THE MODE OF ACTION OF INSECT REPELLENTS I: CHOICE CHAMBER EXPERIMENTS WITH THE GERMAN COCKROACH *BLATTELLA GERMANICA* (L.)

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Apparatus was designed to separate the effects of liquid and vapour phases of insect repellents. It was used to study the sites of action of these two phases on the German cockroach Blattella germanica *(L.). The antennae contain the main sites of repellent receptors with the legs of secondary and the palps of little or no importance. Both the legs and antennae carry receptors for both liquid and vapour repellent phases. The vapour phase appears more effective than the liquid phase, but the combined effect of the two phases is greater than the sum of their individual effects.*

The purpose of this study was to determine the sites of action on insects of insect repellents and to clarify the nature of the senses involved, with particular reference to the common chemical sense. The common chemical sense has been defined by Roys (1954) as a fundamental sensitivity of all nerve tissue to irritant chemical stimuli. This paper is concerned with true repellency or the production of an avoiding response, not with the interference with normal behaviour by repellent chemicals.

There are many empirical methods of evaluating insect repellents (Shepard, 1960). Most of these are designed to test the repellents under the conditions in which they will be used. For example, mosquito repellents are often tested for protection time and degree of protection whilst applied to the human skin under field conditions. In many such tests the repellent is being tested in the presence of attractive factors, and as a preventive against both normal and specialized behaviour, such as blood feeding. Such methods cannot be comparably applied to all insects, nor can they differentiate between compounds which interfere with some behavioural pattern and those compounds which induce active repellency. Since I wished to consider simple repellency, to determine the sites of action on insects and the part played by the liquid and vapour phases of repellents, I chose a method of repellent evaluation that would allow the repellent effect to be tested in the absence of all other known attractive or repellent stimuli.

The simple binary choice test chamber is a commonly used method of testing insect behaviour. Originally, the chamber was used to determine the humidity preferences of insects (Gunn and Cosway, 1938) and has been repeatedly used for that purpose since (Willis and Roth, 1950; Bar-Zeev, 1960). The use of this type of chamber to screen repellents was suggested by Bar-Zeev (1962). The long neglect of the simple choice chamber for testing repellents is not an oversight on the part of repellent workers, but merely because for practical purposes more severe and demanding tests are usually desired for repellent evaluation. The binary-choice chamber is divided into two parts, identical in all ways except for the experimentally introduced variable. Other factors such as temperature and illumination must be the same on both sides of the chamber, so that any deviation from an expected distribution of the insects placed in the chamber can be attributed to the introduced factor.

I designed a variation of this type of chamber to test repellents separately in their two phases, liquid and vapour. By this method, I hoped to separate the repellent effect into contact and olfactory repellency. By using, in this test chamber, insects with some of their appendages painted with nail varnish to block the sense organs, it was hoped to discover which groups of sense organs mediated the response to each of the two phases of the repellent, and to what extent.

EXPERIMENTAL METHODS

Associated with a layer of liquid is a layer of vapour above it, emanating from the liquid. If a liquid repellent is applied to a porous material, sufficient flow of air down through the material will effectively remove this vapour layer. A circular binary-choice test chamber 12 cm in diameter was constructed in the inlet port of a 1.5 kw centrifugal blower (Fig. 1). The outlet port of the blower was vented to the outside of the building. The wire mesh floor of the test chamber was covered with glass fibre cloth, two unconnected halves joined with cellophane tape. One half was treated with repellent by soaking in acetone with a known concentration of repellent in solution, the other half was untreated, soaked merely in pure acetone. Glass fibre cloth has a loose porous construction as well as being insoluble in most organic solvents (most repellents are plasticizers and soften rayon and acetate fibres). The vapour layer associated with the treated cloth could be sucked down by turning the blower on, which maintained a flow through the cloth's surface of about 60 cm/sec. Test insects were prevented from leaving the floor of the chamber by treating the smooth glass walls with polytetrafluorethylene which had a surface too smooth for the insects to climb.

Four arrangements of the test cage were possible: (1) a single layer of cloth in the cage, half treated half untreated, suction off; test conditions for total repellency. (2) a single cloth layer as (1), but the suction fan on; test conditions for contact repellency with a liquid phase only. (3) two layers of cloth; the lower half treated and half untreated, the upper layer entirely untreated, and separated from the lower layer by a 1 mm thick non-absorbent monofilament mesh, of the type used in insect window screens, made of glass fibre 12 x 12 mesh; test conditions for vapour repellency only, since the test insects were kept from contact with the liquid but still exposed to the vapour layer. (4) two cloth layers as (3) but with suction on; test conditions for the total efficiency of the setup. If the apparatus works properly, the insects are not in contact with either liquid or vapour and there should be no repellency.

