) the origin of the soil, dune sand being lowest, soil derived from trachy-andesite intermediate, that derived from dolerite highest; (2) salinity, as given by chloride content, high salinity raising the pH; and (3) humus-content, high humus-content lowering the pH. The humus-content is largely controlled by drainage, poorly-drained soils having high humus-content; soils from shrub communities are also rich in organic matter generally on account of accumulations of dead leaves. *Mesembryanthemum* as a stage in a lithosere also has a soil of high organic content. The W.R.C. is controlled by texture (low for dune sand, high for soils from igneous rocks), and by organic content. The chloride content is governed both by incidence of salt spray (depending on aspect, height and distance from shore), and by leaching, poorly-drained soils tending to have a high salinity.

TABLE 3.

Soil Sample.	Nitrate (mgm. N_2O_5 per gm.).	Phosphate (P_2O_5, parts per million).	NH_3.
Stenotaphrum community, penguin rookery between beach and Camp Hummocks, I ..	0·086	3·03–4·72	+
Stenotaphrum community, High Hummock, I: No birds nesting near this situation ..	0·004	5·76	–
Dune community, mainland at Red Point: No birds present	0·010	3·54	–

Phosphate, nitrate and ammonia content for soil from a penguin rookery, and from similar plant communities where no birds are normally present. Nitrate and phosphate estimated colorimetrically, the former in filtrates from known weights of dry soil within 24 hours of collecting, the latter from known weights of dry soil, calcined and lixiviated. Ammonia was estimated qualitatively with Nessler's Reagent, in filtrates from aliquot parts of soil; + represents a deep colour, – no colour.

TABLE 4.

Life-Form Spectra.

	S.	E.	MM.	M.	N.	N′.	Ch.	H.	G.	HH.	Th.	Number of Species.
Five Islands, I-V—												
(A)	—	—	—	—	5	10	38	22	2	—	23	63
(B)	1	—	—	—	4	7	31	17	1	—	39	81
Five Islands, I, II—												
(A)	—	—	—	—	5	11	32	27	2	—	23	44
(B)	2	—	—	—	4	9	26	21	2	—	36	57
Five Islands, III—												
(A)	—	—	—	—	11	5	52	21	—	—	11	19
(B)	5	—	—	—	9	5	45	18	—	—	18	22
Five Islands, IV—												
(A)	—	—	—	—	8	8	50	13	—	—	21	38
(B)	2	—	—	—	6	6	43	11	—	—	32	47
Five Islands, V—												
(A)	—	—	—	—	0	8·5	50	8·5	—	—	33	12
(B)	—	—	—	—	0	6	44	6	—	—	44	16
Brush Island—												
(A)	—	1·5	—	11	8	13	30	28	1·5	—	7	61
(B)	—	1·5	—	10	8	12	27	26	1·5	—	14	66
Eucalyptus pilularis Association in Bulli District. (B)	—	2	10	7	15	12	12	24	9	—	9	82
'Normal' Spectrum ..	1	3	6	17	20		9	27	3	1	13	400

(A), indigenous species; (B), total species.

S, stem-succulent; E, epiphyte; MM, mega- and meso-phanerophytes; M, microphanerophyte; N, woody nanophanerophyte; N′, other nanophanerophytes (perennial herbs or climbers with growing apex reaching more than 1 foot in height); Ch, chamaephyte; H, hemicryptophyte; G, geophyte; HH, halo- and hydro-phytes; Th, therophyte.

TABLE 5.
Introduced Plants.

	Number of Introduced Species.	Percentage of Species Introduced.	Life-Forms of Species Introduced.	Means of Entry of Species Introduced.
Five Islands, I-V ..	18	22	S 1, Ch 1, Th 16	W 9, F 2, FF 1, X 6
Five Islands, I, II ..	13	23	S 1, Ch 1, Th 11	W 7, F 1, FF 1, X 4
Five Islands, III ..	3	14	S 1, Th 2	W 2, FF 1
Five Islands, IV	9	19	S 1, Ch 1, Th 7	W 5, F 1, FF 1, X 2
Five Islands, V ..	4	2·5	Ch 1, Th 3	W 1, F 1, X 2
Brush Island	5	8	Th 5	W 3, F 1, X 1

For Life-Form abbreviations, see explanation to Table 4.

Means of entry: W, plumed fruit, wind-borne; F, fleshy fruit; FF, fleshy fruit and floating cladode; X, other means.

EXPLANATION OF PLATES XV-XIX.

Plate xv.

Vertical aerial photographs of the Five Islands, scale 10 inches to the mile. A, Islands **I**, **II** and **III**; B, Island **IV**; C, Island **V**. For orientations see Plate xv*a*; for relative positions see Text-figure 1. Sun in approximately north-eastern position; half-tide. July, 1937. Photographs by Adastra Airways Pty., Ltd.

Plate xv*a*.

Outlines of principal communities of vascular plants. For all the islands, bare igneous rock is represented as black. In the transparency to Pl. xv, C (Island **V**), the white areas represent all areas occupied by vascular plants, which are very restricted and scarcely form definite communities. For the other islands, the following symbols are used for the various communities:

Plain white, *Stenotaphrum* community (*Phytolacca* facies with small crosses, *Tetragonia* facies with small black triangles); small circles, *Correa-Westringia* community; tussock symbols, *Scirpus nodosus* community; stippling, *Sporobolus* community; broken diagonal lines, *Mesembryanthemum* community; vertical shading, *Salicornia* community; heavy asterisks, *Lomandra* community; horizontal shading, *Spergularia-Claytonia-Portulaca* community; cross-hatching, loose sand.

Plate xvi.

Vertical aerial photograph of the Five Islands (**I**, **II** and **III**), Midway Reef, and the adjacent coast. Scale 4 inches to the mile. Sun in approximately north-western position; half-tide. August, 1937. Photograph by Adastra Airways Pty., Ltd.

Plate xvii.

A.—Camp Hummocks, **I**: Dune sand held at old level by *Stenotaphrum secundatum* (left) and *Lomandra longifolia* (right); sand elsewhere removed by wind action ('blow-out') to level of underlying recent sandstone. *Scirpus nodosus* tussocks growing in shallow sand accumulations at new level.

B.—Tussocks of *Lomandra longifolia* holding dune sand at old level, north-eastern part of **I**. *Scirpus nodosus* tussocks growing in very shallow soil collected around their bases; 'blow-out' area otherwise devoid of soil.

C.—North-eastern side of **I**, from the west end of **II**, showing drifts of sand removed from the top of **I** by wind action. The area so denuded is visible immediately above the large sand-drift, and to the right of the denuded area Camp Hummocks are seen on the upper parts of the island, where the vegetation has held the sand against erosion.

D.—'Blow-out' area, centre of **I**. Removal of the dune cover has exposed recent sandstone, which has weathered to an irregular surface by water action. Soil absent except for shallow accumulations of sand around tussocks of *Scirpus nodosus*; old shells (*Turbo*

stramineus Gmel., *Cymatium spengleri* Perry) from an aboriginal kitchen-midden have been left behind when the sand around and below them was blown off.

