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SOCIOCHEMICAL ALTERATION OF HONEYBEE HOARDING BEHAVIOR

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Abstract—Hoarding experiments were conducted with honeybees (*Apis mellifera* L.) in cages containing comb treated with either 2-heptanone, isopentyl acetate, citral, or geraniol. 2-Heptanone increased hoarding rates; isopentyl acetate decreased hoarding rates; citral and geraniol had no observed effect.

Key Words—*Apis mellifera*, Hymenoptera, Apidae, 2-heptanone, isopentyl acetate, citral, geraniol, hoarding.

INTRODUCTION

Empty comb in a honeybee (*Apis mellifera* L.) nest plays a major role in influencing the nectar-harvesting activities of honeybees. Substantially increased amounts of empty comb in the nests of colonies during strong nectar flows significantly increase the amount of honey that they store (Rinderer and Baxter, 1978a). Similarly, increased amounts of empty comb result in increased rates of sucrose solution hoarded by caged bees in laboratory experiments (Rinderer and Baxter, 1979, 1980). The stimuli through which comb influences nectar-harvesting are chemical (Rinderer, 1981), and single pheromones often have multiple functions in social insect chemocommunication systems (Blum, 1977). Consequently, knowledge of any effects of known worker honeybee sociochemicals on the hoarding behavior of bees is desirable.

This paper reports the effects of four sociochemicals on the hoarding behavior of bees in laboratory hoarding cages. These small cages (Kulinčević et al., 1973) house bees with a piece of empty comb and feeders containing sucrose solution, water, and pollen substitutes. Bees remove sucrose solution from the feeder and hoard it in the comb (Free and Williams, 1972; Kulinčević

and Rothenbuhler, 1973). Differential amounts of sucrose solution hoarded in these cages are related to differential amounts of honey stored by field colonies. This is true for differences arising primarily from either genotype (Kulinčević et al., 1974; Rothenbuhler et al., 1979) or environment (Rinderer and Baxter, 1978a, 1979; Rinderer et al., 1979).

METHODS AND MATERIALS

In preparation for the experiments, pieces of comb (46.25 cm^2) were placed in desiccators with an atmosphere saturated with either 2-heptanone (2-H), isopentyl acetate (IPA), citral, or geraniol. In each instance, combs increased in weight by ca. 4 g during exposure to volatile chemicals. IPA and 2-H are components of alarm pheromones from honeybee stings and mandibular glands, respectively, which mediate defensive behavior (Boch et al., 1970); geraniol and citral are components of an attractant pheromone from Nasonov glands that mediates orientation and aggregation (see reviews by Blum, 1977; Wilson, 1971). Comb was kept in desiccators for four days and then placed in hoarding cages. Within 4 hr, bees were added to the cages and experimental studies began.

For evaluation of each sociochemical, combs of emerging adult worker bees were obtained from the brood nests of seven colonies. Different colonies were used for evaluating each chemical. The combs of emerging bees were held in an incubator (35°C and 50% relative humidity) until the bees were 0–24 hr old. Groups of 30 bees (Rinderer and Baxter, 1978b) from each colony were then placed into eight cages containing comb treated with the appropriate sociochemical and eight cages containing untreated comb. Each group of cages was placed in a separate incubator (35°C and 50% relative humidity).

Each cage was inspected daily for seven days; the amount of sucrose solution removed from the feeders was measured, and all feeders were replenished.

RESULTS

An inspection of Table 1 reveals that two of the four chemicals, 2-H and IPA, significantly influenced hoarding behavior; 2-H increased hoarding ($P < 0.001$) while IPA decreased it ($P < 0.003$). In all experiments, differences were significant in hoarding rates between colonies. No interactions occurred between the factors of chemical treatment and colony source, which indicates that bees from all colonies responded in similar ways to the chemicals.

