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Research Article

The Effects of Feeding Pollen Cake Containing Royal Jelly on *Bombus terrestris* L. (Hymenoptera: Apidae) Colony Development

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Abstract: This study was performed to determine the effects of feeding pollen cake containing honeybee royal jelly on *Bombus terrestris* colony development. In total, 125 bumblebee queens, 68 of which were fed pollen cake containing 10% royal jelly and 57 of which were fed normal pollen cake, were used. We found that 84% of the queens fed pollen cake containing royal jelly and 82% of those fed normal pollen cake laid eggs; 56% of the queens fed normal pollen cake established colonies, whereas queens fed pollen cake containing royal jelly did not establish any colonies and their larvae died. The results showed that feeding pollen cake containing royal jelly had a negative effect on bumblebee larval development.

Key Words: *Bombus terrestris*, queen, feeding, pollen, royal jelly, colony development

Arı Sütü İlaveli Polen Keki ile Beslemenin *Bombus terrestris* L. (Hymenoptera: Apidae) Arılarında Koloni Gelişimi Üzerine Etkileri

Özet: Bu çalışma, *Bombus terrestris* ana arılarının arı sütü ilaveli polen keki ile beslenmesinin koloni gelişimi üzerine etkilerini belirlemek üzere yürütülmüştür. Araştırmada 68 adedi % 10 arı sütü içeren polen keki, 57 adedi ise normal polen keki ile beslenen toplam 125 adet bombus ana arısı kullanılmıştır. Arı sütü ilaveli polen keki ile beslenen ana arıların % 84'ü, normal polen keki ile beslenen ana arıların % 82'si yumurtlamıştır. Normal polen keki ile beslenen ana arıların % 56'sı koloni oluşturmasına karşın, arı sütü ilaveli polen keki ile beslenen ana arıların koloni oluşturamadıkları ve bütün larvaların öldüğü belirlenmiştir. Araştırmada, bombus ana arılarının arı sütü ilaveli polen keki ile beslenmesinin, bombus arılarının larva gelişimi üzerine negatif bir etki yaptığı saptanmıştır.

Anahtar Sözcükler: *Bombus terrestris*, ana arı, besleme, polen, arı sütü, koloni gelişimi

Introduction

Bumblebees are, in general, annual social insects and valuable pollinators of both crops and native plants. Due to its economic importance in the pollination of greenhouse crops, the bumblebee, *Bombus terrestris*, is the most intensively studied species. The life cycle of bumblebees is different from that of honeybees. Queens emerge from hibernation in spring and individually establish colonies. As soon as the first workers hatch, they help the queen to establish a colony and start foraging for nectar and pollen. Towards the end of the colony cycle, in late summer, sexuals (young queens and males) are produced. After mating, the young queens go into diapause while the founding queen, the workers, and the males of a colony die. Finally, the life cycle of the colony is suspended for a season. The following spring, the queens that survived hibernation give rise to the next generation (1,2).

Early male and progeny queen production in the colonies, as well as mating, managing diapause, and the founding of new colonies are major problems in the year round rearing of bumblebees (2,3). A proportion of queens die in the egg laying stage, some of the remaining queens lay eggs, but some do not. Some of the egg-laying queens do not establish colonies. Significant differences are observed among *Bombus terrestris* colonies in the

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number of workers, males, and progeny queens produced. Furthermore, there is significant variation in the time of the switch and competition point, (3-7). It was estimated that many factors, such as worker density in the nest, worker/larvae ratio, climatic conditions inside and outside the nest, diseases and parasites, the quality of the founder queen, and food, cause these differences (2,6-8).

A *Bombus terrestris* colony containing approximately 80 workers can be used in greenhouse pollination for only 2 months due to the commencement of progeny queen and male production (9). For this reason it is recommended that in commercial rearing, colonies should produce sexuals in the late stage of the colony life cycle and the population of workers should be large. Many authors have reported that the quality, composition, and quantity of pollen used in the rearing of bumblebees under controlled conditions affect colony development, and they support the idea of using fresh pollen collected by honeybees (*Apis mellifera* L.) (10,11).

