THE RESPONSES OF THE HONEY-BEE TO REPELLENT CHEMICALS

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(*Received* 25 *July* 1951)

(With Four Text-figures)

INTRODUCTION

This paper is concerned with the development of techniques for the study of the chemotropic responses of the honey-bee and their application to the study of the effect of adding a repellent to a solution of an attractant.

DEVELOPMENT OF THE BASIC TECHNIQUE

The use of olfactometers such as those used by Marshall (1935) for the study of olfactory responses in the honey-bee has been criticized in that such methods can only measure responses under conditions when active flight and defaecation are inhibited. Further, one does not know how far the responses of such a highly developed social insect are modified by the presence of other bees.

Whilst experiments conducted in the field enable observations to be made on naturally flying bees, they suffer from all the inherent disadvantages of involving a large number of factors which are not under control, and the conditions are therefore difficult to reproduce.

Von Frisch (1934) investigated the extent to which external factors influence the threshold responses of the bee to various sugar solutions. He found that with foraging bees over the range of 10–30° C. temperature changes had little effect, but that variations in the availability of naturally secreted nectar of a sufficiently high concentration materially affected the threshold response; during seasons when there was a good nectar flow the bees would refuse a sugar solution which they would readily accept under less favourable conditions even though, under the latter conditions, there was plenty of food in the hive and the bees were equally well fed.

Any evaluation of the rejection thresholds of unacceptable compounds would naturally depend on the comparison of these thresholds with a standard and constant response and therefore would not be possible where the test bees had access to fluctuating sources of nectar.

Another factor which is difficult to control in such field experiments is the number of bees. The sudden cessation of a nectar flow often results in the swamping of the training table by thousands of bees and accurate observations are rendered impossible.

Beling (1929) installed a small colony in a room for purposes of investigating the time sense of the bees, and this idea of restricting the foraging area of the bees was considered in an attempt to combine the advantages of the olfactometer and field techniques with a minimum of the disadvantages inherent in both.

Preliminary experiments indicated that a greenhouse might serve this purpose, and a small house approximately 12 × 8 ft. was adapted by screening the ventilation lights with muslin and installing circulating fans and heaters which provided temperature control within 5° C.

A small nucleus box containing two frames of bees, brood, and a 2-year-old queen was introduced into the greenhouse, and after a few hours of incessant flying the colony settled down. Dishes of syrup placed within the house were discovered by the foraging, bees, and except that large numbers of bees crowded on the glass of the roof during the hotter parts of the day no unusual activity was observed in the colony. This colony of bees installed in the greenhouse was used in the following experiments and, except for the introduction of a fresh comb containing sealed brood and pollen every fortnight, it was not disturbed.

I. THE EVALUATION OF THE RESPONSE WHEN THE REPELLENT IS INCORPORATED IN THE SOURCE OF ATTRACTION

The response to the stimulus caused by the addition of a chemical to an attractant was measured by obtaining data on the 'rejection threshold' of the foraging population. In this account the term 'rejection threshold' is arbitrarily defined as the range of concentrations of the repellent which, when added to the standard attractant, produces a graded response extending from full acceptance to complete avoidance.

The response was measured when alternatives of the pure attractant were offered either alternating in space or in time.

Experimental lay-out

The chemotropic response was measured by counting the visits of foraging bees to dishes placed on a table in the greenhouse. The number of visits made to the pure attractant was compared with those made to the attractant containing a known concentration of the repellent.

The term 'visit' meant that a bee alighted on a dish and commenced feeding.

Various preliminary experiments were made to test different arrangements of the dishes which consisted of solid watch-glasses holding 5 ml. All patterns involving rectangular shapes were found to be unsuitable because the bees memorized the position of dishes, especially those at the corners, and any complicated pattern had to be avoided because of the difficulty of keeping a large number of dishes under constant observation. It was found that eight dishes (four containing the attractant and four the attractant with repellent) were the maximum that could be effectively observed by one person.

The following method of presenting the dishes to the bees was finally adopted: A turn-table 1 ft. in diameter was geared to a small electric motor so that it made one revolution every 16 min. and the eight dishes were arranged around the edge.

