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Wake-up Treatments for Improving Oviposition and Colony Development of the Bumblebees *Bombus ignitus* and *B. terrestris*

Hyung Joo Yoon*, Kyeong Yong Lee, Samg Beom Lee and In Gyun Park
Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Suwon 441-100, Korea

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Bumblebees are widely used to pollinate crops in greenhouses and fields. Here, we investigated whether different wake-up treatments during a short period of 1~3 days just before indoor rearing has any effects on oviposition and colony development of CO_2-treated *Bombus ignitus* queens and artificially hibernated *B. terrestris* queens The wake-up regimes were defined as 16L for 1 day (16L-1), 16 L per day for 3 days (16L-3), 24L for 1 day (24L-1), or 24D for 1 day (24D-1). Among these wake-up treatments, the oviposition rate and preoviposition period of *B. ignitus* queens reared at 24L-1 were 16.7~25.1% higher and 1.0~3.5 days shorter than other wake-up treatments. *B. terrestris* queens reared at 24L-1 also showed the best results for egg-laying characteristics, which were 8.9~18.8% higher for oviposition and 0.6~3.5 days shorter for preovipostion period than other wake-up treatments. Furthermore, *B. terrestris* queens reared at 24L-1 were 17.5% and 13.8% higher in rate of colony foundation and queen production, respectively, than other wake-up treatments. These results show that the most favorable wake-up treatment just before rearing for egg-laying and colony developmental characteristics of *B. ignitus* and *B. terrestris* queens was 24L-1. Overall, our findings indicate that a wake-up treatment just before rearing was effective for colony initiation and colony development of bumblebee queens.

Key words: Bumblebee, *Bombus ignitus*, *B. terrestris*, Wake-up, Photoperiod, Oviposition, Colony development

Introduction

Bumblebees are an important pollinator of various greenhouse crops and are particularly effective in pollinating tomatoes (Buchmann and Hurley, 1978; Banda and Paxton, 1991; Free, 1993). Worldwide this involves about 95% of all bumblebee sales, although many farmers use bumblebee pollinators for other crops such as pepper, eggplant, watermelon, cucumber, blueberry, apple, and kiwifruit (Velthus and Doorn, 2006). The value of bumblebee-pollinated tomato crops is estimated to be € 12,000 million per year. In 2004, worldwide bumblebee sales reached about one million and about 90% of all bumblebee sales were of *Bombus terrestris* (Velthus and Doorn, 2006).

Techniques for year-round rearing of bumblebees are necessary for commercial applications In year-round rearing of bumblebees, one of the key stages is colony initiation, or the initiation of egg-laying. Asada and Ono (2002) found that providing a short interval between the introduction of foundresses into boxes and the start of egg-laying was an important factor in successfully rearing artificial bumblebees. Yoon *et al.* (2004a) reported that the *B. ignitus* queen that had the earliest first oviposition day could make their colony stronger and resulted in shorter colony formation periods. Thus, the first oviposition day of the founding queen was determined to be a criterion for the selection of highly successful colonies of *B. ignitus*.

The methods for stimulating a founding queen to start egg-laying have been previously reported. Sladen (1912) discovered that bumblebee queens could be stimulated to initiate egg-laying. Plowright and Jay (1966) found that keeping two foundresses together was an effective way of inducing colony initiation in *B. terricola* and *B. ternarius*. Different starting methods, such as queens with a bumblebee worker, honeybee worker, bumblebee pupa and a combination of pupa and worker have been used to stimulate colony initiation (Ptacek, 1985, 1991; Eiinde *et al.* 1991; One *et al.* 1994; Gretenkord and Drescher, 1997;

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*To whom the correspondence addressed
Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Suwon 441-100, Korea.
Tel: +82-31-290-8567; E-mail: Yoonhj1023@korea.kr

Yoon and Kim, 2002a). As other starting methods, Grinfeld and Zacharova (1971) demonstrated that artificial shortening of day length reduced the development cycle of *B. hortorum* L and *B. afrorum* Calam and triggered the production of sexually reproducing individuals. It was reported that the LD 8:16 photoperiod had a stimulating effect on oviposition and sexual production of *B. terrestris* (Tasei and Aupinel, 1994; Yoon *et al*, 2003b). In this study, we investigated whether a wake-up treatment for short periods of 1~3 days at only just before indoor rearing affects oviposition and colony development of the bumblebees *B. ignitus* and *B. terrestris.*

Materials and Methods

Origin of experimental insects

Experimental insects were CO_2-treated 2nd generation *B ignitus* queens and 64-week artificially hibernated 6th generation *B terrestris* queens. The CO_2-narcosis group was exposed to 99% CO_2 for 30 min daily during two consecutive days (Yoon *et al.*, 2003a). The artificially hibernated queens were hibernated for 10 weeks at 2.5°C while preserved in a bottle filled with perlite and kept around 80% R.H After that, the queens were placed in flight cages for three days and then reared at 27 ± 1°C and 65% R.H.

