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Volatiles from Empty Comb Increase Hoarding by the Honey Bee

Substantially increased amounts of empty comb in the nests of honey bees (*Apis mellifera* L.) during periods of abundant nectar secretion and availability, significantly increased the amount of honey the bees stored (Rinderer & Baxter 1978). Also, bees in laboratory hoarding cages (Kulinčević et al. 1973) showed increased rates of sucrose-solution hoarding in the presence of increased amounts of empty comb (Rinderer & Baxter 1979). Laboratory hoarding cages house bees with a piece of empty comb and feeders containing sucrose solution and water. Bees remove the sucrose solution from the feeder and hoard it in the comb (Free & Williams 1972; Kulinčević & Rothenbuhler 1973). Bees in hoarding cages can only occasionally be observed on comb not being used for immediate storage. Nonetheless, the presence of such comb greatly influences hoarding rates. This observation suggests the hypothesis that the stimuli provided by empty comb which lead to increased hoarding are chemical.

Testing this hypothesis required the use of modified hoarding cages. The modification permitted air to be pumped first through a 2-litre Plexiglass box filled with comb pieces and then into hoarding cages just above the comb. Connections between the air pump, the Plexiglass box, and the cages were made with tight-fitting inert plastic tubing. Each cage received 235 cm^2 of air per min from the air flow system throughout the experiments. Control cages received air flows from a similar system, except that the Plexiglass box contained only air. In the first of three experiments, air was passed over empty comb held at 35 C; in the second, air was passed over empty comb held at 5 C and was then warmed to 35 C; in the third, air was passed over comb filled with stored honey held at 35 C. The honey was completely ripened and sealed in the cells with wax by bees. Each experiment was composed of both experimental and control cages of bees.

Combs of emerging adult worker bees were obtained for each experiment from the broodnests of seven colonies to obtain bees for seven different colony-source experimental replications. Different groups of seven colonies were used for each experiment. The combs of emerging bees were held in an incubator (35 C and 50% relative humidity) until the bees were 0 to 24 h old. Bees from each colony were then placed in groups of 30 into five experimental and five control cages. These cages were attached to the appropriate air flow system and placed in an 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 refilled. Data from each experiment on the volume of sucrose solution removed during the seven days were submitted to a two-way analysis of variance.

Bees in cages exposed to volatiles from 35 C empty comb hoarded significantly more sucrose solution ($P < 0.001$) than did bees in control cages (Table I). Differences were significant between the hoarding responses of bees from different colonies ($P < 0.001$). While bees from most colonies increased their hoarding by about 20% over controls, bees from one colony increased their hoarding by more than 100%. This difference resulted in a significant ($P < 0.001$) treatment by colony interaction term in the analysis. Bees exposed to air containing volatiles from cold comb (5 C) or to volatiles from warm comb (35 C) containing honey did not hoard more sucrose solution than control bees exposed to air alone. In both analyses differences were significant between bees from different colonies ($P < 0.005$). Also, in both analyses no interaction occurred between the factors of type of air and the colony-source of bees.

Thus, volatiles from empty comb at a temperature similar to that occurring in the brood area of bee nests increased the hoarding behaviour of bees. These volatiles were not given off in effective amounts by cold empty comb or warm comb that contained stored honey. Probably these volatiles are pheromones incorporated in comb by bees, since increased amounts of freshly made comb effectively increased hoarding (Rinderer & Baxter 1980).

Table I. Analysis of Variance of Millilitres of Sucrose Solution Hoarded by Bees in Response to Airflows Carrying Volatiles from Three Types of Comb

Comb type in experiment	Treatment	ml hoarded $\bar{X} \pm$ SE	Analysis: Source of variation	*df*	*F*	*P*
Empty comb (35 C)	Experimental	17.0 ± 0.5	Treatment	1	28.7	0.001
	Control	12.9 ± 0.3	Colonies	6	13.1	0.001
			Interaction	6	14.9	0.001
			Error	56		
Empty comb (5 C)	Experimental	10.6 ± 0.3	Treatment	1	0.5	NS
	Control	10.0 ± 0.3	Colonies	6	4.7	0.005
			Interaction	6	0.9	NS
			Error	56		
Comb with stored honey (35 C)	Experimental	10.0 ± 0.2	Treatment	1	0.8	NS
	Control	10.4 ± 0.3	Colonies	6	19.8	0.001
			Interaction	6	0.8	NS
			Error	56		

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