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RESEARCH ARTICLE

Effects of CO_2 Narcosis on the Onset of Oviposition and Colony Founding Success of Post Diapausing Bumblebee (*Bombus terrestris*) Queens [1]

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Summary

In year-round rearing of bumblebees, rapid and successful colony production is the major criterion to reduce production costs. In this study, the effect of CO_2 narcosis on the oviposition and colony founding success of post diapausing Bombus terrestris queens were investigated. Mated queens that were kept in the refrigerator at 4.0±0.5°C for two months were randomly allocated to three groups for control, single and double narcosis. The queens in control group were not narcotized, while the queens in single narcosis group were treated once with CO_2 for 30 min and the queens in double narcosis group were treated with CO_2 for 30 minutes two times in consecutive days before introducing in starting boxes. All queens and colonies were kept in standard rearing conditions (27±1°C and 60±5% RH). There were significant differences between the treatment groups in terms of the duration of colony initiation (day) and the percentage of queens that laid eggs. The queens exposed to single and double narcosis started to lay eggs approximately 4.5 and 5 days earlier than the non narcotized queens, respectively. Similarly, 80% of single and double narcotized queens laid eggs, whereas only 45% of non narcotized queens laid eggs. The numbers of workers in the first brood batch, queen mortality rates at the first week and first month (%) were not significantly different between groups. The results showed that single CO_2 narcosis treatment to post diapausing queens can become a useful tool in the mass rearing of *B. terrestris*.

Keywords: *Bumblebee, Bombus terrestris, CO_2 treatment, Colony development*

Bombus Arısı (*Bombus terrestris*) Ana Arılarının Yumurtlamaya Başlaması ve Koloni Oluşturması Üzerine Diyapoz Sonrası CO_2 Uygulamasının Etkileri

Özet

Bombus arılarının yıl boyu yetiştiriciliğinde hızlı ve başarılı koloni üretimi, üretim maliyetini düşürmede temel kriterdir. Bu çalışmada diyapozdan çıkmış Bombus terrestris ana arılarına CO_2 uygulamasının yumurtlamaya başlama ve koloni oluşturma üzerine etkileri incelenmiştir. Çiftleşmiş ana arılar 4.0±0.5°C de iki ay muhafaza edildikten sonra rasgele üç gruba dağıtılmışlardır. Kontrol grubunu oluşturan ana arılar CO_2 narkozu uygulanmaksızın başlatma kutularına yerleştirilirken, bir grup ana arıya 30 dak. süre ile bir kez, diğer grup ana arıya ise 30 dakika süre ile iki kez CO_2 narkozu uygulanmıştır. Bütün ana arılar ve koloniler standart yetiştirme koşullarında tutulmuşlardır (27±1°C ve %60±5 nem). Ana arıların koloni başlatma süreleri (gün) ve yumurtlama oranları (%) bakımından gruplar arasındaki farklılıklar önemli bulunmuştur. Tek ve çift CO_2 narkozu uygulanan ana arılar CO_2 narkozu uygulanmayan ana arılardan sırayla yaklaşık 4.5 ve 5 gün daha erken yumurtlamaya başlamışlardır. Benzer şekilde, CO_2 uygulanmayan ana arıların sadece %45'i yumurtlamışken, hem tek hem de çift CO_2 narkozu uygulanan ana arıların %80'i yumurtlamıştır. İlk kuluçkadaki işçi arı sayısı, ilk hafta ve ilk aydaki ana arı ölüm oranları (%) bakımından gruplar benzer değerler almıştır. Sonuçlar *B. terrestris* arısının kitlesel üretiminde diyapoz sonrası ana arılara bir kez CO_2 uygulamasının faydalı bir yöntem olabileceğini göstermiştir.

Anahtar sözcükler: *Bombus arısı, Bombus terrestris, CO_2 uygulaması, Koloni gelişimi*

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INTRODUCTION

Most bumblebee species are annual social insects and are widely distributed in the temperate regions of the world [1]. 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 the colony die. Finally, colony life cycle is restricted to a season. The following spring, the queens that survived hibernation give rise to the next generation. Thus, queens enter diapause during the summer, which means a diapause duration of six to nine months depending on the spring temperature [2]. However, bumblebees show great ecological flexibility, particularly in terms of diapause responses and duration [3,4]. Diapause is an adaptive strategy for survival in unfavorable environmental conditions and offers obvious advantages to insects that can only utilize small portions of the year for development and reproduction [5].

