

Mating Conditions Favorable for Improving Mating Rate of the Bumblebee, *Bombus ignitus*

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We investigated mating conditions of photoperiod, illumination and temperature during mating periods, care temperature of queen before mating, mating period and number of queen per mating cage to improve mating rate of *Bombus ignitus*. Among photoperiodic regimes of 12L, 14L and 16L during mating periods, queen mated at 14L showed better results than at 12L and 16L in egg-laying characteristics and colony development. In case of illumination during mating periods, intensity of 1000 lux was more effective than at intensity of 100 lux and 2000 lux in mating *B. ignitus* queen. Mating temperature and care temperature of queen before mating favorable for *B. ignitus* queen were 22-25°C and 19°C, respectively. The period need to mating *B. ignitus* queen was 3 days, and the number of queen suitable per mating cage of 55 × 45 × 65 cm was 30.

Key words: Bumblebee, *Bombus ignitus*, Mating, Photoperiod, Illumination, Temperature, Care temperature of queen, Mating cage

Introduction

Bumblebees are an important pollinator of various greenhouse crops, particularly effective in pollinating for the night shade family, which includes tomato and eggplant. (Buchmann and Hurley, 1978; Free, 1993). Bumblebees provide farmers the opportunity to decrease the labor costs of pollination and promise a good yield both in quantity and in quality (Iwasaki, 1995). Introduction of

bumblebees to greenhouses for pollination has become widespread in recent years, and the demand is increasing every year. The large earth bumblebee, *Bombus terrestris*, which is indigenous to Europe, has been artificially introduced throughout the world. Since 1988, *B. terrestris* in portable boxes have been available commercially from European company for crop pollination (Masahiro, 2000). Colonies of *B. terrestris* have already been imported into Korea, Japan, China, Taiwan, Mexico, Chile, Argentina, Uruguay, South Africa, Morocco and Tunisia for development in greenhouses (Dafni 1996). In case of Korea, *B. terrestris* was firstly introduced in 1994.

Using of the bumblebee to greenhouses in Korea causes some anxieties, because it is a foreign species, *B. terrestris*. That is, foreign bumblebees escaping from a greenhouse may have some negative effects, such as competition, on native bumblebees (Ono and Wada, 1996) or cause genetic contamination by cross mating with native bumblebees (Ono, 1997). *B. terrestris* has successfully invaded New Zealand (Macfarland and Gurr 1995), and competitive displacement of native bees and invasion of native vegetation by *B. terrestris* has already been recorded in Tasmania, Austria (Simmens *et al.*, 1993; Hingston and McQuillan 1998; Hingston *et al.*, 2002). In Israel, there has been a decline in the numbers of honeybees and solitary bees on Mt. Carmel associated with expansion of the range of *B. terrestris* during last two decades (Dafni and Shimida 1996). *B. terrestris* has also recently colonized Japan, where it escaped from greenhouses in 1996, after its introduction in 1991 (Goka 1998; Washitani 1998). For this reason, the governments of Canada and the USA prohibit the introduction of foreign bumblebee species, and N. American growers use commercialized native bumblebees (*B. terrestris* and *B. occidentalis*) (de Ruijter, 1997; Asasa and Ono, 2000). *B. terrestris* queen overwintered was trapped at Suwon, Jeongsun, and Mt. Chiak, Korea in early May in 2002 to 2004 (Yoon H. J., personal communication).

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To prevent the above risks, it has been attempted to substitute Korean native bumblebee with the foreign bumblebee. Out of seven Korean native bumblebees tested, *B. ignitus* showed the best results both in artificial multiplication and in pollinating ability, and was selected as the most reliable native species for crop pollination (Yoon *et al.*, 1999b; Yoon and Kim, 2002; Yoon *et al.*, 2002). Though *B. ignitus* has been selected as a reliable species for commercial mass-production of the bees, there are still some unsettled issues. Among these issues, mating environmental conditions must be the most principal factors in rearing of poikilothermal bumblebees, but up until now a few detailed studies on this factor have been reported (Diegham *et al.*, 1994; Tasei *et al.*, 1998; Yoon *et al.*, 2005). Therefore this study was conducted to identify mating conditions most favorable for colony development of *B. ignitus*.