Readings were taken by camera (Fig. 1) to avoid any bias from visual observations. The camera was triggered to take a single frame at the end of 3 minutes by means of a switch on a slow moving kymograph.

Preliminary experiments indicated that the repellent would remain effective at the same level for up to 70 hours with no air flow through the test chamber, and up to 50 hours with air flow. Subsequent experiments were run over shorter periods of time than this (Fig. 2).

Tests were designed to use German cockroaches (*Blattella germanica* (L.)) and the cockroach repellent MGK R-874 (2-hydroxyethyl-n-octyl sulphide), since this is an extremely efficient repellent to cockroaches (Goodhue, 1960). The logic behind the preference for a repellent known to be almost entirely effective is that such a material may be supposed to possess all the characteristics of a 'total' repellent; any less efficient material may be deficient in some aspect of repellency. Only adult male German cockroaches were used, avoiding the possibility of introducing sex attractants into the chamber from female insects; the insects were reared at 23 C in a culture room and the tests conducted in a drakened room at 23 C and relative humidity of 30%-40%; all insects used were first anaesthetized with carbon dioxide, transferred to individual vials and allowed to recover in the test room for 2 hours, whether they had been treated with nail varnish to block their sense receptors or not; the insects were adults between 3 and 10 days old, and were not used more than once. Since cockroaches have a tendency to congregate or clump, readings were taken with only one cockroach in the chamber at a time. For each reading, a roach was dropped on the centre line of the test chamber, allowed to settle for exactly 3 minutes, and a photograph taken of its position. The cockroach was removed and dropped again for a second reading, and so on through

the 10 readings. The cockroach was then discarded. Thus after each reading the insect was thoroughly disturbed, and to this extent the readings may be said to be independent.