Indoor rearing

The basic colony-rearing technique followed that described in Yoon *et al.* (2002b). The queens were reared in three types of cardboard (1.5 mm thick) or plastic boxes each for nest initiation (10.5 × 14.5 × 6.5 cm), colony foundation (21.0 × 21.0 × 15.0 cm), and colony maturation (24.0 × 27.0 × 18.0 cm). Queens were first confined individually in small boxes for colony initiation and remained there until oviposition. To stimulate egg-laying, two narcotized old *B. ignitus* and *B. terrestris* workers, aged 10 ~ 20 days after emergence, were added to each box with a queen (Yoon and Kim, 2002a). When the adults emerged from the first brood, the nest was transferred to a medium box for colony foundation and left there until the number of workers reached 50. The nest was thereafter moved to a big box for further colony development. Forty percent sugar solution with 0.3% sorbic acid and pollen dough were provided ad libitum (Yoon *et al.*, 2005). The pollen dough was made from a sugar solution and pollen (v : v = 1 : 1).

Egg-laying characteristics of *B. ignitus* under different wake-up treatments

To examine the effects of wake-up condition on egg-laying characteristics of *B. ignitus*, the following environmental conditions were provided. The wake-up regimes were defined as control (non-treated light or dark), 16L-1 (light for 16 hrs per day for one day), 16L-3 (light for 16 hrs per day for three days), 24L-1 (light for 24 hrs per day for one day), or 24D-1 (dark for 24 hrs per day for one day). Photoperiodic treatments were administered with fluorescent tubes (TL40D) at an intensity of 600~700 lux on the floor of the flight cages (55 × 45 × 65 cm). On the second day after mating, *B. ignitus* queens were CO_2-treated and then woken up during short periods of 1~3 days at only just before indoor-rearing in the mating cage. After the wake-up treatment, each queen was reared in a climate-controlled room (27 ± 1°C, 65% R.H. and continuous darkness). A total of 30 *B. ignitus* queens were used in this experiment with two replications. Egg-laying characteristics of each colony were estimated by rate of oviposition and preoviposition. The queens that did not oviposit in 40 days were excluded from the number of oviposited colonies (Yoon *et al.*, 2004a)

Colony development of *B. terrestris* under different wake-up treatments

To determine the wake-up conditions that were favorable for colony development of *B. terrestris*, the wake-up regimes and conditions for *B. ignitus* were used. After artificial hibernation, *B. terrestris* queens were placed in flight cages for three days (Yoon *et al.*, 2004b) and then treated with wake-up periods lasting 1~3 days just before indoor-rearing in a mating cage. After the wake-up treatment, each queen was reared in a climate-controlled room (27 ± 1°C, 65% R.H. and continuous darkness). A total of 40 *B. terrestris* queens were used in this experiment with two replications. The developmental ability of each colony was estimated by preoviposition period, rate of oviposition, colony foundation and progeny-queen production. Here, colony foundation was defined as the time period when more than 50 workers emerged from a colony. Statistical analyses were conducted using Chi-square tests and Tukey's pairwise comparison tests (One-way ANOVA) (MINITAB Release 13 for Windows, 2000).

Results and Discussion

Egg-laying characteristics of B. ignitus queens under different wake-up treatments

We investigated whether a wake-up treatment just before indoor-rearing improves egg-laying characteristics of *B. ignitus* (Fig. 1). For the control, 16L-1, 16L-3, 24L-1, and 24D-1 wake-up treatments at 27°C and 65% R.H, the oviposition rate of queens with a wake-up treatment was

