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EFFECT OF EMPTY COMB ON DEFENSIVE BEHAVIOR OF HONEYBEES

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Abstract—Honeybees in colonies with 6.36-m^2 surface area of empty comb responded faster to moving targets and stung the targets more often than bees from colonies with 3.18-m^2 surface area of empty honeycomb. The two groups did not differ significantly in speed of response to alarm pheromone or in number of bees defending the colony. Volatiles from the comb are suggested as primer pheromones for defensive behavior.

Key Words—Honeybees, *Apis mellifera,* Hymenoptera, Apidae, colony defense, stinging, comb volatiles, pheromones.

INTRODUCTION

Rinderer and Baxter (1978) reported that 4.06 m^2 of comb surface area (CSA) of empty beeswax comb suitable for the storage of honey added to a colony of honeybees, *Apis mellifera,* during a period of intensive nectar secretion and availability, significantly increased the amount of nectar gathered and stored as honey, over the quantity stored by a colony given only 1.88 m^2 CSA of empty comb. A possible mechanism for the effect of empty comb on honey production was demonstrated by Rinderer (1981). Caged adult worker honeybees hoarded (stored in comb, see Free and Williams, 1972; Kulincevic and Rothenbuhler, 1973) sucrose solution at an increased rate in the presence of volatiles from warm (35°C) empty comb. Further work (Rinderer, 1982) proposed that differing levels of volatiles from empty comb shifted aspects of honeybee nectar foraging according to seasonally varying conditions of nectar availability.

Results of an experiment comparing colony defense by Africanized and European bees in Venezuela (Collins et al., 1982) led us to postulate that empty comb might also influence defensive behavior. Data from this comparison of ecotypes, analyzed for the effect of empty comb, and from a second experiment designed specifically to determine the influence of empty comb on defensive behavior are presented here.

METHODS AND MATERIALS

For both experiments, a standardized test (Collins and Kubasek, 1982) was used to assess the level of defensive behavior of each colony. Prior to testing, two pictures were taken: one of the bees on the colony around the entrance, and one of the bees flying in front of the colony. The actual test began when 0.8 ml of an artificial alarm pheromone [isopentyl acetate in paraffin oil 1:100 (v/v)] was sprayed above the colony entrance. The time when recruited bees began to issue from the hive was recorded. At 30 sec after spraying, a second pair of photographs was taken, and the colony was given a physical jolt by hitting it with a glass marble (1.9 g, 2.3 cm diam) propelled by a slingshot. A third pair of photographs was taken at 60 sec and two blue suede targets (5 × 5 cm) were moved into place, one 2–6 cm in front of the entrance and one 25 cm farther away. These targets were clipped to the arms of a battery-operated device which swung them up and down through 20 cm about 120 times/min during the 60-sec to 90-sec interval. The time at which the first bee was seen on a moving target was recorded. At 90 sec, the targets were removed and final photographs made. A pair of targets was used only once and replaced for each test sequence. At a later time, the number of stings adhering to each target and the number of bees in each picture were counted.

Ecotype Comparison Experiment. Ten colonies each of Africanized and European bees (900 g; 8500–10,000 workers) were established in either 20-liter (½ standard brood chamber) or 40-liter (standard brood chamber) hives with equal brood and honey and 0.52 m^2 and 1.56 m^2 empty CSA, respectively. After six weeks (during which an unrelated experiment was performed), the colonies were transferred to new, three-comb (43 × 20 cm), 20-liter nucleus colonies with 3-cm entrances and established in one new apiary location near Maturin, Monagas, Venezuela. At the time of transfer, the populations of adult bees and brood were judged to be still equal. On the third and fourth days, the colonies were evaluated using the standardized test, serving as a control for a larger experiment (Collins et al., 1982) comparing colony defense by the two ecotypes. Data were analyzed for ecotype, previous hive volume, and test day by a three-way analysis of variance.