This arrangement was found to be very satisfactory as it constantly changed the position of each dish relative to the hive, and the circular arrangement eliminated 'edge and corner effects'. It also tended to prevent the bees continually returning to one dish having remembered its position.

Experiment 1. *To test the reproducibility of the evaluation of rejection thresholds under greenhouse conditions*

It was considered necessary to determine the variability in the response of the population of foraging bees to a given chemical added to sugar solution.

Method. A large volume of 2M-sucrose solution in distilled water was prepared, and this constituted the standard attractant. This concentration was selected because it was readily acceptable to the bees and yet not so concentrated that it was liable to become viscous by evaporation of water on warm days.

Phenol was selected as a test chemical for this experiment, since, besides being a known bee repellent, it is fairly soluble in water and stable in aqueous solution. Using the stock 2M-sucrose solution, fourteen different concentrations of phenol were made up, ranging from 0·1 to 0·000001%, and each solution was labelled with a code number. This was considered desirable so as to ensure that the observer was not biased in his records by a knowledge of the concentration of the test solution. Each solution was tested separately on the turn-table against the standard sucrose solution, and the responses were measured by recording the total number of visits to the phenol solution when 50, 100 and 200 visits respectively had been made to the pure sucrose. Three complete tests were carried out for each solution over a period of 10 days.

The results are shown in Table 1, and the response to each concentration is expressed as an index of repellency. This index figure is obtained by expressing the number of visits to the phenol solution as a percentage of the number of visits to the pure sucrose and subtracting this figure from 100. Thus the final index figure is directly proportional to the stimulus of the repellent.

Experiment 2. *Repetition of Exp.* 1, *using an alternative technique which obviates errors in observation*

Butler, Finney & Shiele (1943) conducted experiments to determine the responses of the honey-bee to various chemicals, and they related the stimulus to the uptake of sucrose containing the chemical as compared with the uptake of pure sugar. The uptake of solution was measured volumetrically. This technique assumed that the bees when visiting a sugar solution normally drink a fairly constant amount of fluid. The actual experiments were carried out using the cells of a honeycomb.

The tests carried out in Exp. 1 were repeated and the response was measured by an adaptation of the above method. The differences of uptake of the various solutions were obtained by weighing before and after foraging had taken place. The dishes used in Exp. 1 were replaced by eight aluminium containers each holding 5 ml. These were filled with the respective solutions and exposed for varying periods in the greenhouse, the time depending on the activity of the bees. Where possible

Table 1. *Repellency indices at different concentrations; at three successive tests carried out over a period of ten days*

Concentration (%)	29. vi. 50–1. vii. 50 Repellency indices				3. vii. 50–5. vii. 50 Repellency indices				5. vii. 50–8. vii. 50 Repellency indices			
	When 50 visits made to sucrose	100 visits made to sucrose	100–200 visits made to sucrose	200 visits made to sucrose	When 50 visits made to sucrose	100 visits made to sucrose	100–200 visits made to sucrose	200 visits made to sucrose	When 50 visits made to sucrose	100 visits made to sucrose	100–200 visits made to sucrose	200 visits made to sucrose
0·1	100	100	100	100	100	100	100	100	100	100	100	100
0·05	100	100	100	100	100	100	100	100	100	100	100	100
0·025	98	98	99	98·5	100	98	99	98	94	100	96	98
0·010	82	86	84	85	100	97	98	97·5	100	98	99	98·5
0·005	62	57	65	61	58	87	80	83	88	54	50	52
0·001	28	55	73	64	42	53	60	56	42	41	45	43
0·0005	42	50	53	52	8	12	11	12	50	53	49	51
0·00025	12	30	41	35	10	10	34	22	41	51	55	53
0·0001	32	18	17	17	20	31	18	25	40	37	35	36
0·00005	40	29	20	25	72	52	63	57	24	29	10	20
0·00001	42	36	58	47	30	24	50	37	34	39	39	39
0·000005	54	38	27	32	50	40	43	42	46	24	38	31
0·0000025	12	0	−8	−4	4	−4	10	3	0	−1	−1	−1
0·000001	6	0	+6	+3	2	+2	−6	−2	6	+2	−11	−5
0·00000	5	−2	+3	+1	−5	−1	+2	+1	1	+6	−1	+3

a decrease in weight in the pure attractant approximately corresponding to the visits of two hundred bees in Exp. 1 was obtained. This method obviated any errors in observation, and the results only require to be corrected for evaporation. The results are shown in Table 2, and during the tests a number of counts of foraging bees visiting the pure sucrose were made so as to determine the natural variation in uptake per visit. These results are tabulated in Table 3.

