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Oviposition and Colony Development of the Bumblebees, *Bombus ignitus* and *B. terrestris* depending on Different Pollen

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We investigated oviposition and colony development of *Bombus ignitus* and *B. terrestris* depending on different pollen. In five kinds of pollen blend, the colony development in fresh-freezing pollen blend produced in Korea (Korea-FFP) was the best performance, although egg-laying characteristics is lower than that of pollen blend for oviposition imported from Korppert company (Kopport-FOP). The Kopport-FOP proved that it was suitable to use for oviposition of bumblebees. The Korea-FFDP, freezing dried-fresh pollen blend produced in Korea, is lower rather than the Korea-FDP although it is similar to the Kopport-FOP in colony development. It is not efficient to use commercial pollen for bumblebee because it is expensive in cost. The dried pollen blend for honeybee feeding imported from China (China-DP) was not suitable for rearing of bumblebee because it did not form colony although the worker emerged. In types of pollen, the oviposition and colony development of *B. ignitus* were not affected by the fresh-freezing pollen and dried-freezing pollen. This result also indicated that dried pollen, dried in the shade for 5 – 6 days, is possible to use as commercial pollen for bumblebee reproduction.

Key words: Pollen, Bumblebee, *Bombus ignitus*, *Bombus terrestris*, Oviposition, Colony development

Introduction

Bumblebees are eusocial insects and live in colonies of up to a few hundred individuals. Bumblebees do not communicate spatial co-ordinates of food sources as honeybees do with the waggle dance, but successful bumblebee foragers do inform nestmates about the general availability and the scent of rewarding food sources (Dornhaus and Chittka, 1999, 2001).

In artificial mass-rearing of bumblebee, every author used pollen and honey obtained from honeybee hives (Griffin *et al.*, 1991; Ono *et al.*, 1994; Tasei and Aupinel, 1994; Hannan *et al.*, 1998), though the supplying method varied a little among various experiments.

Pollen is used as food by a diverse of flower-feeding animals, providing nutrition through its rich content of proteins, lipids, carbohydrates, as well as vitamins and minerals (Stanley and Linskens, 1974). The nutritive material of pollen is located mainly in the grain's protoplasm, but the inner wall, which may be rich in proteins, and the lipid-rich pollenkitt, which coats the outside of the indigestible outer wall, may also provide nutrients (Stanley and Linskens, 1974; Klungness and Peng, 1984). Since the often thick and sculptured outer wall is highly resistant to chemical degradation (Southworth, 1990), pollen nutrients are removed and digested primarily through the grain's germination pores. The nutritional value of pollen to bees appears to depend on both the chemical composition of the pollen and the digestive equipment of the insect (Herbert *et al.*, 1980; Schmidt and Buchmann, 1985; Loper and Cohen 1987). For bumblebees, pollen is considered to be a non-energetic resource, which primarily provides proteins for female egg production and/or larval growth (Plowright and Pendrel, 1977). Proteins are required for oogenesis in *Bombus terrestris* wokers, and also influence adult size (Sutcliffe and Plowright, 1988; Duchâteau and Velthuis, 1989).

In commercial rearing, bumblebee colonies are normally fed on fresh pollen collected from pollen traps at honeybee hives. However, because fresh pollen is not

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easy to obtain all the year round, dried frozen pollen is a possible alternative. Dried frozen pollen has advantages over fresh pollen in the sense that it is cheaper and is less likely to become contaminated by fungi and other microorganisms (Ribeiro *et al.*, 1996). On the other hand, it is unattractive to queens probably because of its unpalatability (Pomeroy and Plowright, 1980). In fact, many authors have advocated the use of fresh pollen to initiate colonies in captivity (Plowrigt and Jay, 1966; Röseler, 1985). Recently, Ribeiro *et al.* (1996) found that drying pollen, assumed to modify some amino acids, lipids, or vitamins of the pollen, affected the reproductive capacities of *Bombus terrestris* queens. But the degree of impairment depends mainly on the drying methods (Groot, 1953). For this reason, it is recommended that the temperature during the drying process should not exceed 45°C and the moisture should be removed gradually (Chambers, 1990). Therefore, the main objective of this paper is to investigate whether or not different kinds and types of pollen have any effects on oviposition and colony development of *Bombus ignitus* and *B. terrestris.*

Materials and Methods

Origin of experimental insects

Experimental insects were CO_2-treated 2nd generation queens of *Bombus ignitus* and artificially hibernated 2nd *B. terrestris* obtained from *B. ignitus* and *B. terrestris* colonies year-round reared in a controlled climates room (28°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 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 28°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.

