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Effects of different stimulation methods on colony initiation and development of *Bombus terrestris* L. (Hymenoptera: Apidae) queens

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Abstract

The effects of different stimulation methods on colony development of *Bombus terrestris* queens were examined to increase rearing efficiency and reduce production costs. A total of 350 hibernated queens obtained from a commercial company were used. Each queen was placed separately in the starting box and four starting methods were tested: only the queen (q), one queen stimulated with one *B. terrestris* worker (qtw), one honeybee worker (qhw), and one *B. terrestris* pupa (qtp). There were significant differences among the treatments in terms of egg laying, colony production and saleable production ratio, the colony initiation time, the time of the emergence of the first worker, the number of egg cells in the first brood. Starting methods had no significant effect on the other colony characteristics. The highest egg laying ratio, colony production ratio and saleable colony production ratio were found in the qtw group (82.8, 53.3, 39.0%) followed by qtp (72.8, 42.8, 28.5%), qhw (57.1, 28.6, 16.2%) and q (35.7, 17.1, 14.3%) groups, respectively. The queens with qtw treatment developed more egg cells (4.39±0.21) in the first brood and started egg laying (11.75±0.83 days) earlier than in the other treatments. The results showed that adding a *B. terrestris* worker is the best starting method for the mass rearing of *B. terrestris*. Qtp treatment was found to be the second most successful starting method.

Key words: Bumblebee; *Bombus terrestris*; oviposition; stimulation; colony characteristics

INTRODUCTION

Bombus terrestris L. is commercially reared by several companies and widely used as tomato pollinators in greenhouses (Banda and Paxton, 1991). The current worldwide sales of *B. terrestris* have reached some 900,000 colonies per year. It is considered that the relatively high labor costs and often low success rate are major barriers for economic success (Velthuis and Doorn, 2006). In commercial rearing, one of the main criteria is how many of 100 queens can establish colonies of saleable quality (including around 50 workers). Although accurate information is not available, this ratio is estimated to be not more than 50% in commercial companies (Gosterit and Gurel, 2005). The year-round rearing of this species, the rate of egg laying by the queen, and colony foundation are important for commercial breeding since the food quality, queen quality and environmental factors influence the success of colony foundation and egg laying of the queens (Yeninar et al., 2000; Velthuis and Doorn, 2006). Significant variations are seen in the colony initiation time, egg laying ratio and colony production ratio. *B. terrestris* colonies also vary tremendously with regard to the size of the worker population and the production of queens and drones (Beekman and Stratum, 2000).

Several starting methods such as adding a male *B. terrestris* pupa, young honeybee and bumblebee workers, a combination of *B. terrestris* workers and pupa have been described (Ptacek, 1985, 1991; Eijnde et al., 1991; Gretenkord and Drescher, 1997; Kwon et al., 2003) but comparable data related with mass production are limited and, even in the rearing industry, different methods are used to stimulate colony initiation. Therefore, the aim of this study is to investigate the effects of different stimulation methods; a queen with one *B. terrestris* worker, a queen with one honeybee worker, a

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queen with one *B. terrestris* pupa and a queen alone on the development of *B. terrestris* colonies reared under laboratory conditions.

MATERIALS AND METHODS

A total of 350 hibernated queens obtained from a commercial company were used. Each queen was anesthetized with CO_2 once for 30 min and placed into the starting box. Queens were submitted to four starting methods: a queen with one newly emerged *B. terrestris* worker (qtw), a queen with one newly emerged honeybee worker (qhw), a queen with one *B. terrestris* young female pupa (qtp) and the queen alone (q). These workers and pupae were changed every five days in order to prevent competition with the queens. As soon as the first workers emerged, helper workers were removed. All queens and colonies were fed *ad libitum* with sugar syrup and fresh frozen pollen collected from honeybee colonies and kept in climate-controlled rooms at 27±1°C and 65±10% RH. All queens and colonies were kept under identical climatic conditions, and were fed the same pollen and sugar syrup. After all the workers in the first brood had emerged, the colonies were transferred to larger rearing boxes. Clean conditions were maintained inside the boxes.