E.—Erosion of recent sandstone exposed by removal of sand cover, west of Periwinkle Point, **I**. *Scirpus nodosus* tussocks growing as in A, B and D.

F.—Portion of area shown in figure E, where water action has cut through the recent sandstone, exposing a clay soil. This clay is probably the illuvial horizon of a doleritic soil pre-dating cover of the area by dunes. *Mesembryanthemum aequilaterale* growing on left side of cutting.

G.—Bushes of *Correa alba* and *Westringia rosmariniformis*, with *Sporobolus virginicus* in foreground. East end of **II**.

H.—Bushes of *Westringia rosmariniformis*, south end of plateau, **IV**.

I.—Plateau of **IV**, looking north from near southern end. *Mesembryanthemum* community and bushes of *Correa* and *Westringia* in the background, and *Myoporum ellipticum* facies of shrub community on left.

J.—The beach, **I**: *Stenotaphrum secundatum*, with lower limit only 8 feet above mean tide level. Exposed sand with vegetation disturbed by burrows of sea-birds visible in central background. The boat is 16 feet long.

Plate xviii.

A.—Between the beach and Camp Hummocks, **I**: Permanent quadrat in portion of *Stenotaphrum* community disturbed by burrowing activities of sea-birds; looking north-east. Corners of quadrat (side 10 ft.) indicated by black rings. Bushes of *Phytolacca octandra* to the left. Date 18/12/37.

B.—Quadrat of figure A, 24/4/38. Bushes of annual *Phytolacca octandra* dead; about two inches of the surface sand removed by wind action, the isolated patch of *Stenotaphrum* near the centre of the quadrat partly undermined thereby.

C.—North side of **II**, near west end: Large expanse of *Opuntia inermis* killed by poisoning, March, 1936.

D.—*Opuntia* regenerating: Same locality as figure C, August, 1937.

E.—*Opuntia* regenerating: Living cladodes and dead remains, west edge of plateau, **IV**.

F.—*Sporobolus* community, near east end of **II**; on the left, the soil has been blown off, exposing the underlying dolerite. The stick is 1 metre high.

G.—Dense growth of *Scirpus nodosus*, 'blow-out' area near High Hummock, **I**.

H.—*Mesembryanthemum* community, surrounded by *Sporobolus* community, on the higher parts of the west end of **III**. The note-book is 8 inches high.

I.—Central part of plateau, **IV**, March, 1936: *Stephania hernandifolia* with *Plectranthus parviflorus* and species of Compositae. *Correa* and *Westringia* bushes in background.

J.—The same, March, 1938: *Mesembryanthemum aequilaterale* has largely displaced *Stephania*, etc.

Plate xix.

A.—*Salicornia* community, in depression on south side of **III**, near west end. *Sporobolus* and *Mesembryanthemum* communities in background. The note-book is 8 inches high.

B.—*Portulaca oleracea* growing in moist shallow soil in rock-basins, eastern slopes of **IV**.

C.—Gulls (*Bruchigavia novae-hollandiae*) nesting on the western end of **III**; vegetation *Sporobolus virginicus* with *Opuntia*.

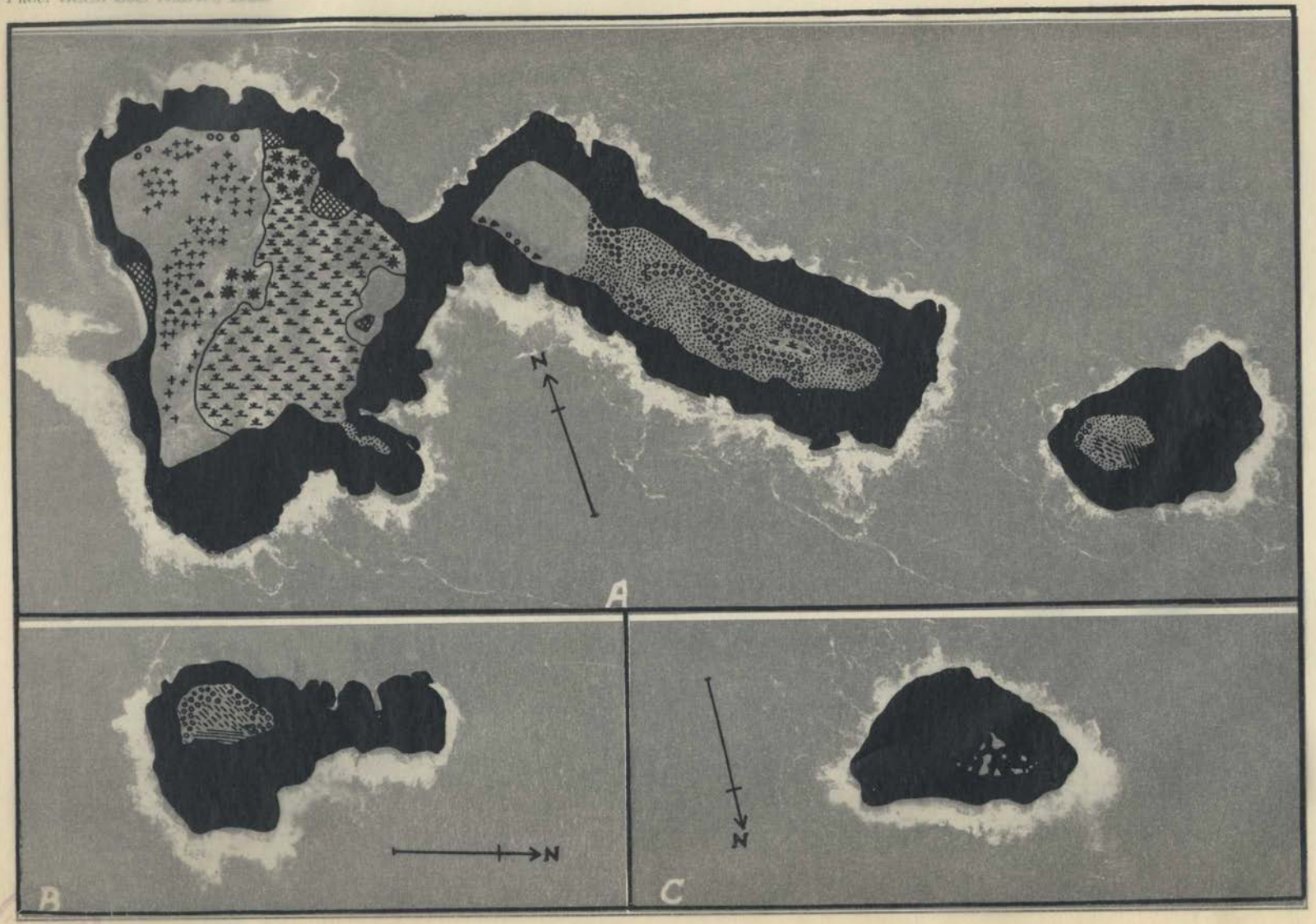
D.—Triangle Pool, **II**: A brackish pool 29 ft. above mean tide level.

E.—Freshwater Springs: Holes dug in the soil of the *Stenotaphrum* community, behind and to the south of the beach, **I**; about 28 ft. above mean tide level, with almost fresh water.

