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Temperature, Photoperiod and Illumination for Mating of the European Bumblebee, *Bombus terrersis*

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To improve mating rate of the bumblebee, *Bombus terrestris*, temperature, photoperiod and illumination during mating periods favorable for *B. terrestris* were investigated. The mating rate of queen mated at 19°C was 92.1%, which was 2.1-5.9% higher than that of 22°C and 25°C. 19°C was more effective than at 22°C and 25°C in death rate during mating periods. The survival rate after hibernation of queen mated at 19°C was 3.0-17.7% higher than that of 22°C and 25°C. At the photoperiod regimes during mating periods, queen mated at 14 L was more effective than 12 L and 16 L in death rate during mating, survival rate after hibernation, and egg-characteristics. In case of illumination during mating periods, intensity of over 1000 lux was suitable for mating *B. terrestris* queen in colony development. Therefore, we supposed that mating temperature favorable for *B. terrestris* was 19°C and photoperiod for mating was 14 L, and illumination was over 1000 lux.

Key words: Bumblebee, *Bombus terrestris*, Mating, Temperature, Photoperiod, Illumination

Introduction

Bumblebees are eusocial insects and live in colonies of up to a few hundred individuals, and have generally one generation per year. Queens are the only caste to overwinter, 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).

Mating is a decisive period in the life of bumblebees. The nuptial flight of *B. terrestris* males consists of patrolling behavior during which secretions from the cephalic labial gland are deposited on marking spots. Males establish nuptial flight circuits by scent-marking plants and they attempt to copulate with young queens encountered on their flight-path (Svensson, 1980; Williams 1991). The active component of male pheromone secreted from the cephalic labial gland has been identified as farnesol. Young queens emit sex pheromones from mandibular glands (Honk *et al.*, 1978; Bergstrom, 1981). A precopulatory ethogram for *B. terrestris* in a cage was produced by Djegham *et al.* (1994). They found that among the male and female behavioral sequences analyzed, antennal inspection by both sexes and queen mobility were key factors in the mating success. Other authors reported on the optimal age for mating in *B. terrestris* (Duchateau, 1985), and the sperm content and transfer of spermatheca (Röseler, 1973; Duvoisin *et al*, 1999) or vasa deferentia (Duchateau and Mariën, 1995). Tasei *et al.* (1998) reported that the relationship between aging, sexual behaviour and sperm production and transfer. Sperm length, sperm storage and mating system characteristics, and bumblebee mating plug to prevents females from remating were also reported (Boris *et al.*, 2001, 2003).

The large earth bumblebee, *B. terrestris*, which is indigenous to Europe, has been artificially introduced through-

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out the world. Since 1988, *B. terrestris* in portable boxes have been available commercially from European company for crop pollination (Mitsuhata, 2000). Colonies of *B. terrestris* have already been imported into Korea, Japan, China, Taiwan, Maxico, Chile, Argentina, Uruguay, South Africa, Morocco and Tunisia for development in greenhouses (Dafni, 1996).

Though *B. terrestris* 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. There is only a few data available on temperature, photoperiod and illumination for mating of *B. terrestris*. Therefore this study was conducted to identify mating conditions most favorable for colony development of *B. terrestris.*

Materials and Methods

Insects

Experimental insects were CO_2-treated and artificial hibernated the 6-7th generation queens of *B. terrestris* reared in a controlled climates room (27±1°C, 65% R.H. and continuous darkness). CO_2-narcosis was exposed to 99% CO_2 for 30 min daily during two consecutive days (Yoon *et al.*, 2003). For artificial hibernation, queens were hibernated for 5 months at 2.5°C to preserve them in a bottle filled with perlite and keep it around 80% R.H. (Yoon, 2003). After that, the queens were placed in flight cages for 3 days and then reared under the 27±1°C, 65% R.H. (Yoon *et al.*, 2004). The basic colony-rearing technique of *B. terrestris* was followed as described previously (Yoon *et al.*, 2002, 2004).

