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THE CHOICE OF DRINKING WATER BY THE HONEYBEE

BY C. G. BUTLER
Entomologist in Charge of Bee Research Laboratory, Rothamsted Experimental Station

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INTRODUCTION

DURING the spring and early summer months, bees collect large quantities of water for use in the hive for such purposes as softening down winter stores, etc.

Hertz (1935) showed without doubt, by means of standard training table experiments, that the honeybee can find pure water by "scent" alone, i.e. that they can distinguish the presence or relative proximity of water by means of the relative water-vapour content of the air. Her experiments give only an approximate indication of how far the "scent" can be distinguished, since the distance from the water surface to the bees is only one of the factors responsible for the water-vapour content of the atmosphere at that distance. Although she demonstrated that bees are sensitive to the water-vapour content of the air, it should not, of course, be assumed that water is sensed by the bee as a "smell". We have no evidence that indicates that the receptors involved are the same as those involved in the perception of ethereal oils, or even that the process is chemical rather than physical. It is not to be doubted, however, that it is a true perception by means of sense organs situated in some portion of the body of the bee. The experiments of Hertz were not concerned as are the ones about to be described, with the water *requirement* of bees.

It is well known that honeybees tend to collect water from many undesirable sources, such as rain-water gutters that are choked with decaying organic matter, on the puddles that form on the top of cow dung and sewage effluent, rather than from a source of clean water provided in the apiary for their use. It was, therefore, considered to be of interest and importance to discover what it is that attracts bees to such sources of water. Obviously something more than the sense of appreciation of the presence of water, as described by Hertz, is involved. Many observations and a few experiments had already been made on this subject, and the results, although somewhat conflicting, tended to show that bees prefer saline water to pure water. For instance, Herrod-Hempsall (1931) and Harrison (1932) both produced evidence to show that bees will readily collect water that is nearly saturated with sodium chloride; on the other hand, Betts (1932), Preuss (1919) and others, came to the conclusion that 1·5% sodium chloride is the greatest concentration palatable to bees and about 0·1% is the optimum concentration. Most observers, in fact, tend to explain the choice of drinking water by the honeybee in terms of the salt content of that water. There are, however, at least four major factors that are likely to be involved: sight, the water perception sense described by Hertz (1935), a perception of various olfactory substances contained in the water, and a gustatory sense once the water has been reached. External physical factors, such as temperature and degree of illumination, also undoubtedly play their part but have been neglected for the purpose of the present study, as they did not vary very greatly throughout each experiment.

METHODS

In order to make this study a "training table", similar to that used by von Frisch and Hertz in so many of their experiments on the senses of the honeybee, was set up in a sunny part of the apiary early in the spring of 1939. This table was painted all over with a matt black enamel in order to conserve as much as possible the heat of the sun's rays, and thirty-six shallow glass Petri dishes 4 in. in diameter were arranged on it in the form of a square. Each dish contained a small quantity of washed dry sand. The bees were first trained to come to this table by offering them very dilute sugar syrup on the sand in all the dishes. Once the bees became used to visiting the table these dishes were replaced by others in which the sand was kept moistened with distilled water. At the same time as many as possible of the natural sources of drinking water, such as rain-water gutters, in the immediate neighbourhood of the apiary were treated with strong lysol or carbolic in order to repel bees from them, thus compelling most of the bees to visit the table in order to collect the water necessary for the requirements of their colonies. After a few days, when the bees had become accustomed to collecting their drinking water at this source, the experiments proper were commenced. By this time thousands of bees were visiting the table daily if weather conditions were favourable. Six different substances at a time were tested against one another on this table and six dishes of each substance were arranged in the form of a Latin square, thus rendering statistical analysis of the results a simple matter. It was found that this modification of the technique usually used in this type of experiment greatly expedited matters, since the significance of the results obtained in an experiment could be checked immediately and the necessity or otherwise of further repetition determined. Six of the dishes on the table in each experiment contained distilled water, so that all the results obtained could be expressed relative to the honeybees' partiality for this substance.

