

# Effect of Climatic Conditions on Pollination Behavior of Honeybees (*Apis mellifera* L.) in the Greenhouse Cultivation of Watermelon (*Citrullus lanatus* L.)

Kyeong Yong Lee<sup>1\*</sup>, Jeonghyeon Lim<sup>2</sup>, Hyung Joo Yoon<sup>1</sup> and Hyeon-Jin Ko<sup>1</sup>

<sup>1</sup>Department of Agricultural Biology, The National Institute of Agricultural Science, RDA, Wanju 55365, Republic of Korea

<sup>2</sup>Fruits & Vegetables Research Institute, The Jeollabuk-do Agricultural Research & Extension Services, Iksan 54591, Republic of Korea

(Received 9 November 2018; Revised 27 November 2018; Accepted 28 November 2018)

## Abstract

We investigated the pollination activity of honeybees (*Apis mellifera* L.) in terms of different climatic conditions in the greenhouse cultivation of watermelons (*Citrullus lanatus* L.) during winter. The aim of the study was to search a climatic condition which effectively can be use honeybees as pollinators during the flowering season of watermelons in winter or early spring. The average climatic conditions inside the greenhouse during the bee activity time (BAT)-between 10:00 and 16:00 in mid-February-were a temperature of 30.4°C, relative humidity of 53.7%, illuminance level of 22,728.4lx, and UV level of 0.233mW/cm<sup>2</sup>. Bee traffic and foraging activity were at their greatest at 10:00 and tended to decrease with time. Male watermelon flowers typically dehisced between 10:00 and 12:00. Climatic conditions were significantly correlated with bee activities, including bee traffic and foraging activity. Bee activities were positively correlated with temperature, illuminance level, and UV level but negatively correlated with relative humidity. Temperature had the greatest effect on honeybee behavior. Among the foraging honeybees, the number of high-flying bees that did not pollinate flowers showed a strong positive correlation with temperature, and the number of bees landing on the flowers showed a positive correlation with the UV level. The temperature range inside greenhouses at which the pollination activities of honeybees can be maintained efficiently during winter watermelon pollination was found to be 21~25°C.

Key words: Honeybee, Climatic conditions, Watermelon, Pollination, Temperature

## INTRODUCTION

The foraging behavior of honeybees (*Apis mellifera* L.), which collect nectar and pollen to maintain their colonies, provides multiple benefits to plant pollination (Young *et al.*, 2007). Thus, honeybees are important pollinators for increasing crop yields - they provide commercial pollination services to plants cultivated in greenhouses, such as strawberries and watermelons, besides pollinating

economical fruit crops such as almond, apples, and blueberries (Free, 1968; Kevan and Baker, 1983; Potts *et al.*, 2010). However, the foraging behavior of honeybees can be affected by factors in the external environment of the colony, such as climatic conditions or plant resources (Fulop and Menzel, 2000; Abou-Shaara, 2014). Predicting the foraging activity of honeybees in response to climatic conditions is critical for planned pollination of crops (Choi, 1987) because honeybee flight is highly related to air

\*Corresponding author. E-mail: ultrataro@korea.kr

temperature (Heinrich, 1979). Hence, many studies have been conducted on the correlations between foraging behavior of honeybees and climatic conditions such as temperature, illuminance level, and wind velocity (Vicens and Bosch, 2000; Puškadija *et al.*, 2007; Clarke and Robert, 2018).

Watermelons (*Citrullus lanatus* L.) are the most cultivated crop in South Korea, with a cultivation area of 12,661 ha in 2017. The greenhouse cultivation area for watermelons is 4 to 5 times larger than the open-field cultivation area, and greenhouse cultivation for watermelons has become common in Gyeongsangnam-do and Jeollanam-do (Ko *et al.*, 2012; Statistics Korea, 2018). Watermelons are dependent on insects for pollination due to their separate male and female flowers (Wien, 1997; Walters, 2005). In greenhouses, because natural pollination by wild bees is inefficient, watermelon cultivators perform hand pollination by human labor or pollination using honeybees for the fruit set of watermelons (RDA, 2014). Due to high labor costs, the use of honeybees for watermelon pollination is increasing gradually. Yoon *et al.* (2017) reported that honeybees used for pollination in 64.8% of the total watermelon cultivation area in 2016.

Since watermelons are high-temperature crops originating from Africa, temperature management is essential when cultivating watermelons in winter or early spring. In particular, daytime ventilation must be sufficient to prevent the temperature inside the greenhouse exceeding the upper temperature limit of watermelons owing to strong solar radiation. In contrast, when the sun sets, the watermelons must be kept warm to prevent them from reaching their lower temperature limit (Lee *et al.*, 2006). This is more challenging in winter and early spring, when solar radiation is insufficient and the level of transmitted UV may vary depending on the chosen covering material of the greenhouse. Because the cultivation of watermelons in winter or early spring is affected by the changing climatic conditions (temperature, humidity, illuminance level, and UV level) inside the greenhouse, the foraging

behavior of honeybees may change greatly according to the climatic conditions - potentially posing a problem for pollination. Therefore, to effectively use honeybees as pollinators in greenhouses during the flowering season of watermelons, we investigated the optimum climatic conditions (temperature, relative humidity, illuminance level, and UV level), for the hourly behaviors of honeybees (bee traffic and foraging activity). Based on our analysis, the factors with the greatest effects on honeybees were derived and the appropriate ranges of climatic conditions for effective pollination were determined.

