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EFFECTS OF TEMPERATURE AND HUMIDITY ON HONEYBEE RESPONSE TO ALARM PHEROMONES*

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Summary

Using a laboratory test, newly emerged worker honeybees (*Apis mellifera*) were evaluated for their response to four alarm pheromones (isobutyl acetate, isopentyl acetate, 2 heptanone, 1 pentanol), under five temperature/humidity regimens (30°C, 30·6°C, 26°7°C, at 45% RH; 35°C at 30% and 85% RH). With a higher temperature, there was increased probability of a response, and the speed, intensity and duration of that response were also greater. Relative humidity seemed to affect only the intensity, higher humidities increasing it. Occurrence of Nasonov fanning as a part of the response by some bees depended largely on the pheromone being tested; the effects of temperature and humidity were less clear-cut.

Introduction

Of the many conditions that probably influence the defensiveness shown by a colony of honeybees, temperature and humidity are usually mentioned. One conclusion reached by the Committee on the African Honey Bee (Michener, 1972) and supported by Gonçalves et al. (1974), was that these bees were gentler in the cooler temperate areas of southern Brazil, or at least more variable in their defensive behaviour, and were consistently defensive in the tropical northern area. Rothenbuhler (1974) further analysed data collected by the Committee on three colonies that were under several temperature conditions; they showed more stinging at higher temperatures.

Following these comments, Brandeburgo et al (1977) compared the behaviour of colonies headed by the same queens in temperate and in tropical parts of Brazil. These results suggested that temperature and humidity affect defensive behaviour, although several secondary climatic features such as differences in the quality and quantity of nectar flow, and the presence of predatory ants, could have influenced the results.

A laboratory test of honeybee defensive behaviour by measuring the response to alarm pheromones was developed by Collins and Rothenbuhler (1978). When it was used to compare two genetically and behaviourally different European lines, the defensive line had faster, longer, and more intensive reactions than did the gentle line (Collins, 1979). In addition, they much more rarely failed to respond.

When bees are tested under controlled laboratory conditions, temperature and humidity can be manipulated, while other factors affecting colony defence are held constant or eliminated. This paper presents the results of an investigation of the effects of temperature and humidity on the responses of honeybees to alarm pheromones.

Methods and Materials

Caged worker brood from three colonies of *Apis mellifera* was allowed to emerge in

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an incubator at 35°C over a 24-h period. The young bees were then placed in small, glass-fronted, wooden test cages, 30 bees/cage, with water and 50% sugar syrup. The cages were spaced out on shelves in a controlled-environment room, and the bees were tested (Collins & Rothenbuhler, 1978) when they were 2-5 days old. Heat in the room was provided by a space heater modified to carry variable currents, controlled by a thermostat. A fan in the heating unit ran continuously. A ceiling fan controlled by a second thermostat cooled the room by drawing air through a filter in the door from an adjacent air-conditioned room. Relative humidity was maintained at 30% by a portable home dehumidifier and at 85% by a spray humidifier. The normal level for the heated room without humidity control was 45% RH. A different sample of 3 cages of bees from each of the same 3 colonies was evaluated at each of three temperatures (26·7, 30·6, 35·0°C ± 1°) and a relative humidity of 45% ± 5%, and at each of two additional relative humidity levels (30% ± 5%, 85% ± 15%) at 35°C.

Each cage of bees was tested using one of four honeybee alarm pheromones: isobutyl acetate (IBA) identified by Blum et al. (1978), isopentyl acetate (IPA) identified by Boch et al. (1962), 2 heptanone (2 HPT) identified by Shearer and Boch (1965), and 1 pentanol (PNT) identified by Blum (personal communication). Each of the four chemicals was diluted with medicinal paraffin to a ratio of 1 : 10. Two drops were presented under the wire floor of the cage on a small piece of cork.

Before a chemical was presented, the number of bees moving about the cage was counted as the initial activity level. The period between presentation of the chemical and a distinct reaction by the bees was measured in seconds. If no reaction was seen within 60 s, 'no response' was recorded.

If a response was seen, its intensity was evaluated subjectively. It was characterized as: 'weak' when only a few bees slightly twitched their wings and moved around a little; 'medium' when more bees made definite flicks of their wings and walked around more; 'strong' when most of the bees made definite synchronous wing and body movements; 'very strong' when the response was frantic or explosive in appearance. The final measurement was the duration of the reaction, i.e. the period in seconds before the bees returned to the original level of activity.