Empty Comb Experiment. Twenty-four colonies of European bees were chosen on the basis of approximately equal numbers of bees (20,000–30,000), equal size brood nests, and equal honey and pollen stores. Each colony was derived from a queen of open-mated mixed commercial stock. These colonies were established at one apiary location near St. Gabriel, Louisiana, after the major honey production season. The colonies were randomly assigned to a treatment group and given empty honey storage comb with 3.18 m^2 CSA (three shallow supers) or 6.36 m^2 CSA (six shallow supers) in addition to their brood nest.

After six weeks (sufficient time for an effect to be significant, as seen in the first experiment), each colony was tested on three days between 10 AM and noon using the standardized test (test 1). Then the honey storage comb was removed, any collected honey was removed, and the two CSA treatment levels were reversed on the same set of 24 colonies. After one week, the colonies were retested three times, again between 10 AM and noon (test 2). A third replicate of three tests was made six weeks after treatment reversal (test 3). Data were transformed to $\log_e$ and analyzed by least-squares analysis of variance.

RESULTS AND DISCUSSION

Ecotype Comparison Experiment. Means of the seven measurements of defensive behavior for each level of empty honey storage comb surface area are presented in Table 1. There was no difference in time to react to the artificial alarm pheromone. However, when a moving target was presented, bees that had been in a hive with more CSA responded approximately twice as fast as bees from the units with less CSA. There were also twice as many stings in targets attacked by these bees.

The influence of more CSA was also seen in the number of bees responding

TABLE 1. MEASUREMENTS OF COLONY DEFENSIVE BEHAVIOR BY HONEYBEES PREVIOUSLY IN 20-LITER OR 40-LITER COLONIES (0.52 m^2 AND 1.56 m^2 CSA, RESPECTIVELY) (ECOTYPE COMPARISON EXPERIMENT)[a]

	Previous hive volume (liter)		
Measurement	20	40	F^b
Time to react (s) to:			
Pheromone	14.9	13.5	0.3
Target	8.5	4.4	4.9*
Total No. stings	4.7	9.5	7.9**
No. of bees on colony front at:			
Pre	3.2	2.5	0.6
30 sec	4.3	9.4	11.0**
60 sec	5.6	12.2	6.7*
90 sec	6.3	7.7	0.9

[a] Values are means from 10 colonies each tested twice.
[b] $df = 1$; $*P < 0.05$; $**P < 0.01$.

after the field test began, at 30 and 60 sec, but not at 90 sec. The more defensive Africanized bees exhibited similar differences when compared with European bees in the small units (Collins et al., 1982). It was proposed that the lack of difference at 90 sec was due to an incomplete count of defending bees. Africanized bees tended to fly off the entrance and attack more readily and, therefore, many did not show up in the photograph. Europeans did not fly as readily. A similar difference might exist between high CSA bees when compared to low CSA bees.

The results of this experiment indicate that honeybee defensive behavior is affected by the presence of empty comb and that the effect remains for a period of time after the comb is removed. Similar stimulation by empty comb has also been shown to alter hoarding behavior by caged adult workers (Rinderer and Baxter, 1979) and honey production in field colonies (Rinderer and Baxter, 1978).

Empty Comb Experiment. The measurements of colony defensive behavior by field colonies with 3.18 m^2 and 6.36 m^2 CSA are shown in Table 2. Six weeks after empty comb was placed on the colonies (combined tests 1 and 3), bees from nests with greater CSA responded to the moving targets faster and stung them twice as much as did bees from small CSA nests. There were no significant differences between treatments for time to react or the number of bees reacting. However, the values for 6.36 m^2 CSA colonies were consistently indicative of greater responsiveness. At one week, it appears that the reversed CSA treatment levels are already affecting the expression of the behavior, as the two treatments are no longer significantly different for any component of the behavior.

In a comparison of the defense test results one week after treatment reversal (test 2) with the results of testing six weeks after treatments were applied (reversed, tests 1 and 3), it appears that the colonies were disrupted by the treatment reversal. More bees are present on the entrance and more stings were counted for test 2 than for tests 1 and 3. A week is more than sufficient time for a colony to settle down after being worked (physically disturbed), and no major differences in temperature, humidity, or foraging behavior were observed. Thus, it seems likely that this difference in behavior may reflect the unsettling effect of having the comb treatments reversed and altering the levels of comb volatiles present.