If the substances to be tested were solid salts they were dissolved in fresh distilled water, and sufficient (10 c.c.) of the substance to be tested was poured into each dish to moisten the sand thoroughly, without allowing any of the solution to remain standing on the surface. The dishes themselves were replaced with clean ones fairly frequently in order to minimize, as far as possible, the chance of a bee selecting a particular dish because of any odour left on it by bees that had previously visited it. The whole table was turned round through 90° at intervals in order that bees should not become conditioned to the position of a particular dish containing some substance they favoured. There is little doubt that the presence of a large number of bees on a dish, perceived by a flying bee both visually and by an olfactory sense, tends to attract others, and to overcome this to some extent the table was frequently cleared of bees throughout the course of an experiment.

The table with the substances to be tested was put out in place of the table with the dishes of distilled water, and every 10 min. a count was taken of the number of bees drinking at each dish. It was found that a bee spends on the average between 3 and 5 min. collecting a load of water, thus by taking counts at 10 min. intervals the same bee was unlikely to be counted twice when on the same journey. Frequently the counting had to be done photographically owing to the large number of bees present at one time and the number of bees alighting and taking off from the dishes.

At the end of an experiment the experimental dishes were again replaced with others containing distilled water, and care was taken to see that there was always abundant distilled water on the table between experiments, since these extended over a period of some weeks.

EXPERIMENTS, RESULTS AND CONCLUSIONS

Two types of experiments were undertaken. First, experiments to determine if the honeybees were seeking water for the substances it might contain in solution and, if so, what those substances were and what was their optimal concentration. Secondly, to try to determine why the honeybee tends to prefer tainted water to pure water, i.e. whether it requires the contained substances or is merely led thither by an olfactory response to these substances.

The following tables clearly show the response of the honeybee to various organic and inorganic salt solutions and to other more complicated substances. These salts were chosen because it was thought that they were likely to be essential to the development of the honeybee and that the water collected might be the chief source of them. In addition, they had all been advocated by practical beekeepers for addition to apiary drinking fountains. As will be seen these two types of experiments overlap to a considerable extent.

In Table I are shown the results obtained in a typical experiment; in this case to determine the preferences, if any, of the honeybee for $N/10$ sodium chloride, $N/10$ ferric chloride, $N/10$ magnesium sulphate, $N/10$ sodium carbonate and $N/10$ sodium phosphate to distilled water. All the experiments were conducted on these lines.

Table I. *Latin square showing the results of a typical experiment carried out continuously between* 11 *a.m. and* 3 *p.m. on* 18 *April* 1939

(The numbers below the substances indicate the number of visits paid to them throughout this period)

Distilled water 99	NaCl $N/10$ 80	$FeCl_3$ $N/10$ 20	$MgSO_4$ $N/10$ 3	Na_2CO_3 $N/10$ 0	Na_2HPO_4 $N/10$ 2
Na_2HPO_4 $N/10$ 1	$MgSO_4$ $N/10$ 5	Distilled water 107	Na_2CO_3 $N/10$ 7	NaCl $N/10$ 76	$FeCl_3$ $N/10$ 25
$FeCl_3$ $N/10$ 16	Na_2CO_3 $N/10$ 3	Na_2HPO_4 $N/10$ 0	NaCl $N/10$ 88	$MgSO_4$ $N/10$ 0	Distilled water 100
$MgSO_4$ $N/10$ 1	Distilled water 86	NaCl $N/10$ 91	Na_2HPO_4 $N/10$ 3	$FeCl_3$ $N/10$ 22	Na_2CO_3 $N/10$ 1
Na_2CO_3 $N/10$ 9	Na_2HPO_4 $N/10$ 1	$MgSO_4$ $N/10$ 0	$FeCl_3$ $N/10$ 11	Distilled water 111	NaCl $N/10$ 77
NaCl $N/10$ 85	$FeCl_3$ $N/10$ 27	Na_2CO_3 $N/10$ 4	Distilled water 121	Na_2HPO_4 $N/10$ 0	$MgSO_4$ $N/10$ 4

The results of various experiments to determine the preference of the honeybee for thirteen different salt solutions are shown in Table II.