Data on time to react, and duration, were analysed by least squares analysis of covariance with initial activity as the independent variable, followed by a least significant difference test, respectively. The analysis of covariance was necessary to eliminate the effects of differing initial activity levels on the response (Collins & Rothenbuhler, 1978). Chi-square was used for comparing the frequencies of occurrence of a reaction and intensities of response.

During the development and use of the test, Nasonov fanning was observed. When stimulated by the alarm pheromone, several of the 30 bees in a cage might begin to fan their wings vigorously while they were in a characteristic alert posture. Some of these fanning bees also exposed the Nasonov gland. No bees were seen to fan with the sting extruded. Such behaviour has also been seen in small observation hives after stimulation by alarm chemicals (Collins, unpublished data).

The frequency of Nasonov fanning as part of the response varied with the chemical being tested. Some pheromones rarely elicited fanning; others very frequently did. The behaviour was also age dependent; newly emerged bees showed it frequently, whereas bees 4 or 6 weeks old showed reduced or no fanning (Collins, 1980). The occurrence of Nasonov fanning during the tests was also recorded, to determine whether temperature and humidity affected it. The data were analysed by a 3-way analysis of variance.

Results

Frequency of response

There were no significant differences between the three colonies or the four chemicals used, so the observations were combined (Table 1). Increasing temperature increased the number of times that a reaction was seen, but changing humidity levels had little or no effect on this character.

TABLE 1. Total number of observations of a reaction (or no reaction) by worker honeybees to four alarm pheromones, during a 60-s test period.

	Reaction	*No reaction*
Temperature (°C)		
35	258	12
30·6	195	93
26·7	122	158
	χ^2 = 172·66[a]	df = 2
Relative humidity (%)		
85	222	15
45	258	12
30	274	14
	χ^2 = 1·00[b]	df = 2

a = significant at P < 0·005; *b* = not significant

Time to react and duration

There were no significant differences between colonies or between chemicals in time to react or duration of reaction. Time to react was significantly shorter (P <0·005) at the higher temperatures, that is, the bees reacted more slowly at 26·7° than at 35° and 30·6° (Table 2). In addition, the duration of reaction was longer (P <0·05) at the two higher temperatures. There were no significant differences between the three levels of humidity for these two measures.

TABLE 2. Least squares mean (± standard error) of time to react and duration of reaction by worker honeybees to four alarm pheromones, adjusted for initial activity level.

		Time to react (s)	*Duration of reaction (s)*
with 45% RH	*Temp. (°C)*		
	35	5·3 ± 3·9	39·2 ± 2·9
	30·6	6·5 ± 3·3	42·2 ± 2·4
	26·7	13·2[a] ± 3·4	28·2[b] ± 1·9
with 35°C	*RH (%)*		
	85	4·8 ± 1·0	47·5 ± 3·5
	45	5·3 ± 3·9	39·2 ± 2·9
	30	4·9 ± 2·1	43·4 ± 2·9

a = significantly different from other two values at P < 0·01
b = significantly different from other two values at P < 0·05

Intensity of the response

Although no colony differences were seen for the level of intensity of the response, the four chemicals gave significantly different results. Comparisons were made within

each chemical treatment: higher temperature was consistently associated with more reactions judged as medium and strong rather than weak (Table 3), and higher relative humidity was also associated with somewhat more intense responses (Table 4).

TABLE 3. Intensity of reaction by worker honeybees to four alarm pheromones measured at three temperatures.
The pheromones had significantly different effects ($\chi^2 = 43{\cdot}98$, $P < 0{\cdot}01$) and are presented with individual analyses.

		Intensity				
	Temp. (°C)	*Weak*	*Medium*	*Strong*	*Very strong*	*Total*
IBA	35	21	34	10	0	65
	30·6	19	16	4	1	40
	26·7	20	10	0	0	30
				$\chi^2=52{\cdot}63$	df=6	
IPA	35	2	26	43	0	71
	30·6	8	31	20	1	60
	26·7	21	14	1	0	36
				$\chi^2=107{\cdot}40$	df=6	
2HPT	35	4	27	30	0	61
	30·6	17	14	6	0	37
	26·7	15	11	0	0	26
				$\chi^2=92{\cdot}87$	df=4	
PNT	35	5	30	26	0	61
	30·6	23	26	9	0	58
	26·7	29	1	0	0	30
				$\chi^2=117{\cdot}07$	df=4	

a = significant at $P < 0{\cdot}01$

TABLE 4. Intensity of reaction by worker honeybees to four alarm pheromones measured at three relative humidities.
The pheromones had significantly different effects ($\chi^2 = 119{\cdot}68$, $P < 0{\cdot}01$) and are presented with individual analyses.