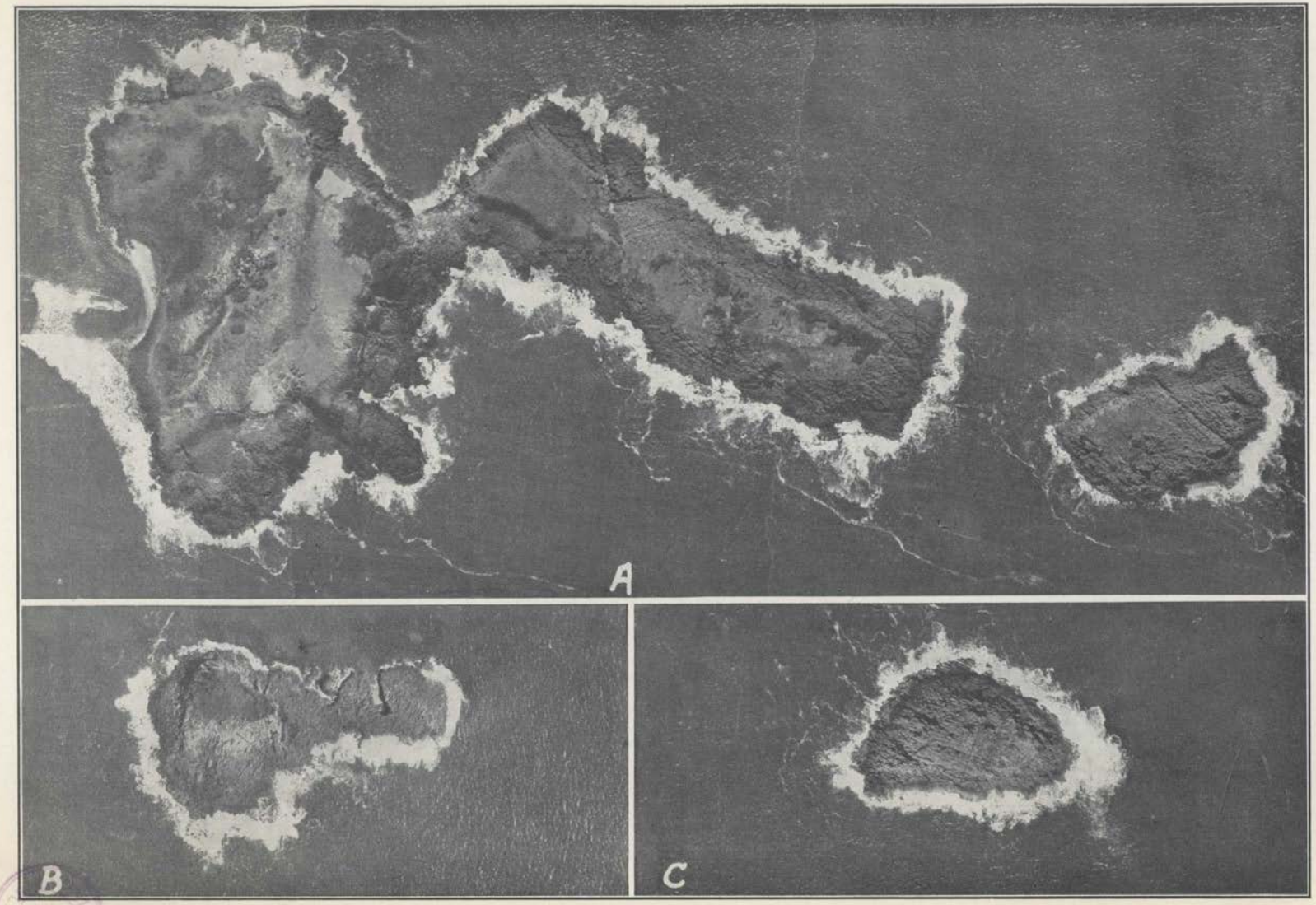
F.—Pool in hollow in the dolerite, Periwinkle Point, **I**. Elevation 40 ft. above mean tide level, but with high salinity, due to infrequent overflow and heavy incidence of spray. Periwinkles (*Nodilittorina tuberculata*) occur in and around this pool.

G.—Phragmites Soak, **I**: A pool at the bottom of the sand-drift, through which water percolates. Although less than 25 ft. above mean tide level, constant run-off ensures low salinity of the water. The vegetation of the sand-drift is *Stenotaphrum secundatum* (with stolons hanging down over the rocks), and *Phragmites communis*. The note-book is 8 inches high.

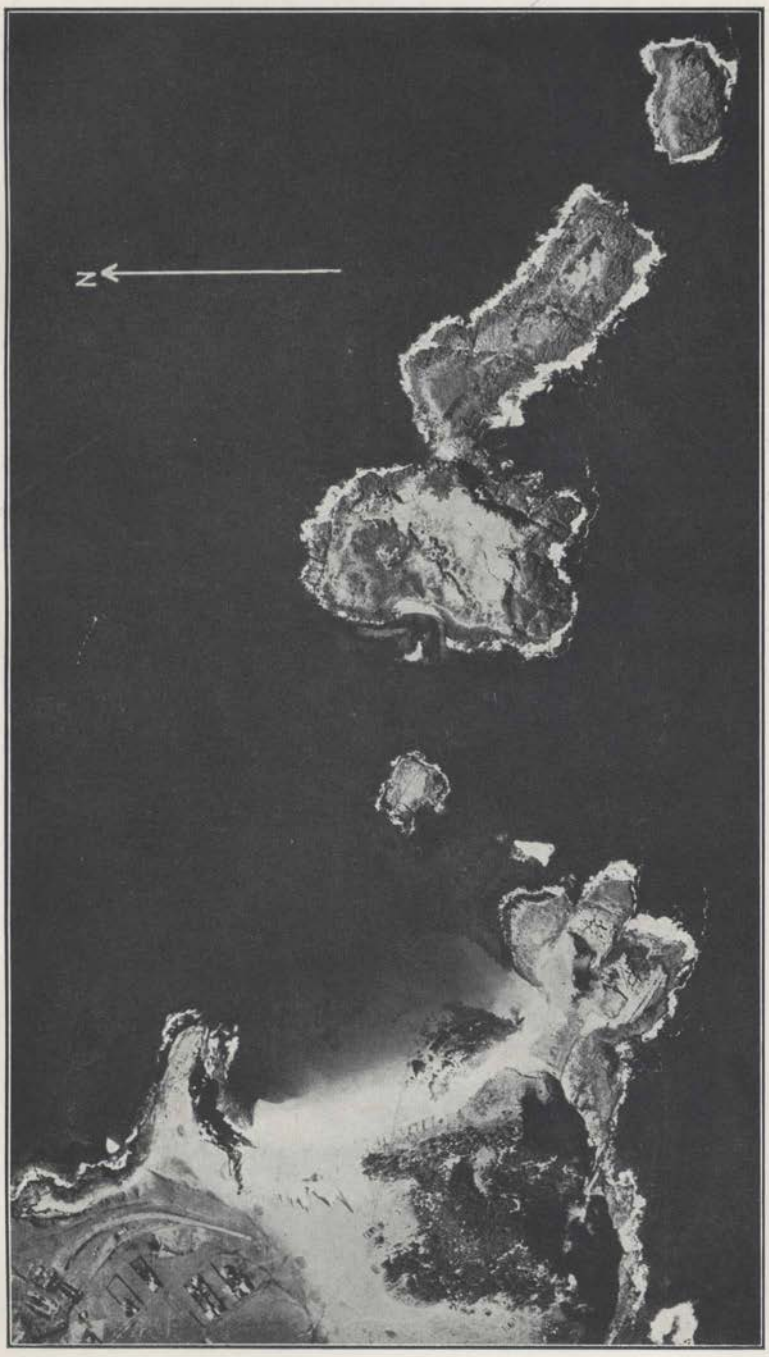
H.—A pool below that shown in the preceding figure, with slightly higher salinity. *Scirpus cernuus* (in rectangle) growing in wet sand in pool, *S. nodosus* in drier situation above. The characteristic crevices in the dolerite are apparent in this photograph.



