J Chem Ecol (2010) 36:1241–1243
DOI 10.1007/s10886-010-9866-3

RAPID COMMUNICATIONS

High Concentration of Nectar Quercetin Enhances Worker Resistance to Queen's Signals in Bees

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Received: 9 June 2010 / Accepted: 20 September 2010 / Published online: 30 September 2010

Abstract In honeybee colonies, pheromones produced by the host's queen inhibit worker reproductive potential and queen rearing. Here, we showed that worker bees fed with syrup containing high concentrations of the phenolic quercetin are likely to initiate ovarian development and to build many queen cells in their colony throughout the feeding trial. Workers fed syrup containing high levels of quercetin were aggresive against their queen. Our study suggests that increased phenolic compounds in nectar could enhance worker bee resistance to queen signals in honeybee colonies.

Key Words Honeybees · Nectar · Phenolics · Quercetin · Queen bee pheromones · Worker bee reproduction

Introduction

Environmental stresses ranging from lack of water to altered weather increase the levels of phenolics in various plant tissues (Simmonds, 2001). Such stress-induced phenolics can be found in various parts of plants including nectar, and may be incorporated into honey. Honey produced during extreme weather usually contains high concentrations of plant phenolics (Kenjerić et al., 2007). Floral phenolics affect honeybee feeding and foraging activities (Liu et al., 2007). However, it is not known whether phenolics affect social organization in honeybee colonies.

Honeybees have a complex social organization that is controlled by pheromones. For example, queen mandibular pheromone (QMP) inhibits worker ovary development (Hoover et al., 2003) and prevents the rearing of new queens (Winston et al., 1991). Increased phenolics in nectar can alter the response threshold of worker bees to sugars (Liu et al., 2007; Liu and Liu, 2010). We hypothesized that increased phenolics in nectar may alter bee responses to the QMP that controls reproduction in a colony.

Methods and Materials

Honey Bees and Feeding Procedure We conducted feeding trials at the experimental farm of the Institute of Sericulture and Apiculture (23°24′N, 103°17′E, 1260 m elevation) in July and August of 2008. At that time, few flowers were available in the field. Nine multi-patrilinial colonies of *Apis mellifera* were used for our feeding trial. We used acaricide to kill bee mites in each colony. To diminish the effects of colony size and genetic background on the results, we mixed broods of all colonies to equalize adult (10,000 bees) and brood (1000 cm^2) populations for each colony before the feeding trial. These food-deprived colonies were fed simultaneously in flight cages (25×10×5 m). Quercetin (95%) was obtained from Shanghai Healthjoy Chemical Co. Ltd,

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Three colonies were fed with 30% (w/w) sucrose syrup (hereafter "S-fed"), three fed with the same syrup containing 0.005% (w/w) quercetin (P1-fed), and the others fed the same syrup with 0.01% quercetin (P2-fed). Nectars contain about 0.0005% quercetin (Kenjerić et al., 2007), and honey contains about 2–12 mg phenolics/100 g (Yaoa et al., 2005). Here, we focused on the post-ingestive effect of nectar phenolics on workers and used quercetin concentrations within the typical range for natural honey. Each day, 200 g of test solution and 20 mg of pollen cake (corn pollen moulded into a doughy consistency using a 50% sucrose solution) were supplied to each colony. During the period of the feeding trial, water was available *ad libitum* for the caged colonies. The feeding trial lasted 15 days.

Colony Inspection and Worker Bees' Ovarian Examination Daily inspection of colonies was made twice a day, once in the morning (09:00–11:30) and again in the afternoon (12:00–14:30). At each time, each colony was observed for 10 min to record egg laying by the queen and in-hive worker bee activities such as building cells and tending brood. This procedure provides 'snapshot' data about the reproductive status in each colony.

