

## PAPER DETAILS

TITLE: Farkli Sicaklik Uygulamalarinin Bombus ( Bombus terrestris L.) Arisinin Koloni Gelisimi  
Üzerine Etkileri

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PAGES: 75-78

ORIGINAL PDF URL: <https://dergipark.org.tr/tr/download/article-file/18096>

## EFFECTS OF TEMPERATURE TREATMENTS ON THE BUMBLEBEE (*Bombus terrestris* L.) COLONY DEVELOPMENT\*

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Accepted 29 April 2008

### Abstract

The effects of three temperature treatments on the colony development characteristics of *Bombus terrestris* L. were investigated. A total of 300 queens overwintered in artificial conditions were used. Queens were randomly subjected to one of three temperature treatments at  $24\pm 0.5$  °C,  $27\pm 0.5$  °C or  $30\pm 0.5$  °C under constant humidity of  $60 \pm 5$  %. All queens and colonies were furnished with unlimited amounts of sugar solution and fresh pollen collected from honeybee colonies. There were significant differences between treatments in terms of egg laying, the ratios of colony production, saleable colony production and progeny queen production, the colony initiation time, the number of egg cells in first brood, and the timing of first worker emergence. The highest egg laying, colony production and saleable colony production ratios were found at the  $30\pm 0.5$  °C treatment (86 %, 48 %, 34 %) followed by  $27\pm 0.5$  °C (42 %, 19 %, 13 %) and  $24\pm 0.5$  °C (43 %, 14%, 12 %) treatments respectively. Similarly, at the  $30\pm 0.5$  °C queens developed more egg cells ( $4.72\pm 0.22$ ) in the first brood and started egg laying ( $12.47 \pm 0.78$  days) earlier than in the other treatments. The results show that  $30\pm 0.5$  °C is the best temperature condition for colony development of bumblebee (*B. terrestris*).

**Keywords:** *Bombus terrestris*, rearing techniques, temperature, colony characteristics

### Farklı Sıcaklık Uygulamalarının *Bombus (Bombus terrestris* L.) Arısının Koloni Gelişimi Üzerine Etkileri

#### Özet

Bu çalışmada *Bombus terrestris* L. arısının koloni gelişim özellikleri üzerine üç farklı sıcaklık uygulamasının etkileri karşılaştırılmıştır. Yapay koşullarda kışlatılmış toplam 300 adet ana arı %  $60 \pm 5$  nem içeren  $24\pm 0.5$  °C,  $27\pm 0.5$  °C ve  $30\pm 0.5$  °C 'lik besleme odalarına rasgele ve eşit sayıda dağıtılmıştır. Bütün ana arılar ve koloniler sınırsız biçimde şeker şurubu ve polenle beslenmişlerdir. Periyodik gözlemlerle koloni gelişim özellikleri saptanmıştır. Yumurtlama, koloni oluşturma, satılabilir koloni oluşturma ve ana arı üretim oranları, ilk yumurtlama zamanı, ilk kuluçkadaki yumurta hücresi sayısı ve ilk işçi arı çıkış zamanı bakımından sıcaklık grupları arasında gözlemlenen farklılıklar önemli bulunmuştur. En yüksek yumurtlama, koloni oluşturma ve satılabilir koloni oluşturma oranları  $30\pm 0.5$  °C de tutulan grupta (sırasıyla, % 86, % 48, % 34) gözlenmiş bu grubu  $27\pm 0.5$  °C (% 42, % 19, % 13) ve  $24\pm 0.5$  °C (% 43, % 14, % 12) de bulunan gruplar izlemiştir. Benzer şekilde  $30\pm 0.5$  °C de tutulan ana arılar diğer sıcaklık gruplardakilerden daha erken yumurtlamaya başlamışlar ( $12.47 \pm 0.78$  günde) ve ilk kuluçkada daha fazla yumurta hücresi ( $4.72\pm 0.22$  adet ) oluşturmuşlardır. Araştırma sonucunda *B. terrestris* arısının koloni gelişimi için en uygun sıcaklık uygulamasının  $30\pm 0.5$  °C olduğunu belirlenmiştir.

**Anahtar Kelimeler:** *Bombus terrestris*, Yetiştirme Teknikleri, Sıcaklık, Koloni Özellikleri

### 1. Introduction

Due to its economic importance for pollination of greenhouse crops, the bumblebee *Bombus terrestris* L. is reared on a large scale under controlled conditions since it was introduced in the greenhouse production of tomatoes in the Netherlands

and Belgium (Beekman et al., 2000). The current worldwide sales of *B. terrestris* have reached some 900 000 colonies per year. This species produces large colonies and adapts quite well to artificial conditions (Velthuis and Doorn, 2006). Although the

\* This study was supported by the Scientific Research Projects Units of Akdeniz University (No: 2005.01.0104.006)

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development of production techniques has been rapid since its start in 1988, there is still a problem in commercial rearing to maximize the quality and profitability of artificially reared colonies (Hughes, 1996).