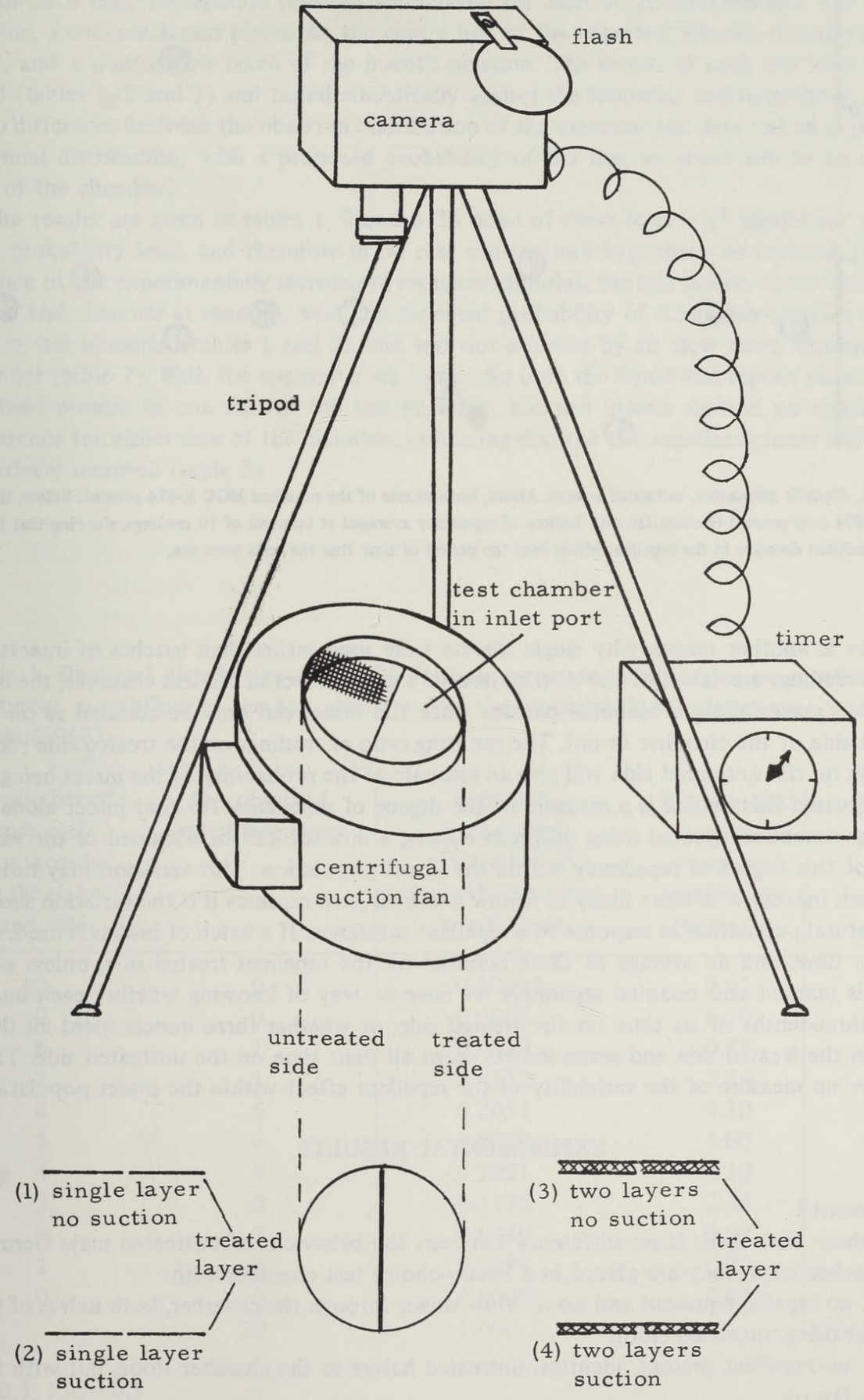

Figure 1. Sketch of the choice-chamber apparatus, and chamber floor arrangements for separating the repellent phases: (1) liquid and vapour present, (2) liquid only present, (3) vapour only present, (4) neither phase present (control).

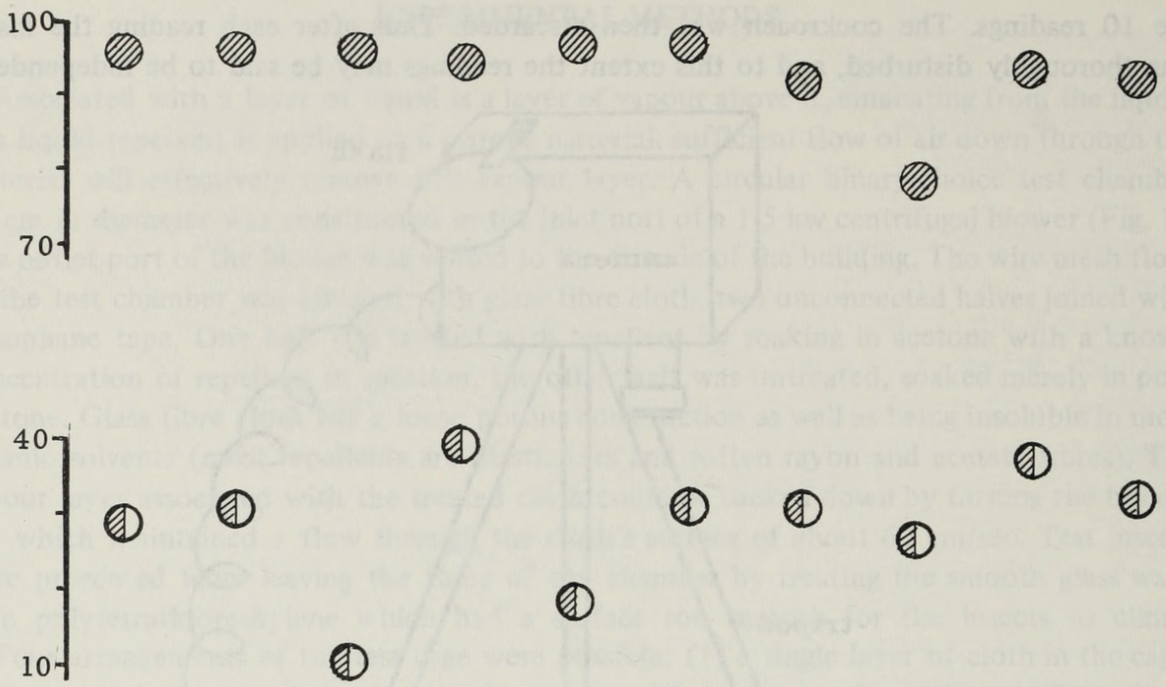

Figure 2. ***Blattella germanica*****, untreated insects. Above, both phases of the repellent MGK R-874 present. Below, liquid MGK R-874 only present (suction fan on). Indices of repellency averaged at intervals of 10 readings, showing that there is no consistent decrease in the repellent effect over the period of time that the tests were run.**

