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CO_2-Narcosis Time Favorable for Colony Development in the Bumblebee Queen, *Bombus terrestris*

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As a means for year-round rearing of bumblebee, CO_2-narcosis time favorable for colony development was identified in *Bombus terrestris*. CO_2-narcosis time divided into five classes: 11 days of adult emergence (A-11), the day of adult emergence (A-0), late pupal stage (LP), middle pupal sta ge (MP), and early pupal stage (EP). In egg-laying characteristics, the oviposition rate of LP, A-11 and A-0 was over 76.0%, but that of MP and EP was less than 61.1%. At the same time, the days needed to first oviposition shortened to 9.8 - 10.5 days in A-11, A-0 and LP, comparing to 13.7 - 16.1 days in MP and EP. The rate of colony foundation, progeny-queen produced and period of colony foundation of A-11 were the best results in among those at different CO_2-treatment time. The number of worker produced was 109.2 - 110.5 in A-11, LP and A-11, comparing to 82.0 - 86.8 in MP and EP. Also, the number of progeny-queen produced of A-11, A-0 and LP was 36.1, 41.0 and 71.3, respectively, which corresponded to 1.5 - 3.1 fold higher than MP and EP. Taken these together, CO_2-narcosis time favorable for colony development was determined to be 11 days of adult emergence. Also, the day of adult emergence and late pupal stage showed a positive effect on the ovipostion and colony development in CO_2-narcosis time.

Key words: Bumblebee, *Bombus terrestirs*, CO_2-narcosis, Time, Oviposition, Colony development

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Introduction

Bumblebees have generally one generation per year. Queens are the only caste to overwinter (enter diapause), and workers and males die during late summer and early autumn, respectively. In early spring queens that overwintered leave their hibernation sites. The queen builds up a store of pollen and lays her first batch eggs into the pollen mass after searching a suitable site to found a colony. As soon as the workers of the first brood have emerged they take over the foraging actives of the queen, who from now on spends her time predominantly on the laying of eggs. In the late summer, many males and new queens are produced and only mated queens hibernate and emerge in spring (Heinrich, 1979, Duchateau and Velthusis, 1988).

It is essential for year-round mass rearing of bumblebee, which undergoes one generation per year, to break diapause. Since mass-rearing of *Bombus terrestris* began in 1987 to supply glasshouse tomato grower in Europe, bumblebee producers have used several methods to rear colonies year-round, despite the long ovarian diapause of bumblebee (Alford, 1975). Röseler and Röseler (1984) demonstrated that narcotizing prediapausing *B. terrestris* queens with carbon dioxide (a 30 min narcosis repeated twice) would start laying eggs within a week. Part of the great commercial development of bumblebee rearing is due to Röseler's narcosis method which was also recommended by van den Eijnde *et al.* (1991) who described how to produce colonies of *B. terrestris* artificially. But this method had also many side-effects. Pormeroy and Plowright (1979) found that induced ejection of larvae by bumblebee workers in narcotized colonies. Röseler (1985) reported the emergence of some males among the first workers batch. Carbon dioxide treated bumblebee queens sometimes produce males instead of workers and their nests may be of smaller size than those of overwintered queens (Tasei, 1994; Yoon *et al.*, 2003).

In previous study, we reported concentration of CO_2 treatment and time after mating of *Bombus ignitus* queens (Yoon *et al.*, 2003). In this study we first conducted to identify CO_2 narcosis time favorable for colony development of *B. terrestris*.

Materials and Methods

Origin of experimental insects

Experimental insects were artificially hibernated 5^{th} - 7^{th} generation queens obtained from *B. terrestris* colonies year-round reared in a controlled climates room (28°C, 65% R.H. and continuous darkness). Experimental insects obtained from 5 colonies, which have over 80 progeny-queens.