Materials and Methods

Origin of experimental insects

Experimental insects were CO₂-treated and artificial hibernated 2nd generation queens of *B. ignitus* reared in a controlled climates room (27 ± 1°C, 65% R.H. and continuous darkness). CO₂-narcosis was exposed to 99% CO₂ for 30 min daily during two consecutive days (Yoon *et al.*, 2003). For artificial hibernation, queens were hibernated for 10 weeks at 2.5°C to preserve them in a bottle filled with perlite and keep it around 80% R.H. After that, the queens were placed in flight cages for 3 days and then reared under the 27 ± 1°C, 65% R.H.

Indoor rearing

The basic colony-rearing technique was followed as described in Yoon *et al.* (2002). The queens were reared in three types of cardboard (1.5 mm thick) boxes each for nest initiation (10.5 × 14.5 × 6.5 cm: small box), colony foundation (21.0 × 21.0 × 15.0 cm: medium box), and colony maturation (24.0 × 27.0 × 18.0 cm: large box). Each box had a wire net window on its lid for ventilation. The sizes of these windows were 5.5 × 6.5 cm, 7.0 × 14.0 cm and 10.0 × 20.0 cm, respectively. 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. ignitus* and *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.

Forty percent sugar solution and pollen dough were provided *ad libitum*. The pollen dough was made from sugar solution and pollen (v:v=1:1).

Colony development of *B. ignitus* at different photoperiods during mating periods

To examine the photoperiod during mating periods favorable for colony development of *B. ignitus*, the following environmental conditions were provided. The photoperiodic regimes during mating periods were defined as 12L (light for 12 hrs per day), 14L (light for 14 hrs per day) and 16L (light for 16 hrs per day). Environmental conditions in mating room were maintained at 23-24°C, 65% R.H., and the intensity of 2000 lux. According to Yoon *et al.* (1999a), thirty five-day-old males and ninety ten-day-old virgin queen were introduced a wooden mating cage (55 × 45 × 65 cm) with wire mesh during one week. At the second day after mating, *B. ignitus* queen was treated CO₂-narcosis, and then reared in a controlled climates room (27 ± 1°C, 65% R.H. and continuous darkness). The number of *B. ignitus* queens allotted to this experiment was 30 and 3 replications. The developmental ability of each colony was estimated by death rate during mating period, rate of oviposition, rate of worker emergence, colony foundation and progeny-queen production. Colony foundation here indicates that more than 50 workers emerged in a colony. The queens that did not oviposit in 60 days were excluded from the number of oviposited colonies.

And also, the 2nd generation queens produced from the difference photoperiods during mating were hibernated during one month at 2.5°C, and examined survival rate and rate of weight loss. The number of *B. ignitus* queens allotted to this experiment was 30.

Colony development of *B. ignitus* at different illuminations during mating periods

To investigate the illumination during mating periods favorable for colony development of *B. ignitus*, the illuminations were defined as 100 lux, 1000 lux, and 2000 lux. Environmental conditions in mating room were maintained at 23-24°C, 65% R.H., and 14L. The number of queens allotted to this experiment was 30 and 3 replications. After mating, queen was treated CO₂ narcosis, and then reared to investigate developmental ability of colony. The developmental ability of each colony was estimated by death rate during mating periods, rate of oviposition, preoviposition period and rate of worker emergence.