Exps. 1 and 2 were successive tests carried out in the greenhouse with a fairly constant population of foraging bees extending over a period of 3 weeks. A visual inspection of the results shows that the response to the high concentration of phenol in all the tests was very constant. Both methods revealed an increase in variance with decrease in concentration, but the observational method used in the first experiment showed that from 0·000005 to 0·0000025 % there was a rapid falling off in the bees' response to the stimulus of the phenol. The fact that this falling off was not so clearly revealed by the weighing method is partly explained by the results shown in Table 3, which show that there is a normal variation in uptake when bees are foraging from a sucrose solution. The S.E. was calculated at ±0·239 g. sucrose solution per hundred visits of the bee.

It would appear that the observational method gives a more accurate determination of the full range of concentrations involved in the rejection thresholds than is obtained by the weighing method, though the latter might prove useful where a rapid determination of approximate values was required.

There was no evidence of any definite trend in the results over the 3-week period which could suggest that the bees were becoming conditioned in their responses.

It was expected that the results would have revealed a relationship between concentration and response of a sigmoid nature, but this did not happen. In approximately the range 0·0001 to 0·00001 % a slight increase in repellency occurs; this is most marked in Table 1 but is also evident in Table 2.

A comparison of the indices for each experiment in Table 1 at the successive points of 50, 100 and 200 visits to the pure syrup indicates that where only the limits of the response are required, no advantage is obtained by continuing the test beyond the point when 50 visits have been made.

Experiment 3. The determination of data relevant to the rejection thresholds of a number of chemicals, some of which are known insect repellents

Using the method described in Exp. 1, information on the rejection threshold was determined for the following chemicals, viz. phenol, dimethylphthalate, acetic acid, Rutgers 612, isopropyl cinnamate and Teepol (a commercial detergent).

Insoluble oils such as dimethyl phthalate were emulsified using cyclohexylamine dodecylsulphate at a concentration of 0·03 % w/v. This was chosen as preliminary experiments indicated that some detergents such as Teepol were active repellents, whilst CHDS at this concentration did not elicit any response from the bees.

The results as shown in Table 4 indicate that at a concentration of 1 % all the compounds tested were equally unacceptable, and that as the concentration decreased the repellency of each solution also decreased. The results further show

Table 2. *Results of Exp. 2 corrected for evaporation*

w/v Concentration (%)	Wt. of 2M-sucrose consumed	Wt. of sucrose + phenol consumed	$\frac{\text{Wt. of sucrose + phenol}}{\text{Wt. of sucrose}} = X$	Repellency index = 100 − (100 × X)
		Date 10. vii. 50–12. vii. 50		
0·05	16·491	0·000	0·0	100
0·025	12·226	0·000	0·0	100
0·010	6·682	0·480	0·01	99
0·005	12·220	4·060	0·31	69
0·001	8·330	4·310	0·52	48
0·0005	9·980	5·380	0·53	47
0·00025	7·750	6·570	0·86	14
0·0001	10·710	8·880	0·83	17
0·00005	6·030	4·440	0·74	26
0·00001	9·926	8·910	0·90	10
0·000005	11·230	8·880	0·79	21
0·0000025	7·860	9·290	1·19	−19
0·000001	12·256	10·491	0·87	+13
0·000000	6·001	7·340	1·22	−22
		Date 13. vii. 50–15. vii. 50		
0·05	11·290	0·000	0·0	100
0·025	14·100	0·000	0·0	100
0·010	15·670	1·070	0·06	94
0·005	15·845	4·930	0·31	69
0·001	12·718	4·780	0·37	63
0·0005	21·140	10·770	0·51	49
0·00025	6·670	4·080	0·61	39
0·0001	9·550	6·280	0·66	34
0·00005	10·230	6·280	0·62	38
0·00001	8·050	6·290	0·78	22
0·000005	10·330	12·590	1·21	−21
0·0000025	9·210	8·890	0·96	+ 4
0·000001	8·860	6·605	0·74	26
0·000000	6·120	5·280	0·87	13
		Date 15. vii. 50–16. vii. 50		
0·05	9·250	0·000	0·0	100
0·025	14·142	0·000	0·0	100
0·010	6·780	0·250	0·03	97
0·005	7·195	2·050	0·28	72
0·001	6·215	2·140	0·34	66
0·0005	6·650	3·460	0·52	48
0·00025	8·340	3·105	0·37	63
0·0001	11·340	6·255	0·55	45
0·00005	7·750	4·730	0·61	39
0·00001	6·710	3·325	0·50	50
0·000005	12·350	7·740	0·63	37
0·0000025	8·720	6·680	0·78	22
0·000001	4·850	4·790	0·99	1
0·000000	10·340	9·980	0·96	4