The main factor in the differentiation of worker and queen honeybees is that queens are fed royal jelly, which is synthesized and then secreted by the hypopharyngeal glands of nurse bees. It is a thick, very nutritious, milky-white creamy liquid, which is fed to all honeybee larvae for 3 days, but lifelong to the queen larva and queen. The sole feeding of a honeybee queen larva with royal jelly leads to the development of a queen, makes her fertile, and perhaps contributes to her increased life span of up to 7 years, in contrast to workers whose life expectancy ranges from 7 weeks to 7 months. Furthermore, royal jelly allows the honeybee queen to produce 1500-2000 eggs per day, which is 2.5 times her own body weight (12). In contrast, bumblebees (unlike honeybees) do not produce royal jelly to feed larvae (13,14); therefore, it remains unknown what that the effects of adding royal jelly to bumblebee diets are on the number of workers, males, and progeny queens produced. It was reported that queens fed pollen cake containing 5% royal jelly established colonies, but there wasn't an increase in the number of workers, males, or progeny queens produced (15). Consequently, the main objective of this study was to investigate if feeding pollen cake containing 10% royal jelly affects egg laying performance of queens, the number of individuals, and other *Bombus terrestris* colony traits.

Materials and Methods

The study included 125 *Bombus terrestris* queens that emerged from diapause, but did not establish a nest. The queens were collected between December 20 and 25, 2001 from the flowers of *Arbutus unedo* L. (Ericaceae), numbered, and put singly into starting boxes with 2 compartments (an $8 \times 8 \times 4$-cm nest compartment and a $6 \times 8 \times 2$-cm feeding and defecation compartment) in a climate controlled room (28-30 °C, 60%-65%, RH, and continuous darkness) (2,3). To stimulate egg laying, 1 callow *Bombus terrestris* worker and its pupae were added to each queen (16). These workers were changed every 5 days until the first workers emerged, in order to prevent competition with the queen.

During the experiment, in addition to unlimited sugar syrup (sugar:water, 1:1), 2 kinds of diet were used to feed the queens and their colonies; the first was normal pollen cake (100% pollen) and the other was pollen cake containing royal jelly (90% pollen and 10% royal jelly). Both kinds of pollen cake were molded into a doughy consistency using honey. The royal jelly and pollen used were produced by honeybees and were preserved at –18 °C until used. The honeybees collected pollen mainly from *Sinapis arvensis* L. (Brassicaceae), *Cistus* spp. (Cistaceae), and *Papaver rhoeas* L. (Papaveraceae) plants. Pure, fresh royal jelly was used in the preparation of the cakes containing royal jelly. In all, 57 of the 125 queens used in this study were fed normal pollen cake and 68 were fed pollen cake containing royal jelly ad libitum. The nests were checked every day and syrup and pollen were replaced when necessary.

A daily note was made of the queens that laid eggs, did not lay eggs, or died. After the first workers emerged, the colonies were transferred to larger rearing boxes with 2 compartments (a $30 \times 21 \times 17$-cm nest compartment and a $9 \times 12 \times 9$-cm feeding and defecation compartment). In each experimental colony we noted the time of first egg laying, the time the first workers, males, and progeny queens emerged, the switch and competition points, and the number of workers, males, and progeny queens produced. During observations, dead bees in the colonies were counted and removed in order to determine the number of workers, males, and progeny queens.

The competition point was calculated from time the first workers emerged (social phase initiation), the switch point was calculated from the time the queens first laid

eggs, and other characteristics were calculated after the queens were put into the starting boxes. The timing of the switch point was calculated as follows:

Timing of switch point = (time of first male emergence - development time of the male) - time the founder queen first laid eggs; or timing of switch point = (time of first progeny queen emergence - development time of the progeny queen) - time the founder queen first laid eggs (17).

The competition point was recognized by such worker behaviors as egg robbing, egg laying, and attacking the founder queen, and by founder queen behaviors, such as oophagy (17).

Statistical analysis was conducted with the Minitab (18) statistical program. To determine group differences t-test was used and descriptive statistics were given. Test for proportion was used to compare the egg laying ratio of the queens.