Mating rate and survival rate after hibernation of *B. terrestris* at different mating temperatures

To examine the optimum mating temperature of *B. terrestris*, 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 14 L, 65% R.H., and the intensity of 2000 lux with fluorescent tubes. For mating, thirty virgin queens and ninety males were introduced a wooden mating cage (55×45×65 cm) with wire mesh during one week. Queens used for the experiment were five-days-old and the males ten-days-old, which is the optimal age for mating (Duchateau, 1985; Tasei *et al.*, 1998; Yoon *et al.*, 1999). In this experiment, we allotted to 30 queens and 3 replications. Mating rate was investigated 09:00 to 17:00 at the first day of mating periods. Queens mated at mating temperatures of 19°C, 22°C, and 25°C were hibernated for 3 months, and investigated survival rate and rate of weight loss after hibernation.

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

To examine a suitable photoperiod for colony development of *B. terrestris* during mating periods, the following environmental conditions were provided. The photoperiodic regimes during mating periods were defined as 12 L (light for 12 hrs per day), 14 L (light for 14 hrs per day) and 16 L (light for 16 hrs per day). Environmental conditions in mating room were maintained at 22-23°C, 65% R.H., and the intensity of 2000 lux. The number of queens allotted to this experiment was 30 and 3 replications. After mating, *B. terrestris* queens were hibernated for 5 months at 2.5°C, and then reared in a controlled climates room (27±1°C, 65% R.H. and continuous darkness) to investigate survival rate after hibernation and developmental ability of colony. The developmental ability of each colony was estimated by death rate during mating period, preoviposition period, and rate of oviposition, 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 within 40 days were excluded from the number of oviposited colonies.

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

The illumination favorable for *B. terrestris* during mating periods was investigated. The illuminations were defined as 100 lux, 1000 lux, and 2000 lux with fluorescent tubes. The number of queens allotted to this experiment was 30. Environmental conditions in mating room were maintained at 22-23°C, 65% R.H. and 14 L. At two days after mating, queens were treated CO_2 narcosis, and then reared to investigate developmental ability of colony. The developmental ability of colony at different illuminations during mating periods was estimated as above colony development of *B. terrestris* at different photoperiods

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

Results and Discussion

Mating rate and survival rate after hibernation of *B. terrestris* at different mating temperatures

To examine optimum mating temperature of *B. terrestris*, we investigated mating rate and survival rate after artificial hibernation (Fig. 1). The death rate at 19°C, 22°C, and

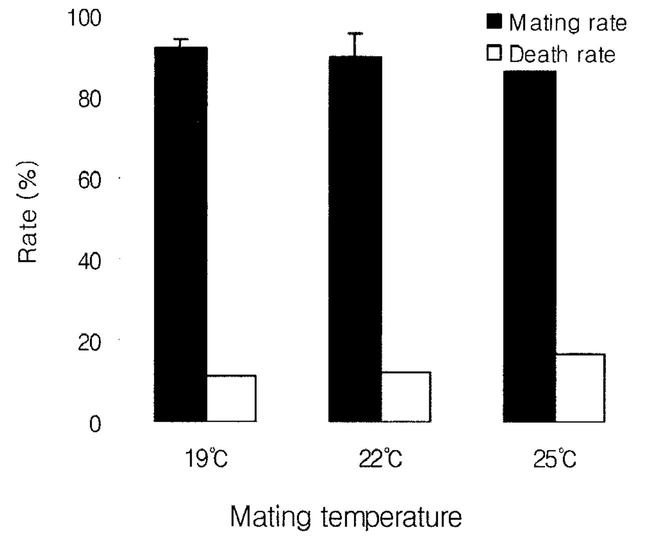

Fig. 1. Mating rate and death rate of *Bombus terrestris* queen at different mating temperatures. Mating rate was investigated 09 : 00 to 17 : 00 at the first day of mating periods. There were no significant in rate of mating and death rate of *B. terrestris* at $p<0.05$ by Tukey's pairwise comparison test and Chi-square test.