Table 3. *Weights of 2M-sucrose consumed by bees in 100 visits*

g.	g.	
4·12	4·14	Mean = 4·92
5·70	4·78	S.E. = ±0·239 g.
5·05	5·62	
5·07		

that the range of concentrations included in the rejection threshold varied considerably with the different chemicals.

Isopropyl cinnamate appeared to become attractive at a dilution of 0·0025 %, but observations during the experiments suggested that emulsions of this type need very careful treatment as they tend to alter in form on dilution, and this may produce anomalous results.

Table 4

Chemical	Repellency index at the following concentrations (%)									
	1	0·1	0·05	0·01	0·005	0·0025	0·001	0·0001	0·00001	0·000001
Phenol	100	100	100	93	65	.	54	29	41	6
Acetic acid	100	61	47	46	2	−5	.	.	.	.
Rutgers 612	100	100	74	51	21	5	.	.	.	.
Dimethyl-phthalate	100	96	.	57	10	2	4	.	.	.
Teepol	100	100	94	80	61	58	39	10	2	.
Isopropyl cinnamate	100	95	.	68	16	−55	−6	.	.	.

All concentrations were volume/volume relationships.

Experiment 4. The evaluation of the threshold response when an alternative food source was available in time but not in space

In the previous experiments the bees were simultaneously offered two solutions of the attractant, one of which contained the repellent material, and it might be expected that if the pure attractant was removed, the bees would accept a much higher concentration of the repellent material.

The experimental method used in the previous experiments was adapted to investigate this effect. Metal covers were used to cover four of the dishes on the turn-table, and in this way it was arranged that the bees only had access to one solution (see Fig. 1). The number of visits to these dishes were recorded for a fixed time and the metal covers transferred to the other four dishes. The method is best illustrated by the following example:

No. of visits by bees in 5 min. to 2M-sucrose	31
No. of visits by bees in 5 min. to 2M-sucrose and 0·03 % phenol	10
No. of visits by bees in 5 min. to 2M-sucrose	40
No. of visits by bees in 5 min. to 2M-sucrose and 0·03 % phenol	16

The above technique was used to test the following chemicals: phenol, D.M.P., Rutgers 612, Teepol and acetic acid; the results are shown in Table 5.

The rejection thresholds indicated by this experiment are quite different from those in Exp. 3; all the concentrations involved are higher, though the various chemicals retain approximately the same order of effectiveness. In both experiments phenol was the most active repellent, and the lowest concentration involved in the rejection threshold changed from 0·000001 to 0·01 % when no alternative food source was present.

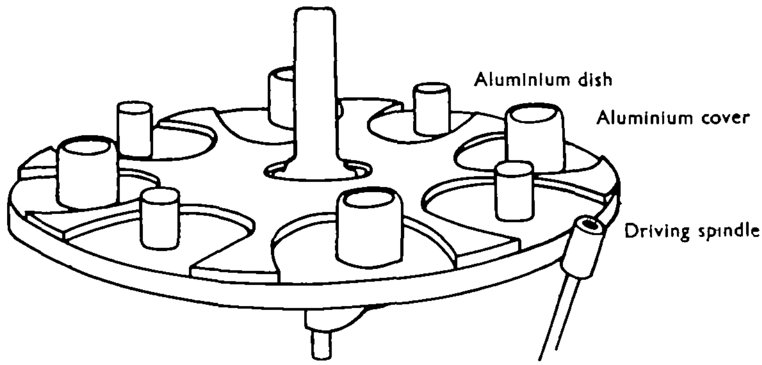

Fig. 1. Turn-table with dishes arranged as for Experiment 4.