## MATERIALS AND METHODS

### Insects and crops

These experiments were conducted at Wolchon-ri, Haman-gun, Gyeongsangnam-do, Korea (35.3°N, 128.3°E) from February 13 to 23, 2017. The test plot consisted of three greenhouse units (660m<sup>2</sup>/unit). For watermelons (*Citrullus vulgaris* S.), we used the “Speed-honey” cultivar for forcing culture (Nongwoo Bio, Suwon, Gyeonggi, Korea). On December 28, 2016, the watermelons were planted (154 plants per test plot) with 40cm spacing between each one. To compare pollination effects, three-way cross system honeybees (*Apis mellifera* L., 7,500 bees/hive), selected by the Rural Development Administration, were deployed on February 13, 2017 (Lee *et al.*, 2014).

### Climatic conditions in watermelon greenhouses

To investigate climatic conditions in the greenhouses, data loggers (Illuminance UV recorder TR-74Ui; T&D Co.; Matsumoto, Nagano, Japan) were installed 50 m from the entrance of the three test plots and 1.3m above-ground. The installed data loggers recorded the temperature, relative humidity, illuminance level, and UV-level inside the greenhouses at 10 min intervals from February 21 to 23, 2017.

**Table 1.** Climatic conditions in the greenhouse

Statistics**	Temperature (°C)		Relative humidity (%)		Illuminance level (lux)		UV level (mW/cm <sup>2</sup> )	
	Diurnal	BAT*	Diurnal	BAT	Diurnal	BAT	Diurnal	BAT
Mean	15.0±0.1 <sup>z</sup>	30.4±3.8	70.8±16.5	53.7±4.2	9,607±2,040	22,728±7,641	0.098±0.030	0.233±0.090
Maximum	39.2±1.6	39.2±1.6	95.2±5.4	76.3±5.8	43,826±13,888	43,826±3,888	0.420±0.199	0.420±0.199
Minimum	-0.4±0.2	22.5±3.2	40.3±4.4	40.3±4.4	0	7,049±4,047	0	0.070±0.029

<sup>z</sup>Mean ± SD.

\*BAT: bee activity time, 10:00-16:00.

\*\*Survey period: February 21~23, 2017.

**Table 2.** Honeybee traffic during the day in the greenhouse

Diurnal time	N	Incoming (Bees)	Outgoing (Bees)	Total traffic (Bees)
10:00	3	58.0±6.9 <sup>az</sup>	55.3±8.9 <sup>a</sup>	113.3±1.9 <sup>a</sup>
12:00	3	33.0±6.3 <sup>b</sup>	33.8±9.4 <sup>ab</sup>	66.8±15.5 <sup>b</sup>
14:00	3	46.0±4.1 <sup>ab</sup>	37.3±15.1 <sup>ab</sup>	83.3±19.2 <sup>ab</sup>
16:00	3	30.0±8.7 <sup>b</sup>	20.3±6.4 <sup>b</sup>	47.4±13.8 <sup>b</sup>

<sup>z</sup>Different letters after the “mean ± SD” indicate that data were significant difference among times within one-way ANOVA test and Tukey’s HSD ( $p < 0.05$ ).

### Pollinating activity and foraging characteristics

To investigate the characteristics of honeybees’ pollination activity, bee traffic through the hive entrance and foraging activity were examined at 2 h intervals from 10:00 to 16:00. The bee traffic was defined as the number of worker bees entering and exiting the hives for 5 min in each interval. The foraging activity was defined as the number of bees landing on watermelon flowers in the test plot and the number of bees flying at 1.6 m or higher for 10 min. Foraging characteristics that were investigated were the time spent on the flowers (during which the honeybees collected nectar or pollen) and the time taken to move from one flower to another. To check the dehiscence of male flowers, stamens were rubbed against filter paper at various points during the day and the amount of pollen transfer was categorized into three levels (strong, medium, weak).

### Change in bee activities according to climatic conditions inside greenhouses

To check the effects of the climatic conditions inside the greenhouses on bee activity, multiple regression analysis was conducted between temperature, relative humidity, illuminance level, UV level, bee traffic, and foraging activity to determine the most significant factors for bee

activity. In addition, partial correlation analysis was conducted for the number of bees flying high without exhibiting pollination behavior, and the number of bees landing on flowers in the context of the climatic conditions. From this, the most highly correlated climatic conditions were selected. A regression analysis was completed for the selected climatic conditions, foraging activity, and number of high-flying bees. The ranges of climatic conditions for which the pollinating activity was at its highest were derived from the regression analysis.