		Intensity				
	Relative humidity(%)	*Weak*	*Medium*	*Strong*	*Very strong*	*Total*
IBA	*85*	*6*	*31*	*7*	*0*	*54*
	45	*21*	*34*	*10*	*0*	*65*
	30	*29*	*31*	*7*	*0*	*67*
				$\chi^2=19{\cdot}31a$	df=4	
IPA	85	1	17	36	3	57
	45	2	26	43	0	71
	30	8	29	28	5	70
				$\chi^2=17{\cdot}26b$	df=6	
2HPT	85	0	15	38	10	63
	45	5	24	32	0	61
	30	14	22	30	4	70
				$\chi^2=31{\cdot}39a$	df=6	
PNT	85	6	20	22	0	48
	45	4	9	28	0	61
	30	12	36	19	0	67
				$\chi^2=12{\cdot}68b$	df=4	

a = signficant at $P < 0{\cdot}01$; *b* = significant at $P < 0{\cdot}05$

Frequency of fanning

Significant differences were found between the four chemicals ($P < 0{\cdot}01$) and between the three temperatures ($P < 0{\cdot}01$), but not between the three humidities. There were also significant ($P < 0{\cdot}05$) interactions between the two environmental conditions and the chemicals. The differing effects of temperatures with different chemicals can be seen in Table 5, those of humidity in Table 6. In general fanning occurred much less frequently with IBA and IPA than with 2HPT and PNT.

TABLE 5. Mean number of worker honeybees fanning for each pheromone, tested at three temperatures (RH 45%).
Means followed by the same letter are not significantly different at $P < 0{\cdot}01$.

	Temperature (°C)		
Pheromone	35	30·6	26·7
IBA	1·7*a*	6·3*a*	0·0*a*
IPA	5·3*a*	17·7*b*	1·3*a*
2HPT	16·3*b*	4·3*a*	1·0*a*
PNT	20·0*b*	15·0*b*	2·3*a*

TABLE 6. Mean number of worker honeybees fanning for each pheromone, tested at three relative humidities (35°C).
Means followed by the same letter are not significantly different at $P < 0{\cdot}05$.

	Relative humidity (%)		
Chemical	85	45	30
IBA	0·0*a*	1·7*a*	0·7*a*
IPA	0·3*a*	5·3*b*	5·0*b*
2HPT	22·3*d*	16·3*cd*	7·3*b*
PNT	13·3*c*	20·0*d*	11·0*bc*

Discussion

It is certainly not unexpected that changes in temperature rather dramatically alter the expression of response to alarm pheromones by worker honeybees. That humidity levels seemed to influence only the intensity with which bees responsible to alarm pheromones is more surprising. However, one can predict that bees will be likely to show their quickest, most vigorous and most long-lasting colony defensive behaviour under hot (30+°C), humid (85+% RH) conditions. This could be all the year in humid tropical areas, or during summer months in humid temperate areas. However, many other factors influence colony defence, from the genotypes of the bees to microclimatic differences in colony sites. In the field, temperature and humidity are not separate from the milieu in which they occur.

What function Nasonov fanning plays in response to alarm pheromones remains to be clearly defined. Scenting with fanning at the hive entrance (Sladen, 1902; Renner, 1960) probably guides bees to the hive and to other bees of their colony. The same behaviour is also important for marking desirable foraging sites and for enabling a swarm to cluster quickly (Carr & Levin, 1967). Four pheromones of the scent gland have been identified (geraniol, nerolic acid, geranic acid, citral), all of which are attractive to worker honeybees (Butler & Calam, 1969). Perhaps release of these scent pheromones during colony defence, i.e., following release of alarm pher-

omones, serves to muster the defending bees around the colony. In other laboratory cage tests, duration of the response to alarm chemicals was positively correlated with the frequency of fanning (Collins, unpublished data). Possibly, these or other components of the Nasonov secretion maintain arousal to alarm pheromones. It is equally possible, however, that scent fanning at such times has no, or another, function.

Whatever the function may be, only some of the sting-associated alarm chemicals induced Nasonov fanning (IBA was very ineffective). Among those that did, the effect of temperature and humidity varied somewhat according to the chemical being tested. Perhaps different alarm pheromones stimulate Nasonov fanning under different conditions.

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