There is another reason why single insects were used rather than batches of insects. If several readings are taken of the distribution of a single insect in the test chamber, the data produced must follow a binomial pattern since the insect can only be counted as on the treated side of the chamber or not. The resulting ratio of readings on the treated side versus readings on the untreated side will give an estimate of the probability of the insect being on the untreated side, which is a measure of the degree of repellency for that insect alone. If the experiment is repeated using different insects, a measure can be obtained of the variability of this degree of repellency within the insect population. This variation may not be binomial; indeed, it is more likely to follow a normal pattern, since it is the variation shown by a natural population in response to a repellent substance. If a batch of insects is used, say 10 at a time, and an average of three counted on the repellent treated side, unless each insect is marked and counted separately we have no way of knowing whether each insect spent three-tenths of its time on the treated side, or whether three insects spent all their time on the treated side and seven insects spent all their time on the untreated side. Thus we have no measure of the variability of the repellent effect within the insect population.

EXPERIMENTAL RESULTS

Experiment I

To show that there is no difference between the behaviour of untreated male German cockroaches when they are placed in a binary-choice test chamber with:

1. no repellent present and no air flow down through the chamber, both halves of the chamber being untreated cloth;

2. no repellent present, identical untreated halves to the chamber floor, but with the suction fan on;

3. two layers of cloth separated by glass fibre mesh, half the lower layer treated with

repellent (MGK R-874), suction fan on; these are the conditions for testing the efficiency of the apparatus (see Fig. 1).

For each test, 10 separate readings were taken on each of 20 cockroaches. For every reading, a cockroach was placed on the centre line of the chamber, allowed to settle in the dark, and a photograph taken of the insect's position. The results of each test were tabulated (tables 1, 2 and 3) and tested statistically against the following null hypothesis: there is no difference between the observed distribution of the experimental data and an expected binomial distribution, with a proposed probability of 0.5 that an insect will be on either side of the chamber.

The results are given in tables 1, 2 and 3. In none of these tests is χ^2 significant at the 0.05 probability level, and therefore in no case can the null hypothesis be rejected. In the absence of the experimentally introduced repellent stimulus, the test insects chose their side of the test chamber at random, with the expected probability of 0.5 (table 1). This distribution was binomial (tables 1 and 2), and was not affected by air flow down through the chamber (table 2). With the apparatus set to remove both the liquid and vapour phases of a repellent present in one side of the test chamber, the test insects showed no significant preference for either side of the chamber, indicating that the two repellent phases had been effectively removed (table 3).

Table 1. Binomial distribution fit for 20 *Blattella germanica* adult males, no repellent, no treatment, no airflow in the test chamber. For the binomial fit calculations see Steel and Torrie (1960).

N = number of times each insect recorded on the right hand side	O = observed insect frequency	Expected probability $p^N(1-p)^{10-N}$ times the binomial coefficients	E = expected frequency = expected probability times 20	$\frac{(O-E)^2}{E}$
10	0	0.00098	0.02	0.02
9	0	0.0098	0.20	0.20
8	1	0.0439	0.88	0.16
7	1	0.1172	2.34	0.77
6	6	0.2051	4.10	0.88
5	6	0.2500	5.00	0.20
4	4	0.2051	4.10	0.00
3	0	0.1172	2.34	2.34
2	2	0.0439	0.88	1.42
1	0	0.0098	0.20	0.20
0	0	0.00098	0.02	0.02
	20	1.000		$\chi^2 = 6.21$

$p = 0.5$, $1 - p = 0.5$

10 degrees of freedom (only one degree of freedom is lost, since p was not estimated)

Table 2. Binomial distribution fit for 20 *Blattella germanica* adult males, no repellent, no treatment, but with the suction fan on; i.e., airflow down through the chamber.