Bumblebees are important pollinators of many crops and wild flowers. Currently five species of bumblebees are reared commercially on a large scale. Due to its economic importance for pollination of greenhouse crops, the Eurasian bumblebee *Bombus terrestris* L. is the most prominent species reared under controlled conditions [4]. Although the development of management techniques has been rapid since its start in 1987, there is still a problem in commercial rearing to maximize the quality and profitability of commercial colonies [6]. It is considered that the relatively high labor costs and often low success rate are major problems for commercial rearing [7]. Colony initiation, queen rearing, mating and breaking of diapause are the major stages in the commercial rearing of bumblebees [6,7].

The long hibernation period, the single colony cycle per year, the variability in the social structure of the colonies and the lack of product shelf life are major obstacles in the year-round rearing of bumblebees [6]. *Bombus terrestris* producers today have developed their own rearing systems and have used several methods to rear colonies year-round, despite the long ovarian diapause of this species [7,8]. The storage of hibernating queens at low temperature for several months to mimic diapause has been widely used in the commercial rearing. Another important finding by Röseler [9] was that a CO_2 treatment could also be used to inhibit diapause as well as to activate overwintered queens. In bumblebee mass-rearing, mated queens are stored at temperatures between 1 - 5°C for different durations based on the demand by producers. After this process, the queens may receive CO_2 narcosis [7,9]. However, there has been limited research on the effects of CO_2 narcosis on the onset of oviposition and colony founding of post diapausing *B. terrestris* queens. In this study hibernated queens were divided into three groups; non-narcotized queens, queens exposed to a single narcosis with CO_2 for 30 min and queens exposed to a double narcosis with CO_2 for 30 min. Our aim was to compare the effects of narcotization on the onset of oviposition and development of colonies founded by post diapausing *B. terrestris* queens to find out the best method for mass rearing.

MATERIAL and METHODS

A total of 70 queens were collected from *Bombus terrestris* colonies kept in Animal Science Laboratory at Akdeniz University. Approximately 7 days old queens and 12 days old males were put together in plastic cage with mash walls (queen to male ration: 1:1.5). After successful matings, the queens were kept in the refrigerator at 4.0±0.5°C for two months. After that, hibernated queens were placed in flight cage for 5 days by providing sugar syrup and pollen. Then, the queens were randomly allocated to three groups of 20 queens each for control, single and double narcosis. The queens in single narcosis group were treated once with CO_2 for 30 min, while the queens in double narcosis group were treated with CO_2 for 30 minutes two times in consecutive days before introducing in starting boxes. The queens in control group were not narcotized. We narcotized the queens as described by Röseler [9]. The queens were put in a glass jar and exposed to CO_2 until they became immobilized. Then, the all queens were kept altogether in the closed jar for 30 min.

After narcotization process, each queen was placed separately in the small starting box and kept in captivity under standard rearing conditions (27±1°C and 60±5% RH). The queens and the colonies were fed with sugar syrup (approximately 1:1 water: sugar) and fresh pollen collected from honeybee colonies ad-libitum through the experimental period. Each queen was attended by two newly emerged honey bee workers to stimulate egg laying [10]. Honey bee workers were changed every week until the emergence of the first *B. terrestris* workers. When the workers from the first brood batch emerged, the colonies were transferred to larger plastic boxes. Clean conditions were maintained inside the boxes. Each nest box was observed daily for the duration of the experiment. The date of onset of oviposition of each queen in a starting box was recorded in order to determine the duration of colony initiation of queens (interval from installing a queen into starting box to onset of oviposition). The numbers of workers emerged from first brood batch in every starting box were also recorded. The colony founding success (%) was defined as the ability of a queen to produce at least 10 adult workers. Therefore, the queens that produced fewer than 10 workers were not considered to be successful colony producers [11]. In each group, percentage of queens that laid eggs, queen mortality rates at the first week and month were calculated as follows;

Percentage of queens that laid eggs (%): The number of queens that produced eggs / total number of queens x 100.

Queen mortality rate at first week (%): The number of dead queens during the first week after introducing in starting boxes/total number of queens x 100.

Queen mortality rate at first month (%): The number of dead queens during the first month after introducing in starting boxes/total number of queens x 100.

Statistical Analysis

Proportional data were analyzed by the Chi-square test. Data on the duration of colony initiation and the number of workers in the first brood were analyzed by ANOVA and the mean values were compared by Duncan multiple comparison test (SPSS version 12.01).

RESULTS

The means of the queen and colony development characteristics of control group and experimental groups are shown in *Table 1*. Our results revealed several similarities and differences between the non narcotized, single narcotized and double narcotized groups. The first important difference between groups is the interval from installing a queen into starting box to onset of oviposition (the duration of colony initiation). Single and double narcotized queens started to lay eggs 6.69 and 6.06 days, respectively, whereas non-narcotized queens (11.22 days) started to lay eggs about 4.5 days and 5 days later than single and double narcotized queens, respectively ($F=21.03$, $df=2$, $P=0.0001$). The second important difference we found between groups is the percentage of queens that laid eggs (%). Similarly, 80% of single and double narcotized queens laid eggs, whereas only 45% of non narcotized queens laid eggs ($\chi^2=7.548$, $df=2$, $P=0.023$). Colony founding successes of single and double narcotized queens were higher (50%) compared with non narcotized queens (35%), but the difference was not significant ($\chi^2=1.212$, $df=2$, $P=0.054$). The numbers of workers emerged from first brood batch did not vary significantly between groups. Queen mortality rates at the first week and at the first month were also similar between groups.