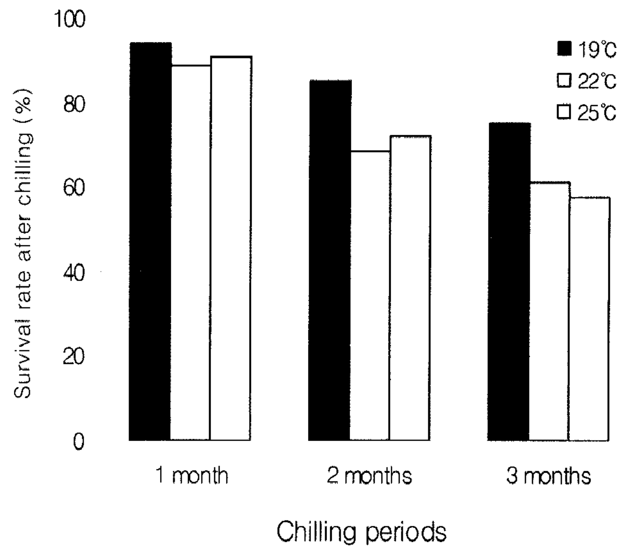

Fig. 2. Survival rate after artificial hibernation of *B. terrestris* queen mated at different mating temperatures during mating periods. There was statistically significant difference in survival rate of 2 months and 3 months after hibernation of *B. terrestris* at different mating temperatures at $p<0.05$ by Chi-square test. Thirty queens and 3 replications were allotted for mating temperature regimes

25°C during mating periods was 11.1 - 16.7%, which was not affected by mating temperature (Chi-square test: $x^2=1.346$, DF=2, $p=0.510$). In *B. ignitus*, the death rate during mating period (7 days) is 10.0 - 12.2%(Yoon *et al.*, 2007). The mating rate of queen mated at 19°C was 92.1%, which was 2.1 - 5.9% higher than those of 22°C and 25°C, and low in order of 22°C and 25°C. The higher temperature is, the lower mating rate is. There was no statistical difference at mating temperatures, 19°C, 22°C, and 25°C (Tukey's pairwise comparison test: F=2, 13, DF=2, 6, $p=0.200$), but statistically significant difference between 19°C and 25°C (F=24.17, DF=1, 4, $p=0.008$). Fig. 2 showed the survival rate after artificial hibernation examined with the 2nd generation queens produced from different mating temperatures. The survival rate after one month, 2 months and 3 months at 19°C was high as 93.7%, 85.0% and 75.0%, respectively, and it was 3.0 - 17.7% higher than that of 22°C and 25°C. The survival rate after 2 months and 3 months was affected by mating temperature (2 months: $x^2=6.519$, DF=2, $p=0.038$; 3 months: $x^2=6.019$, DF=2, $p=0.049$). The change of weight loss at before and after hibernation was examined. The rate of weight loss after artificial hibernation during 3 months was 9.4 - 11.4% (Table 1). Horber (1961) found an average weight loss during hibernation was 151.3 mg. Furthermore he proved that surviving queens were heavier than queens which died during hibernation. Alford (1969 a, b) also found 57% water in bumblebee queen following hibernation and a reduction of about 50% in both live weight and dried weight. The fat makes up an average of 34% of the total dry weight of the bumblebee queens prior to hibernation and 80% of this fat is absorbed during hibernation. A well developed fat body in a queen bumblebee will thus be of importance to safe hibernation. With above results about mating rate, death rate during mating periods and survival rate after hibernation of *B.*

Table 1. Change of weight after artificial hibernation of *B. terrestris* queen mated at different mating temperatures during mating periods

Mating temperature (°C)	n	Change of weight in artificial hibernation		
		Before (g)	After (g)	Rate of weight loss (%)
19	59	0.85±0.09	0.77±0.08	9.4
22	48	0.97±0.10	0.86±0.09	11.3
25	43	0.96±0.08	0.86±0.08	11.4

1) The period of artificial hibernation was 3 months.
2) n means the number of surveyed.

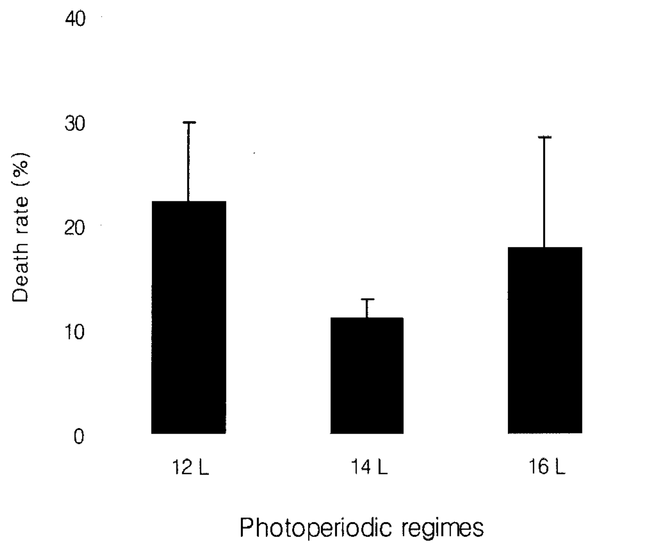

Fig. 3. Death rate of *B. terrestris* queen at different photoperiods during mating periods. There was no significant difference in death rate of *B. terrestris* queen at $p<0.05$ by Tukey's pairwise comparison test.