Table II. *Showing the preference of the honeybee for various salt solutions as compared with distilled water*

(The substances used in each experiment are arranged in order of preference, and the number of visits to each given)

Substances arranged in order of decreasing preference → (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
Rain water from choked gutter 218	Distilled water 128	NH_4Cl $N/10$ 51	KI $N/10$ 27	$CaCl_2$ $N/10$ 13	Na_2CO_3 $N/10$ 6	I
Distilled water 497	$MgCl_2$ $N/40$ 209	NaCl $N/5$ 164	$MgCl_2$ $N/20$ 135	$MgCl_2$ $N/10$ 54	$MgCl_2$ $N/5$ 28	II
Distilled water 624	NaCl $N/10$ 497	$FeCl_3$ $N/10$ 121	Na_2CO_3 $N/10$ 24	$MgSO_4$ $N/10$ 13	Na_2HPO_4 $N/10$ 7	III

From the results shown in Table II it appears that the honeybee prefers distilled water to any of the salt solutions offered. It was considered, however, that it was improbable that the optimal concentrations of these salts, from the bee's point of view, had been used, therefore further experiments were carried out in which the three salts, sodium chloride, ammonium chloride and magnesium chloride, which at deci-normal concentrations the bees had seemed to prefer to others, were each offered at five different concentrations. Similar experiments were also undertaken with potassium iodide, since several beekeepers have claimed that the bees both like and obtain benefit from this substance when it is added to their drinking water. The results of these experiments are shown in Table III.

Table III. *Showing the preference of the honeybee for certain concentrations of sodium chloride, magnesium chloride, ammonium chloride and potassium iodide*

Substances in order of decreasing preference → (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
NaCl *N*/40 966	NaCl *N*/80 926	NaCl *N*/20 759	Distilled water 579	NaCl *N*/160 567	NaCl *N*/10 387	I
Distilled water 502	$MgCl_2$ *N*/160 496	$MgCl_2$ *N*/80 234	$MgCl_2$ *N*/40 189	$MgCl_2$ *N*/20 111	$MgCl_2$ *N*/10 57	II
NH_4Cl *N*/40 318	NH_4Cl *N*/80 290	Distilled water 263	NH_4Cl *N*/160 249	NH_4Cl *N*/20 210	NH_4Cl *N*/10 106	III
Distilled water 130	KI *N*/80 48	KI *N*/160 46	KI *N*/40 28	KI *N*/20 21	KI *N*/10 13	IV

As will be seen from the results given in Table III the honeybee definitely appears to prefer *N*/40, *N*/80 and *N*/20 sodium chloride, in the order stated, to distilled water, and is probably unable to appreciate the difference between *N*/160 sodium chloride and distilled water, but definitely prefers distilled water to *N*/10 sodium chloride. Similarly, the honeybee prefers *N*/40 ammonium chloride and probably *N*/80 ammonium chloride to distilled water, and finds it more difficult to distinguish between distilled water and *N*/160 ammonium chloride, or else does not actively dislike the latter solution.

The honeybee does not appear to distinguish clearly between *N*/160 magnesium chloride and distilled water, but prefers the latter to higher concentrations of this salt. In the case of potassium iodide the honeybee appears quite definitely to dislike all the concentrations of this substance that were offered to it.

The above results appear to indicate that the honeybee in a state of nature does not seek water for the contained salts, since none of the solutions tested with the exception of dilute sodium and ammonium chloride solutions had any particular attraction.