Fifty 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* and *B. terrestris* depending on different pollen

To examine the effect of kinds of pollen blend on oviposition and colony development of *B. terrestris*, five different pollen blend were supplied *ad libittum* to the queens of *B. terrestris*: a general commercial fresh pollens blend (Kopport-CFP) and for oviposition (Kopport-FOP) imported from Korppert company in Netherland, dried pollen blend for honeybee feeding imported from China (China-DP), a fresh pollen blend (Korea-FFP), and freezing dried fresh pollen blend (Korea-FFDP), collected by honeybees in South and Middle part of Korea. Those were frozen at −70°C until before used in experimental. A fresh-pollen blend collected in Korea contained pollens collected from various flowers including *Actindia arguta*, Quercus, *Rosa polyantha*, *Weigela subsessillis*, *Securinega suffraticosa* and *Humulus japonicus*. Although kinds of pollen differed in composition, they could be considered of good quality. The numbers of *B. terrestris* queens allotted to this experiment were 30 and the average weight of queens in kinds of pollen was 0.67 to 0.71 g. However there was no statistical difference between them (Tukey's pairwise comparison test: $F = 1.16$, $df = 2, 23$, $p = 0.332$).

In the other hand, we investigate three types of pollen, one fresh and another dried, the other mixed with fresh and dried pollen (1: 1). Pollen was *Actindia arguta* collected from apiary located in middle part of Korea. The collected fresh pure pollen was frozen immediately at −70°C and dried pure pollen was dried in the shade for 5 – 6 days, and then kept frozen immediately at −70°C as the fresh pollen. The numbers of *B. ignitus* queens allotted to this experiment were 45.

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. ignitus* and *B. terrestris* by effect of kinds and types of pollen. 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

Oviposition and colony development of *B. terrestris* depending on different pollen blend

We investigated the effect of five kinds of pollen blend on oviposition characteristics, oviposition rate and preoviposition periods, of artificially hibernated *B. terrestris* queen (Fig. 1). The nutritional value of the pollens can be estimated by measuring egg laying. The rate of oviposition of fresh-freezing pollens blend for oviposition imported from Kopport (Kopport-FOP) showed the best performance as 83.9% among the other pollens and decreased in the order of the fresh-freezing pollen blend of Korea (Korea-FFP, 80.0%), and freezing-dried-fresh pollen blend of Korea (Korea-FFDP, 77.4%), a general commercial fresh-freezing pollen blend imported from Kopport company (Kopport-CFP, 71.0%), and dried-freezing pollen blend for honeybee feeding imported from China (China-DP, 66.7%). There was no significant differences in the oviposition rate of *B. terresris* queen in affect of kinds of pollen blend at $p < 0.05$ by Chi-square test ($x^2 = 3.943$, df = 4, $p = 0.414$) (Fig. 1). In case of preoviposition period, the Kopport-FOP was 10.7 days, which was also 2.1 – 4.9 days earlier than that of the other kinds of pollen groups (Fig. 1). But we observed on significant differences between the treatment of kinds of pollen blend at $p < 0.05$ by Tukey's pairwise comparison test (F = 1.06, df = 4.108, $p = 0.381$). In the egg laying characteristics, the Kopport-FOP showed the best results among the other pollens although statistical significant differences between the treatment, and was suitable to use for oviposition of bumblebees.