TABLE 1. HOARDING RESPONSE (ML OF SUCROSE SOLUTION) OF BEES CAGED WITH COMB TREATED WITH SOCIOCHEMICALS

Sociochemical treatment	$\bar{X} \pm SE$[a]	Analysis of variance			
		Source of variation	*df*	*F*	*P*<
2-Heptanone	15.7 ± 0.3	T[b]	1	16.44	0.001
Control	12.5 ± 0.2	C	6	17.04	0.001
		I	6	1.7	NS
Isopentyl acetate	13.7 ± 0.2	T	1	9.20	0.003
Control	16.2 ± 0.3	C	6	8.81	0.001
		I	6	1.27	NS
Citral	10.3 ± 0.1	T	1	0.86	NS
Control	10.7 ± 0.2	C	6	8.26	0.001
		I	6	1.12	NS
Geraniol	14.8 ± 0.3	T	1	0.42	NS
Control	15.4 ± 0.3	C	6	15.34	0.001
		I	6	0.51	NS

[a]Each mean is calculated from 8 replicate measurements from each of 7 colonies.
[b]T = treatment; C = colonies; I = interaction.

DISCUSSION

The presence of two compounds resulted in a change in hoarding and the presence of the other two did not, even though all four compounds are known to be pheromones that are perceived by honeybees. Also, of the two chemicals that did affect hoarding, one increased and one decreased it. Therefore, the results are unlikely to be caused by general chemical irritation, nor are the two responses likely to arise from a similar effect since the responses were directionally different. Rather, the effect of each chemical is likely to have its origins in a separate adaptive feature of the natural history of bees.

Bees in hoarding cages exhibit a defensive response under brief exposure to IPA vapors (Collins and Rothenbuhler, 1978). In the experiment reported here bees were continually exposed to IPA vapors and continually exposed to the hoarding stimuli provided by comb. Limited olfactory habituation coupled with the role of IPA in defense probably underlie the reduction of hoarding by bees continually exposed to IPA. The chemical tends to draw bees away from other activities into defense and, with some efficiency, continues to do so.

In a natural setting, IPA plays a major role in colony-defensive episodes. During such episodes, large numbers of bees may be temporarily drawn from other activities into defense, at least in part by the action of IPA (Maschwitz, 1964; Boch et al., 1962). Bees in a colony, while providing each other with

chemical stimuli, which release a defensive response, apparently do not provide each other with stimuli which terminate that response. Rather, termination has its beginnings when the intruder ceases to provide appropriate stimuli. Included among these stimuli is the presence of IPA released from stings delivered to the intruder.

When presented with 2-H, bees exhibit defensive responses similar to those elicited by IPA under both laboratory (Collins, 1980) and field conditions (Boch and Shearer, 1971). 2-H is regarded as an alarm pheromone that is secondary in function to IPA (Boch et al., 1970), even though 2-H is produced in quantities ranging to 40 μg (Boch and Shearer, 1967), whereas IPA is produced in quantities ranging to 5 μg (Boch and Shearer, 1966). This differential production suggests that 2-H has additional roles in which it functions as a primary behavioral mediator. In large quantities, 2-H repels bees (Simpson, 1966). This response occurs in the natural history of honeybees, since foraging bees apparently label exhausted food sources with 2-H that marks them as "empty" (Nuñez, 1967). While this function of 2-H explains, at least in part, the presence of large amounts of 2-H in foraging bees, it does not satisfactorily explain the presence of large amounts of 2-H in guard bees (Crewe and Hastings, 1976).

Perhaps 2-H released during defensive episodes by guard bees not only functions as a releaser pheromone with a secondary alarm role, but also functions as a primer pheromone which physiologically alters the bees and thereby increases the intensity of nectar-harvesting activities by foragers. Nectar foraging is regulated chemically through the action of volatiles from empty comb (Rinderer, 1981). Similar laboratory hoarding responses are evoked by both 2-H and comb volatiles. If this effect by 2-H occurs in the field, it would serve to increase the intensity of nectar-harvesting after a defensive episode and thereby stimulate a colony to regain losses incurred through the actions of intruders.

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