Table 5

Repellent	Concentration (%)	No. of visits to pure sucrose (S)	No. of visits to sucrose and repellent ($S+R$)	R.I. $= 100 - \frac{S+R}{S} \times 100$
Acetic acid	0·8	71	0	100
	0·7	62	5	92
	0·6	58	26	55
	0·5	61	35	44
	0·4	53	35	34
	0·3	59	56	5
	0·2	68	69	0
Rutgers 612	0·2	52	0	100
	0·18	63	0	100
	0·125	58	15	74
	0·1	68	47	38
	0·09	53	45	15
	0·08	64	60	6
Dimethylphthalate	0·5	60	0	100
	0·1	49	5	90
	0·09	48	28	42
	0·08	72	43	41
	0·07	68	43	37
	0·06	73	47	36
	0·05	55	60	−9
Phenol	0·05	79	0	100
	0·04	77	4	95
	0·03	71	25	65
	0·02	67	63	6
	0·01	68	70	−1
Teepol	0·5	103	0	100
	0·4	33	3	94
	0·3	50	7	86
	0·2	56	1	98
	0·1	59	20	68
	0·09	47	23	49
	0·08	52	29	45
	0·07	61	37	40
	0·06	73	40	45
	0·05	80	65	19

Experiment 5. *The effect of varying the concentration of the attractant*

The last experiment was repeated using only phenol and acetic acid as test chemicals. These were made up in 1 M-sucrose solution, and this concentration was also used as the standard attractant. The bees did not forage as actively as they had done in the previous experiments, but the results as shown in Table 6 indicate yet a third set of figures relevant to the rejection threshold, lower in concentration than when the 2 M-sucrose was used.

Table 6. *Repellency index when solutions are made up in* 1 M-*sucrose*

	Concentration (%)	Repellency index calculated as in Table 5
Phenol	0·05	100
	0·02	77
	0·01	66
	0·009	37
	0·007	17
	0·006	2
Acetic acid	0·6	87
	0·5	69
	0·4	72
	0·3	70
	0·1	50
	0·09	37
	0·07	10
	0·06	8

Experiment 6. *To determine whether bees having once sampled a repellent solution are conditioned not to return*

Teepol, a commercial detergent, was found to have some repellent properties, and Exp. 3 showed that the concentration giving a repellency index of approximately 50 was determined to be 0·0025 %.

The eight dishes, four containing sucrose and Teepol and the other four with plain sucrose, were placed in the greenhouse. All the bees visiting the plain sucrose were marked white, and those visiting the repellent were marked red. Those visiting both solutions received red and white marks. This was continued for 3 hr. until about 150 bees had been marked. The dishes were refilled and all the bees' visits were recorded as red, white, red and white or unmarked (UM.). This was continued for 2 hr. and the results are shown in Table 7. The total number of visits to the plain sucrose solution is approximately twice the number made to the sucrose and Teepol, indicating an index of repellency of 58, which corresponds to the original figure at this concentration.

Table 7. *Repellency index as determined for* 0·0025 % *solution of Teepol* 56

Plain sucrose				Sucrose and Teepol 0·0025 %			
R.	W.	R.W.	UM.	R.	W.	R.W.	UM.
6	28	72	46	3	4	34	23
Total 152				Total 64			

The visits made by the various groups of bees (red, white, red and white, and unmarked) to the two solutions follow the same distribution, though at different levels, with the exception of the bees which had only received a white mark. These had made twenty-eight visits to the plain sucrose and only four to the repellent. This result might be due to the sudden crowding of bees on to a particular dish during the experiment, in which case the discrepancy would have disappeared if the experiment had been continued longer.