### Statistical analysis

An one-way ANOVA test was carried out to verify the differences between the climatic conditions (air temperature, relative humidity, illuminance level, and UV level) and the following characteristics: bee traffic (including incoming and outgoing activity), foraging activity (including landing on flowers), number of high-flying bees, time spent on a flower, and time spent traveling from one flower to another. Post-hoc analysis was carried out using Tukey’s HSD. Particularly for correlation analysis, Spearman partial correlation was performed after normality was verified through the Kolmogorov-Smirnov test. After performing correlation

**Table 3.** Foraging activity of the honeybee during the day in the greenhouse

Diurnal time	N	Landing on flower (Bees)	Flying high (Bees)	Total foraging activity (Bees)
10:00	3	21.0 ± 6.9 <sup>az</sup>	7.8 ± 3.1 <sup>ab</sup>	28.8 ± 9.5 <sup>a</sup>
12:00	3	13.3 ± 3.1 <sup>ab</sup>	4.2 ± 0.8 <sup>b</sup>	17.4 ± 3.8 <sup>ab</sup>
14:00	3	8.6 ± 1.4 <sup>b</sup>	13.6 ± 2.8 <sup>a</sup>	20.7 ± 3.7 <sup>ab</sup>
16:00	3	4.3 ± 2.0 <sup>b</sup>	8.6 ± 4.3 <sup>ab</sup>	11.6 ± 4.4 <sup>b</sup>

<sup>a</sup>Different letters after the “mean ± SD” indicate that data were significant difference among times within one-way ANOVA test and Tukey’s HSD ( $p < 0.05$ ).

**Table 4.** Time spent by *A. mellifera* on flowers and moving from flower to flower during the day in greenhouse

Diurnal time	N	Time spent on flower (Second)	Time spent moving from flower to flower (Second)
10:00	20	14.5 ± 11.3 <sup>az</sup>	3.7 ± 2.2 <sup>a</sup>
12:00	20	10.6 ± 7.8 <sup>ab</sup>	3.0 ± 2.0 <sup>ab</sup>
14:00	60	8.8 ± 7.2 <sup>ab</sup>	2.6 ± 2.1 <sup>ab</sup>
16:00	6	5.8 ± 2.8 <sup>b</sup>	1.5 ± 0.8 <sup>b</sup>
Total	106	10.1 ± 8.3	2.8 ± 2.1

<sup>a</sup>Different letters after the “mean ± SD” indicate that data were significant difference among times within one-way ANOVA test and Tukey’s HSD ( $p < 0.05$ ).

**Table 5.** Amount of pollen staining filter paper during the day

Diurnal time	N	Stained pollen on filter paper		
		Day 1*	Day 2	Day 3
10:00	10	- <sup>y</sup>	⊙	⊙
12:00	10	-	○	⊙
14:00	10	○ <sup>z</sup>	×	○
16:00	10	×	×	×

\*survey period

<sup>y</sup>“-” means that the test was not conducted.

<sup>z</sup>“⊙”, “○” and “×” mean that pollen strongly, medium and weakly stained the filter paper, respectively.

analysis, the regression equation was derived by regression analysis if a significant correlation was confirmed. For all statistical analyses, the statistics package SPSS PASW 22.0 for Windows (IBM, Chicago, IL, USA) was used.