N = number of times each insect recorded on the right hand side	O = observed insect frequency	Expected probability $p^N(1-p)^{10-N}$ times the binomial coefficients	E = expected frequency = expected probability times 20	$\frac{(O-E)^2}{E}$
10	0	0.00098	0.02	0.02
9	0	0.0098	0.20	0.20
8	2	0.0439	0.88	0.16
7	2	0.1172	2.34	0.05
6	4	0.2051	4.10	0.00
5	5	0.2500	5.00	0.00
4	4	0.2051	4.10	0.00
3	1	0.1172	2.34	0.77
2	2	0.0439	0.88	1.42
1	0	0.0098	0.20	0.20
0	0	0.00098	0.02	0.02
	20			$\chi^2 = 2.84$

$p = 0.5$, $1 - p = 0.5$
10 degrees of freedom (only one degree of freedom is lost, since p was not estimated).

Table 3. Binomial distribution fit for 20 *Blattella germanica* adult males, repellent MGK R-874 present in the lower left layer of the choice chamber floor. Suction fan on, and the insects separated from the repellent by a layer of fibre mesh and a second layer of cloth. Control conditions for the removal of both the liquid and vapour phases of repellent.

N = number of times each insect recorded on the untreated side	O = observed insect frequency	Expected probability $p^N(1-p)^{10-N}$ times the binomial coefficients	E = expected frequency = expected probability times 20	$\frac{(O-E)^2}{E}$
10	0	0.00098	0.02	0.02
9	0	0.0098	0.20	3.20
8	1	0.0439	0.88	0.16
7	1	0.1172	2.34	0.77
6	2	0.2051	4.10	0.88
5	6	0.2500	5.00	0.20
4	4	0.2051	4.10	0.00
3	4	0.1172	2.34	0.05
2	1	0.0439	0.88	0.16
1	0	0.0098	0.20	0.20
0	0	0.00098	0.02	0.02
	20	1.000		$\chi^2 = 5.86$

$p = 0.5$, $1 - p = 0.5$
10 degrees of freedom (only one degree of freedom is lost, since p was not estimated).

Experiment II

This experiment was designed to test the response of treated and untreated German cockroaches to various phases of the repellent MGK R-874 (purity 96.4%). It was hoped to answer the following questions. Can the repellent effect be partitioned into a vapour effect and a liquid effect? Which receptor sites on the insect respond to repellent, and to what extent? Is there an association between the receptor sites and the repellent phases; i.e., do the legs mostly respond to liquid and the antennae to vapour?

Ten readings were taken for each of the 20 separate insects used in each treatment combination. The experiment was designed as a 3 x 4 factorial, and the results analysed by standard analysis of variance procedures. The controls for the experimental design were not included in the main analysis, but treated separately (experiment I) because the analysis of variance presumes a common error variance. In the experimental readings there were two sources of error variation, the binomial variation present in the test chamber readings on each insect (sampling error), and the variation in the response of different insects from the population to the repellent stimulus. An assumption of the analysis is that all measured variables are normally independently distributed. Since the basic readings were binomial, they were transformed by the arcsin $\sqrt{X}$ transformation (Steel and Torrie, 1960), giving data which is approximately normal.

A randomization procedure was carried out on the treatment combinations to minimize error, and the design was as follows.

A: repellent phase treatments. a = 3.
- A_1 liquid repellent only
- A_2 vapour repellent only
- A_3 liquid plus vapour repellent.

B: insect treatments. b = 4.
- B_1 palps exposed (legs and antennae blocked)
- B_2 legs exposed (palps and antennae blocked)
- B_3 antennae exposed (legs and palps blocked)
- B_4 untreated, all sensory areas exposed.

R: 20 male cockroaches used per treatment. r = 20.
10 readings taken on each insect, $y = \sin^{-1}\sqrt{X}$ where
X = recordings of each insect on untreated side as a proportion.

The gross data and results are summarized in table 4. Since interaction was statistically significant when compared with the error term, the main effects were compared with the interaction, showing that overall only factor A repellent phase was significant. The interaction means that in this experiment the two factors, repellent phase and insect treatment did not act independently of each other; and that for meaningful interpretation of the data, the effect of each treatment must be examined separately; such effects are known as simple effects.