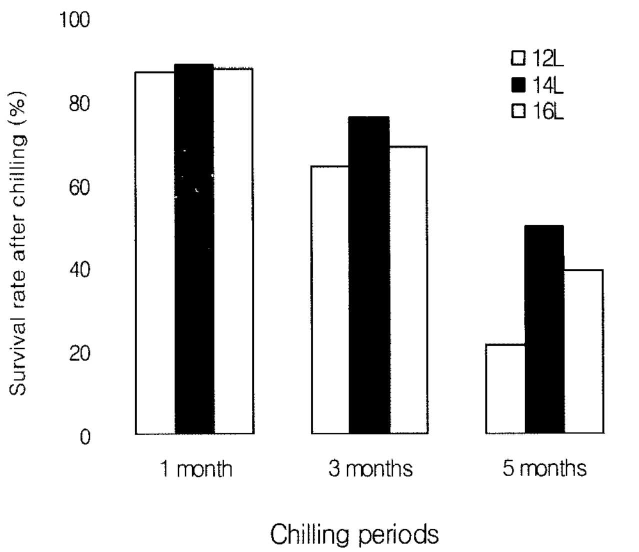

Fig. 4. Survival rate after artificial hibernation of *B. terrestris* queen mated at different photoperiod durings mating periods. There was statistically significant difference in survival rate of queen *B. terrestris* hibernated for 5 months at different mating temperatures at $p<0.05$ by Chi-square test. Thirty queens and 3 replications were allotted for this mating photoperiod regimes

terrestris queen, we think that 19°C was more effective than at 22°C and 25°C in mating temperature of *B. terrestris*. The result was similar to a previous report on the western bumblebee, *B. terrestris*, which was mated in 20°C (Djegham *et al.*, 1994) and 18°C (Tasei *et al.*, 1998). But it was different with that of *B. ignitus*, which was the highest at 25°C and low in order of 22°C and 19°C although mating rate was low than that of *B. terrestris* (Yoon *et al.*, 2007). 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. terrestris* at different photoperiods during mating periods

It was investigated the photoperiod favorable for colony development of *B. terrestris* during mating periods. In the photoperiodic regimes of 12 L, 14 L and 16 L under in 22 - 23°C, 65% R.H., and the intensity of 2000 lux, the death rate of queen mated at 14 L during mating periods was 11.0%, which was 6.8 - 11.2% lower than that of 12 L and 16 L although there was no statistical difference ($F=1.58$, $DF=2, 6$, $p=0.280$) (Fig. 3). The survival rate after hibernation during 1, 3 and 5 months was high at 14 L as 88.7%, 76.2% and 50.0%, respectively and low in order of 16 L and 12 L. There was statistically significant difference in 5 months at different photoperiods ($x^2=24.021$, $DF=2$, $p=0.001$) (Fig. 4). The change of weight loss at before and after artificial hibernation was examined. The rate of weight loss after artificial hibernation during 3 months was 8.3 - 11.8%, 5 months is 20.2 - 24.7% (Table 2). Hoem (1972) reported that the weight losses occurred during the first half of the hibernation period after which time the body weight increased. Significant, positive correlations were found between the body weight

Table 2. Change of weight after artificial hibernation of *B. terrestris* queen at different photoperiod during mating periods

Chilling periods (month)	Photoperiod regimes	n	Change of weight in artificial hibernation		
			Before (g)	After (g)	Rate of weight loss (%)
3	12 L	47	0.84±0.11	0.78±0.11	8.3
	14 L	62	0.85±0.10	0.75±0.09	11.8
	16 L	50	0.85±0.09	0.77±0.08	9.4
5	12 L	14	0.85±0.14	0.65±0.12	23.5
	14 L	31	0.85±0.09	0.64±0.08	24.7
	16 L	24	0.84±0.19	0.67±0.08	20.2

1) n means the number of surveyed.