DISCUSSION

In commercial rearing of bumblebees, rapid colony initiation and successful colony production are the major criteria to reduce production costs. Several methods have been described to rear colonies in captivity [12,13]. Although *B. terrestris* queen can produce a colony without a period of cold storage (diapause) or CO_2 narcosis [4,14], this method has not been used in mass rearing, because non-diapausing queens do not establish high quality colonies for pollination purpose. In commercial bumblebee rearing industry, it is vital to plan the production within tight limits to avoid both over and underproduction [6]. A substantial prerequisite for year-round rearing is, therefore, to control diapause in captivity and to carry out an optimal diapause process suitable for the mass rearing of *B. terrestris*.

Under laboratory conditions, many researchers stored their queens at a temperature between 1-5°C to simulate hibernating conditions [3,4,15,16]. Previous studies also showed that queen survival during diapause (cold storage conditions) is strongly determined by the weight of the queen and the diapause duration [3,15]. Röseler [9] found another successful method that a CO_2 treatment could also be used to circumvent or break diapause. Tasei [8] reported that the timing of CO_2 narcosis after mating did not affect the delays of egg-laying within the range of 5-30 days. Yoon et al.[17] investigated the effect of CO_2 treatment on interrupting diapause, oviposition and colony development of *Bombus ignitus* and reported that CO_2 treatment showed a positive effect on the oviposition and colony development. They found that the days needed to first oviposition shortened to 17-18 days in CO_2 treated queens, comparing to 30 days in CO_2 untreated queens and CO_2 treatment at the second day after mating was appropriate to the oviposition and colony development. Yoon et al.[18] also found that CO_2 narcosis time favorable for colony development was 11 days of adult emergence in *B. terrestris*. Our results are not directly comparable with these results because queens were not narcotized with CO_2 after cold storage in these previous studies.

Röseler [9] also recommended that CO_2 narcosis induces egg formation in bumblebee queens not only prior to hibernation but also after hibernation. There are a few studies about cold storage in combination with CO_2 treatment [9,12,15]. Our finding that CO_2 narcosis of post diapausing queens was

Table 1. *The characteristics of non-narcotized, single narcotized and double narcotized queens and the colonies founded by these queens*
Tablo 1. *Narkozlanmayan, bir kez narkozlanan ve iki kez narkozlanan ana arıların ve bu ana arıların kurdukları kolonilerin özellikleri*

Queen and Colony Characteristics	Non Narcotized Queens	Single Narcotized Queens	Double Narcotized Queens
Duration of colony initiation of queens (day; mean ± s.e.)	11.22 ± 1.29^{A} (n=9)	6.69 ± 0.28^{B} (n=16)	6.06 ± 0.23^{B} (n=16)
Number of workers in first brood batch (mean ± s.e.)	9.29±1.35 (n=7)	10.10±1.49 (n=10)	10.20±1.08 (n=10)
Percentage of queens that laid eggs (%)	45^{a} (n=20)	80^{b} (n=20)	80^{b} (n=20)
Colony founding success of queens (%)	35 (n=20)	50 (n=20)	50 (n=20)
Queen mortality rate at first week (%)	10 (n=20)	10 (n=20)	10 (n=20)
Queen mortality rate at first month (%)	20 (n=20)	20 (n=20)	20 (n=20)

Different letters denote significant differences between means within a row (A, B: P<0.01; a, b: P<0.05)

effective to stimulate oviposition and colony initiation is consistent with these earlier studies [9,12,15]. Gosterit and Gurel [15] reported that hibernating queens for 45 days at 4.5°C and then anesthetizing them with CO_2 appears to be the best diapause regime for the mass rearing of *B. terrestris.* Similarly, Gretenkord and Drescher [12] found that after a short hibernation period (up to 3 month), CO_2 treatments of 30 min gave the best result.

In this study, if non-narcotized queens are compared with narcotized queens, there are differences in queen and colony characteristics whereas no difference is found between single and double narcotized queens. According to our results, single narcosis is sufficient to shorten the duration of onset of oviposition. It seems that second CO_2 narcosis is not necessary. In conclusion, our results have shown that single CO_2 narcosis of post diapausing queens can become a useful tool in the mass rearing of *B. terrestris* colonies.

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