## RESULTS

### Climatic conditions inside greenhouses during the watermelon flowering period

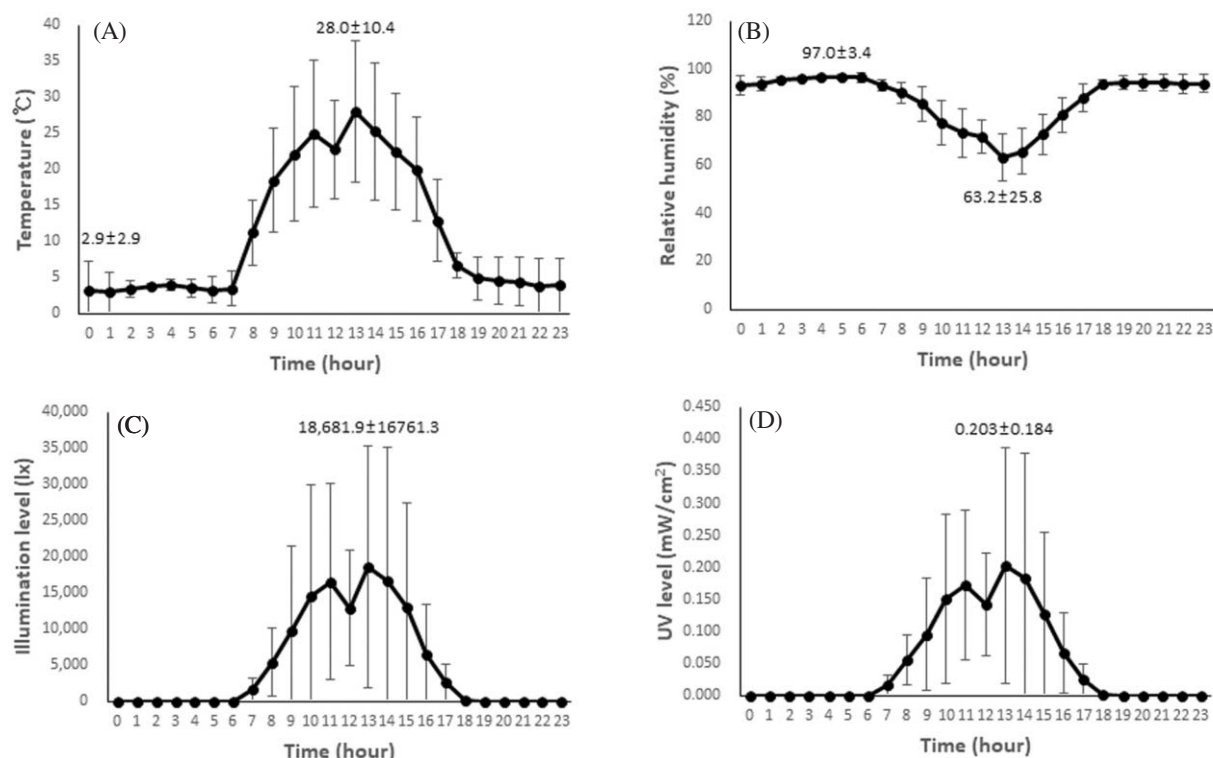
For three days of the watermelon flowering period, the average temperature was  $15.0 \pm 0.1^\circ\text{C}$ , relative humidity was  $70.8 \pm 16.5\%$ , illuminance level was  $9,607 \pm 2,040\text{lx}$ , and UV level was  $0.098 \pm 0.030\text{mW/cm}^2$ . The climatic

conditions during the bee activity time (BAT), from 10:00 to 16:00, were an average temperature of  $30.4 \pm 3.8^\circ\text{C}$ , relative humidity of  $53.7 \pm 4.2\%$ , illuminance level of  $22,728 \pm 7,642\text{lx}$ , and UV level of  $0.233 \pm 0.090\text{mW/cm}^2$ . The range of each climatic condition was as follows: the temperature ranged from  $39.2^\circ\text{C}$  to  $-0.4^\circ\text{C}$ , with a daily temperature difference of  $39.6^\circ\text{C}$ , the humidity ranged from  $95.2 \pm 5.4\%$  to  $40.3 \pm 4.4\%$ , the illuminance level reached its peak at  $43,826 \pm 13,888\text{lx}$ , and the UV level reached its peak at  $0.420 \pm 0.199\text{mW/cm}^2$  (Table 1). Daily changes of climatic conditions were then investigated: the temperature began to rise at 7:00 in the morning, maintained from 10:00 to 16:00, then sharply decreased after 16:00; the highest temperature was at 13:00 ( $28.0 \pm$

**Table 6.** Correlations between climatic conditions and bee activity

Bee activity	Air temperature		Relative humidity		Illuminance level		UV level	
	R	<i>p</i>	R	<i>p</i>	R	<i>p</i>	R	<i>p</i>
Bee traffic	0.465* <sup>z</sup>	0.069	0.937	0.135	0.307	0.248	-0.168	0.533
Foraging behavior	0.488*	0.055	0.287	0.281	-0.316	0.234	0.275	0.302
Landing on flower	0.556**	0.025	0.375	0.152	-0.244	0.362	0.125	0.645
High flying	-0.198	0.463	0.094	0.729	-0.129	0.633	0.453*	0.078

<sup>z</sup>Spearman partial correlation coefficients were calculated with bee activity and greenhouse environmental parameters as controlling variables. As each set of values was used to calculate two correlations, “\*\*” and “\*” indicate a significant correlation at the  $p < 0.05$  or  $p < 0.1$  level, respectively (2-tailed).