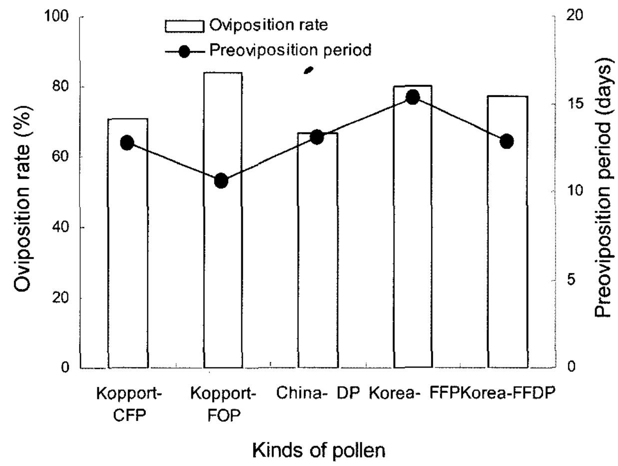

Fig. 1. Oviposition rate and preoviposition period of *Bombus terrestris* at kinds of pollen blend. Pollen was used a commercial pollen blend. Abbreviations: Kopport-CFP, commercial fresh pollen imported from Kopport company; Kopport-FOP, fresh-oviposition pollen imported from Kopport company; China-DP, drying pollen imported from China for honeybee feeding; Korea-FFP, fresh-freezing pollen produced in Korea; Korea-FFDP, fresh-freeing dryed pollen produced in Korea. For the statistical analysis, a Chi-square test and Tukey's pairwise comparison test were used. There were no significant at oviposition rate and preoviposition period of *B. terrestris* at kinds of pollen. The numbers of *B. terrestris* queens allotted to this experiment were 30.

The colony development, rates of worker emergence, colony foundation and progeny-queen production, by kinds of pollen blend was investigated (Fig. 2). The rate of worker emergence of Korea-FFP was the highest among the other treatments as 67.7% and the Kopport-CFP was the lowest among the other pollens as 19.4%. The rate of worker emergence of *B. terrestris* was significantly different by kinds of pollen blend ($x^2 = 16.288$ df = 4, p = 0.003) (Fig. 2). In case of the colony foundation rate, the Korea-FFP showed the best performance as 33.3%, which was 1.3 – 33.3 fold higher than that of the other kinds of pollen groups and decreased in the order of the Kopport-FOP, Korea-FFDP, Kopport-FOP and China-DP. The

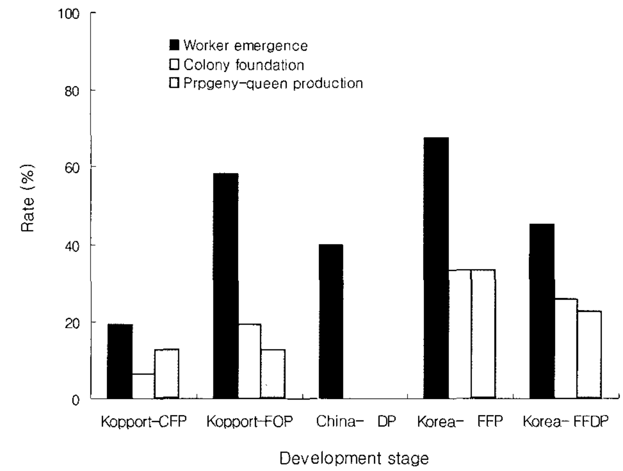

Fig. 2. Comparison of colony development of *B. terrestris* at kinds of pollen blend. For abbreviations, see legend to Fig. 1. For the statistical analysis, a Chi-square test was used for each developmental stage: $x^2 = 16.288$, $p > 0.05$ for worker emergence; $x^2 = 16.092$ $p > 0.05$ for colony foundation; $x^2 = 13.616$, $p > 0.001$ for progeny-queen production.