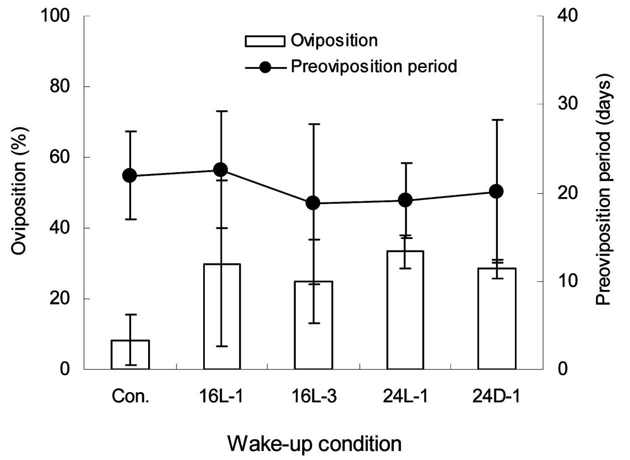

Fig. 1. Oviposition rate and preoviposition period of *B ignitus* reared under different wake-up treatments. Abbreviations: Con = non-treated light or dark; 16L-1 = light for 16 hrs per day for one day; 16L-3 = light for 16 hrs per day for three days; 24L-1 = light for 24 hrs per day for one day; and 24D-1 = dark for 24 hrs per day for one day. Thirty queens and two replications were allotted for wake-up regimes. There were no significant differences in oviposition rate and preoviposition period of *B. ignitus* at different wake-up conditions at $p < 0.05$ using Tukey's pairwise comparison test.

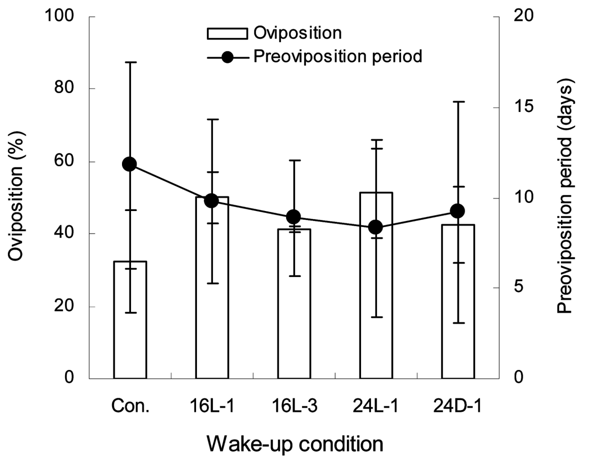

Fig. 2. Oviposition rate and preoviposition period of *B terrestris* reared under different wake-up treatments. For abbreviations, see legend to Fig. 1. Forty queens and two replications were allotted for wake-up regimes. There were no significant differences in oviposition rate and preoviposition period of *B. terrestris* at different wake-up conditions at $p<0.05$ using Tukey's pairwise comparison test.

16.7~21.7% higher than that of controls (non-treated light or dark). Among these wake-up treatments, the oviposition rate of queens reared at 24L-1 was the highest (33.4 ±4.7% higher than controls) followed by 16L-1, 24D-1 and 16L-3, although there was no statistically significant difference between treatments (Tukey's pairwise comparison test: F=0.12, DF=4, 5, *p*=0.970) (Fig. 1).

The preoviposition periods for different wake-up treatments showed a similar pattern as the rate of oviposition. The preoviposition periods of queens with wake-up treatments, except 16L-1, were 2.5~3.9 days shorter than that of the controls. The average preoviposition period of queens reared at 16L-3 was 18.7±9.1 days followed by 24L-1, 24D-1 and 16L-1. There was no statistically significant difference in preoviposition period between the different wake-up treatments (Tukey's pairwise comparison test: F=2.05, DF=4, 87, *p*=0.095) (Fig. 1). Asada and Ono (2002) found that the first oviposition day and date of first worker emergence had significant effects on the number of workers and queen progeny produced in laboratory-reared *B. ignitus* and *B. hypocrite* colonies, because earlier oviposition by foundresses would result in earlier colony development. Yoon and Kim (2003c) showed that oviposition rates of *B. ignitus* queens were higher in LD 8:16 and 16:8 than in 0:24 conditions among photoperiodic regimes during rearing.

We conclude that a wake-up treatment just before rearing is more effective than no treatment at improving egg-laying characteristics of *B. ignitus*. Furthermore, among the wake-up regimes foundation queens reared at 24L-1 showed the best performance in egg-laying characteristics, which are oviposition and preoviposition period.

Egg-laying characteristics of *B. terrestris* queens under different wake-up treatments

We investigated the effect of wake-up treatment on ovipositon rate and preovipostion period of artificially hibernated *B. terrestris* queens (Fig. 2). The oviposition rate of queens under the 16L-1, 16L-3, 24L-1 and 24D-1 treatments was 8.9~18.8% higher than that of queens exposed to the control. Among the four wake-up treatments, the oviposition rate of queens reared at 24L-1 was highest (51.3±12.4%), followed by 16L-1, 24D-1 and 16L-3. There was no statistically significant difference in oviposition rate between different wake-up treatments (Tukey's pairwise comparison test: F=0.12, DF=4, 5, *p*=0.970) (Fig. 2).