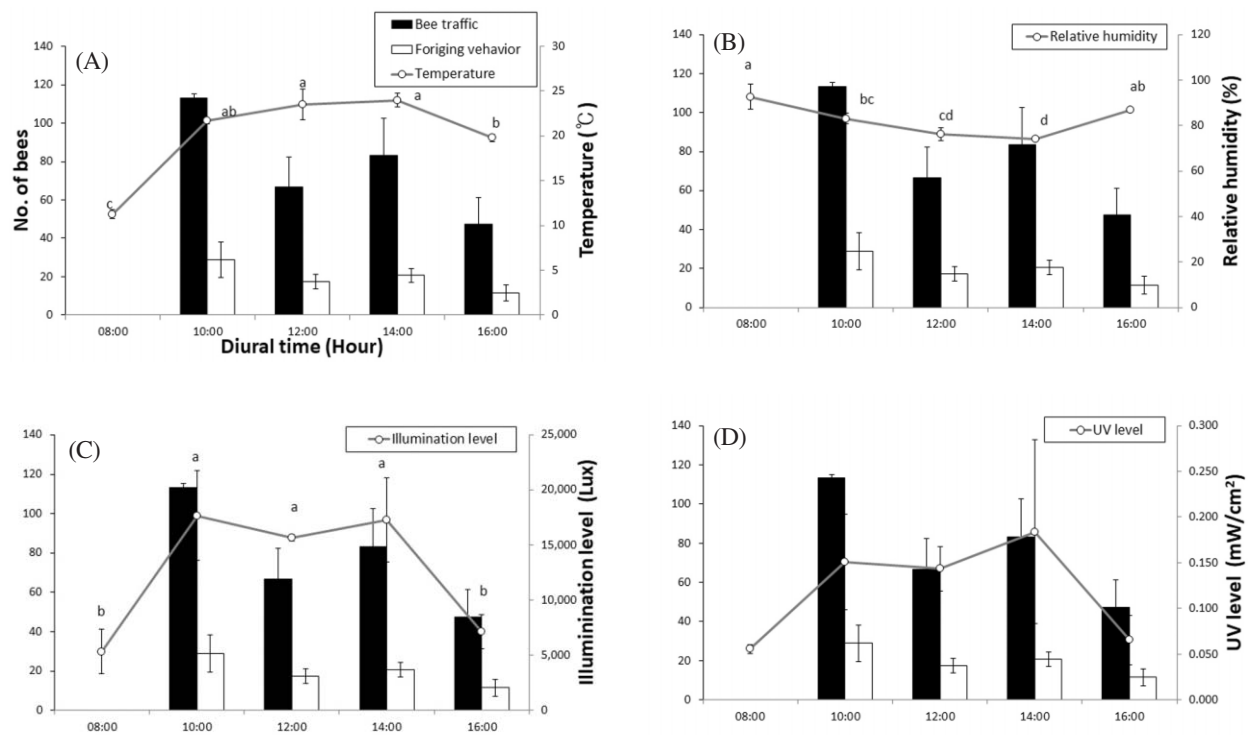
**Fig. 1.** Climatic condition during the survey period (February 21-23, 2017) in the watermelon greenhouse: (A) temperature, (B) relative humidity, (C) illuminance level, and (D) UV level.

10.4°C) and the lowest temperature was at 1:00 ( $2.9 \pm 2.9^\circ\text{C}$ ) (Fig. 1A). The relative humidity stayed at 90~97% from 18:00 to 06:00, began to decrease at 7:00, then increased again after 13:00. The highest humidity was  $97.0 \pm 3.4\%$  at 5:00, and the lowest humidity was  $63.2 \pm 25.8\%$  at 13:00. In general, the relative humidity tended to be inversely related to the temperature (Fig. 1B). The illuminance began to increase from 7:00 and continued until 13:00, then it began to decrease before sharply decreasing after 15:00. The highest illuminance level was

$18,681.9 \pm 16,761.3\text{lx}$  at 13:00. This pattern was generally similar to the temperature change (Fig. 1C). The pattern of the UV level almost coincided with the pattern of the illuminance level (Fig. 1D). The highest UV level was  $0.203 \pm 0.184\text{mW}/\text{cm}^2$  at 13:00.

### Bee activity during the day

Bee traffic showed significant differences throughout the day (one-way ANOVA test:  $F_{3,8} = 11.624$ ,  $p = 0.003$ ; Table 2). The bee traffic peaked at 10:00 ( $113.3 \pm 1.9$  bees) and



**Fig. 2.** Climatic conditions including (A) temperature, (B) relative humidity, (C) illumination, (D) UV level, and pollination activity including bee traffic and foraging behavior of *A. mellifera* in the watermelon greenhouse. Different letters above the empty cycle indicate significant difference among times within Welch's ANOVA and Tukey's HSD ( $p < 0.05$ ).

was at its lowest at 16:00 ( $47.4 \pm 13.8$ ), a 2.4-fold difference. The numbers of incoming and outgoing bees showed similar patterns as the bee traffic (incoming:  $F_{3,8} = 10.986$ ,  $p = 0.022$ ; outgoing:  $F_{3,8} = 5.713$ ,  $p = 0.022$ ). Regarding foraging activity, the number of bees landing on flowers, the number of high-flying bees, and the total foraging activity over time showed statistically significant differences (landing on flowers:  $F_{3,8} = 9.604$ ,  $p = 0.005$ ; flying high:  $F_{3,8} = 5.029$ ,  $p = 0.030$ ; total foraging activity:  $F_{3,8} = 9.604$ ,  $p = 0.005$ ; Table 3). The total foraging activity and number of bees landing on flowers were highest at 10:00, followed by 12:00, 14:00, and 16:00. Meanwhile, the number of high-flying bees was at its highest at 14:00, which was significantly higher (1.5-3.2-fold higher) than at other points during the day. When the diurnal time was examined together with other climatic conditions (temperature, illuminance level, humidity, and UV level), the bee traffic showed a similar pattern as the illuminance

level, but the foraging activity showed no similar pattern to any of the climatic conditions (Fig. 2). The time spent on flowers and the time spent moving from one flower to another were the longest at 10:00, then significantly decreased thereafter (Welch's ANOVA test: time spent on flowers  $F_{3,27.47} = 4.029$ ,  $p = 0.017$ ; time spent moving from one flower to another:  $F_{3,20.638} = 4.166$ ,  $p = 0.019$ ; Table 4). The analysis confirmed a negative correlation between the diurnal time and the time spent on flowers as well as time spent moving from one flower to another (time spent on flower:  $R = -0.223$ ,  $p = 0.022$ ; time spent moving from one flower to another  $R = -0.281$ ,  $p = 0.008$ ). The pollen mainly dehiscence between 10:00 and 12:00; after 14:00, the pollen was sparse or not observable (Table 5).