China-DP didn't have colony emerged more than 50 workers in a colony at all although the rate of worker emergence was 40.0%. The colony foundation rate of *B. terrestris* was very affected by kinds of pollen blend (x^2 = 16.092, df = 4, p = 0.003) (Fig. 2). The rate of progeny-queen production was also compared depending on kinds of pollen. As shown in Fig. 2, the rate of progeny-queen production of the Korea-FFP was 33.3% and this value was also 1.5 – 33.3 fold higher than that of the kinds of pollen. The China-DP resulted in a zero protein efficacy in progeny-queen production. There was statistically differences in the progeny-queen production rate of *B. terrestris* queen in effect by pollen at $p < 0.05$ by Chi-square test (x^2 = 13.616, df = 4, p = 0.009).

Table 1 shows the duration up to colony foundation and adult emergence from *B. terrestris* queen at kinds of pollen blend. The duration up to colony foundation of the Korea-FFP was 58.8 days. It was 7.2 – 16.2 days shorter than that of the other four kinds of pollens and longed in the order of the Korea-FFDP, Kopport-CFP and Kopport-FOP. But the colony foundation period was not affected by kinds of pollen blend (F = 2.53, df = 3, 19, p = 0.088). The period up to first worker emergence was 26.1 days, which was 6.9 – 12.7 days shorter in the Korea-FFP than that of the other four kinds of pollens. In normal colony, the worker emerges within 25 days but emerged a little late in this experiment (Yoon *et al.*, 2003). The period of first worker emergence was significantly difference at $p <$ 0.001 by Tukey's pairwise comparison test (F = 6.58, df = 4, 60, p = 0.001). The period up to first male emergence was the shortest at the China-DP as 58.5 days and the latest in the Kopport-CFP. However there was no statistical difference between them (F = 0.61, df = 4, 37, p = 0.657). Besides, the period of first queen emergence was not either affected by kinds of pollen blend although the period of queen emergence at the Kopport-CFP was 3.7 – 17.3 days shorter than that of the other kinds of pollen blend (F = 2.24, df = 3, 21, p = 0.114).

The number of adults produced from *B. terresrtris* queen at kinds of pollen blend was surveyed with more than 50 workers emerged colonies (Table 2). The numbers of worker produced at four kinds of pollen except the China-DP was 81.5 – 99.0 (F = 1.02, df = 3, 20, p = 0.406). In case of the number of males produced, the Korea-FFDP

Table 1. Duration up to preoviposition, colony foundation, and adult emergence from queens of *B. terrestris* at kinds of pollen blend

Kinds of pollen blend	n[a]	Colony Foundation (days)[b]	First adult emergence (days)[b]					
			n[a]	Worker	n[a]	Male	n[a]	Queen
Kopport-CFP	22	71.7 ± 25.7	2	38.3 ± 6.4 [a]	4	83.5 ± 19.0	4	76.0 ± 16.2
Kopport-FOP	26	75.0 ± 7.8	6	33.0 ± 5.8 [ab]	12	71.1 ± 14.5	4	93.3 ± 16.7
China-DP	19	-	-	38.8 ± 10.6 [a]	2	58.5 ± 3.5	-	-
Korea-FFP	23	58.8 ± 9.2	8	26.1 ± 6.2 [bc]	4	70.7 ± 20.5	10	79.7 ± 12.4
Korea-FFDP	23	66.0 ± 7.5	8	33.0 ± 7.2 [abc]	5	71.0 ± 23.6	7	93.3 ± 14.4

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.05$ by Tukey's pairwise comparison test.

Table 2. Number of adults produced from queens of *B. terrestris* at kinds of pollen blend

Kinds of pollen blend	Number of adults produced						n[a]	Longevity of foundation queen (days)[b]
	n[a]	Worker[b]	n[a]	Male[b]	n[a]	Queen[b]		
Kopport-CFP	2	99.0 ± 7.1	2	127.0 ± 0.0	4	14.8 ± 7.6	22	65.2 ± 34.1 [abc]
Kopport-FOP	6	89.0 ± 15.1	6	156.0 ± 40.1	5	10.0 ± 9.9	22	80.9 ± 36.3 [ab]
China-DP	-	-	-	-	-	-	21	61.5 ± 32.5 [ac]
Korea-FFP	8	87.5 ± 16.4	8	198.1 ± 66.4	5	10.0 ± 9.3	22	93.1 ± 34.7 [ab]
Korea-FFDP	8	81.5 ± 8.9	8	211.8 ± 73.8	8	27.6 ± 22.9	19	90.4 ± 43.2 [ab]