Results

During the experiment, 84% of the queens fed pollen cake containing royal jelly and 82% of the queens fed normal pollen cake laid eggs. The difference in the egg laying ratio between both groups was not significant (test for proportion, $P > 0.05$). These results show that the addition of royal jelly to the bumblebee diet did not influence the ability of the queens to start the first brood or their survival.

Another important result of experiment is that rearing conditions, pollen, sugar syrup, and all other possible factors did not affect larval development, but that the addition of royal jelly to pollen cake had a negative effect on *Bombus terrestris* larval development. The larvae belonging to the first brood in the group fed pollen cake containing royal jelly died after the queens fed them. Although the larvae died, the queens continued to lay eggs; however, in the larval period, the color of the larvae turned to black and they died, and no adult bees emerged from the eggs of the queens fed pollen cake containing royal jelly. For this reason only the colony initiation time and the number of egg cells in the first brood were determined in this group (Table 1). Differences between both groups in terms of the colony initiation time and the number of egg cells in the first brood were not significant (t-test, $P > 0.05$).

The queens in the group fed normal pollen cake established colonies. After being placed in the starting boxes the mean time of emergence of the first workers, first males, and first progeny queens was of 36.5 ± 1.30, 68.5 ± 4.39, and 89.7 ± 5.72 days, respectively. The other characteristics of the colonies established by the queens fed normal pollen cake are shown in Table 2.

Discussion

We investigated if the addition of royal jelly to the diet of *Bombus terrestris* affects egg laying performance of

Table 1. Performance of the queens fed pollen cake containing royal jelly and those fed normal pollen cake.

		Time of first egg laying (days)	Egg cells in first brood	Colony establishment ratio
	n	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	%
Normal pollen cake	47	7.6 ± 0.81	4.7 ± 0.29	55
Pollen cake with R J	57	9.5 ± 0.74	4.5 ± 0.31	0

Table 2. Characteristics of colony development in the colonies established by queens fed normal pollen cake.

	n	$\bar{x} \pm S_{\bar{x}}$	Min	Max
Switch point (days)	14	36.0 ± 2.27	4	34
Competition point (days)	19	45.1 ± 3.21	31	76
Number of workers	21	197.6 ± 23.50	27	392
Number of males	17	132.4 ± 26.90	13	442
Number of queens	9	60.8 ± 12.70	9	120

queens, number of individuals produced, and other colony characteristics. Yeninar (15) reported that the addition of 5% royal jelly to the bumblebee diet did not improve egg laying of queens or other colony traits. Increasing the royal jelly concentration to 10% did not affect egg laying of queens, but affected the larval development in our study. Although the queens were fed pollen cake containing 10% royal jelly laid eggs, the larvae belonging to this group died after the queens fed them. It is clear that royal jelly has a negative effect on *Bombus terrestris* larval development. This could not have been the result of using poor quality royal jelly, as fresh, pure royal jelly was used in the preparation of the pollen cake containing royal jelly.

In *Bombus terrestris,* larval diet does not include royal jelly (13,14). At the start of the colony, the queen feeds the larvae until the first workers emerge. The queen has glandular secretions, which she can transfer to larvae during feeding. In this case, the glandular secretions of the queens increase the nutritional value of the larvae's food (14). It is not known if mixing royal jelly with these glandular secretions of the queen has a toxic effect. Royal jelly has a strong antibiotic effect and can, therefore, probably cause problems related to the feeding of larvae.

Balanced nutrition is the most important dietary factor for effective growth in insects and optimal balance frequently changes with species, sex, and age or stage of development (19). In one experiment, 45% of *Agria affinis* larvae fed a balanced diet reached maturity, while those fed an unbalanced diet remained as first instars or died (20). Therefore, it is possible that pollen cake containing 10% royal jelly is nutritionally unbalanced for *Bombus terrestris* larvae and prevents their effective growth.

As a result, royal jelly used for feeding honeybee queens and larvae has a negative effect on *Bombus terrestris* larval development. No workers were produced in the nests of queens fed pollen cake containing 10% royal jelly, while those fed normal pollen cake produced workers, males, and progeny. The feeding metabolism of the larvae and the composition of royal jelly should be examined in detail in order to better understand this negative effect.

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