### Bee activity by climatic conditions

According to the results of the regression analysis, the bee traffic changed according to the climatic conditions

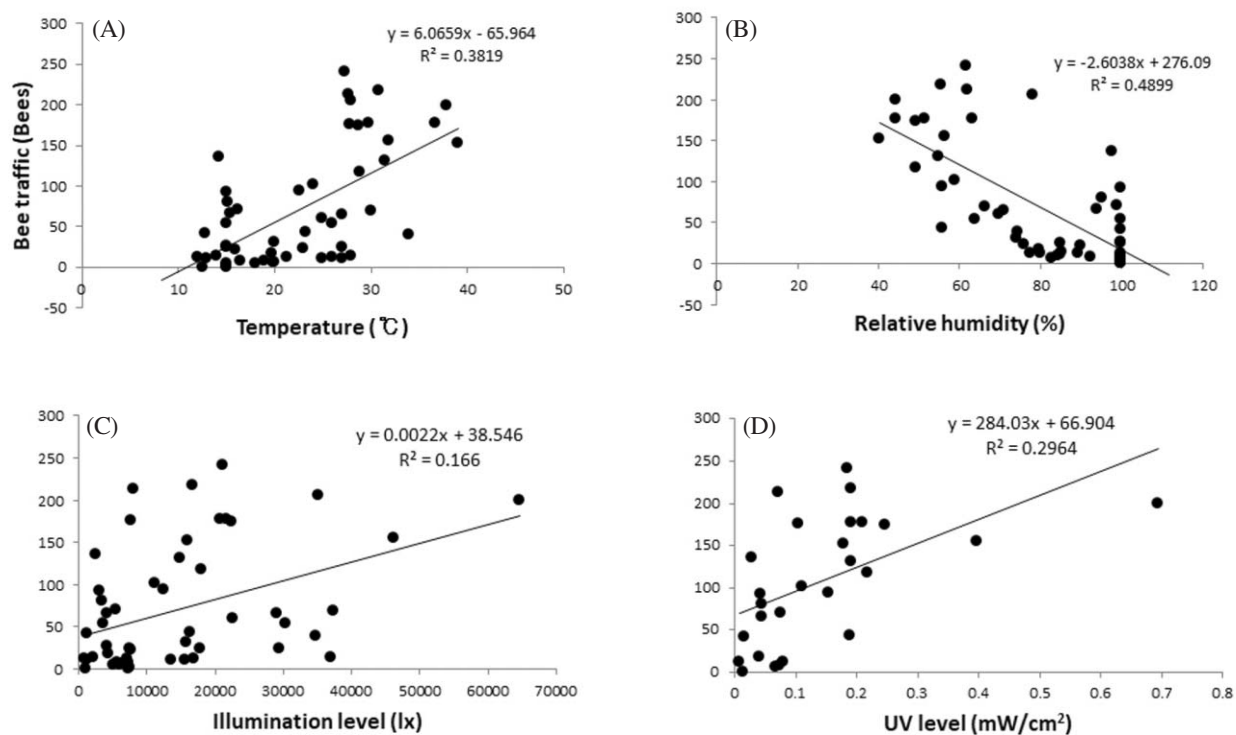


Fig. 3. Relationship between bee traffic and climatic condition: (A) temperature, (B) relative humidity, (C) illumination level, and (D) UV level.

inside the greenhouse (Fig. 3). The temperature, illuminance level, and UV level showed positive correlations with the bee traffic (temperature ANOVA test:  $F_{1,46}=22.544$ ,  $p=0.0001$ ,  $R^2=0.314$ ,  $DW=1.320$ ; illuminance:  $F_{1,46}=7.964$ ,  $p=0.007$ ,  $R^2=0.166$ ,  $DW=1.392$ ; UV level:  $F_{1,23}=8.880$ ,  $p=0.007$ ,  $R^2=0.296$ ,  $DW=2.281$ ). In contrast, the relative humidity showed a negative correlation with bee traffic ( $F_{1,46}=36.630$ ,  $p=0.0001$ ,  $R^2=0.489$ ,  $DW=1.602$ ; Fig. 3B). As with the bee traffic, the foraging activity also showed positive correlations with temperature, illuminance level, and UV level (temperature:  $F_{1,50}=18.548$ ,  $p=0.0001$ ,  $R^2=0.271$ ,  $DW=1.487$ ; illuminance:  $F_{1,50}=11.288$ ,  $p=0.002$ ,  $R^2=0.184$ ,  $DW=1.494$ ; UV level:  $F_{1,50}=11.288$ ,  $p=0.002$ ,  $DW=1.556$ ; Fig. 4). In contrast, the relative humidity showed a negative correlation with the foraging activity ( $F_{1,50}=12.991$ ,  $p=0.001$ ,  $R^2=0.190$ ,  $DW=1.654$ ; Fig. 4B). The ranges of the climatic conditions during the BAT were 12.1~39.8°C for temperature, 40~100% for