Colony development of *B. ignitus* at different mating temperatures

To examine the optimum mating temperature in the col-

ony development of *B. ignitus*, the mating temperature regimes were defined as 19°C, 22°C, and 25°C with results reported in Yoon *et al* (2005). Copulation room was maintained at 14L, 65% R.H., and the intensity of 2000 lux. In this experiment, we allotted to CO₂-treated 30 queens and 3 replications. The developmental ability of each colony was estimated by death rate during mating period, mating rate, egg-laying characteristics, rate of worker emergence, colony foundation and progeny-queen production. Mating rate was investigated 09:00 to 17:00 at the first day of mating periods. The survival rate and rate of weight loss after artificial hibernation were also examined with the 2nd generation queens produced from the difference mating temperatures. The number of *B. ignitus* queens allotted to this experiment was 30.

Colony development of *B. ignitus* at different care temperatures of queen before mating

To examine a suitable care temperature for colony development of *B. ignitus* queen before mating, care temperature of queen were defined as 19°C, 22°C and 25°C. Copulation room was maintained at 23-24°C, 14L, 65% R.H., and the intensity of 2000 lux. The number of CO₂-treated queens allotted to this experiment was 30 and 3 replications. The developmental ability of each colony was estimated by death rate during mating period, egg-laying characteristics, rate of worker emergence, colony foundation and progeny-queen production. The survival rate and rate of weight loss after artificial hibernation were also examined with the 2nd generation queens produced from the photoperiod during mating. The number of *B. ignitus* queens allotted to this experiment was 30.

Colony development of *B. ignitus* at different mating periods

To investigate mating period favorable for colony development of *B. ignitus*, mating periods were defined as 3 days, 5 days and 7 days. Conditions of mating room were maintained at 23-24°C, 65% R.H., 14L and the intensity of 2000 lux. The number of queens allotted to this experiment was 30 and 3 replications. The developmental ability of each colony was estimated by rate of oviposition, preoviposition period and rate of worker emergence.

Colony development of *B. ignitus* at the number of queen per mating cage

To examine the number of adequate queen per mating cage (55 × 45 × 65 cm) for colony development of *B. ignitus*, the number of queen per mating cage was defined as 10, 20 and 30. Conditions of mating room were maintained at 23-24°C, 65% R.H., 14L and the intensity of 2000 lux. The number of CO₂-treated queens allotted to

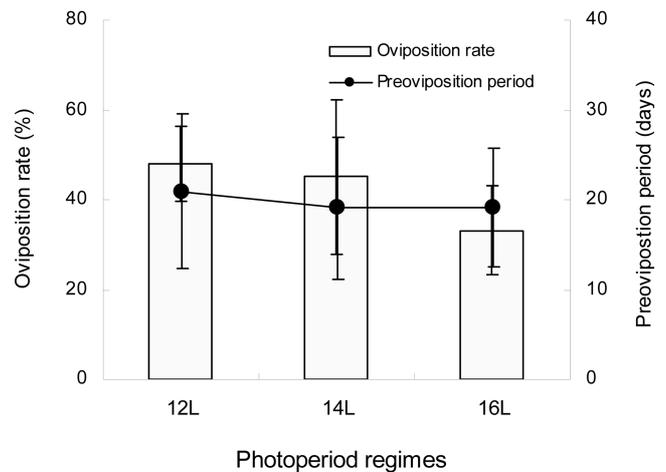


Fig. 1. Oviposition rate and preoviposition period of *Bombus ignitus* at different photoperiods during mating periods. Mating periods were 7 days. There were no significant differences in oviposition rate and preoviposition period of *B. ignitus* at different photoperiods during mating periods at $p < 0.05$ by Tukey's pairwise comparison test.

this experiment was 30 and 3 replications. The developmental ability of each colony was estimated by death rate during mating periods, rate of oviposition, preoviposition period and rate of worker emergence.

Statistical analysis was done with Chi-square test and Tukey's pairwise comparison test (MINITAB Release 13 for Windows, 2000).