Indoor rearing

The basic colony-rearing technique was followed as described in Yoon *et al.* (2002). The queens were reared in three types of plastic boxes each for nest initiation (8.0 × 11.0 × 8.0 cm: small box), colony foundation (15.0 × 16.5 × 11.0 cm: medium box), and colony maturation (22.0 × 27.0 × 14.0 cm: large box). Queens were first confined individually in small boxes for colony initiation and remained there until oviposition. To stimulate the egg laying, two narcotized old *B. terrestris* worker 10 – 20 days aged after emergence was added to each queen (Yoon and Kim, 2002). 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 the big box for further colony development. Fifty percent sugar solution and pollen dough were provided *ad libitum*. The pollen dough was made from 50% sugar solution and fresh pollen collected from an apiary (v : v = 1 : 1). For mating, newly emerged queens and males were collected from the colonies and maintained in a box until mating and one six-day-old virgin queens were placed in flight cages (55.0 × 65.0 × 40.0 cm) in mating room (uncontrolled photoperiod and 24 – 25°C) with one ten-day-old virgin males from other colonies during one week.

Colony developmental characteristics of *B. terrestis* queen by CO_2-treatmnent time

To identify CO_2 narcosis time favorable for oviposition and colony development of *B. terrestrs*, the experimental regimes of CO_2-narcosis time were defined as 11 days of adult emergence (A-11), the day of adult emergence (A-0), late pupal stage, pupae 7 – 9 days (LP), middle pupal stage, pupae 4 – 6 days (MP) and early pupal stage, pupae 1 – 3 days (EP). CO_2 narcosis of A-11 and A-0 was exposed to 99% CO_2 for 30 min daily during two consecutive days in flask (Yoon *et al.*, 2003) and CO_2 narcosis of LP-, MP- and EP-time in colonies themselves sealed with plastic bag. In the interval queens and colonies remained in the dark at 23 – 24°C. Queens finished CO_2 treatment were weighed and confined individually in small boxes for colony initiation. The numbers of queens allotted to stage of A-11, A-0, LP, MP and EP were 34, 25, 23, 35 and 36 respectively. The developmental ability of each colony was estimated by rate of oviposition, colony foundation and progeny-queen foundation, production of progeny, and period up to first adult emergence. Colony foundation here indicates that more than 50 workers emerged in a colony. Period up to first adult emergence designates the duration from the first oviposition to the first adult-emergence. The queens that did not oviposit in 60 days were excluded from the number of oviposited colonies.

Statistical analysis was done with Chi-square test and Tukey's pairwise comparison test (MINITAB Release 13 for Windows, 2000). The Chi-square test was used to compare colony development of *B. terrestris* by CO_2 treatment time. Tukey's pairwise comparison test was used to examine the durations until colony foundation and first adult emergence, as well as the number of adults produced.

Results and Discussion

Egg-laying characteristics of *B. terrestris* queen by CO_2-treatment time

We investigated relationship between CO_2-narcosis time and egg-laying characteristics of *B. terrestris* queen (Fig. 1). In CO_2-narcosis time of five groups of 11 days of adult emergence (A-11), the day of adult emergence (A-0), late pupal stage (LP), middle pupal stage (MP) and early pupal stage (EP), the oviposition rate of LP- time queen showed the best performance as 91.3% among other regimes and decreased in the order of the A-11- (Control), A-0-, EP- and MP-time queen, 82.3%, 80.0%, 75.0% and 74.3%, respectively. But there was no significant differences in oviposition rate of bumblebee queen in different CO_2-treatment time at $p < 0.05$ by Chi-square test ($x^2 = 3.194$, df = 4, $p = 0.526$) (Fig. 1). In case of the oviposition rate within 20 days, the LP- time queen showed also the best performance as 78.3% among other regimes, which was 24% higher than that of EP-time queen, and decreased in the order of the A-11-, A-0-, EP- and MP-time queen. But the oviposition rate within 20 days of *B. terrestris* queen was not affected by CO_2-treatment time ($x^2 = 6.777$, df = 4, $p = 0.148$) (Fig. 1). Yoon *et al.* (2004) reported that col-