The preoviposition period of queens with wake-up treatments were 2.0~3.5 days shorter than that of queens exposed to the control. Among the four wake-up treatments, the preoviposition period of queens reared at 24L-1 was shortest (8.3±5.0 days), followed by 16L-3, 24D-1 and 16L-1. There were no statistically significant differences in preoviposition period of *B. terrestris* queens with different wake-up treatments (Tukey's pairwise comparison test, F= 1.53, DF=4, 136, p=0.196) (Fig. 2). In view of the above results, queens with a wake-up treatment had better egg-laying characteristics than queens exposed to the control, although there was no statistically significant difference. Among queens with a wake-up treatment, queens reared at 24L-1 showed the best performance in egg-laying characteristics. The effect of wake-up treatment on egg-laying characteristics of *B. terrestris* queens showed a similar pattern as that of *B. ignitus* queens. Although egg-laying is only one small step in the process of successfully rearing a bumblebee, it is a key factor in the large-scale production of commercial colonies and can reduce production costs. Yoon *et al.* (2004a) reported that the queen that had the earliest first oviposition day could make a colony stronger and could make the colony formation period shorter. The first oviposition day was an important factor in the successful artificial rearing of bumblebees. Overwintered *B. terrestris* queens showed higher rates of oviposition under the LD 8:16 photoperiod compared to the LD 0:24 and 16:8 photoperiods (Yoon *et al.*, 2003b). Yoon *et al.* (2003b) also mentioned that preoviposition period was shorter in the same photoperiodic regime.

Colony development of *B. terrestris* queens under different wake-up treatments

Colony development of *B. terrestris* queens under different wake-up treatments was investigated (Fig. 3). For the rate of colony foundation, which is one of the main criteria for colony quality in commercial rearing, the 24L-1 queens showed the best performance, 17.5±10.6%, which was 2.1-2.8-fold higher than that of queens under other wake-up treatments, followed by 16L-1, 24D-1, control and 16L-3 queens, at 8.8±1.8%, 7.5±0.0%, 7.5±3.5% and 6.3±1.8%, respectively. There was no statistically significant difference in colony foundation rate of *B. terrestris* queens under different wake-up treatments (Tukey's pairwise comparison test, F= 1.58, DF=4, 5, p=0.310). As shown in Fig. 3, the rate of progeny-queen production of the 24L-1 queens was 13.8±8.96%; this value was 0.8~2.2 fold higher than the 16L-1, 24D-1, 16L-3 and control queens. However, progeny-queen production rate of *B. terrestris* queens was not affected by wake-up treatment (F= 0.82, DF=4, 5, p=0.565).

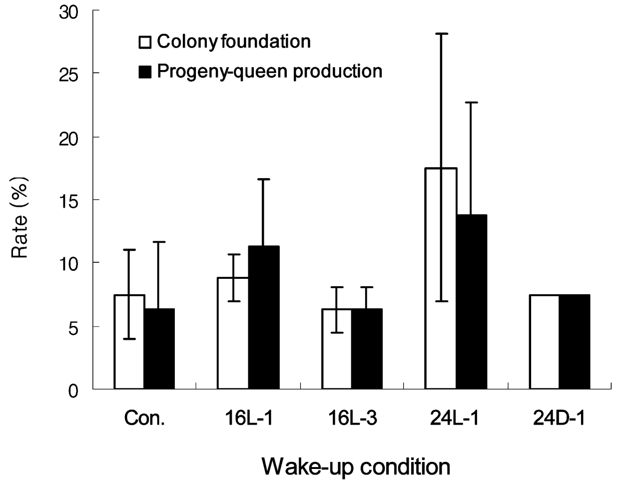

Fig. 3. The colony development of *B. terrestris* reared under different wake-up treatments. For abbreviations, see legend to Fig. 1. Forty queens and two replications were allotted for wake-up regimes. There were no significant differences in rate of colony foundation and progeny-queen production of *B. terrestris* at different wake-up conditions at $p<0.05$ using Tukey's pairwise comparison test.

Table 1. First adult emergence of *B. terrestris* indoor–reared under different wake-up treatments

Wake-up treatment	First adult emergence (days)					
	n	worker	n	male	n	Queen
Con.	6	28.2±8.5	5	57.2±24.3	4	76.5±16.0
16L-1	17	29.4±6.1	8	61.8±17.7	6	72.3±26.5
16L-3	12	29.0±6.9	5	55.2±14.0	4	73.3±11.9
24L-1	18	27.6±6.6	17	57.8±23.5	9	68.3±5.1
24D-1	15	29.6±8.5	11	54.2±14.9	4	63.3±13.0

1) n = number of colonies surveyed.
2) For abbreviations, see legend to Fig. 1.
3) There were no significant differences in first adult emergence of *B. terrestris* at different wake-up conditions at $p<0.05$ using Tukey's pairwise comparison test.