relative humidity, 914~64,700lx for illuminance level, and 0.009~0.694mW/cm<sup>2</sup> for UV level.

#### Climatic conditions affecting bee activity

According to the results of the multiple regression analysis (variable selection procedure: stepwise selection), the factor that had the largest effect on bee traffic and foraging activity was temperature. Furthermore, when the regression coefficients of climatic conditions - bee traffic and climatic conditions - foraging activity were compared, the bee traffic had a higher correlation with the climatic conditions than with foraging activity. However, temperature, relative humidity, illuminance level, and UV level showed higher correlations with each other ( $R>0.8$ ). Therefore, partial correlation analysis was conducted to examine the correlations between the climatic conditions and bee activity (Table 6). Temperature and high-flying behavior had a clear significant correlation ( $p<0.05$ ), so a second-order regression equation was derived. At a

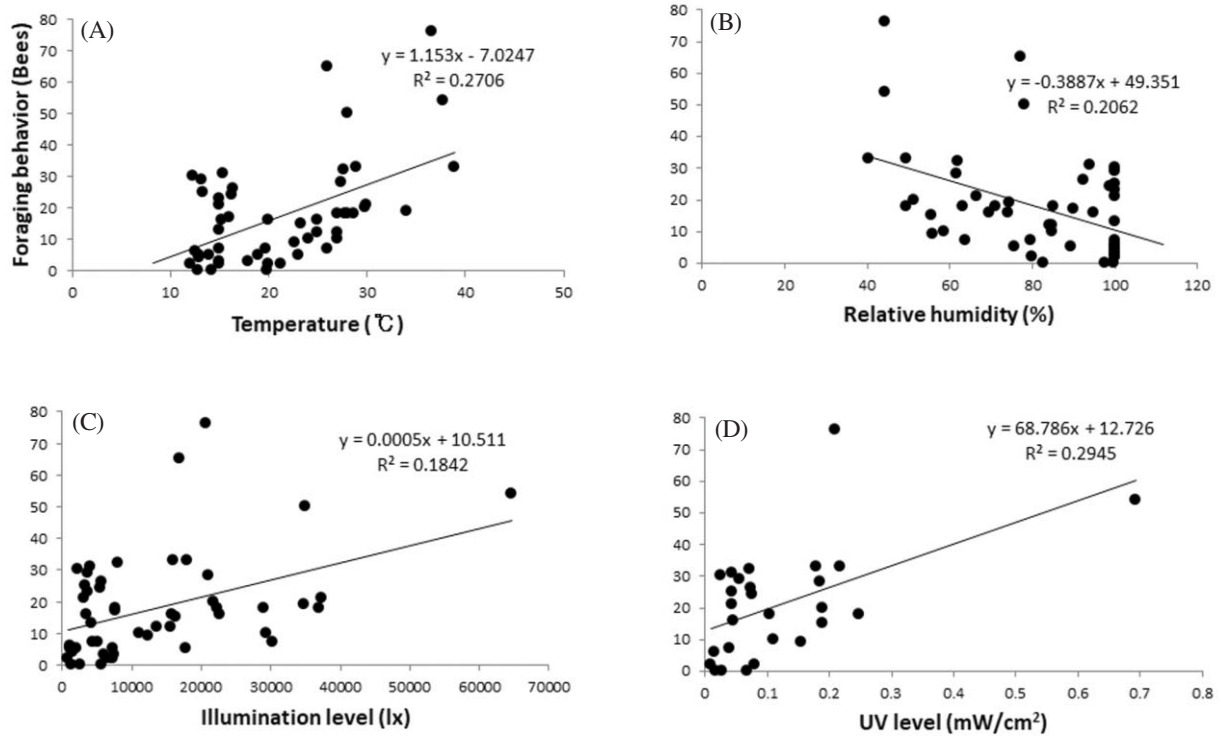


Fig. 4. Relationship between foraging behavior and climatic condition: (A) temperature, (B) relative humidity, (C) illumination level, and (D) UV level.