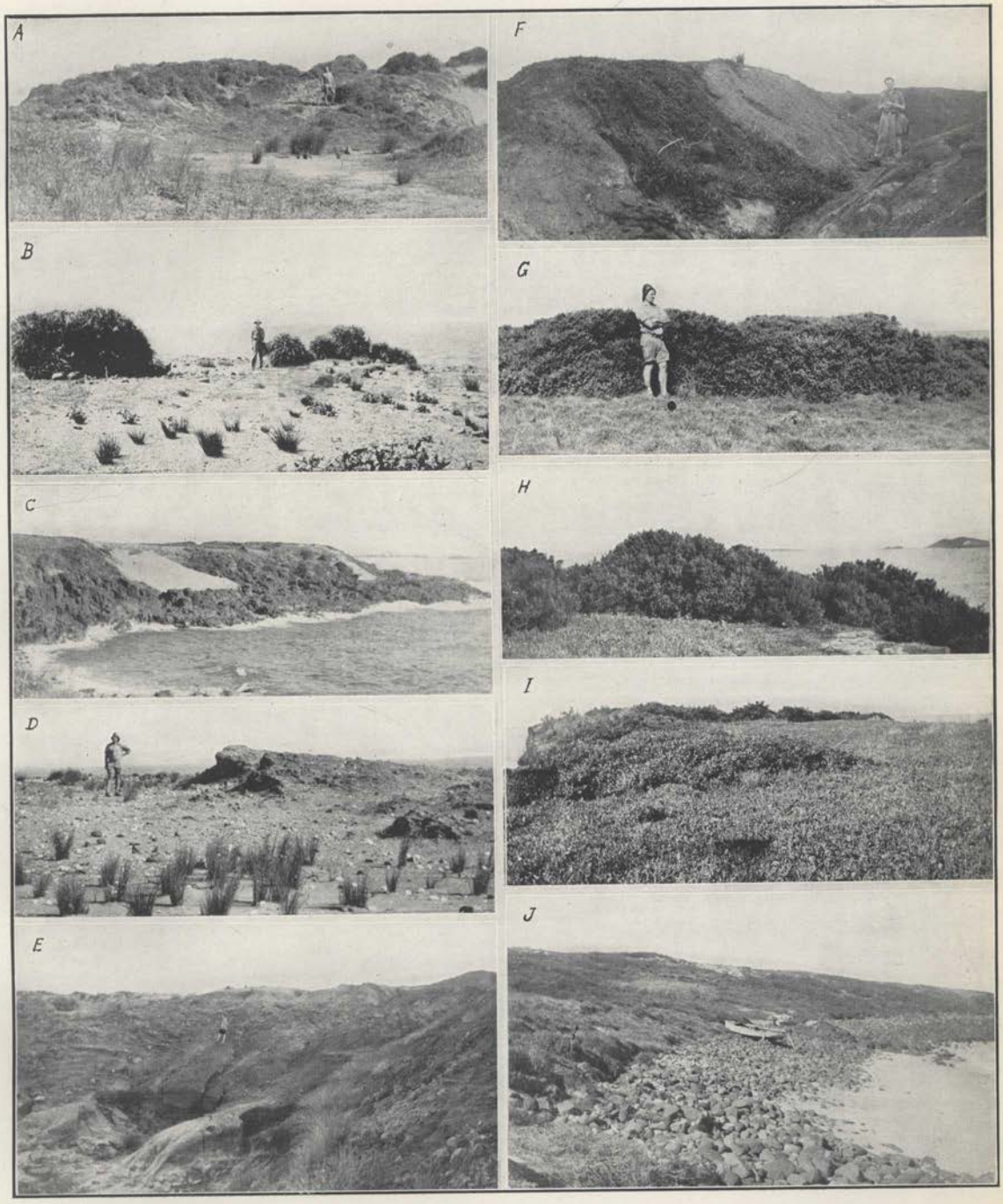
Aerial photographs of the Five Islands.



Aerial photographs of the Five Islands.



Aerial photograph of three of the Five Islands,
Midway Reef, and the adjacent coast.



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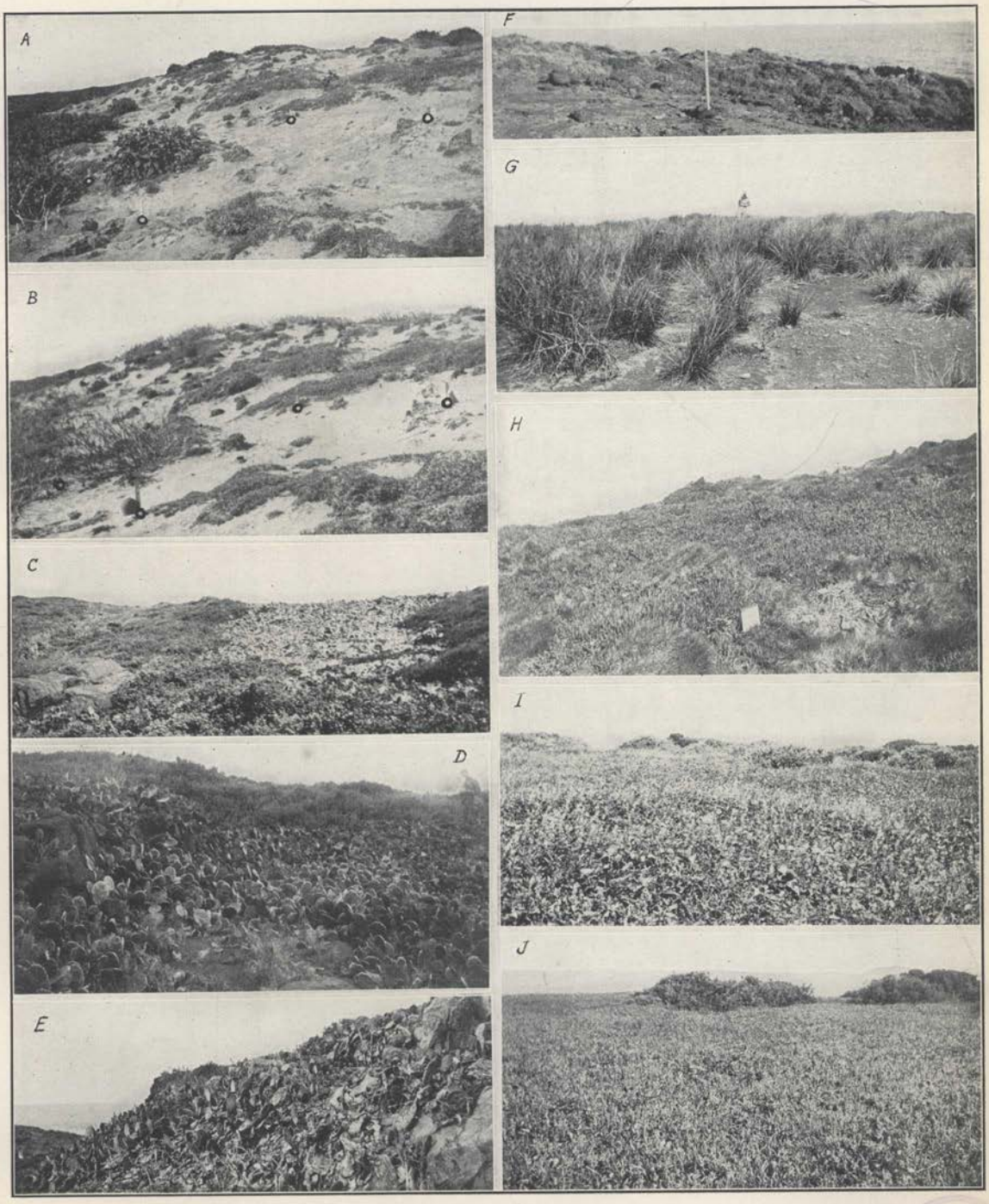