The relatively high labor cost and often low success rate are major barriers for economic success. *B. terrestris* colonies show much variation in the number of workers, males, and queens produced (Beekman and Stratum, 2000). Significant variations are also seen in the colony initiation time, and colony production ratio. These characteristics are important criteria in year round rearing of *B. terrestris* and affected by several factors such as the food quality, and climate in the rearing room. Several researchers recommended various rearing temperature conditions from 24 °C to 31°C (Plowright and Jay, 1966; Beekman and Stratum, 2000; Jie et al., 2005) but 27°C-28°C were often determined to be the favorable rearing temperatures in *B. terrestris* mass rearing (Duchateau and Velthuis, 1988; Velthuis, 2002; Velthuis and Doorn, 2006). However, there has been limited research on the effects of high and low temperature regimes on the colony development patterns of this species. In this work, our aims were to compare the effects of three temperature regimes (24°C, 27°C and 30°C) on the colony development characteristics of *B. terrestris* and to find out optimal temperature condition for mass rearing of bumblebee.

## 2. Materials and Methods

A total of 300 *Bombus terrestris* queens overwintered in artificial conditions were used. Each queen was anesthetized with CO<sub>2</sub> once for 30 min and put singly into starting box. Queens were subjected to one of three temperature treatments at 24±0.5 °C, 27±0.5 °C or 30±0.5 °C under constant humidity of 60 ± 5 %. Each treatment comprised 100 queens. All queens and colonies were furnished with unlimited amounts of sugar solution (50% water: 50 % sugar) and fresh pollen collected from honeybee colonies. The initiation of egg laying of each queen was stimulated by adding a young helper worker (*B. terrestris*)

into starting box. The worker in starting box was changed with a new one to prevent worker from competing with queen (Gurel and Gosterit, 2008). As soon as the first workers emerged, helper worker was removed and the colony was transferred to a larger box.

Colony development was tracked by direct daily observation. The colony initiation time (the time interval from installing queen to the queen's first egg laying), the timing of the first worker emergence, 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 progeny queen production ratio were recorded by periodical observations. During the observations, the dead bees in the colonies were counted and noted in order to determine the total number of workers, males and queens produced in the colonies. 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. A bumblebee colony used for crop pollination in greenhouses must contain a healthy queen and at least 50 workers. Colonies that produced 50 or more workers were accepted 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 or by founder queen behavior such as oophagy. The switch point was calculated by subtracting of the development time of the male from the time between the first worker emergence and first male emergence. 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 x 100. Colony traits of three groups were compared by one-way ANOVA and Duncan multiple comparison test (SPSS). The proportions were compared by t- test.

## 3. Results

Table 1 shows that 86 % of the queens

kept at  $30 \pm 0.5$  °C laid eggs. However, only 43 % and 42 % of the queens kept at  $24 \pm 0.5$  °C and  $27 \pm 0.5$  °C laid eggs, respectively. Similarly, the highest colony production ratio was obtained at  $30 \pm 0.5$  °C treatment (48%) followed by  $27 \pm 0.5$  °C (19 %) and  $24 \pm 0.5$  °C (14%) treatments. There were significant differences between treatments in terms of egg laying, colony production, saleable colony production and progeny queen production ratios ( $p < 0.01$ ).

The means of the some colony characteristics for the three temperature regimes are shown in Table 2. The colony

initiation time, the number of egg cells in first brood, and the timing of first worker emergence varied significantly between treatments ( $p < 0.01$ ,  $p < 0.01$ ,  $p < 0.05$  respectively). However, the number of workers in the first brood, the switch and competition points, the total number of workers, males, and queens produced by each colony did not vary significantly between the three temperature treatments. Queens at  $30 \pm 0.5$  °C, started laying eggs  $12.47 \pm 0.78$  days after they were placed in the starting boxes, whereas it took queens an average  $16.81 \pm 1.31$  days at

Table 1. Percentages of Queens that Laid Eggs, Founded Colonies and Saleable Colonies under Different Temperature Conditions

Queen Characteristics	Temperature Regimes		
	24 °C	27 °C	30 °C
Egg laying ratio (%)	43 a (n=100)	42 a (n=100)	86 b (n=100)
Colony production ratio (%)	14 a (n=100)	19 a (n=100)	48 b (n=100)
Saleable colony production ratio (%)	12 a (n=100)	13 a (n=100)	34 b (n=100)
Progeny queen production ratio (%)	35.71 a (n=14)	63.15 ab (n=19)	79.16 b (n=48)