[a]n means the number of colony surveyed.
[b]The figures stand for means ± SD. Means followed by different letters in the same column are significantly different at $p < 0.05$ by Tukey's pairwise comparison test.
There were no significant in number of adults produced from queens *B. terrestris* at kinds of pollen

was 211.8 numbers and these values were 13.7 – 84.8 numbers more than that of the other treatments. But there was no statistical difference between them at $p < 0.05$ (F = 1.63, df = 3, 20, $p = 0.214$). In case of the number of queens produced, which is an important point in year-round rearing of bumblebee, the Korea-FFDP queen produced 27.6 numbers, which corresponded to 1.9 – 2.8 fold of that of three kinds of pollen blend. However there was no statistical difference between them (F = 2.49, df = 3, 18, $p = 0.086$). The longevity of foundation queens in the Korea-FFDP was 93.1 days, which is 2.7 – 31.6 days longer than that of the other pollens. There was statistically difference at kinds of pollen blend (F = 3.33, df = 4, 101, $p = 0.013$).

Above results showed the colony development (e.g., rate of worker emergence, colony foundation and progeny-queen production *et al.*) in the Korea-FFP was the best performance among five kinds of pollen blend, although egg-laying characteristics is lower than that of the Kopport-FOP. The Kopport-FOP proved that it was suitable to use for oviposition of bumblebees. The Korea-FFDP, freezing-dried-fresh pollen blend, is lower rather than the Korea-FDP although it is similar to the Kopport-FOP in colony development. So, we think it is not efficient to use commercial pollen for bumblebee because it is expensive. Our results also indicated the China-DP was not suitable for rearing of bumblebee because it did not form colony although the worker emerged.

The origin of the pollen diet affected reproductive process of *B. terrestris* micro-colonies. The main developmental steps were completed differently over the range of pollen diets, with effects varying in their intensity. The unifloral pollen is a significantly lower egg production than for the commercial blend (Gënissel *et al.*, 2002). More male progeny are produced by small queenless colonies when fed on pollen with a high protein rate (Regali and Rasmont, 1995).

Oviposition and colony development of *B. ignitus* depending on types of pollen

The affect between types of pollen and oviposition characteristics of *B. ignitus* queen was investigated (Fig. 3). Pollen was used common pure pollen, *Actinidia arguta.* The rate of oviposition in the mixed frozen pollen with fresh- and dried- freezing pollen (FFP+DFP) was 88.6%, which was 6.8 – 13.6% higher than that of the fresh-freezing pollen (FFP) and the dried-freezing pollen (DFP). The rate of oviposition of the DFP is a little lower than that of the FFP. However, there was no significant difference in oviposition rate of *B. ignitus* queen at types of pollen at $p < 0.05$ by Chi-square test ($x^2 = 2.750$, df = 2, $p = 0.253$) (Fig. 3). The preoviposition period in three types of pollens was 18.3 – 19.8 days, which was not differences between the treatment of kinds of pollen at $p < 0.05$ by Tukey's pairwise comparison test (F = 1.06, df = 4.108, $p = 0.381$). In the egg laying characteristics, we don't observed significant differences between the treatments. The colony developmental characteristics by types of pollen were investigated (Fig. 4). The rate of worker emergence of the DFP was 2.4% higher than that of the FFP and the FFP+DFP but was not affected by types of pollen