Results and Discussion

Comparison of colony development of *B. ignitus* at different photoperiods during mating periods

It was investigated the photoperiod favorable for colony development of *B. ignitus* during mating periods. In the photoperiodic regimes of 12L, 14L and 16L under in 23-24°C, 65% R.H. and the intensity of 2000 lux, the oviposition rate of queen mated at 16L during mating periods was 11.9-14.7% lower than that of 12L and 16L, but there was no statistical difference (Tukey's pairwise comparison test: $F = 1.18$, $DF = 2, 6$, $p = 0.370$) (Fig. 1). The preoviposition periods in the photoperiodic regimes of 12L, 14L and 16L were 19.1-20.9 days and also no statistical difference ($F = 0.57$, $DF = 2, 91$, $p = 0.568$) (Fig. 1). The death rate during mating periods was also not affected by photoperiod (Table 1). The rate of worker emergence of queen mated at 14L was 4.7-6.9% higher than that of 12L and 16L although there was no statistical difference ($F = 0.53$, $DF = 2, 6$, $p = 0.611$) (Table. 1). The colony foundation and progeny-queen production were also showed a similar tendency with the rate of worker emergence (Table 1).

Table 1. Death rate and colony development of *B. ignitus* at different photoperiod during mating periods

Photoperiod regimes	Death rate during mating periods (%)	Rate (%)		
		Worker emergence	Colony foundation	Progeny-queen production
12L	12.2±6.9	10.1±4.1	2.6±2.3	1.2±2.1
14L	11.1±1.9	14.8±3.3	2.5±2.1	2.5±4.3
16L	13.3±5.8	7.9±4.4	0.0±0.0	0.0±0.0

1)There were no significant differences in death rate and colony development of *B. ignitus* at different photoperiods during mating periods at $p < 0.05$ by Tukey's pairwise comparison test.

Table 2. Survival rate and change of weight after artificial hibernation of *B. ignitus* at different photoperiod during mating periods

Photoperiod regimes	Survival rate (%)	Change of weight in artificial hibernation			
		n	Before (g)	After (g)	Rate of weight loss (%)
12L	72.0	14	0.80±0.05	0.76±0.04	5.0
14L	82.1	13	0.86±0.09	0.81±0.09	5.8
16L	69.2	11	0.79±0.07	0.76±0.05	3.8

1)The period of artificial hibernation was one month.

2)There were no significant differences in survival rate and change of weight after artificial hibernation of *B. ignitus* at different photoperiods during mating periods at $p < 0.05$ by a Chi-square test and Tukey's pairwise comparison test.

3)n means the number of surveyed.

Table 3. Colony development of *B. ignitus* at different illuminations during mating periods

Illumination (lux)	Death rate during mating periods (%)	Preoviposition period (day)	Rate (%)	
			Oviposition	Worker emergence
100	13.4±4.7	19.6±7.5	50.2±8.2	3.9±0.2
1000	8.4±2.3	19.2±7.3	51.2±11.9	7.3±0.2
2000	18.4±2.3	18.6±6.2	46.8±1.1	4.1±3.0

1)There was no significant differences in colony development of *B. ignitus* at different illuminations during mating periods at $p < 0.05$ by Tukey's pairwise comparison test.

The survival rate and rate of weight loss after artificial hibernation were examined with the 2nd generation queens produced from different photoperiods during mating periods (Table 2). The survival rate after artificial hibernation during one month was high at 14L as 82.0% and low in order of 12L and 16L. The rate of weight loss after artificial hibernation during one month was 3.8-5.8%.

With above results, we supposed that 14L at photoperiodic regimes during mating periods was more effective than that of 12L and 16L in colony development of *B. ignitus*. Ono (1997) reported that mating efficiency of *B. ignitus*, *B. hypocirta hypocrite* and *B. hypocirta saporicensis* was increased by controlling the light-dark condition (2L-22D) and temperature (22°C at L, 17°C at D) using a low temperature incubator.