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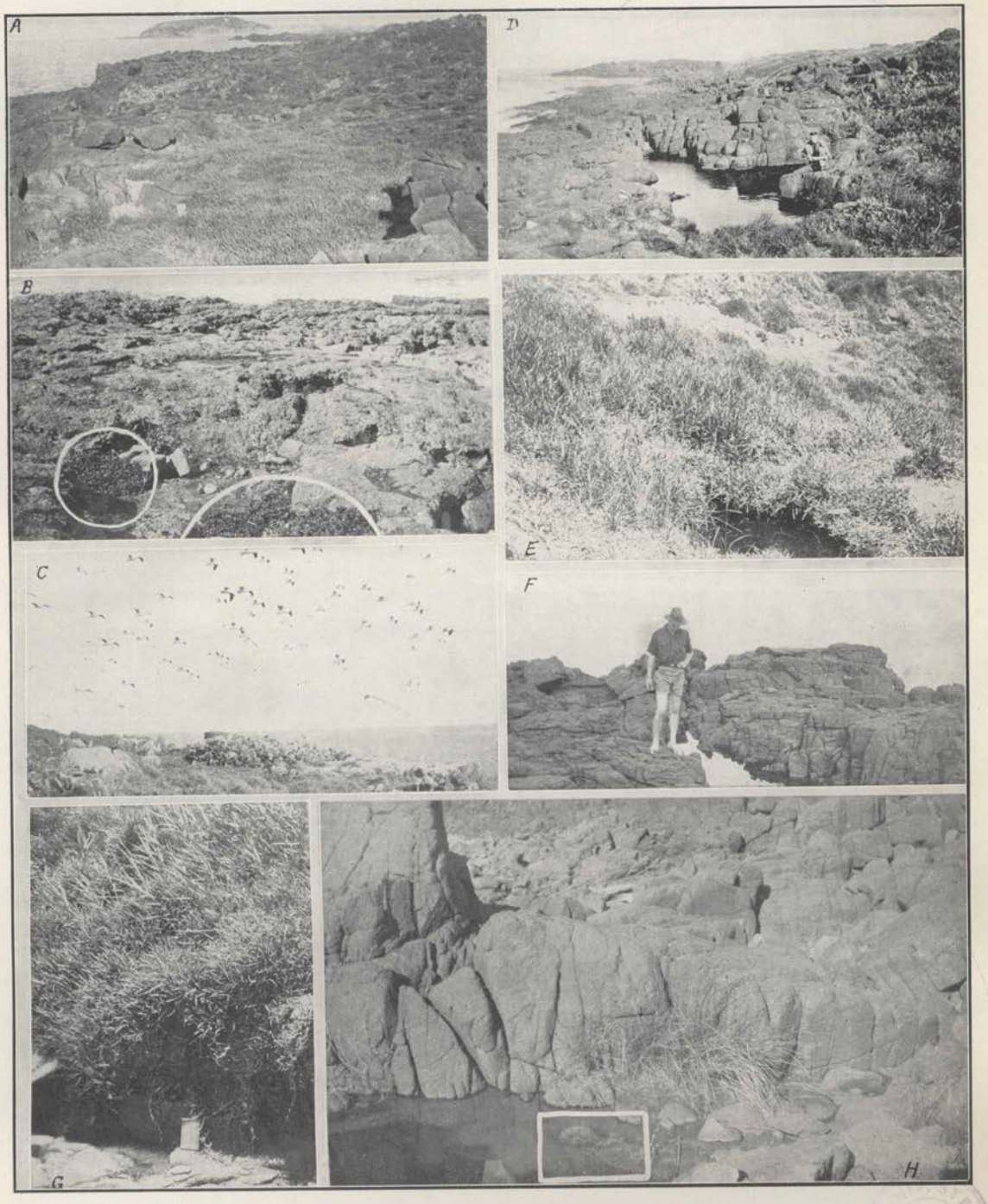

Ecology of the Five Islands.

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The Present Status of D.D.T. as an Insecticide

By

G. A. H. Helson and D. F. Waterhouse,
Division of Economic Entomology, C.S.I.R., Canberra, A.C.T.

The new insecticide D.D.T. has aroused very great interest since its possibilities were first publicized early in 1944, largely in connexion with its effect on insect vectors of human disease. More recently, attention has been drawn to the possibility of its wide usefulness against agricultural, horticultural and domestic insect pests. In this article, some attempt will be made to indicate briefly its present status as an insecticide.

The initials D.D.T. stand for the chemical compound p, p-dichlor-diphenyl-trichlorethane. This material was first synthesized in 1874, but its value as an insecticide was not recognized until Müller, a Swiss research worker, discovered that it had insecticidal properties. His work was confirmed by Wiesmann.

The great value of D.D.T. as an insecticide is due to its unusual combination of the following properties : its effectiveness in extremely low concentrations, its action both as a contact poison and as a stomach poison, and its stability or resistance to breakdown, which results in it retaining insecticidal action for prolonged periods.

In 1942, the Geigy Company of Switzerland took out two British patents, and in 1943 an American patent, covering the use of D.D.T.-containing insecticides. Large-scale manufacture is now in progress both in England and America, and Australia is also producing substantial amounts.