The alternative explanation is that some of the bees were more prone either to avoid the repellent or to concentrate on the plain syrup, and if this was the case the response of the bees is not entirely uniform and some conditioning had taken place,

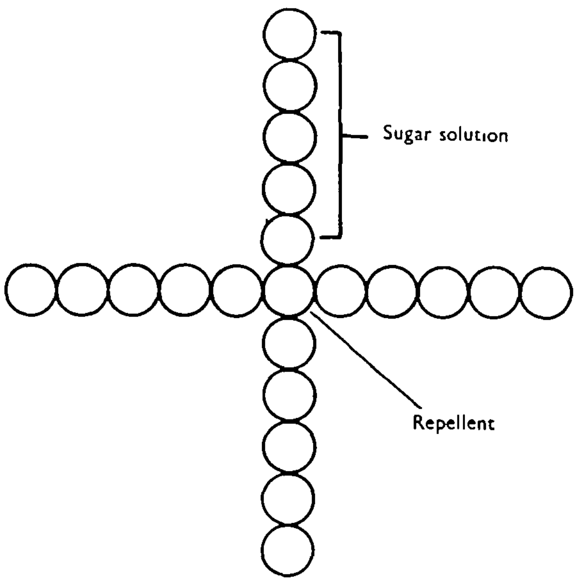

Fig. 2.

though it would appear from this and previous experiments that such a variation in response by a small proportion of the bees did not seriously affect the reproducibility of the results.

II. THE MEASUREMENT OF THE RESPONSE OF THE BEE TO A CHEMICAL IN THE VICINITY OF AN ATTRACTANT

Experimental methods

Twenty-one dishes, each having a capacity of 5 ml. and an external diameter of 2·5 cm., were arranged in the shape of a cross as shown in Fig. 2.

The repellent was placed in the central dish and the others were filled with a 2M-sucrose solution. The dishes were placed on a tray on the training table in the greenhouse. The number of visits to each of the four groups of four dishes equidistant from the centre was counted. A bee would often drink at one dish and then at another of the same row—this would be counted as two visits. The fifth group of dishes, i.e. those at the end of each arm, used to remove 'edge' effects caused by their position at the end of the row, and visits to the group were not counted in the assay. The experiment was terminated when 100 visits had been made to the fourth group of dishes. The whole tray bearing the dishes was moved through 45° every 15 min. to avoid the bees remembering the position of any particular dish and returning to it.

Table 8. *Number of visits*

Concentration of cresol (%)	Dish no. 1	Dish no. 2	Dish no. 3	Dish no. 4
0·0	103	118	91	100
0·25	100	105	121	100
0·50	53	78	100	100
1·0	37	93	97	100
50·0	33	63	75	100
100	23	71	90	100

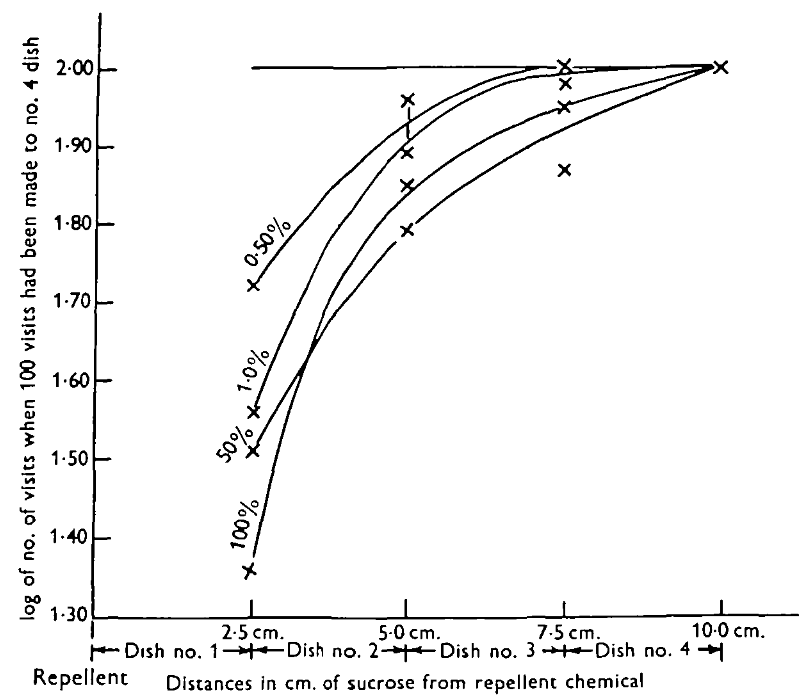

Fig. 3.