Comparison of colony development of *B. ignitus* at different illuminations during mating periods

We investigated the illumination favorable for colony development of *B. ignitus* during mating periods (Table 3). Among illuminations of 100 lux, 1000 lux and 2000 lux, the death rate during mating periods of 1000 lux was 8.4%, which was 1.6-2.2 times lower than that of 100 lux and 2000 lux., and low in order of 100 lux and 2000 lux. But it was not affected by the illumination during mating periods (Tukey's pairwise comparison test: $F = 4.50$, $DF = 2, 6$, $p = 0.125$) (Table 3). The preoviposition periods in the intensity of 100 lux, 1000 lux and 2000 lux were 18.6-19.6 days. The rate of oviposition and worker emergence of queens mated at 1000 lux were 51.2% and 7.3%, respectively, which is a little higher than those at 100 lux and 2000 lux. But there was no statistical difference (Table 3). Above results showed that the illumination

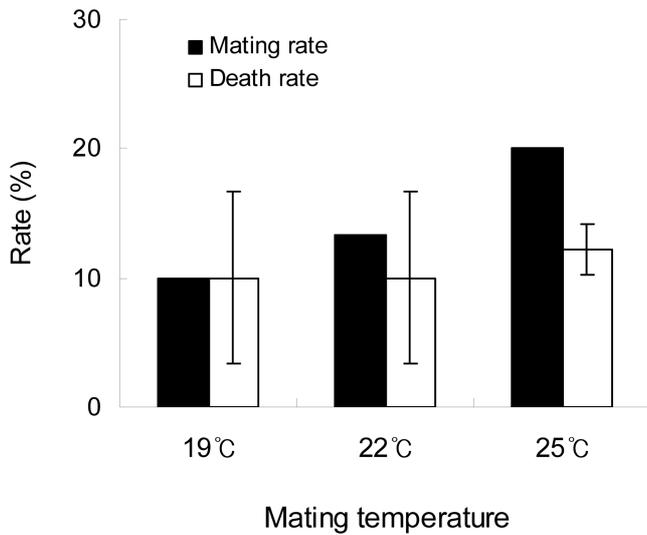


Fig. 2. Mating rate and death rate of *B. ignitus* at different mating temperatures. Mating rate was investigated 09:00 to 17:00 at the first day of mating periods. There were no significant differences in rate of mating and death rate of *B. ignitus* at different mating temperatures at $p < 0.05$ by Tukey's pairwise comparison test and Chi-square test.

favorable for colony development of *B. ignitus* during mating periods is over 1000 lux.

B. terrestris was mated in copulation room maintained as 20°C, 70% R.H. and light intensity of 2000 lux with halogen lamp (Djegham *et al.*, 1994). It is not clear whether the upper discrepancy on the illumination during mating periods is caused by species-specific characteristics, difference of light source or insufficient investigation into the optimum illumination.

Comparison of colony development of *B. ignitus* at different mating temperatures

To examine optimum mating temperature at colony development of *B. ignitus*, we defined mating temperature regimes as 19°C, 22°C, and 25°C. The mating rate of queen mated at 25°C was 6.7-10.0% higher than that of 22°C and 19°C, and low in order of 22°C and 19°C. But there was no statistical difference (Chi-square test: $\chi^2 = 1.259$, $DF = 2, 6$, $p = 0.533$) (Fig. 2). The death rate