Values marked with different letters in each row were significantly different by t test ( $p < 0.01$ )

Table 2. Colony Development Characteristics under Different Temperature Conditions

Colony Characteristics	Temperature Regimes			P F
	24 °C	27 °C	30 °C	
Colony initiation time (days)	$18.49 \pm 1.34$ a (n = 43)	$16.81 \pm 1.31$ a (n = 42)	$12.47 \pm 0.78$ b (n = 86)	0.000 9.373
Number of egg cells in first brood	$2.31 \pm 0.22$ a (n = 42)	$3.51 \pm 0.24$ b (n = 41)	$4.72 \pm 0.22$ c (n = 85)	0.000 26.359
First worker emergence (days)	$55.14 \pm 3.53$ a (n = 14)	$50.90 \pm 1.81$ ab (n = 21)	$47.53 \pm 1.40$ b (n = 58)	0.047 3.158
Number of workers in first brood	$6.83 \pm 0.57$ (n = 12)	$8.10 \pm 0.93$ (n = 20)	$7.30 \pm 0.62$ (n = 53)	0.667 0.407
Switch point (days)	$28.80 \pm 4.83$ (n = 10)	$16.00 \pm 5.08$ (n = 14)	$15.17 \pm 3.46$ (n = 42)	0.180 1.764
Competition point (days)	$39.69 \pm 3.37$ (n = 13)	$36.00 \pm 1.60$ (n = 18)	$38.98 \pm 1.45$ (n = 47)	0.479 0.743
Total number of workers	$102.71 \pm 14.30$ (n = 14)	$77.91 \pm 11.46$ (n = 22)	$72.77 \pm 7.12$ (n = 57)	0.180 1.748
Total number of males	$37.82 \pm 13.60$ (n = 11)	$48.41 \pm 12.81$ (n = 17)	$34.96 \pm 4.66$ (n = 46)	0.487 0.728
Total number of queens	$20.20 \pm 6.89$ (n = 5)	$13.08 \pm 3.73$ (n = 12)	$17.37 \pm 2.03$ (n = 38)	0.496 0.711

Values marked with different letters in each row were significantly different by Duncan test

All data show the mean  $\pm$  SE.

27±0.5 °C and 18.49 ±1.34 days at 24±0.5 °C treatments to start egg laying. The number of egg cells in the first brood increased significantly with increasing temperature condition. On average, 4.72±0.22 egg cells were produced in the first brood at 30±0.5 °C treatment while only 3.51±0.24 and 2.31 ± 0.22 egg cells were produced in the first brood at 27 ±0.5 °C and 24±0.5 °C treatments, respectively.

#### 4. Discussion

Colony initiation, queen rearing, mating and control of diapause are the major stages in the commercial rearing of bumblebees. Bumblebee producers today have developed their own rearing system, which is essential to maintain the queens in a suitable environment for brood rearing all year round. (Hughes, 1996; Velthuis and Doorn, 2006). Generally temperature seems to range from 24 °C to 30 °C and humidity from % 50 to % 65. In this study, we compared the effects of three temperature regimes (24°C, 27°C and 30°C) on the colony development characteristics of *B. terrestris* under constant humidity of 60 ± 5 %. Egg laying, colony production, saleable colony production and progeny queen production ratios, the colony initiation time, the number of egg cells in first brood, and the first worker emergence time were significantly affected by temperature regimes. Queens from 30±0.5 °C treatment produced approximately 29 % and 34 % more colonies and 21% and 22 % more saleable colonies than those from 27±0.5 °C and 24±0.5 °C temperature regimes, respectively. They also laid more egg cells in the first brood and started egg laying earlier than other two treatments. Similarly, Jie et al. (2005) found that egg laying and colony production ratios were highest at 30°C ± 0.5 under constant humidity of 60 % compared with other four temperatures (24 °C ± 0.5, 26 °C ± 0.5, 28 °C ± 0.5, 32 °C ± 0.5) and 30°C ± 0.5 was appropriate temperature regime for rearing *B. terrestris* colonies in China. However, Yoon et al. (2002) compared 23 °C, 27 °C and 30 °C under a constant humidity of 65 % and found that 27 °C and 65 % R. H. were the

favorable temperature and humidity conditions for colony development of *Bombus ignitus* in indoor rearing. Although 27°C and 28°C were determined to be the favorable rearing temperatures in *B. terrestris* mass rearing in the earlier studies (Duchateau and Velthuis, 1988; Velthuis, 2002; Velthuis and Doorn, 2006), the results presented here show that 30±0.5 °C is the best temperature condition for colony development of *B. terrestris*.

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