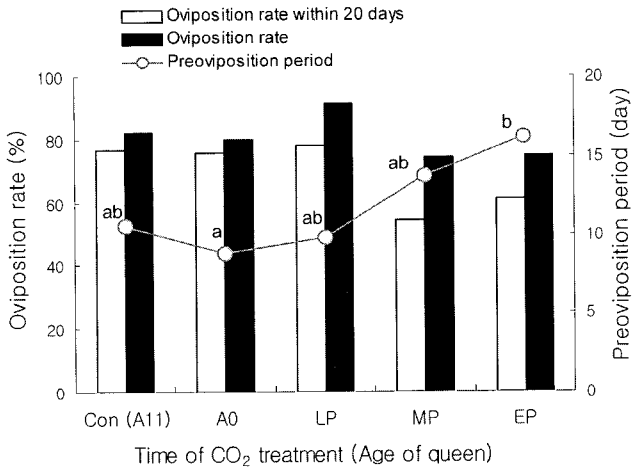

Fig. 1. Egg-laying characteristics of indoor-reared *Bombus ter restris* at different time of CO_2 treatment. Abbreviations: A-11 11 days of emergence; A-0, the day of emergence; LP, Lat pupal stage, pupae 7 – 9 days; MP, Mid-pupal stage, pupae 4 - 6 days; EP, Early pupal stage, pupae 1 – 3 days. For the statisti cal analysis, Tukey's pairwise comparison test and Chi-squar test were used. Oviposition rate; Chi-square test: $x^2 = 3.194$ $p = 0.526$, Oviposition rate within 20 day after rearing; Chi square test: $x^2 = 6.777$, $p = 0.148$, Preoviposition period; Tukey' pairwise comparison test: F = 2, 77, $p = 0.031$.

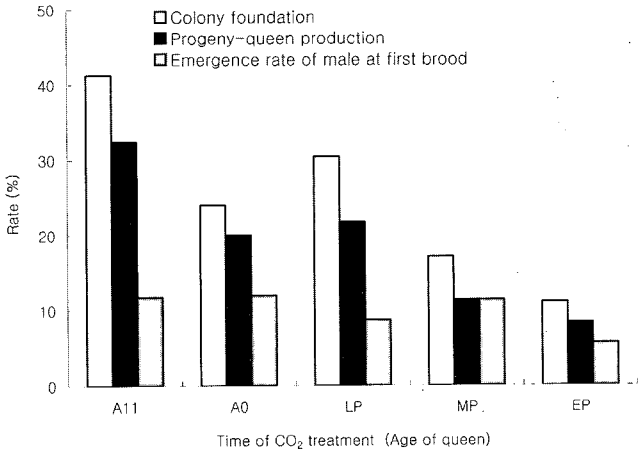

Fig. 2. Colony development of indoor-reared *Bombus terrestris* at different time of CO_2 treatment. For abbreviations, see legend to Fig. 1. For the statistical analysis, Chi-square test was used. There was significant difference in rate of colony foundation at $p < 0.05$.

ony development of queens laid eggs within 20 days were better than those of queens ovipostited after 20 days. So, discard queens ovipostited after 20 days seems to be economic in year-round mass rearing of bumblebees. As shown in Fig. 1, the duration up to preoviposition at the A-0- and LP-time queen was 8.8 days and 9.8 days, respectively, and these were 6.3 – 7.3 days shorter than that of the EP-time queen. The significant difference was detected between CO_2-narcosis time at $p < 0.05$ by Tukey's pairwise comparison test (F = 2.77, df = 4, 121, $p = 0.031$). Insect metabolism is influenced in various ways by carbon dioxide exposure and by other environmental factor. In *B. terrestris*, Larrere *et al.* (1993) observed that neurosecretory cells of diapausing queens resumed their activity at 72 hrs after CO_2-narcosis. With results of above egg-laying characteristics, we confirmed that CO_2-treatment at A-0- and LP-time *B. terrestris* queen is as effective as at A-11 time queen which treated Carbon dioxide generally in all CO_2-narcosis experiment (Yoon *et al.*, 2003).