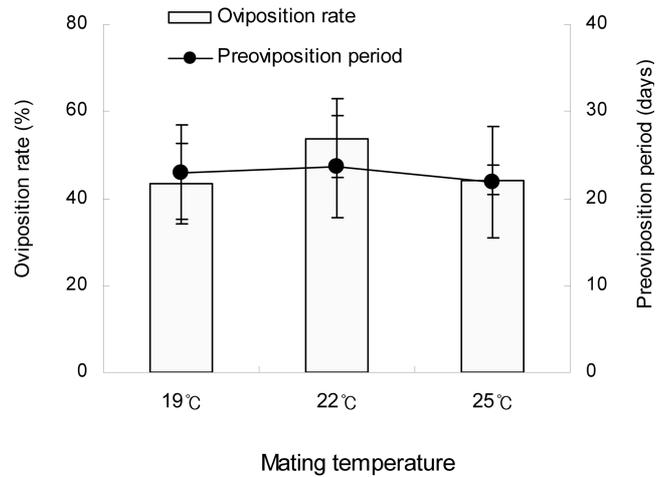


Fig. 3. Oviposition rate and preoviposition period of *B. ignitus* at different mating temperature. There were no significant differences in oviposition rate and preoviposition period of *B. ignitus* at different mating temperatures at $p < 0.05$ by Tukey's pairwise comparison test.

during mating periods was 10.0-12.2%, which was also not affected by mating temperatures (Tukey's pairwise comparison test: $F = 0.16$, $DF = 2, 6$, $p = 0.859$).

In egg-laying characteristics, the oviposition rate of queen mated at 22°C was 53.8% and it was 9.6-10.3% higher than that of 25°C and 19°C (Fig. 3). The preoviposition period was 21.9-23.0 days and also no statistical difference (Fig. 3). In case of colony development, the rate of worker emergence of queen mated at 22°C was 2.5-9.1% higher than that of 25°C and 19°C although there was no statistical difference (Table. 4). The colony foundation and progeny-queen production were high at 22°C and 25°C than at 19°C.

Table 5 showed the survival rate and change of weight loss at before and after artificial hibernation examined with the 2nd generation queens produced from different mating temperatures. The survival rate after artificial hibernation during one month was low at 22°C as 64.2% although rate of weight loss was lower than that of 25°C and 19°C, but there was no statistical difference (Chi-square test: $\chi^2 = 1.065$, $DF = 2, 6$, $p = 0.587$). The rate of weight loss after artificial hibernation during one month

Table 4. Colony development of *B. ignitus* at different mating temperatures during mating periods

Mating temperature (°C)	Rate (%)		
	Worker emergence	Colony foundation	Progeny-queen production
19	9.5 ± 2.9	0.0 ± 0.0	0.0 ± 0.0
22	18.6 ± 6.5	1.2 ± 2.1	3.7 ± 0.3
25	16.4 ± 4.3	1.3 ± 2.2	3.8 ± 6.6

1) There was no significant differences in colony development of *B. ignitus* at different mating temperatures during mating periods at $p < 0.05$ by Tukey's pairwise comparison test.

Table 5. Survival rate and change of weight after artificial hibernation of *B. ignitus* at different mating temperatures during mating periods

Mating temperature (°C)	Survival rate (%)	Change of weight in artificial hibernation			
		n	Before (g)	After (g)	Rate of weight loss (%)
19	72.1	11	0.83 ± 0.17	0.79 ± 0.06	5.3
22	64.2	12	0.80 ± 0.14	0.78 ± 0.09	2.5
25	76.9	17	0.80 ± 0.09	0.76 ± 0.07	5.0

1)The period of artificial hibernation was one month.

2)There were no significant differences in survival rate and change of weight after artificial hibernation of *B. ignitus* at different mating temperatures during mating periods at $p < 0.05$ by a Chi-square test and Tukey's pairwise comparison test.

3)n means the number of surveyed.

Table 6. Colony development of *B. ignitus* in different care temperatures of queen before mating

Care temperature (°C)	Death rate at mating periods (%)	Rate (%)		
		Worker emergence	Colony foundation	Progeny-queen production
19	17.8 ± 5.1	16.7	11.1	5.6
22	12.2 ± 7.7	9.1	0.0	0.0
25	18.9 ± 7.0	5.9	0.0	0.0

1)There was no significant differences in colony development of *B. ignitus* at different mating care temperatures of queen before mating at $p < 0.05$ by Tukey's pairwise comparison test and Chi-square test.

was 2.5-5.3%.