After colonies were fed for 15 d, more than 300 foraging bees from each colony were captured at the hive entrance. The sampled bees were killed with 70% ethanol, and their ovaries were examined after fixing the insect to a wax board with an insect pin through the thorax. Under a microscope, the 3rd and 4th metasomal segments were separated using two pairs of forceps. The digestive tract was removed to reveal the ovaries. Ovaries were scored as: 0 = ovarioles not visible, 1 = ovarioles clearly visible, or 2 = small eggs or full-size eggs present. For each sample, the bees with each ovarian score were recorded as n_1 (score = 0), n_2 (score = 1), and n_3 (score = 2), and the weighted means of the ovarian scores were calculated as

Table 1 Effect of quercetin in nectar on ovarian development of worker bees

Colony[a]	Number of examined bees	Number of bees with each ovarian score[b]			Weighted mean of ovarian score
		0	1	2	
S-fed	306	176	117	13	0.47
S-fed	309	145	148	16	0.58
S-fed	302	180	115	7	0.43
P1-fed	301	128	162	11	0.61
P1-fed	309	157	137	15	0.54
P1-fed	316	132	171	13	0.62
P2-fed	318	96	183	39	0.82
P2-fed	306	82	183	41	0.89
P2-fed	301	89	177	35	0.82

[a] S-fed, P1-fed and P2-fed represent the colonies fed 30% sucrose syrup, the same syrup containing 0.005 and 0.01% quercetin, respectively.

[b] Ovaries were scored as: *0* ovarioles not visible, *1* ovarioles clearly visible, or *2* small eggs or full-size eggs present.