Colony development was tracked by direct daily observation. The colony initiation time (queen's first egg laying), the time of emergence of the first worker, the number of workers and egg cells in the first brood, the switch and competition point, the total number of workers, males and queens produced by each colony, egg laying ratio, colony production ratio, saleable colony production ratio, and mortality ratio were recorded by periodical observations. During the observations, dead bees in the colonies were counted and noted in order to determine the total number of workers, males and queens produced. In this study, a minimum of ten workers was the criterion used to define a colony. Queens that produced fewer than ten workers were not considered to be colony producers. One healthy queen and a minimum of 50 workers are necessary in *B. terrestris* colonies used for pollination in greenhouses. Colonies that produced 50 or more workers were evaluated as saleable colonies. The competition point between queens and workers was recognized by workers' behavior, such as egg robbing, egg laying and attacking the founder queen, and by founder queen behavior such as oophagy. The switch point was calculated by subtracting the development time of the male from the time between the first worker emergence and first male emergence. The progeny queen production ratio was calculated as follows: the number of queens that produced a progeny queen/the number of queens that produced a colony×100. For statistical analysis, colony traits belonging to four groups were analyzed by ANOVA and treatment means were compared using the Tukey-Kramer HSD test (SAS). Proportional data were analyzed using the χ^2 test.

RESULTS

Percentage of queens that produced eggs, colonies and sexuals

The highest egg laying ratio, colony production ratio and saleable colony production ratio were found in the qtw group (82.8, 53.3, 39.0%) followed by qtp (72.8, 42.8, 28.5%), qhw (57.1, 28.6, 16.2%) and q group (35.7, 17.1, 14.3%) respectively (Table 1). There were significant differences among treatments in terms of egg laying, colony production and saleable production ratio according to the chi-square values ($p<0.01$, $\chi^2=46.99$, 30.66, 20.08, df=3). The proportion of colonies which produced progeny queens varied from 43.7% to 66.6%; however, there was no significant difference between treatments ($\chi^2=1.47$, $p=0.689$).

Colony characteristics of *B. terrestris* according to starting methods

The means of the some colony developmental characteristics for the four starting methods are shown in Table 2. We found that the colony initiation time, the time of emergence of the first worker, and the number of egg cells in the first brood varied significantly among treatments ($p<0.05$). Staring methods, however, had no significant effect on the further aspects of colony development. The number of workers in the first brood, the switch and competition point, and the total number of workers, males and queens produced by each colony, were not statistically different among the four starting groups. In qtw and qtp groups, queens started laying eggs 11.75±0.83, 11.96±1.31 days, respectively, after they were placed in the starting

Table 1. Percentage of queens that laid eggs, founded colonies and saleable colonies depending on the starting treatments

Queen characteristics	Treatments				χ^2
	Only queen (q)	Queen with *B. terrestris* pupa (qtp)	Queen with honeybee worker (qhw)	Queen with *B. terrestris* worker (qtw)	
Egg laying ratio (%)	35.7 (n=70)	72.8 (n=70)	57.1 (n=105)	82.8 (n=105)	46.99 p=0.000
Colony production ratio (%)	17.1 (n=70)	42.8 (n=70)	28.6 (n=105)	53.3 (n=105)	30.66 p=0.000
Saleable colony production ratio (%)	14.3 (n=70)	28.5 (n=70)	16.2 (n=105)	39.0 (n=105)	20.08 p=0.000
Mortality ratio (%)	11.4 (n=70)	8.6 (n=70)	19.0 (n=105)	11.4 (n=105)	4.94 p=0.176
Progeny queen production ratio (%)	66.6 (n=12)	53.3 (n=29)	43.7 (n=30)	55.1 (n=58)	1.47 p=0.689

n: number of queens or colonies.