Forms in which D.D.T. may be Used.

D.D.T. may be applied either as a dust or as a spray ; it has no fumigant action.

Dusts.

Dusts containing from 0·5 to 10 per cent. D.D.T. on an inert carrier have been used extensively both in experiments and in the actual control of insects. Commercial D.D.T. cakes readily on grinding or mixing unless 75 per cent. or more of an inert carrier (such as pyrophyllite, talc, or kaolin) is present. It is probable that most manufacturers will provide for the sale of dusts containing 10 per cent. D.D.T. or less, prepared either by thorough mechanical mixing or by spraying a solution of D.D.T. on to the agitated powder, this latter process resulting in most, if not all, of the particles being coated with the insecticide.

Dusts have been used extensively to control the human body louse, which is the vector of typhus, since, in dust form, D.D.T. is readily applied without harm to infested individuals by blowing the powder between the clothing and the body of the wearer. Dusts have also been used in some situations for the control of mosquito larvæ, because of the availability of dusting machinery hitherto used to distribute the less effective paris green over the breeding grounds. For this purpose, however, D.D.T. dusts have now been largely replaced by oil solutions.

In the initial stages of testing D.D.T. as an agricultural insecticide in Australia during 1944-45, dusts were largely used because, at the time, this was the most convenient method of application. These dusts consisted of 90 per cent. pyrophyllite (a naturally occurring mixture of pinite and siliceous pyrophyllite) and 10 per cent. D.D.T. Kaolin or talc may also be used as the inert carrier. These carriers should be neutral, since alkaline dusts may react chemically with D.D.T., and at least 90 per cent. should pass through a 200 mesh British standard sieve.

Sprays.

D.D.T. may be applied as a spray in the form of a suspension in water (in which its solubility is extremely low), as a solution in organic solvents, or as an emulsion.

(*a*) *Suspension in Water.* D.D.T., in contrast to the dust mixture of 90 parts pyrophyllite and 10 parts D.D.T. already mentioned, is not readily wetted by water. This mixture, primarily designed for application as a dust, has also been used to produce a spray by agitation with water. A mixture of D.D.T. and soap powder is also readily wetted by water, but, to prevent excessive loss by run-off, not more than 1 per cent. of soap should be used.

D.D.T. may also be dissolved in a water-miscible solvent, such as methyl or ethyl alcohol, acetone, or pyridine. When these solutions are diluted with water, D.D.T. is thrown out into fine suspension. Acetone, which dissolves about 50 per cent. D.D.T., is not a suitable solvent, however, because the particles of D.D.T. thrown out on dilution tend to aggregate. The suspension resulting from the dilution of a solution of D.D.T. in ethyl alcohol is excellent, but the solubility of D.D.T. in this solvent is low (about 1·5 per cent.), consequently excessive amounts of alcohol must be used to obtain an aqueous mixture containing an adequate concentration of D.D.T.

(*b*) *Solutions in Organic Solvents.* D.D.T. is soluble in many organic solvents, some of which are useful for household sprays and for other purposes where toxicity of the carrier to the insect substrate is not a factor. Aromatic compounds are often better solvents than aliphatic compounds, and there are some materials and mixtures of materials which will dissolve at least an equal weight of D.D.T. For household sprays kerosene is used as the solvent, while for spraying swamps for the control of mosquito larvæ the less volatile diesolene is used. Kerosene is also a convenient solvent for D.D.T. when surfaces are treated to obtain a deposit of D.D.T. for residual effect.

(*c*) *Emulsions.* A number of emulsions have been used abroad for the control of insects of medical importance (e.g., for impregnation of clothing against body louse), and also against agricultural pests. Since some of the solvents and emulsifiers used in these formulations were not readily available in Australia, it was necessary to test materials which were available. Earlier work by C.S.I.R. had shown that a solvent naphtha emulsion at concentrations below 1 per cent. solvent naphtha was non-injurious to potato plants. It was found that solvent naphtha would dissolve about 20 per cent. D.D.T. at ordinary temperatures, and that a satisfactory emulsion could be made with this solution. Tests showed this emulsion to be highly toxic to many insects, such as the larvæ of the potato moth (*Gnorimoschema operculella*). A stock solution for the preparation of this emulsion is made by dissolving 1 lb. of D.D.T. in five pints of solvent naphtha (boiling range 90-190° C.) and adding to this half a pint of an emulsifier (the sodium salt of an alkyl naphthalene sulphonic acid mixed with pine oil). This quantity of stock solution, when diluted with water to 100 gallons, produces a spray containing 0·1 per cent. D.D.T.

Attempts to incorporate D.D.T. in some white oil emulsions have not been satisfactory, since the conventional petroleum oils seldom dissolve more than 5 per cent. D.D.T. In order to increase the concentration of D.D.T. in these oils, it is necessary to add a material such as xylene, in which D.D.T. is very soluble. When these stock white oil concentrates containing D.D.T. are diluted to the final spray strength, it is often found that D.D.T. is thrown out of solution and adheres to the sides of the containing vessel. Another disadvantage of D.D.T.-white oil emulsions is suggested by the inhibition of growth observed in potted French bean seedlings treated in the glass-house, and by reports of foliage injury in apples.

Emulsions have also been prepared using eucalyptus oils as solvents for D.D.T. These oils dissolve about 25 per cent. D.D.T. at ordinary temperatures and very

stable emulsions may be produced. These emulsions show promise in the experiments already carried out, but further work is necessary.

Most emulsions so far prepared suffer from one or more defects. For instance, solvent naphtha obtained by distillation from coal tar is very variable in composition, and is thus not a suitable solvent for the preparation of a uniform emulsion. Investigations are proceeding to evolve more satisfactory formulations for preparing uniform D.D.T. emulsions, and it is probable that solvent naphtha will be replaced by other materials, the composition of which is less variable.

Methods of Application.

Many of the methods of application of D.D.T. are the same as those used for other insecticides, namely the conventional spray and dust gun. Although the apparatus used is extremely varied and cannot be reviewed here, brief mention should be made of the use of the aeroplane. The extremely low dosages of D.D.T. required for the control of many insects make it possible for an aircraft to carry a paying load of insecticide containing D.D.T., although this would not be possible with many other insecticides. Allied aircraft have been used extensively in various theatres of war to spray inaccessible swamps or large areas of country with D.D.T. in oil for the control of mosquito larvæ. Aeroplane application has been so satisfactory for this purpose that it is certain that this means will be adopted also for other insects where large areas of country are involved.