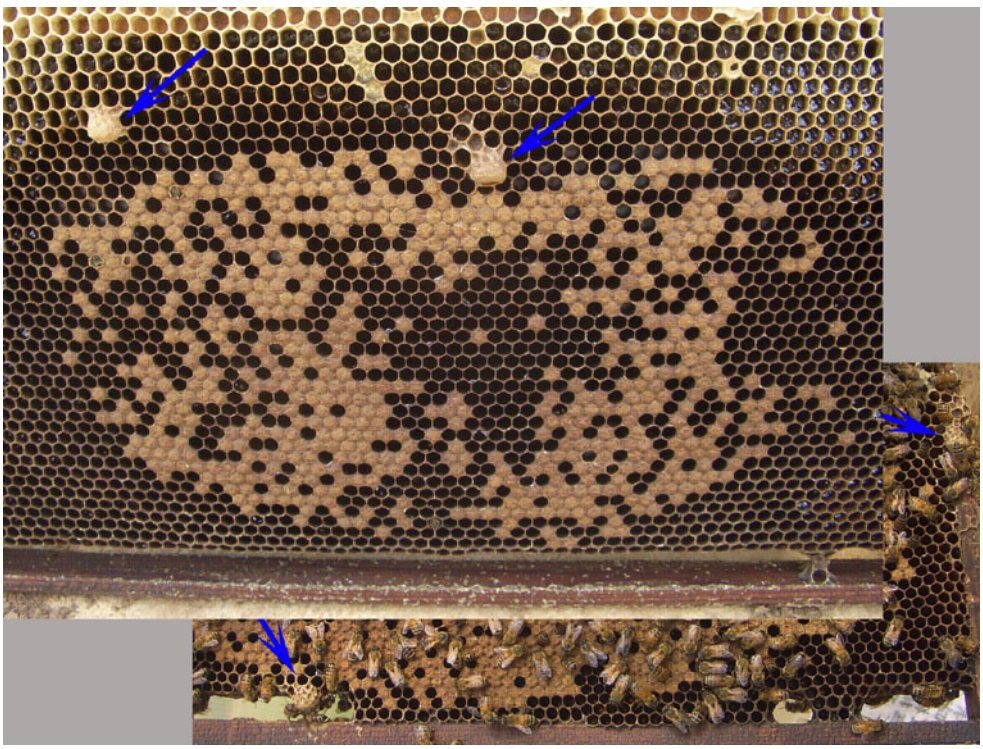

Fig. 1 Production of queen-like worker bees in colonies of *Apis mellifera* fed with quercetin-laced syrup. The colonies continuously built queen cells throughout the experiment when they were fed with 30% sucrose syrup containing 0.01% quercetin. Queen cells are typically concentrated in the bottom of the brood nest, but colonies fed quercetin-laced syrup built queen cells that were randomly distributed near the center and periphery of the brood nest. In contrast, control colonies fed a diet of pure 30% sucrose syrup with no phenolics did not produce any queen cells during the experiment. *Arrows* indicate the locations of queen cells in the quercetin-treated colonies

$(n_1\times0+n_2\times1+n_3\times2)/(n_1+n_2+n_3)$. The weighted means of the ovarian scores were compared between the S- and P-fed colonies using one-way ANOVA, followed by *post hoc* tests (least significant difference, LSD).

Results and Discussion

After the colonies were fed for 2 to 3 days, 3–11 queen cells emerged in each of the P2-fed colonies (Fig. 1). Some queen cells were torn down by worker bees, but new ones were re-built in the same or in different locations. The queen cells were not positioned in the bottom of the brood, but were randomly distributed throughout the brood nest (Fig. 1). Workers challenged their queens at least two times in each P2-fed colony during the first 3 days of the feeding trail. The S-fed and P1-fed colonies produced no queen cells during the experimental period.

Although workers in each colony were developed from a single mixed brood, experimental colonies had different levels of ovarian development 15 days after starting the feeding trial. In the P2-fed colonies, 12.4% of the worker bees had full-size eggs, and 58.7% had activated ovaries (Table 1). However, only 3.9% of the workers in the S-fed colonies and 4.2% in the P1-fed colonies contained small eggs in their ovarioles, and 41.4% and 50.8% had activated ovaries, respectively (Table 1). Ovarian scores were significantly different among three groups ($F_{2,8}$=25.52, P=0.001). The worker bees had higher ovarian scores in the P2-fed colonies than in the P1-fed and S-fed colonies (LSD, P<0.001 for comparisons between P2-fed and S-fed and between P2-fed and P1-fed). No differences were found between P1-fed and S-fed colonies (P>0.05). Thus, high levels of quercetin caused adult bees to become queen-like.

The excessive numbers of queen cells and well-developed ovarian workers suggested that high concentrations of quercetin in nectar disturbed the pheromonal communication system in honeybee colonies. Quercetin supplementation may affect the production of bee brood and queen pheromones. However, workers began to attack queens and built queen cells only a few days after the trial started. It is unlikely that queens and broods were affected by nectar phenolics during the very early stages of the feeding trial, as they did not directly feed on honey. Another possibility is that nectar phenolics altered worker responses to queen pheromone. The major components of QMP are not volatile, and are transmitted through worker-worker physical contact after workers lick QMP from the queen (Naumann et al., 1991). Worker gustatory receptors are involved in the QMP transmission, that nectar phenolics can modulate bee gustatory responsiveness (Liu et al., 2007; Liu and Liu, 2010). Thus, nectar phenolics such as quercetin might alter bee gustatory response thresholds, disturbing the effective transmission of QMP within a colony.

Although nest homeostasis and bee enzymes can partly detoxify or break down phenolics during the processing of nectar by worker bees (Mao et al., 2009), honey does contain phenolics especially when nectar and pollen were collected during extreme weather (Kenjerić et al., 2007). Extreme weather events are becoming more common due to global climate change (Easterling et al., 2000), suggesting that honeybee populations are at risk of altered social organization due to changes in plant composition in the future.

Acknowledgement We thank Xuewen Zhang for assistance in the feeding trail. We also thank Dr. David Roubik and three anonymous reviewers for comments on earlier version of the manuscript. This work was supported by the National Basic Research Program of China (2007CB411603) and the National Natural Science Foundation of China (30870445).

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