Table IV. *Showing the preference of the honeybee for rain water, cow-dung water and urine and some of their constituents over distilled water*

Substances in order of decreasing preference → (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
Rain-water distillate 678	Rain-water residual salt 343	Distilled water 318	Rain-water distillate + charcoal 316	NaCl $N/10$ 223	NaCl $N/5$ 95	I
Cow-dung water distillate 396	Cow-dung water 371	Cow-dung water distillate + charcoal 243	Distilled water 198	NaCl $N/10$ 132	Cow-dung water residual salts 31	II
Urine distillate 737	Distilled water 408	NaCl $N/10$ 282	NH_4Cl $N/10$ 166	Urine 93	Urine residual salts 21	III

In the next series of experiments, the results of which are shown in Table IV above, direct attempts were made to discover what causes the honeybee to tend to prefer rain water from a leaf-choked gutter, water that has collected on top of a cow dung and urine to a source of clean water provided in the apiary. That this preference does exist has been reported by many observers. It has also been noted many times in the apiary in which this work was carried out. An instance of bees much preferring rain water from a gutter choked with decaying organic matter to pure water is shown experimentally in Table II, Exp. 1. For the purpose of these experiments large samples of the three substances mentioned above were collected. A sample of each was immediately evaporated to dryness over a very gently heated sand-bath, the distillate being condensed and collected. The minimum of heat was used, since it was regarded as most important to try to prevent the decomposition by heat of any of the contained substances. The residual salts thus produced were carefully redissolved in a bulk of distilled water equal in volume to the amount of the substance (500 c.c.) originally taken. All solutions were carefully filtered at atmospheric or slightly reduced pressure. These two solutions, the residual salt solution and the distillate, produced from these three substances were then offered to the bees together with distilled water. In every case the bees showed a marked preference for the distillate over distilled water. Only in the case of the rain water, where the salt concentration was presumably very low, was the residual salt solution preferred to distilled water.

In the experiments with the urine and the cow-dung water, the original substances were also offered alongside their derivatives. The bees showed a marked preference for the cow-dung water over the distilled water, but, in the case of the urine the bees flew over it in a large group but very few of them collected it. This is in complete accordance with observations made in the field. Although bees have been observed to be strongly attracted by urine they will seldom collect it, unless it is considerably diluted. A few experiments were commenced using different dilutions of urine, but the relatively short period of the season during which large numbers of bees collect water prevented their completion. So far as they went, however, they definitely tended to show experimentally that bees will collect urine that has been considerably diluted in preference to distilled water. The same thing seems to be true of cow-dung water, although the experiment given in Table IV does not show it. Field observations, however, showed that although bees are often strongly attracted to small puddles of water that collect on the top of cow dung they would only collect it from the older pats, by which time it had, presumably, become more dilute. The cow-dung water used in these experiments was collected from old pats that had been exposed to the weather for about 10 days or more.

An interesting feature of these experiments was the result obtained when samples of rain-water distillate and also cow-dung water distillate were allowed to stand with animal charcoal for 36 hr. at room temperature. When the resulting supernatant fluid was offered to the bees it appeared in the case of the rain water that the bees were no longer able to distinguish between it and distilled water, and in the case of cow-dung water distillate the preference was very markedly reduced. It would therefore appear that some olfactory substance or substances contained in the distillates produced from rain water, cow-dung water and urine, is the factor responsible for attracting the bees to these substances in preference to distilled water. Further, in the case of the rain-water sample and to a large extent the cow-dung water sample, these volatile substances can be absorbed on animal charcoal, in which case the resulting supernatant fluid is found to have lost its strong attraction for the honeybee. The conclusion can therefore be drawn that the honeybee is, in nature, attracted to these sources of water supply by the combined influence of the water perception sense described by Hertz (1935) together with a true olfactory sense. Sight may in nature also play a part in water discrimination, though the possible operation of this sense was, so far as possible, eliminated in the experiments described above. Once the honeybee has found a source of drinking water that it prefers to others in the immediate vicinity of its colony, by means of these senses, it is probably kept there by a gustatory sense, since it has been shown that the honeybee prefers sundry dilute salt solutions to pure water. It is almost certain that in this way a complicated conditioned reflex is set up, an expression of the so-called "memory" of the honeybee, which causes it to visit one particular source of water many times in preference to all others. That this conditioned reflex can persist over a considerable period of time is clearly demonstrated by the fact that honeybees will in the spring visit the site of a drinking fountain, that they used in the previous autumn, even though the fountain had been removed.