Before going on to the simple effects, the nature of the interaction was examined to see if its meaning could be understood in terms of the experiment. An interaction can be expressed as a function of the regression characteristics of the treatment means. Tukey (1949) has dealt with this type of problem and devised an approach, even though the levels of each factor are not orthogonal. If the treatment means for each level of a factor are averaged over all levels of the other factor, factor level means are obtained, ($\overline{A}$ and $\overline{B}$ in table 5). These are estimates of proportions, and for ease of calculation were transferred into deviations from the overall treatment mean, giving the x_A and x_B values in table 5. The experimental treatment means are denoted as $\overline{y}$ values. Using the x values as the basis for linear regression equations, theoretical sums of squares can be calculated for the linear

regression of A on B, B on A, and for the A-linear B-linear interaction, which is a measure of the extent to which the two regressions are not additive but multiplicative. The A-linear B-linear sum of squares comes to 2853.8, which is significant. A multiple regression equation based on the linear additive and linear multiplicative sums of squares was estimated as: $\hat{y} = x_A + x_B + 0.1x_Ax_B + 56.48$ (all figures in the transformed range). The $\hat{y}$'s are estimates of the treatment means $\bar{y}$ (see table 6). Table 6 also shows the residues ($\bar{y} - \hat{y}$) of the treatment means not attributable to linear additive and multiplicative regression. These residues would include any effect due a particular association between two specific levels of the main factors, such as between the vapour phase of repellent and the antennae. These residues are all non-significant, both individually and collectively. This indicates that there is no significant correlation between any particular group of sense organs and any particular phase of repellent.

Table 4. Analysis of variance table for experiment II, and a summary of the results. Values shown are based on transformed data.

	A_1 liquid repellent	A_2 vapour repellent	A_3 liquid and vapour repellent
B_1 palps only exposed	$\Sigma y =$ 929.0 $\Sigma y =$ 46494.64	968.4 51035.12	993.6 55583.24
B_2 legs only exposed	980.0 53073.04	1090.0 63864.88	1172.0 72020.24
B_3 antennae only exposed	1067.0 60857.56	1243.6 80918.32	1395.8 99569.96
B_4 legs, palps and antennae exposed (untreated)	1036.0 56448.20	1098.6 61180.56	1581.2 127078.16

Source	degrees of freedom	sums of squares	mean square	F
Treatments	(ab-1) = 11	(20591.5)		
A	a-1 = 2	8246.8	4123.4	5.63*
B	b-1 = 3	7949.3	2650.0	3.62
AB	(a-1) (b-1) = 6	4395.4	732.6	3.99*
Error	ab (r-1) = 228	41909.2	183.8	
Total	rab-1 = 239	62500.7		

*significant at 0.05 probability level.

Table 5. Transformed treatment means (denoted as $\bar{y}$) for three levels of repellent factor, A_1 liquid, A_2 vapour, A_3 liquid plus vapour; and four levels of sense organ treatment, B_1 palps only, B_2 legs only, B_3 antennae only, B_4 all sense organs exposed. Average effects of a factor at each level of the other factor are shown under $\bar{A}$ and $\bar{B}$, x_A and x_B are the deviations of $\bar{A}$ and $\bar{B}$ from the overall mean 56.48. Untransformed values for these means, i.e., percent of insects on the untreated side, are given in brackets.

	A_1	A_2	A_3	$\bar{B}$	x_B
B_1	46.46 (52.55)	48.42 (55.95)	49.68 (58.13)	48.19 (55.56)	-8.29
B_2	49.00 (56.96)	54.50 (66.28)	58.60 (72.86)	54.03 (65.50)	-2.45
B_3	53.35 (64.36)	62.18 (78.21)	69.79 (88.06)	61.77 (77.63)	5.29
B_4	51.80 (61.75)	54.93 (66.98)	79.06 (96.40)	61.93 (77.86)	5.45
$\bar{A}$	50.15 (58.94)	55.01 (67.12)	64.28 (81.17)	56.48 (69.50) overall mean	
x_A	-6.33	-1.47	7.80		

Table 6. Estimates ($\hat{y}$) of the transformed experimental means ($\bar{y}$, see table 5), based on the multiple regression equation $\hat{y} = x_A + x_B + 0.1x_Ax_B + 56.48$. This equation was estimated from the experimental sums of squares. The non-significant residues ($\bar{y}$-$\hat{y}$) include the contributions due to any particular association between a repellent phase A, and an insect treatment B. The term $0.1x_Ax_B$ accounts for most of the significant interaction noted in the main analysis (table 4). The increase in the repellent effect due to insect treatment is greater if accompanied by an increase in the repellent effect due to repellent phase.