Solutions in organic solvents are not generally suitable for application to agricultural crops with the standard appliances now in use, because the solvent is often toxic to plants. However, it is probable that some will find extensive use if the aerosol method of application can be satisfactorily adapted for this purpose. An aerosol is a suspension of fine solid or liquid particles in air, which means that both dusts and sprays may be regarded as aerosols if the particle size is small enough. One convenient method of producing liquid aerosols is to mix the insecticide materials with a gas, such as freon (dichlorodifluoromethane) or methyl chloride, which liquefies fairly readily under pressure at ordinary temperatures. This mixture is stored in a cylinder fitted with a capillary tube. When the capillary tube is opened, the pressure of the gas inside the cylinder forces the liquid out. At the orifice of the tube the liquefied gas volatilizes and, in doing so, disperses the insecticide in a very finely divided form. Cylinders containing from 1 to 15 lb. of a mixture consisting of liquefied gas, lubricating oil and pyrethrins, with or without D.D.T. and sesame oil (a pyrethrin activator) have been used very extensively, and with great success, by the armed forces, principally for the control of mosquitoes and flies. Thus, a discharge of four seconds' duration from a 1 lb. aerosol bomb, as these cylinders have been called, will kill most, if not all, mosquitoes in a 1,000 cubic foot tent or room. This 1 lb. bomb is very compact, occupying much less space and being considerably lighter than the equivalent amount of the more conventional type of insecticide spray based on kerosene. Its peace-time applications will be many, for the aerosol is effective both under confined conditions and in the open. Thus a 1 lb. bomb contains sufficient insecticide to kill adult mosquitoes in an acre of country, the insecticide being released as the operator walks up and down through the area which it is desired to disinfest.

The aerosol formulæ originally developed for the armed forces are now in process of modification for use on agricultural crops, and suitable machinery for their application is being developed. Some of these developments have been described in the literature. A suitable machine for some crops consists of a container for the aerosol mounted on a light frame carried on bicycle wheels. The container is connected to a number of special delivery nozzles which are mounted on two booms, one behind the other, adjustable for height and width of row. The aerosol is discharged into a canvas hood covering the framework of the machine. This hood temporarily

confines the aerosol in the vicinity of the plants and enables the machine to be used under gentle wind conditions. In one series of experiments, approximately 10 lb. of aerosol were used per acre, and this was delivered from a machine travelling at a speed of three to five miles per hour. This method of application, with its advantages of lightness of machinery, cheapness of construction and ease of manipulation, particularly for market garden crops, has a very great future.

The Toxicity of D.D.T. to Insects.

Agricultural Insects.

An extensive programme of research on the use of D.D.T. against agricultural and horticultural pests is in progress in many parts of the world, including Australia. D.D.T. has been reported to be very effective against a wide range of insects, but there are some notable exceptions ; for example, the cotton boll weevil (*Anthonomus grandis*), the cotton aphid (*Aphis gossypii*), the cotton leaf worm (*Alabama argillacea*), the Mexican bean beetle (*Epilachna varivestis*) and the Mexican butterfly (*Anostrepha ludens*). This list will doubtless increase as further work is carried out. It is also interesting to note that mites are not affected, whereas their insect predators may be. Thus, in some fruit-growing areas serious outbreaks of red spider have followed the experimental use of D.D.T. for the control of codling moth, because the predaceous Coccinellid beetles which normally keep the numbers of mites down at a very low level were killed by the D.D.T. treatment.

The results of preliminary experiments carried out by the Council for Scientific and Industrial Research have shown that D.D.T. is very effective against a large number of agricultural and horticultural pests. This includes certain species which could not be adequately controlled by insecticides used previously. On the other hand, a few pests proved to be resistant to D.D.T. in the preparations and concentrations used.

Experience gained in trials with dusts and sprays containing D.D.T. is summarized below. In most cases the dusts were applied at strengths of 2 per cent D.D.T. or lower and pyrophyllite was used as a diluent. The spray used was generally the D.D.T.-solvent naphtha emulsion diluted to contain from 0·05 to 0·2 per cent. D.D.T. This spray was not injurious to the plants.

TABLE I.

Insects against which D.D.T. was Effective as a Dust or a Spray.

D.D.T. Dust.	D.D.T. Spray.
Green vegetable bug (*Nezara viridula*).	Green vegetable bug (*Nezara viridula*).
Potato moth (*Gnorimoschema operculella*).	Potato moth (*Gnorimoschema operculella*).
Cabbage moth (*Plutella maculipennis*).	Cabbage moth (*Plutella maculipennis*).
Cabbage butterfly (*Pieris rapæ*).	Cabbage butterfly (*Pieris rapæ*).
Cabbage centre grub (*Hellula undalis*).	Oriental peach moth (*Cydia molesta*).
Cabbage cluster grub (*Crocidolomia binotalis*).	Codling moth (*Cydia pomonella*).
Common cluster grub (*Prodenia litura*).	Pear slug (*Caliroa limacina*).
Corn earworm (*Heliothis armigera*).	Green jassid (*Empoasca fabæ*).
Green peach aphis (*Myzus persicæ*).	Bean aphis (*Doralis fabæ*).
Potato aphis (*Macrosiphum gei*).	Black peach aphis (*Anuraphis persicæ-niger*).

The D.D.T. dust and the D.D.T. spray used were ineffective against the cabbage aphis (*Brevicoryne brassicæ*) while the spray was ineffective against the woolly aphis (*Eriosoma lanigerum*) and red spider (*Tetranychus urticæ*).

A great deal of work still remains to be done to discover all the pest which can be controlled, those which are not affected, and the best means of application to crops. Another important aspect which must be explored fully is the effect of D.D.T. upon insect parasites and predators which may be destroyed by its use. It is possible that the indirect effects so produced upon the population densities of their hosts may make necessary some modification of the manner of application used, or even warrant the discontinuance of the use of D.D.T. against certain pests. Bees

are also affected, but, on the evidence available at present, if insecticides containing D.D.T. are applied to plants in flower, there is no reason to expect that the danger to bees visiting the flowers would be greater than if materials containing arsenicals were used. As far as possible, however, treatment of plants in flower should be avoided.

Insects of Medical, Veterinary and Household Importance.

A great deal of work has been done during the war years on this group of insects, particularly against the principal insect vectors of disease, namely mosquitoes (malaria, dengue fever, etc.), lice (typhus) and flies (dysentery, etc.).

(*a*) *Dusts.* Dusts containing D.D.T. have been found to be very effective against surface-feeding (Anopheline) mosquito larvæ, lice and cockroaches. Thus, D.D.T., which is from 10 to 100 times as toxic to Anopheline larvæ as Paris green, exercises effective control when used at the extremely light dosage of 0·1 lb. or less of D.D.T. per acre. A 2 per cent. D.D.T. dust was used in some of this work. However, the difficulty of producing D.D.T. dusts sufficiently fine to be swallowed by the larvæ, and the usually greater ease of application of oily solutions of D.D.T., have resulted in oils being the preferred vehicle in most cases.

As mentioned earlier, dusts containing 5 or 10 per cent. D.D.T. are in use for the control of lice, either for disinfestation, or as a protective measure. Following the outbreak of typhus in Naples in January, 1944, over 1,300,000 civilians were dusted, and within three weeks the outbreak was completely under control. This is the first occasion in medical history that a typhus outbreak has been arrested in mid-winter. Promising results have also been obtained in experiments with lice infesting other animals. D.D.T. dusts are very effective against fleas, both on humans and on animals, and protection is afforded against reinfestation so long as the dust remains on the body.

Cockroaches, which are comparatively resistant to many poisons, can be controlled by dusts containing 10 per cent. D.D.T., lower concentrations giving less satisfactory results, especially against the German cockroach (*Blatella germanica*), which is probably one of the most difficult species to control.