Colony developmental characteristics of *B. terrestris* queen by CO_2-treatmnent time

The relationship between CO_2-treatment time and colony development of *B. terresris* queen was investigated (Fig. 2). In the colony foundation rate, the A-11-time queen (control) showed the best performance as 41.2 %, which was 3.7 fold higher than that of EP-time queen, and decreased in the order of the LP-, A-0-, MP- and EP-time queen, 30.4%, 24.0%, 17.1% and 11.1%, respectively. There was statistically significant difference in colony foundation rate of *B. terresris* queen in different CO_2-treatment time at $p < 0.05$ by Chi-square test ($x^2 = 10.147$, df = 4, $p = 0.038$). As shown in Fig. 2, the rate of progeny-queen production of the A-11-time queen was 32.4% and this value was also 2.8 – 3.9 fold higher than those at the MP- and EP-time queen. But progeny-queen production rate of bumblebee queen was not affected by CO_2-treatment time ($x^2 = 6.902$, df = 4, $p = 0.141$). In case of emergence rate of male at first brood in normal colony, that of EP-time queen was the lowest as 5.6%, which was 1.5 – 2.1 fold lower than that of other CO_2-treatment time. However, the oviposition rate within 20 days of *B. terrestris* queen was not affected by CO_2-treatment time ($x^2 = 1.155$, df = 4, $p = 0.885$). Röseler (1985) and Yoon *et al.* (2003) reported the emergence of some males among the first workers batch as one of side effects of CO_2-treatment.

Table 1 shows durations up to colony foundation and adult emergence of *B. terrestris* at different time of CO_2 treatment. Durations up to colony foundation at the the A-11-time queen was 61.3 days, which was 15.7 days shorter than that of EP-time queen. That at the A-0-, LP- and MP-time queen was 65.1 – 67.0 days. The period of colony foundation was not statistical difference (F = 1.63, df = 4, 29, $p = 0.192$). The period up to first worker emergence of the A-11-time queen was the shortest among other CO_2 treatment time as 26.1 days, which was 11.1 days shorter than that of MP-time queen, and prolonged in

Table 1. Durations up to colony foundation and adult emergence of indoor-reared *Bombus terrestris* at different time of CO_2 treatment

Time of CO_2 treatment (Age of queen)	n	Colony foundation (days)	First adult emergence					
			n	Worker	n	Male	n	Queen
Con (A-11)	12	61.3 ± 6.7	16	26.1 ± 4.7 a	17	51.2 ± 24.6	13	77.5 ± 15.4
A-0	5	66.0 ± 13.2	7	30.0 ± 5.0 ab	7	49.8 ± 28.7	4	79.3 ± 6.9
LP	8	65.1 ± 15.5	6	27.3 ± 5.6 ab	5	46.7 ± 28.6	4	85.0 ± 15.1
MP	5	67.0 ± 15.5	13	37.2 ± 14.4 b	10	41.0 ± 24.2	4	77.0 ± 8.9
EP	4	77.0 ± 11.6	4	33.6 ± 7.7 ab	8	52.1 ± 24.7	3	80.7 ± 2.1

1) For abbreviations, see legend to Fig. 1.
2) For the statistical analysis, Tukey's pairwise comparison test was used. There was significant difference in durations up to worker emergence colony foundation at $p < 0.05$.

the order of the LP-, A-0-, EP- and MP-time queen. Statistically significant difference was not detected between CO_2 treatment and period of first worker emergence at $p < 0.05$ by Tukey's pairwise comparison test ($F = 3.37$, $df = 4, 48$, $p = 0.016$). The period up to first male emergence at the A-11- and EP-time queen was also 10.2 – 11.1 days longer than that of MP-time. But there was no statistical difference between them ($F = 0.95$, $df = 4, 40$, $p = 0.444$). Besides, the period of first emergence queens, which was 77.0 – 85.0 days, was also not affected by the CO_2 treatment time ($F = 0.29$, $df = 4, 23$, $p = 0.884$). First emergence of worker, male and queen of overwintered *B. ignitus* was 18 – 22 days, 60 – 74 days and 61 – 80 days, respectively (Yoon *et al.*, 1999).