		A_1 liquid repellent	A_2 vapour repellent	A_3 liquid and vapour repellent	x_B
B_1 palps only exposed	$\hat{y}$	47.11	47.94	50.01	-8.29
	$\bar{y}$-$\hat{y}$	-0.65	0.48	-0.33	
B_2 legs only exposed	$\hat{y}$	49.16	52.92	59.92	-2.45
	$\bar{y}$-$\hat{y}$	-0.16	1.58	-1.32	
B_3 antennae only exposed	$\hat{y}$	52.09	59.52	73.70	5.29
	$\bar{y}$-$\hat{y}$	1.26	2.66	-3.91	
B_4 untreated	$\hat{y}$	52.15	59.66	73.98	5.45
	$\bar{y}$-$\hat{y}$	-0.35	-4.73	5.08	
	x_A	-6.33	-1.47	7.80	

The simple effects are a measure of the effect of each level of each factor examined separately over all levels of the other factor. Table 7 shows these effects. Factor A, repellent phase had a significant effect when the test insects were untreated or had their antennae exposed. The repellent phase was not significant when the insects used had only the legs or palps exposed. Factor B, insect treatment, had a significant effect when the insects were exposed to the vapour phase of repellent or to both phases together. The insect treatment was not significant when the insects were exposed to liquid alone. In table 8, the treatment means are arranged in order of magnitude and classified according to levels of significance, based on Duncan's multiple range test.

In addition to the main analysis, the simple effects of repellent phase were analysed separately, including the control from table 1, (table 9). Duncan's multiple range test was also applied to these treatment means.

The significant differences between the treatment means for a factor at fixed levels of the other factor, based on Duncan's test, are summarized in table 10. This completes the analysis. The conclusions are as follows.

Table 7. Simple treatment effects. Figures in the transformed range. The effect of differing repellent phases A is significant for B_3 (insects with the antennae exposed) and B_4 (untreated insects). The effect of the differing insect treatments B is significant for A_2 (exposure to repellent vapour) and A_3 (liquid plus vapour together).

Source	degrees of freedom	sums of squares	mean square	F
A in B_1	2	105.3	52.7	0.29
A in B_2	2	928.2	464.1	2.53
A in B_3	2	2707.8	1353.9	7.37*
A in B_4	2	8901.1	4450.5	24.21*
A + AB	8	12642.4		
B in A_1	3	558.1	186.0	1.01
B in A_2	3	1902.1	634.0	3.45*
B in A_3	3	9883.9	3294.6	17.92*
B + AB	9	12344.1		
Error			183.8	

*significant at 0.05 level

Table 8. Significance levels for the 12 treatment means. Treatments which are not significantly different from each other have the same number opposite.

Treatment	average % insects on untreated side	transformed treatment means	significance levels
A_3 B_4	96.40	79.06	(1)
A_3 B_3	88.06	69.79	(2)
A_2 B_3	78.21	62.18	(2) (3)
A_3 B_2	72.86	58.60	(3) (4)
A_2 B_4	66.98	54.93	(3) (4) (5)
A_2 B_2	66.28	54.50	(3) (4) (5)
A_1 B_3	64.36	53.35	(3) (4) (5)
A_1 B_4	61.75	51.80	(4) (5)
A_3 B_1	58.13	49.68	(4) (5)
A_1 B_2	56.96	49.00	(5)
A_2 B_1	55.95	48.42	(5)
A_1 B_1	52.55	46.46	(5)

A_1 liquid repellent

A_2 vapour repellent

A_3 liquid and vapour repellent

B_1 palps exposed

B_2 legs exposed

B_3 antennae exposed

B_4 untreated insects

The effect of different repellent phases

With untreated insects, the shown repellent effect was greatest for both phases of repellent together. There was no significant difference between the effect produced by liquid repellent alone and vapour repellent alone, but all repellent treatments caused a significantly greater effect than the control without repellent.

For insects with only the antennae exposed and the legs and palps covered, the effect of both repellent phases together was significantly greater than for liquid alone, but not than for vapour alone. There was no significant difference between the effect of the liquid and vapour phases of repellent.

For insects with either the legs only exposed or palps only exposed, differences in repellent phase treatment had no significant effect.

The effect of insect treatment (blocking groups of sense organs with nail varnish)

With both phases of repellent present together, all four insect treatments were significantly different from each other. In order of descending repellent effect, they were: untreated insects; antennae exposed; legs exposed; palps exposed.