Table V, shown above, gives a final summary of the order of preferences of the honeybee for various solutions. If an arbitrary factor 10 is used to express the degree of appreciation of distilled water shown by the honeybee, the degree of preference shown for all other solutions used in these experiments can also be expressed by means of a factor. This factor has been calculated by taking the mean number of visits paid in all experiments to each substance and dividing this by the mean

Table V. *Showing the order of preference of various drinking waters. The various drinking waters have also been given a factor which shows the degree of preference compared with distilled water which has been given the arbitrary factor* 10

Substance	Factor
Rain-water distillate	21
Cow-dung water distillate	20
Cow-dung water	19
Urine distillate	18
Rain water from gutter	17
NaCl $N/40$	
NaCl $N/80$	16
NaCl $N/20$	13
NH_4Cl $N/40$	12
Cow-dung water distillate + charcoal	
Rain-water residual salts	11
NH_4Cl $N/80$	
Rain-water distillate + charcoal	10
$MgCl_2$ $N/160$	
NaCl $N/160$	
Distilled water	
NH_4Cl $N/160$	9
NH_4Cl $N/20$	8
NaCl $N/10$	7
$MgCl_2$ $N/80$	5
NH_4Cl $N/10$	4
$MgCl_2$ $N/40$	
KI $N/80$	
KI $N/160$	
NaCl $N/5$	3
NH_4Cl $N/5$	2
$MgCl_2$ $N/20$	
$FeCl_3$ $N/10$	
Urine	
Cow-dung residual salts	
KI $N/40$	
KI $N/20$	
KI $N/10$	1
$MgCl_2$ $N/10$	
Urine residual salts	
$CaCl_2$ $N/10$	0
Na_2CO_3 $N/10$	
$MgCl_2$ $N/5$	
$MgSO_4$ $N/10$	
Na_2HPO_4 $N/10$	

number of visits paid in the same experiments to distilled water—the figure for sodium chloride $N/40$ calculated in this way comes to approximately 1·7. Both sides of the equation were then multiplied by 10 which gives distilled water a factor of 10 in each case, and in the above example gives sodium chloride $N/40$ a factor of 17. In this way, for example, the preference shown by the honeybee for $N/40$ sodium chloride solution to distilled water can be expressed reasonably accurately by the following ratio:

$$\frac{N/40\ \text{NaCl}}{\text{Distilled water}} : \frac{17}{10}.$$

Several of the experiments have been statistically analysed and differences between substances found to be very significant. Though the remaining experiments have not been fully analysed, inspection of the figures shows most of the differences to be very clear cut, and there is little doubt of their significance.

SUMMARY

A brief review of the literature on this subject is given. By means of "training table" experiments in which use was made of the Latin square system in order to allow of rapid statistical analysis of the results obtained, it was shown that:

(1) The honeybee prefers dilute sodium chloride and ammonium chloride solutions to distilled water.

(2) It does not prefer concentrations of these salts higher than *N*/20 solutions and solutions of various other salts to distilled water.

(3) The honeybee appears unable to distinguish between *N*/160 sodium chloride or *N*/160 ammonium chloride and distilled water.

(4) The honeybee is probably largely attracted to such sources of drinking water as rain water from gutters choked with decaying organic matter, sewage effluent, etc.: by a water perception sense coupled with an olfactory appreciation of various volatile substances contained in these sources of water.

(5) The volatile substances present in the distillates from the various naturally occurring solutions examined could be absorbed on to animal charcoal to a large extent, in which case the resulting supernatant fluid was found to have lost its great attraction for the honeybee and was no longer clearly distinguished from distilled water.

(6) The salts contained in these sources of drinking water do not appear to play an important part in attracting the bee thither.

(7) It was found possible to express the preference shown by the honeybee for various solutions by means of numerical factors based on distilled water having an arbitrary factor of 10. Forty different solutions have been arranged in order of preference by this means.

I am greatly indebted to the Statistical Department, Rothamsted Experimental Station, for assistance in analysing the results obtained in these experiments. I should also like to take this opportunity of thanking my predecessor, Mr D. M. T. Morland, who suggested that I should undertake an investigation of this problem, and my colleagues in the Rothamsted Entomological and Bee Research Laboratories.

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