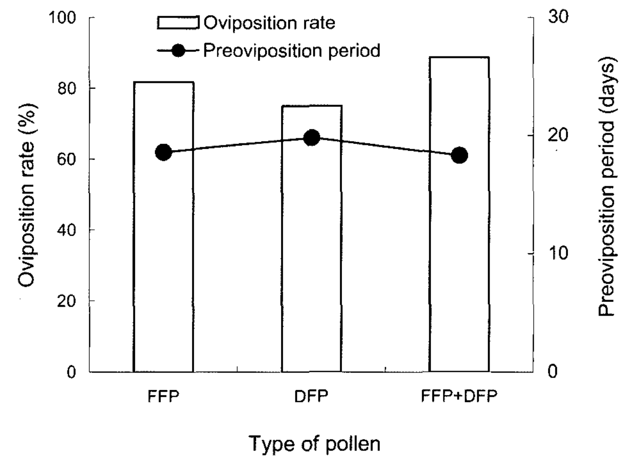

Fig. 3. Oviposition rate and preoviposition period of *B. ignitus* at types of pollen. Pollen was used common pure pollen, *Actinidia arguta*. Abbreviations: FFP, fresh-freezing pollen ; DFP, dried freezing pollen; FFP+DFP, mixed pollen with fresh and dried pollen (1: 1). There were no significant in oviposition rate and preoviposition period of *B ignitus* at types of pollen at $p < 0.05$ by Chi-square test and Tukey's pairwise comparison test. Forty four queens were allotted for each experiment.

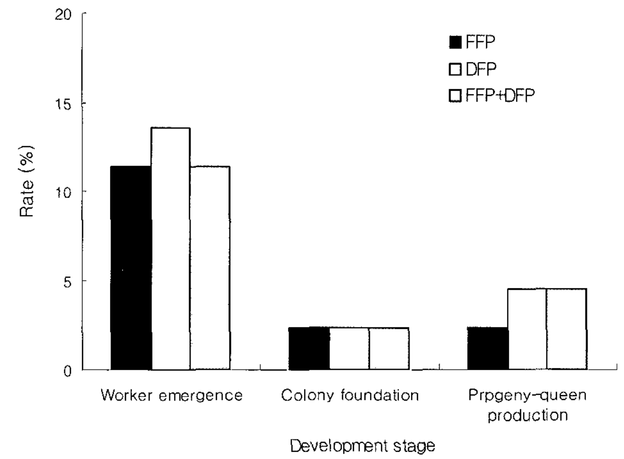

Fig. 4. Comparison of colony development of queen of *B. ignites* at type of pollen. Pollen was used common pure pollen, *Actinidia arguta*. For abbreviations, see legend to Fig. 3. There were no significant in each developmental stage of *B ignitus* at type of pollen at $p < 0.05$ by Chi-square test.

(x^2 = 16.288 df = 4, p = 0.003) (Fig. 4). The colony foundation rate of *B. ignitus* in three types of pollen was the same as 2.3% (x^2 = 0.000, df = 2, p = 1.000). The rate of progeny-queen production was also compared depending on types of pollen. As shown in Fig. 4, the rate of progeny-queen production in the FFP was lower than other treatments but not affected by kinds of pollen (x^2 = 0.416, df = 2, p = 0.812).

Above results showed that the oviposition and colony development of *B. ignitus* were not affect by the fresh-freezing pollen and dried-freezing pollen. This result also indicated that dried pollen, dried in the shade for 5 – 6 days, is possible to use commercial pollen for bumblebee reproduction. Because dried frozen pollen has advantages in the sense that it is cheaper and is less likely to become contaminated by fungi and other microorganisms than fresh pollen (Ribeiro *et al.*, 1996). However, Riberio *et al.* (1999) also reported that the pattern of colony development, and the number and biomass of workers and males were similar for colonies fed on fresh-frozen pollen (FFP) and dried-frozen pollen (DFP). But queens reared on DFP were smaller, had lower biomass, higher mortality and produced smaller colonies than queens reared on FFP.

In conclusion, the present results indicate that colony development in the Korea-FFP was the best performance among five kinds of pollen blend, although egg-laying characteristics is lower than that of the Kopport-FOP. The Kopport-FOP proved that it was suitable to use for oviposition of bumblebees. The China-DP was not suitable to rearing of bumblebee because it was not formed colony although the worker emerged. In types of pollen, the oviposition and colony development of *B. ignitus* were not affect by the fresh-freezing pollen and dried- freezing pollen.

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