Table 2. Colony development characteristics depending on the starting treatments

Colony characteristics	Treatments			
	Only queen (q)	Queen with *B. terrestris* pupa (qtp)	Queen with honeybee worker (qhw)	Queen with *B. terrestris* worker (qtw)
Colony initiation times (days)[a]	21.72±1.82 a (n=25)	11.96±1.31 b (n=51)	15.43±1.07 b (n=56)	11.75±0.83 b (n=87)
Number of egg cells in first brood[a]	3.04±0.31 b (n=25)	3.39±0.21 b (n=51)	3.66±0.23 ab (n=56)	4.39±0.21 a (n=87)
First worker emergence (days)[a]	49.33±1.78 ab (n=12)	47.00±2.32 ab (n=30)	51.34±1.44 a (n=32)	40.17±1.02 b (n=58)
Number of workers in first brood	6.42±1.03 (n=12)	7.55±0.78 (n=29)	7.27±0.49 (n=30)	7.81±0.67 (n=58)
Switch point (days)	26.00±2.82 (n=9)	21.52±2.56 (n=23)	18.32±2.92 (n=22)	21.37±2.09 (n=41)
Competition point (days)	39.58±2.35 (n=12)	33.00±2.61 (n=24)	33.36±1.71 (n=25)	36.69±1.27 (n=49)
Total number of workers	87.70±13.50 (n=12)	74.00±10.10 (n=30)	69.70±10.50 (n=32)	82.98±10.50 (n=58)
Total number of males	49.70±11.00 (n=9)	27.83±4.25 (n=23)	33.83±7.44 (n=24)	34.33±5.56 (n=46)
Total number of queens	13.25±4.04 (n=8)	14.38±3.84 (n=16)	13.64±3.76 (n=14)	12.97±1.87 (n=32)

All data show the means±SE.
[a] Values marked with different letters in each row were significantly different by Tukey-Kramer HSD test (p<0.05).

boxes, whereas it took queens an average 15.43±1.07 days to start egg laying in the qhw group and 21.72±1.82 days in the q group, which were significantly longer than the qtw and qtp groups. On average, 4.39±0.21 egg cells were produced in the first brood in the qtp group followed by qhw (3.66±0.23), qtp (3.39±0.21) and q (3.04±0.31) groups.

DISCUSSION

In year-round rearing of bumblebees, rapid colony initiation and successful colony production are the major criteria to reduce production costs. Sladen (1912) discovered that bumblebee queens could be stimulated to initiate egg laying. Different starting methods, such as queens with a bumblebee worker, honeybee worker, bumblebee pupa and a combination of pupa and worker are used to stimulate colony initiation. Ptacek (1985, 1991) and Eijnde et al. (1991) used honeybee workers instead of bumblebee workers and obtained good results in *B. terrestris* queens with this method. However, Gretenkord and Drescher (1997) compared several stimulating methods and found that a combination of *B. terrestris* workers and larvae was the most successful method to stimulate *B. terrestris* queens. Yoon and Kim (2002) investigated the effects of a worker bee, bee cocoon and egg-cup on oviposition and colony foundation of the *B. ignitus* queen and found that two callow workers of *B. ignitus* and *B. terrestris* had the most remarkable effects on the oviposition rate and colony development. Kwon et al. (2003) examined the stimulating effect of the age and fixation angle of a male pupa on *B. terrestris* queens and found that providing one horizontally fixed young (1–2 days) male pupa to a single queen resulted in the production of good colonies. This method is less appropriate for bumblebee rearing on a commercial scale, because fixation of the pupa and the determination of pupa age are difficult.

In this study, we tested and compared the effects of different stimulating methods on the colony development of *B. terrestris* queens. Although all queens and colonies were kept under identical climatic conditions, and were fed the same pollen and sugar water, there were significant differences in egg laying, colony production and the saleable production ratio, the colony initiation time, the time of emergence of the first worker, and the number of egg cells in the first brood among treatments. Queens from qtw (queen with a *B. terrestris* worker) treatment produced approximately 10, 25, 36% more colonies and 10, 23, 25% more saleable colonies than those from qtp (queen with *B. terrestris* pupa), qhw (queen with honeybee worker) and q (queen only) treatments, respectively. The queens with qtw treatment developed more egg cells in the first brood and started egg laying earlier than with other treatments. Asada and Ono (2002) found that the time of colony initiation and first worker emergence had a significant effect on the number of workers and progeny queens in laboratory-reared *B. ignitus* and *B. hypocrita* colonies, and they reported that these two parameters provide an effective and practical method for identifying normal bumblebee colonies in the early stages of commercial mass production; however, in our study, after the first workers emerged, the starting methods had no significant effect on further aspects of colony development. In conclusion, the results showed that adding a *B. terrestris* worker is the best stimulating method for the commercial mass rearing of *B. terrestris*.

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