With above results about death rate during mating periods, mating rate, egg-laying characteristics, rate of worker emergence, colony foundation and progeny-queen production of *B. ignitus* queen, we think that 22°C and 25°C were more effective than at 19°C in mating temperature of *B. ignitus*. The Western bumblebee, *B. terrestris* was mated in 20°C (Djegham *et al.*, 1994) and 18°C (Tasei *et*

al., 1998). It is not clear whether the upper difference on mating temperature is caused by species-specific characteristics. The reason for this should be investigated through further examinations.

Comparison of colony development of *B. ignitus* at different care temperatures of queen before mating

It was investigated the care temperature of queen before mating favorable for colony development of *B. ignitus*. Among care temperatures of queen before mating of 19°C, 22°C and 25°C, the oviposition rate of queen cared at 19°C was 51.2%, which is 7.5-12.6% higher than that of 22°C and 25°C, but there was no statistical difference (Tukey's pairwise comparison test: $F = 1.29$, $DF = 2, 6$, $p = 0.343$) (Fig. 4). The preoviposition periods were 19.7-20.6 days and also no statistical difference ($F = 0.06$, $DF = 2, 83$, $p = 0.939$) (Fig. 4). The death rate during mating periods was low at 22°C as 12.2%, and low in order of 19°C and 25°C (Table 6). Generally for the death rate during mating periods, the higher the temperature is, the more the death rate is. The rate of worker emergence of queen cared at 19°C was 7.6-10.8% higher than that of 22°C and 25°C. The colony foundation and progeny-queen production were also showed a similar tendency with the rate of worker emergence (Table 6).

The survival rate and rate of weight loss after artificial hibernation were examined with the 2nd generation queens produced from a different photoperiods during mating

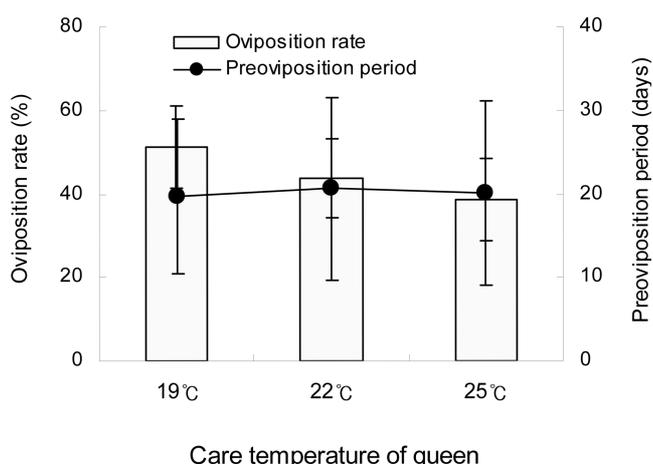


Fig. 4. Oviposition rate and preoviposition period of *B. ignitus* at different care temperatures of queen before mating. There were no significant differences in oviposition rate and preoviposition period of *B. ignitus* at different care temperatures of queen before mating at $p < 0.05$ by Tukey's pairwise comparison test.

Table 7. Survival rate and change of weight after artificial hibernation of *B. ignitus* at different care temperatures of queen before mating

Care temperature (°C)	Survival rate (%)	Change of weight in artificial hibernation			
		n	Before (g)	After (g)	Rate of weight loss (%)
19°C	85.0	20	0.78 ± 0.07	0.74 ± 0.06	5.1
22°C	96.0	22	0.81 ± 0.07	0.76 ± 0.06	6.2
25°C	96.0	19	0.82 ± 0.06	0.78 ± 0.08	4.5

1)The period of artificial hibernation was one month.

2)There were no significant differences in survival rate and change of weight after artificial hibernation of *B. ignitus* at different care temperatures of queen before mating at $p < 0.05$ by a Chi-square test and Tukey's pairwise comparison test.

3)n means the number of surveyed.