The results obtained from a series of tests using emulsions of various strengths of cresol (mixed isomers) in the central dish are shown in Table 8 and in Fig. 3. A preliminary test was first made without the repellent, as a control. The experiments were all done on the same day and took about 2 hr. each.

Fig. 3 shows that the bees' response to the stimulus varied with the concentration and that, assuming all the bees drank at the first group of dishes at the farthest point from the cresol, it appeared that until the concentration exceeded 0·25 % the bees showed no response to the presence of the stimulus at a greater distance than 2·5 cm., but as the concentration increased the distance over which a response was elicited also increased.

The experiment was repeated with other repellents, e.g. oil of citronella and creosote, and the results are shown in Table 9.

The tests indicate a possible technique for measuring this type of response, and it was felt that the replacement of the rows of dishes by a long narrow trough would allow for more accurate measurements to be made and would also remove a difficulty due to the fact that when the level of solution had been lowered in one dish it appeared to be more attractive to the foraging bees.

Table 9. *Visits to dishes*

Chemical	Concentration (%)	1	2	3	4
Oil of citronella	100	33	51	92	100
Oil of citronella	1	69	85	85	100
Creosote (crude)	1	35	75	76	100
Teepol (mixtures of sodium salts of secondary alkyl sulphates)	100	23	87	81	100
Isopropyl cinnamate	100	8	17	56	100

GENERAL DISCUSSION

The three different sets of data obtained during the experiments on the rejection thresholds of phenol and acetic acid are compared graphically in Fig. 4. The graphs indicate that the concentrations which completely repel all the bees were very similar under the three different experimental conditions, viz. with an alternative of pure sucrose in space, with an alternative of pure sucrose in time, (*a*) using 2 M-sucrose and (*b*) 1 M. When the last two conditions applied, both the curves approximate to a straight line. It is interesting to note that the three different conditions appear to exert a similar effect on the bees' responses to both chemicals. These variations in response, especially the increase in repellency shown with phenol over the range 0·0001–0·00001 % concentration, and also a similar trend being apparent for acetic acid, appear to be relevant to the problem mentioned by Dethier (1948) of whether gaseous mixtures act as repellent odours through the medium of localised olfactory receptors or as irritants through the medium of the more generalised common chemical sense.

For the purposes of this discussion we can define the olfactory, gustatory and common chemical senses as follows:

The olfactory sense is that stimulated by the chemical in gaseous form.

The gustatory sense is that stimulated by the chemical in solution.

The common chemical sense is that stimulated by chemicals acting as irritants and always produces an avoidance reaction. It must be mentioned here that there is some doubt as to whether, in the case of the honey-bee, it is permissible to consider the olfactory and gustatory senses separately (Butler 1949), and also that there are still wide gaps in our knowledge on chemoreception in insects.

In the experiments described in this paper it is likely that only the olfactory and common chemical senses were involved. The gustatory sense is only stimulated when actual contact with the test solution is made, and observations during the experiments indicated that nearly all the bees which alighted on the dishes drank some solution after inserting their proboscis and were thus counted. Very occasionally a bee would dip in its proboscis then fly away.

When the bees were offered a solution of the attractant which contained the repellent it may be assumed that at the lower concentrations of the latter only the olfactory sense organs would be stimulated. When the concentration of the repellent is progressively increased it is conceivable that a point is reached where the