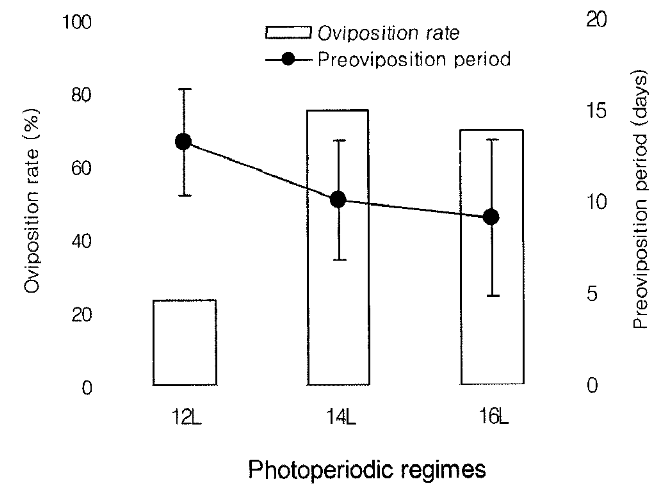

Fig. 5. Oviposition rate and preoviposition period after hibernation of *B. terrestris* queen mated at different photoperiods during mating periods. The period of artificial hibernation was 5 months. There were significant differences in oviposition rate of *B. terrestris* at $p<0.05$ by Chi-square test.

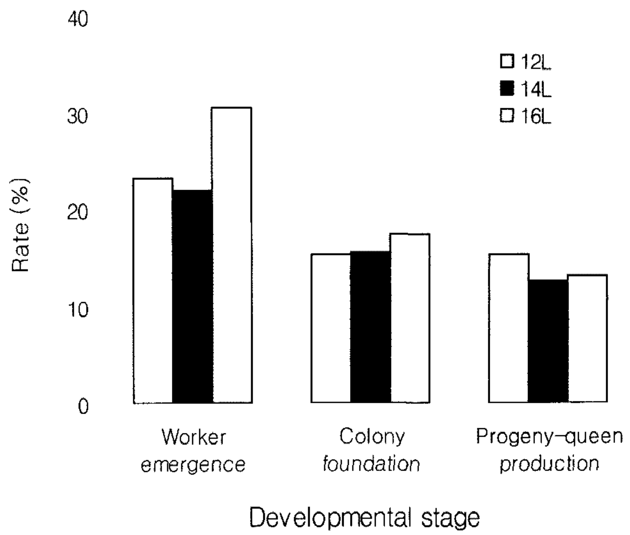

Fig. 6. The colony development after hibernation of *B. terrestris* queen at different photoperiod during mating periods. There were no significant differences in rate of worker emergence, colony foundation and progeny-queen production of *B. terrestris* at different mating temperatures at $p<0.05$ by Chi-square test.

of the queens and the length of the period of survival during and after hibernation respectively. The weight of *B. terrestris* queens prior to hibernation varied from 400 mg to 1000 mg and after hibernation 300 mg to 900 mg. Similarly the weight of *B. lapidarius* queens prior to hibernation was 250 - 850 mg and after hibernation 250 - 750 mg.

The egg-characteristics and colony development of *B. terrestris* at different mating photoperiods were investigated. The oviposition rate of queen mated at 14 L during mating periods was 75.0%, which was 5.4 - 51.8% higher than that of 12 L and 16 L. The oviposition rate was affected by photoperiod during mating ($x^2=11.319$, DF=2, $p=0.003$) (Fig. 5). The preoviposition periods in the photoperiodic regimes of 12 L, 14 L and 16 L were 9.1 - 13.3 days (F=1.62, DF=2, 35, $p=0.212$) (Fig. 5). In the rate of worker emergence, queen mated at 16 L was 30.4%, which was 7.3 - 8.5% higher than that of 12 L and 14 L although there was no statistical difference ($x^2=2.684$, DF=2, $p=0.493$) (Fig. 6). The colony foundation and progeny-queen production were 15.4 - 17.4% and 12.5 - 15.4%, respectively (colony foundation: $x^2=0.054$, DF=2, $p=0.973$; progeny-queen production: $x^2=0.068$, DF=2, $p=0.967$) (Fig. 6).

With above results, we supposed that 14 L at photoperiodic regimes during mating periods was more effective than that of 12 L and 16 L in death rate during mating, survival rate after hibernation, and egg-characteristics although colony development was not different. This result showed a similar tendency with that of *B. ignitus* (Yoon *et al.*, 2007). In nature, bumblebee was mostly mated at the late summer to early autumn and Korean photoperiod at this session was about 14 L.