Table 8. Colony development of *B. ignitus* at different mating periods

Mating period (days)	Preoviposition period (days)	Rate (%)	
		Oviposition	Worker emergence
3	23.1 ± 6.9	52.9 ± 6.8	11.4 ± 5.7
5	23.1 ± 8.8	48.0 ± 28.2	10.0 ± 2.8
7	19.6 ± 8.1	44.7 ± 12.7	5.4 ± 7.6

1)There was no significant differences in colony development of *B. ignitus* at different mating periods of queen before mating at $p < 0.05$ by Tukey's pairwise comparison test.

periods (Table 7). The survival rate after artificial hibernation during one month was high at 22°C and 25°C as 96.0% and 96.0%, respectively. The rate of weight loss after artificial hibernation during one month was 4.5-6.2%.

With above results, we supposed that 19°C at care temperature before mating was more effective than those of 22°C and 25°C in egg-laying characteristics and colony development of *B. ignitus*, except the survival rate after artificial hibernation during one month.

Colony development of *B. ignitus* at different mating periods and the number of queen per mating cage

We investigated appropriate mating periods and the num-

ber of queen per mating cage for egg-laying characteristics and rate of worker emergence of *B. ignitus* (Table 8 and Table 9). Among the preoviposition periods of 3 days, 5 days and 7 days, queen mated during 7 days oviposited 3.5 days earlier than queen mated during 3- 5 days. But in the case of the rate of oviposition and worker emergence, queen mated during 3 days of queens mated was higher than queen mated during 5-7 days, which were 52.9% and 11.43%, respectively (Table 8). Above results showed that 3 days was appropriate mating periods for colony development of *B. ignitus*. Generally, the European bumblebee, *B. terrestris* was mated during 7 days (Djegham *et al.*, 1994; Tasei *et al.*, 1998).

The death rate at the number of queen in mating cage of 55 × 45 × 65 cm was 7.5-10.0%, and there was no statistical difference (Tukey's pairwise comparison test: $F = 0.36$, $DF = 2, 6$, $p = 0.722$) (Table 9). The preoviposition period at mating cage with 10 queens and 30 males was 16.4 days and it was 4.5-4.7 days earlier than that at mating cage with 30 queens and 60 queens. The oviposition rate is high at mating cage with 30 queens, the rate of worker emergence was high at mating cage with 10 and 30 queens although there was no statistical difference (Table 4). The colony foundation and progeny-queen production were high at 22°C and 25°C than at 19°C. In above results, we supposed that the number of queen suitable per mating cage of 55 × 45 × 65 cm size was 30.

Table 9. Colony development of *B. ignitus* at the number of queen per mating cage

No. of queen/mating cage	Death rate of mating period (%)	Preoviposition period (day)	Rate (%)	
			Oviposition	Worker emergence
10	10.0 ± 0.0	16.4 ± 7.7	50.0 ± 39.2	11.1 ± 15.7
20	7.5 ± 3.5	20.9 ± 7.7	48.7 ± 1.84	5.5 ± 0.2
30	10.0 ± 4.7	21.1 ± 6.7	66.5 ± 7.0	10.9 ± 10.0

1)Mating cage size was 55 × 45 × 65 cm.

2)There was no significant differences in colony development of *B. ignitus* at the number of queen per mating cage at $p < 0.05$ by Tukey's pairwise comparison test.

In view of the results so far archived, mating conditions favorable for colony development of *B. ignitus* were as follows. At the photoperiod regimes during mating periods, queen mated at 14L was more effective than 12L and 16L in egg-laying characteristics and colony development. In case of illumination during mating periods, intensity of 1000 lux was suitable for mating *B. ignitus* queen than at 100 lux and 200 lux. Mating temperature and care temperature of queen before mating favorable for *B. ignitus* queen were 22-25°C and 19°C, respectively. The period need to mating *B. ignitus* queen was 3 days, and the number of queen suitable per mating cage of 55 × 45 × 65 cm was 30.

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