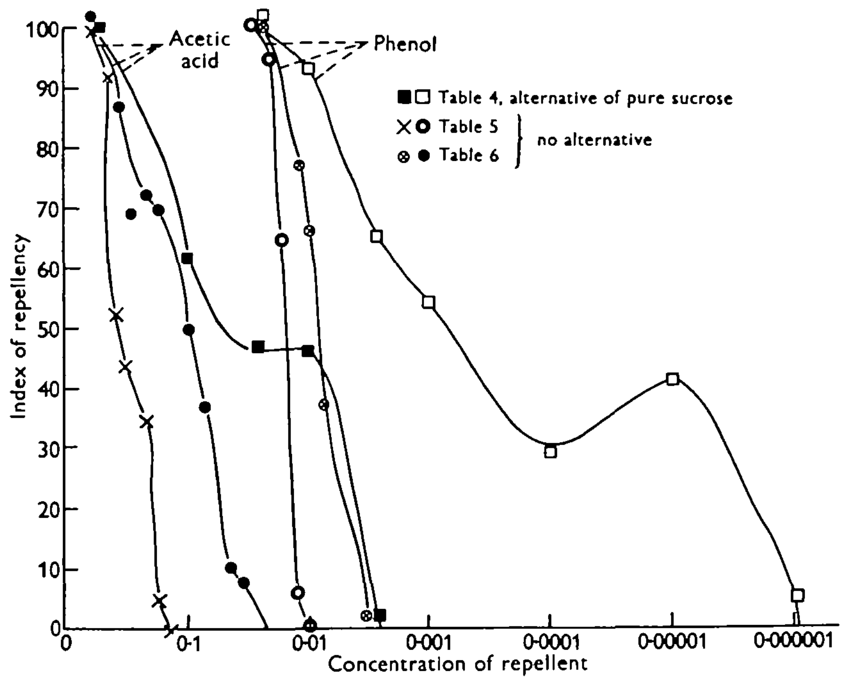

Fig. 4.

olfactory senses cease to be effective. (A parallel situation is commonly observed in humans who can only detect certain odours at low concentrations.) Further increases in concentration results in the stimulation of the common chemical senses by the repellent acting as an irritant.

When a choice of solution was available (Exps. 1–3) the trends of the graphs for acetic acid and phenol may be interpreted as indicating the insect's response to two stimuli, one caused by the lower concentrations affecting the olfactory sense organs and the other at the higher concentrations causing an irritation of the common chemical sense. With phenol, and less markedly so with acetic acid, it would appear that with a progressive increase in concentration the olfactory sense ceases to be effective before the concentration is high enough for the chemical to act as an irritant. Hence the falling off in the response which occurs between 0·00001 and 0·0001 % phenol.

If the choice in space is removed (Exps. 4, 5) it would appear that the olfactory sense ceases to elicit a response, as there is now only a conflict between the stimulus inherent in the worker bee to collect food and the power of the chemical to act as an irritant and hence elicit an avoidance reaction. Under these circumstances it would be expected that the range of concentrations defining the rejection threshold would be much narrower and the experimental evidence confirms this expectation. In Exp. 5 when the strength of the attractant is decreased the phenol and acetic acid become more repellent but follow the same type of response curve. As in Exp. 4, the type of curves obtained when no choice is available support the hypothesis that under these conditions only one sense is being stimulated.

Other experiments not quoted here showed that in a large apiary when there was little food in the hives and no sources of nectar available in the field, higher concentrations of phenol than 0·025 % are accepted, but here the urge to collect food was probably much greater and there was an exceptionally large population of foraging bees.

SUMMARY

1. This paper is concerned, first, with the development of a technique for maintaining a population of honey-bees (*Apis mellifera*) under conditions where it does not have access to naturally secreted nectar, but is allowed free flying conditions, and secondly with the application of this technique to the study of the chemotropic responses of bees to repellent chemicals.

2. Two types of stimuli are considered:

(*a*) The effect of the addition of the repellent to an attractant (sucrose solution).

(*b*) The effect of the repellent in the vapour phase in the vicinity of the attractant.

In (*a*) techniques are described for measuring the response with the standard attractant alternating in time and space.

3. Data collected on the rejection thresholds of acetic acid and phenol showed that whilst little variation in response occurred at the higher concentrations of the repellent, the offering of the standard attractant alternating in time as opposed to space greatly modified the response. Tests were carried out to determine the reproducibility of the results.

4. It would appear that for a repellent chemical to be capable of effectively reducing the number of visits to a source of food, it must be capable of irritating the common chemical senses of the bee.

I am indebted to the Agricultural Research Council for a grant which has made this work possible; also to Dr C. G. Butler and Dr C. Potter of Rothamsted Experimental Station for helpful advice and criticism. I am especially grateful to Mrs W. Heatherington, B.Sc., and Miss M. Burgoyne for giving valuable assistance with the experimental work.

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