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

We investigated the illumination favorable for colony

Table 3. Death rate during mating period, preoviposition period and colony foundation period after CO_2 treatment of *B. terrestris* queen mated at different illuminations during mating periods

Illumination (lux)	Death rate during mating period (%)	n	Preoviposition period (days)	n	Colony foundation period (days)
100	20.0	20	12.0±8.4	5	67.0±13.0
1000	20.0	25	12.1±6.0	8	59.5±11.2
2000	16.7	26	8.0±5.6	8	60.0±13.8

1) There were no significant differences in death rate during mating period, preoviposition period and colony foundation period of *B. terrestris* at different illuminations during mating periods at $p<0.05$ by Tukey's pairwise comparison test and Chi-square test.
2) n means the number of surveyed.
3) Thirty queens and 3 replications were allotted for this experiment.

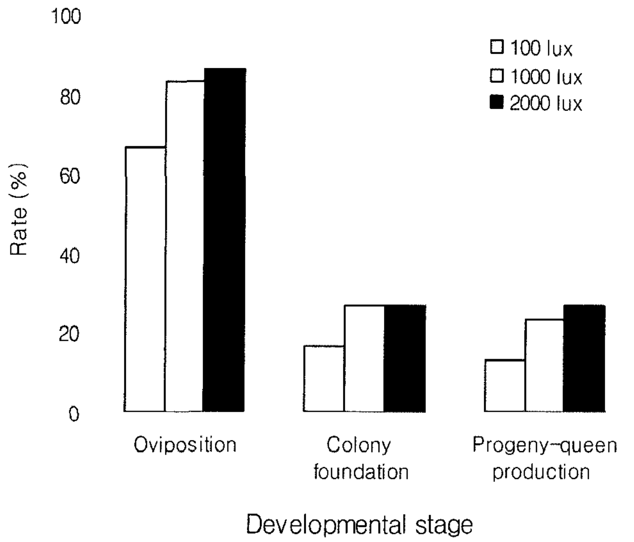

Fig. 7. Colony development after CO_2 treatment of *B. terrestris* queen mated at different illuminations during mating periods. There was no significant difference in colony development of *B. terrestris* at different illuminations during mating periods at $p<0.05$ by Chi-square test.

development of *B. terrestris* during mating periods. Among illuminations of 100 lux, 1000 lux and 2000 lux, the death rate during mating periods at 2000 lux was 16.7%, which was 3.3% lower than that of 100 lux and 1000 lux. But it was not affected by the illumination during mating periods (x^2=0.145, DF=2, p=0.930) (Table 3). The preoviposition periods of 2000 lux was 8.0 days, which was also 4.0-4.1 days earlier than that of 100 lux and 1000 lux (F=1.62, DF=2, 35, p=0.212). In the period of colony foundation, queen mated at 100 lux was 7.0-7.5 days later than that of 1000 lux and 2000 lux (F=0.49, DF=2, 16, p=0.49) (Table 3). In case of colony development, the rate of oviposition, colony foundation and progeny-queen production of queens mated at 2000 lux were 86.7%, 26.7% and 26.7%, respectively, which was 1.3-2.0 fold higher than that at 100 lux. But colony development of queen mated at 1000 lux was a similar tendency with that of 2000 lux. The colony development of *B. terrestris* was not affected by illumination during mating (rate of oviposition: x^2=0.460, DF=2, p=0.144; rate of colony foundation: x^2=0.723, DF=2, p=0.697; rate of progeny-queen production: x^2=1.459, DF=2, p=0.482) (Fig. 7). Above results showed that the illumination favorable for colony development of *B. terrestris* during mating periods is over 1000 lux.

Djegham *et al.* (1994) reported that *B. terrestris* was mated in copulation room maintained as 20°C, 70% R.H. and light intensity of 2000 lux with halogen lamp.

In view of the results so far archived, mating conditions favorable for colony development of *B. terrestris* were as follows. Mating temperature favorable for *B. terrestris* queen were 19°C. 19°C was more effective than at 22°C and 25°C in mating temperature of *B. terrestris*. At the photoperiod regimes during mating periods, queen mated at 14 L was more effective than 12 L and 16 L in death rate during mating, survival rate after hibernation, and egg-characteristics. In case of illumination during mating periods, intensity of over 1000 lux was suitable for mating *B. terrestris* queen than at 100 lux in colony development.

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