The relationship between number of adults produced and CO_2 narcosis time was investigated with more than 50 workers emerged colonies (Table 2). The numbers of worker at the A-11-, A-0- and LP-time was about 110, which was 22 – 24 numbers more than those at MP- and EP-CO_2 narcosis but there was no statistical difference at $p < 0.05$ by Tukey's pairwise comparison test ($F = 0.86$, $df = 4, 28$, $p = 0.499$). In case of the number of males produced at different CO_2 treatment time, the A-11-, A-0- and LP-time queen was about 229 – 236 numbers and MP- and EP-CO_2 narcosis time queen was 125 – 142 numbers. But there was no statistical difference between them at $p < 0.05$ ($F = 1.14$, $df = 4, 20$, $p = 0.366$). In case of the number of queens produced, which is an important point in year-round rearing of bumblebee, the EP-CO_2 narcosis time queen produced 51.6 ± 34.4 numbers, which corresponded to 1.7 – 2.2 fold of those of the EP- and MP-CO_2 narcosis time queen. However, the number of queen produced from *B. terrestris* queen was not detected statistical difference by the CO_2 treatment time ($F = 2.43$, $df = 2, 13$, $p = 0.127$).

In view of above colony developmental characteristics results, the favorable time of CO_2-narcosis was determined to be 11 days of adult emergence. And also A-0- and LP-time *B. terrestris* queen is as effective as A-11 time queen in CO_2 treatment. Röseler (1985) reported that CO_2 narcosis time is one day after mating and CO_2 treatment queens became very active and flew in the gauze cage. According to Tasei (1994) the timing of CO_2 narcosis after mating did not affect the delays of egg-laying within the range of 5 – 30 days. In case of *Bombus hypocrita* and *B. ignitus*, the timing of CO_2 narcosis is 2 – 4

Table 2. Number of adults produced from queen of indoor-reared *Bombus terrestris* at different time of CO_2 treatment

Time of CO_2 treatment (Age of queen)	Number of adults produced					
	n	Worker	n	Male	n	Queen
Con (A-11)	12	109.2 ± 36.4	10	236.2 ± 103.7	11	36.1 ± 28.1
A-0	6	110.5 ± 44.1	5	233.8 ± 120.9	5	41.0 ± 31.6
LP	6	109.0 ± 19.1	5	228.8 ± 79.6	5	51.6 ± 34.4
MP	5	82.0 ± 28.2	3	125.0 ± 41.6	4	29.8 ± 36.8
EP	4	86.8 ± 42.3	2	142.0 ± 24.0	3	23.3 ± 15.9

1) For abbreviations, see legend to Fig. 1.
2) For the statistical analysis, Tukey's pairwise comparison test was used. There was no significant difference in number of adults produced at $p < 0.05$.

days after mating (Asada and Ono, 1997). As physiological effects of CO_2 narcosis, Röseler and Röseler (1984) demonstrated that narcotizing prediapausing queens with carbon dioxide (a 30 min narcosis repeated twice) inhibited the formation of fat reserves, increased the size of juvenile hormone *in vitro*, and induced oogensis. In the honeybee, histological studies brought evidence of CO_2 effects on neurosecretory cells of brain, *corpora allata* and juvenile hormone more titres (Nicolas, 1989).

In conclusion, the present results indicate the favorable time of CO_2-narcosis was determined to be 11 days of adult emergence. Also, the day of adult emergence and late pupal stage has the same efficacy on the ovipostion and colony development in CO_2-narcosis time. However CO_2 treatment at late pupal stage is insufficient to produce commercial grade bumblebee colony in spite its capability for promating oviposition, because method of CO_2 treatment is less efficient than adult stage.

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