Rinderer (1981) concluded that it was the volatiles from empty comb at temperatures similar to that of the normal brood nest which increased hoarding behavior, rather than any actual contact with the combs. He proposed that these volatiles are pheromones incorporated into the comb by the bees themselves. Our results show that the presence of empty comb in a colony enhances the level of defensive behavior. Comb volatiles may function as primer pheromones in defensive behavior, altering the physiology of worker bees such that they are more responsive to primary stimuli eliciting colony defense (Collins et al., 1980).

TABLE 2. MEASUREMENTS (LEAST-SQUARES MEANS ± STANDARD ERROR) OF DEFENSIVE BEHAVIOR BY HONEYBEES EXPOSED TO TWO LEVELS OF EMPTY COMB[a]

		Comb surface area (m^2)		
Behavioral component	Test[b]	3.18	6.36	F^c
Time(s) to react to:				
Pheromone	1	9.9 ± 1.0	8.9 ± 0.7	
	2	10.6 ± 0.7	9.4 ± 0.9	1.23
	3	9.7 ± 1.0	7.9 ± 0.8	
	1 + 3	9.7 ± 0.6	8.6 ± 0.5	1.15
		F^d 2.63		
Target	1	8.2 ± 1.0	6.4 ± 0.8	
	2	7.9 ± 0.9	7.5 ± 1.0	0.77
	3	8.8 ± 1.0	5.5 ± 0.8	
	1 + 3	8.5 ± 0.6	6.0 ± 0.6	8.85**
		F^d 0.16		
Total No. stings	1	9.5 ± 1.5	18.3 ± 2.7	
	2	13.4 ± 2.4	20.4 ± 3.5	2.39
	3	4.2 ± 1.0	12.5 ± 2.2	
	1 + 3	7.0 ± 1.4	15.4 ± 1.4	31.62**
		F^d 2.98**		
Total No. bees:	1	198.5 ± 20.9	181.5 ± 14.7	
On colony front	2	248.8 ± 19.2	253.5 ± 32.2	0.95
	3	102.5 ± 10.5	143.7 ± 21.6	
	1 + 3	145.7 ± 12.6	161.4 ± 12.7	1.49
		F^d 104.49**		
Flying in front of colony	1	48.8 ± 5.7	60.4 ± 6.6	
	2	61.1 ± 6.1	69.4 ± 9.8	0.18
	3	57.4 ± 7.6	60.6 ± 6.8	
	1 + 3	54.6 ± 5.0	59.8 ± 4.9	2.40
		F^d 0.44		

[a]Data for each mean are from 12 colonies each tested three times.
[b]Test 1, 6 weeks after empty comb was added to colonies; test 2, 1 week after reversal of treatments; test 3, 6 weeks after reversal of treatments.
[c]$df = 1$; $^{**}P < 0.01$; comparison of treatment; 3.18 CSA vs. 6.36 CSA.
[d]$df = 1$; $^{**}P < 0.01$; comparison of tests; one week after treatment (test 2) vs. six weeks after treatment (tests 1 + 3).

It is not surprising that comb volatiles regulate both foraging and defensive behavior. Established feral colonies with large amounts of empty comb would normally have only comparatively small amounts of stored honey (i.e., early spring, prior to a nectar flow but after brood rearing has resumed). Such colonies would clearly benefit by foraging intensively at high-quality nectar sources if they can be found and at the same time vigorously defending their limited reserve of honey. Normally colonies in nests with less empty comb can be expected to have more stored honey (i.e., after a major honey flow). Such colonies, in defense-eliciting situations not requiring massive responses, may benefit from reduced defense responses which result in fewer bees lost in colony defense. At such times, surviving bees may be more important to a colony than the loss of small amounts of honey from plentiful reserves. Although less empty comb reduces intensity of defense, it does not eliminate it. Presumably, with adequately strong stimulation, such colonies are well able to defend themselves in critical circumstances.

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