Table 1 shows adult emergence of *B. terrestris* at different wake-up treatments. The period up to first worker emergence for the 24L-1 queens was the shortest among all wake-up treatments (27.6±6.6 days), followed by control, 16L-3, 16L-1 and 24D-1 queens. No statistically significant difference was detected between wake-up treatment and period of first worker emergence (Tukey's pairwise comparison test, F = 0.21, df = 4, 63, p = 0.932). The period up to first male emergence, which was 54.2~ 61.8 days, was not statistically different between wake-up treatments (F = 0.15, df = 4, 41, p = 0.960). Similarly, the period of first queen emergence, which was 68.3~76.5 days, was also not affected by the wake-up treatment (F = 0.29, df = 4, 23, p = 0.884). The first emergence of workers, males and queens of *B. terrestris* that were artificially hibernated for 4 months was 26.8~29.8 days, 52.1~57.9 days and 68.2~76.3 days, respectively (Yoon *et al.*, 2004).

The relationship between the number of adults produced and wake-up treatment was investigated by comparing the time it took for more than 50 workers to emerge from each colony (Table 2). The numbers of workers reared

Table 2. Number of adults produced from foundation queens of *B. terrestris* indoor–reared under different wake-up treatments

Wake-up treatment	Number of adults produced					
	n	worker	n	male	n	Queen
Con.	4	100.0 ± 8.8	4	144.8 ± 40.2	4	10.0 ± 8.9
16L-1	6	99.2 ± 10.2	6	160.8 ± 44.1	6	9.2 ± 12.6
16L-3	5	99.4 ± 7.6	4	141.4 ± 38.3	4	7.3 ± 7.1
24L-1	11	105.8 ± 20.0	11	145.3 ± 40.1	9	24.8 ± 19.7
24D-1	5	100.6 ± 19.8	4	164.6 ± 33.9	4	10.8 ± 6.2

1) n = number of colonies surveyed.

2) For abbreviations, see legend to Fig. 1.

3) There were no significant differences in first adult emergence of *B. terrestris* at different wake-up conditions at $p < 0.05$ using Tukey's pairwise comparison test.

under the 24L-1 treatment was 105.8 ± 19.9, which was 5.2~6.2 times that of other wake-up treatments; nevertheless, there was no statistically significant difference between wake-up treatments (Tukey's pairwise comparison test, F = 0.27, df = 4, 26, $p = 0.897$). Worker production is important in evaluating the potential pollination efficiency of an insect pollinator. For the number of males produced at different wake-up treatments, which was 141.4-164.6, there was no statistically significant difference between wake-up treatments (F = 0.63, df = 4, 25, $p = 0.648$). For the number of queens produced, which is an important factor in year-round rearing of bumblebees, the 24L-1 queens produced 24.8 ± 19.7; this corresponded to 2.3-3.4-fold increase over queens in other treatments. However, the number of queens produced from *B. terrestris* queens was not statistically different between wake-up treatments (F = 1.84, df = 4, 22, $p = 0.158$).

In view of the above colony developmental characteristics results, the most favorable wake-up treatment was 24L-1 at just before rearing. In addition, having a wake-up regime at just before rearing was more effective than not having one in colony developmental characteristics of *B. terrestris* queens. Although photoperiodic treatment was continued throughout rearing, Grinfeld and Zacharova (1971) demonstrated that artificial shortening of day length reduced the development cycle of *B. hortorum* L and *B. afrorum* Calam, and triggered the production of sexuals. However, the *B. terrestris* queens reared in an LD 8:16 photoperiod had greater capacity for colony development than queens reared in LD 2:24 and 16:8 photoperiods (Yoon *et al*., 2003b). It is not clear whether the upper discrepancy that the effect of photoperiodic regimes on oviposition and colony development of *Bombus* spp. is caused by a species-specific characteristic or insufficient investigation on the optimum photoperiod and period. Also, the intensities and wavelengths of light are important parameters for reproduction and development. Further experiment can clarify this issue.

In conclusion, the present results indicate that the most favorable wake-up treatment at just before rearing was determined to be 24L-1. Furthermore, the wake-up treatment was more effective than no treatment in egg-laying and colony developmental characteristics of bumblebee queens.

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