Table 9. Analysis of variance for untreated insects. Repellent phase treatments are the same as in the main analysis in experiment II, but include also the no repellent control.

	mean % of insects on untreated side	$\overline{y}$ transformed treatment means	Σy	Σy^2
A_0 no repellent	49.48	44.70	894.0	41489.12
A_1 liquid repellent	61.75	51.80	1036.0	56448.20
A_2 vapour repellent	66.98	54.93	1098.6	61180.56
A_3 liquid and vapour repellent	96.40	79.06	1581.2	127078.16
Source	degrees of freedom	sums of squares	mean square	F
repellent phase treatments	3	13354.2	4451.4	46.9*
error	76	7210.6	94.9	
	79	20564.8		

*significant at 0.05 probability

With repellent vapour only present, insects with the palps only exposed were significantly less repelled than insects with legs, antennae, or all sense organs exposed. There was no significant difference between these last three treatments.

With repellent liquid only present, there was no significant difference shown due to insect treatment. Some answers may be made to the questions posed on page 345. The removal by the experimental apparatus of either the liquid or vapour phase of repellent did reduce the repellent effect, but still left it greater than the control. The vapour phase seemed more effective than the liquid, but in no case could this be declared significant. The legs and antennae were both shown to be capable of responding to repellent, but the palps were not. The response produced by the antennae was greater than that shown by the legs. although this was only significant with both phases of repellent present. No qualitative differences could be shown between the responses of the various groups of sense organs to the two phases of repellent. All observed differences could be explained in quantitative terms; i.e., as the repellent effect connected with repellent phase increased from liquid to vapour to both phases, the importance of the sensitivity of the sense organ groups was increased geometrically as well as arithmetically. This fits well with the simple morphological observation that there are more sense organs on the antennae of German cockroaches than on the legs, and more on the legs than on the palps. It also indicates that there is little qualitative difference between these groups of receptors. If there are separate receptors involved in the perception of repellent vapours and repellent liquids, they do not seem to be confined to separate areas.

As far as the repellent response is concerned, no distinctions could be made between olfaction, gustation or the common chemical sense.

Table 10. Duncan's multiple range significance levels for all significant simple effects from the main analysis (table 7) and the separate analysis with control (table 9). Treatments which are not significantly different from each other share the same number opposite.

Treatment	average % insects on untreated	transformed treatment means	significance levels
A_3 B_4	96.40	79.40	(1)
A_2 B_4	66.98	54.93	(2)
A_1 B_4	61.75	51.80	(2)
A_0 B_4	49.48	44.70	(3)

4 repellent phase treatments, untreated insects

A_3 B_3	88.06	69.79	(1)
A_2 B_3	78.21	62.18	(1) (2)
A_1 B_3	64.36	53.35	(2)

3 repellent phase treatments, insects with antennae exposed

A_3 B_4	96.40	79.06	(1)
A_3 B_3	88.06	69.79	(2)
A_3 B_2	72.86	58.60	(3)
A_3 B_1	58.13	49.68	(4)

4 insect treatments, liquid and vapour repellent present

A_2 B_3	78.21	62.18	(1)
A_2 B_4	66.98	54.93	(1)
A_2 B_2	66.28	54.50	(1)
A_2 B_1	55.95	48.42	(2)

4 insect treatments, vapour repellent only present

factor A, repellent phases
A_0 no repellent
A_1 liquid
A_2 vapour
A_3 liquid and vapour

factor B, insect treatment
B_1 palps active
B_2 legs active
B_3 antennae active
B_4 all groups active

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REFERENCES

Bar-Zeev, M. 1960. The reaction of mosquitoes to moisture and high humidity. Entomologia exp. appl. 3: 198-211.

Bar-Zeev, M. 1962. A rapid method for screening and evaluating mosquito repellents. Bull. ent. Res. 53: 521-528.

Goodhue, L. D. 1960. New techniques for screening cockroach repellents. J. econ. Ent. 53(5): 805-810.

Gunn, D. L. and C. A. Cosway. 1938. The temperature and humidity relations of the cockroach. V. Humidity preference. J. exp. Biol. 15: 555-563.

Roys, C. 1954. Olfactory nerve potentials a direct measure of chemoreception in insects. Ann. N. Y. Acad. Sci. 58: 250-255.

Shepard, H. H. 1960. Methods of testing chemicals on insects. Burgess Publ. Co., Minn.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. New York, N. Y. 481 pp.

Tukey, J. W. 1949. One degree of freedom for non-additivity. Biometrics 5: 232-242.

Willis, E. R. and L. M. Roth. 1950. Humidity reactions of *Tribolium castaneum* (Herbst.) J. exp. Zool. 115: 561-587.