(*b*) *Sprays.* Sprays containing D.D.T. have been used both to produce direct effects on the insects hit by the droplets, and also to obtain residual films of D.D.T. on surfaces where the insects to be controlled will come in contact with the poison.

Sprays containing 0·5 per cent. D.D.T. in kerosene are extremely effective against adult flies and mosquitoes, although the onset of toxic symptoms following application of the spray is slow. This disadvantage may be remedied by the incorporation in the spray of a rapid knockdown agent, for instance, pyrethrins or certain organic thiocyanates. Such sprays are effective also against many other insects, although there are some exceptions, for instance, the German cockroach mentioned above.

Perhaps even more important in many cases than the initial contact effect of a D.D.T. spray is the fact that surfaces receiving a sufficient deposit of spray retain their toxicity for considerable periods. The D.D.T. can be applied by spray-gun or brush, either as a kerosene emulsion or incorporated in paint. The amount required in paint has been found to vary from 0·5 per cent. in oil-bound water paints to 5·0 per cent. in oil paints. A house fly resting on a surface treated with D.D.T. at the rate of 50 mg. per sq. ft. absorbs sufficient poison in a few seconds to kill it, although the first symptoms may not appear for 15 minutes or more, and death may not occur for several hours.

The application of D.D.T. to kitchen walls, wire screens and other places favoured by flies is a very effective means of reducing fly nuisance. Because of its low volatility and resistance to breakdown, D.D.T. will remain effective for many months. Even

when rooms are treated with D.D.T., it is still desirable to use wire screens because, if flies are abundant and are continually entering, there will always be some flying about which have not been present long enough to become affected. All flies in treated premises should, however, die overnight.

Contact with treated surfaces will kill many other insects, although the period of contact and the concentration required vary somewhat. These insects include adult mosquitoes, bed bugs, lice and fleas (treated clothing) and silverfish. Treated surfaces are slightly repellent to some insects (e.g., some mosquitoes, flies, cockroaches) in the sense that the insects do not rest on them as long as on untreated surfaces, although the effect is only noticed following contact of the insect with the surface. The absence of any distance repellent effect is to be expected from the very low volatility of D.D.T. Eradication of bed bugs can be achieved by treating surfaces over which the bugs must pass on the way to or from their hiding places.

Because D.D.T. in solution in oil may be toxic to the animal it is best applied as an emulsion. D.D.T. emulsions have given very promising results in the control of buffalo fly and cattle tick in Queensland, and it is probable that the residual effect of the spray is an extremely important factor. Thus, in recent experiments all parasitic stages of the cattle tick were killed with one application of an emulsion containing 2 per cent. D.D.T., and this treatment prevented reinfestation for a period of about 12 days. Against the buffalo fly it was found that when each beast in a herd was sprayed with 2 per cent. D.D.T. emulsion on an area 12 in. $\times$ 18 in. on either side of the shoulders, adequate control of the flies resulted, although the treated hair did not remain toxic for as long on the living animal as on a piece of dead hide. Much work, however, still remains to be done in the selection of a satisfactory emulsion for application to animals, and in obtaining adequate proof that the D.D.T. does not harm them in any way.

Oils containing 5 per cent. D.D.T., and also emulsions, have been applied in spray form for the control of mosquito larvæ, since application of a liquid is often the most convenient way of dispersing a small amount of material uniformly over a large area. The D.D.T. is applied either from the ground by hand or power spray-guns, or from low-flying aircraft. As little as one to two quarts of oil solution is required per acre, which may be contrasted with the 18 to 20 gallons of oil often used per acre before the discovery of D.D.T. Oil solutions at one to two quarts per acre are used against the surface-feeding Anopheline mosquito larvæ, but are less effective against bottom-feeding Culicine mosquito larvæ, against which D.D.T. emulsions are used. Of the latter group of mosquitoes, the larvæ of the vector of dengue fever (*Aedes ægypti*) are killed under certain conditions by D.D.T. concentrations as low as one part in one hundred million parts of water. In direct contrast is the lack of effect of D.D.T. dusts, solutions, or emulsions upon the larvæ (or maggots) of flies, such as the Australian sheep blowfly (*Lucilia cuprina*) and the house fly (*Musca domestica*). The possibility of using D.D.T. against the sheep blowfly is still being examined, but it is clear that it will have to be employed against the adults, and not the maggots.

D.D.T. is ineffective against many species of mites attacking man and domestic animals.

The Toxicity of D.D.T. to Higher Animals and to Plants.

No instances of toxic symptoms in man have been recorded following the very extensive use of D.D.T. in the dry state, either as a 10 per cent. dust, or to impregnate clothing for the control of body-infesting insects. In experiments with laboratory animals, D.D.T. has been shown to be capable of absorption in toxic quantities through the skin from solutions or emulsions. It is desirable, therefore, to avoid prolonged skin contact with D.D.T. in these vehicles, particularly if the concentration

of D.D.T. is high. Little danger is expected from the occasional inhalation of fly spray, or from contamination of food by it.

The information at present available suggests that when D.D.T. is used on agricultural and horticultural crops for the control of pests it will not be any more dangerous to man than some insecticides now in common use, for instance lead arsenate and fluorine compounds.

Ingestion of comparatively large quantities of D.D.T. may lead to toxic symptoms or death in laboratory animals. It is desirable, therefore, to avoid excessive contamination of human food or eating utensils.

The permissible limit of food contamination has not yet been determined, but on present indications it appears that this limit may be set about seven parts per million.

D.D.T. poisoning affects the central nervous system and the liver, and the possibility of a cumulative effect has not yet been eliminated. It is for the latter reason that further work is necessary before it is safe to recommend the application of massive doses at regular intervals to animals, for instance, to cattle for the control of cattle tick or buffalo fly.

D.D.T. in the form of a dust mixed with pyrophyllite, talc or kaolin may be safely applied to crops with the exception of members of the cucurbit family, which have been found susceptible to injury. Aerosols developed for mosquito control are not suitable for application to crops, but a suitable mixture which has proved satisfactory during the last three years contains 5 per cent. each of D.D.T., cyclohexanone and lubricating oil (SAE 10), acetone 35 per cent., and methyl chloride 50 per cent. The acetone is phytotoxic, so care must be taken not to overdose crops. As already mentioned, some D.D.T.-white oil emulsions tested caused inhibition of growth in potted French bean seedlings in the glasshouse. The D.D.T.-solvent naphtha emulsion already mentioned appears quite safe to use on crops.

References.

Owing to lack of space, it is not possible to list the many references to published and unpublished work which have been drawn upon for the present article. Results of C.S.I.R. experiments will shortly commence to appear in C.S.I.R. publications. References to all articles published on D.D.T. up to April, 1944, are given by Roark (Roark, R. C., U.S.D.A., June, 1944), and a supplementary list includes all publications up till December, 1944 (Roark, R. C., U.S.D.A. E660, May, 1945). A great deal of information has been published in the *Journal of Economic Entomology*, particularly the February, 1944, and April, 1945, issues. These sources should be consulted by those desiring detailed information.

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