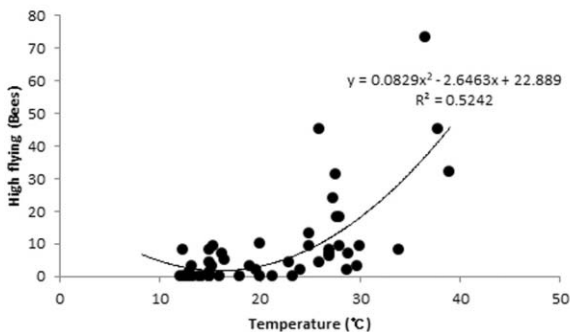


Fig. 5. Relationship between temperature in the greenhouse and high-flying behavior of *A. mellifera*.

significance level of less than 0.1, correlations were confirmed between temperature and bee traffic/foraging activity, and between UV level and landing on flowers.

## DISCUSSION

The flowering of watermelons is influenced by temperature more so than day length (RDA 2014). The

optimal growing temperature for watermelons is 25~30°C, but the temperature at which the largest number of flowers bloom on the main stem is 30~35°C (Sedgley and Buttrose, 1978). Furthermore, the dehiscence time of male flowers is critical for the fruit setting of watermelons. In general, the male flowers begin to dehisce at temperatures greater than or equal to 15°C and their fertility peaks within 3 h of dehiscence. Therefore, the effective fertilization time falls mainly in the morning. In this study, bee traffic and foraging activity of the honeybees reached a maximum at 10:00 in the morning, suggesting that sufficient pollination of watermelon flowers can be expected at the observed level and timing of bee activity.

Bee activity over time can change by flowering and climatic conditions of the pollen source (Heinrich, 1979; Abou-Shaara, 2014). As shown in Fig. 2, bee foraging does not coincide with the change in climatic conditions, seemingly because the flowering situation affects bee activity more than climatic conditions. As shown by bee



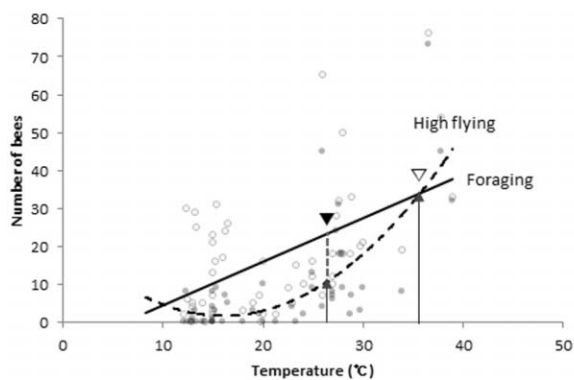


Fig. 6. Comparison between the regression equation of air temperature to foraging behavior and the regression equation of temperature to high-flying behavior. The blue arrow point ( $\uparrow$ ) indicates the number of foraging bees. The red dotted line ( $;$ ) indicates the estimate of the number of pollinating bees. The filled triangle ( $\blacktriangledown$ ) indicates the air temperature at which the rate of high-flying behavior was 50% of the total foraging behavior. The empty triangle ( $\nabla$ ) indicates the temperature at which high-flying behavior and total foraging behavior were equal.

traffic and foraging activity (Table 3), the number of flower landings was greatest at 10:00 and tended to decrease over the course of the day. Furthermore, pollen generally began to appear after 12:00 (Table 5). The time spent on flowers to collect pollen was the longest in the morning and also tended to decrease over the course of the day (Table 4). Statistical analysis is difficult because the amount of pollen was not quantified, but it can be reasonably inferred that the amount of pollen would have affected bee activity. Graham (1993) and Konzmann and Lunau (2014) reported that the foraging activity of honeybees can vary by the quantity and quality of the pollen and nectar.

Even when excluding the effects of the flowering situation, the climatic conditions (temperature, illuminance level, humidity, and UV level) demonstrated significant correlations with bee activity. Both bee traffic and foraging activity had positive correlations with temperature, illuminance level, and UV level, and a negative correlation with relative humidity. This means that if the flowering time varies depending upon the planting time of watermelons, the lengths of day and night can become

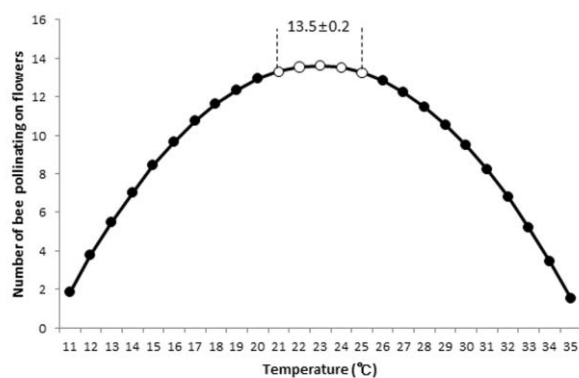


Fig. 7. Prediction of the number of bees pollinating watermelon flowers in the greenhouse based on air temperature. The empty circles ( $\circ$ ) indicate the largest number of pollinating bees in the range of 21–25°C.

different, and thus the amount of bee activity for one day can also be different. Therefore, the bee density must be adjusted during over the course of the flowering time of greenhouse watermelons. Furthermore, the determination coefficient of the regression analysis was more affected by climatic conditions than by foraging activity, because bee activity is inferred from bee traffic - which is mainly influenced by temperature (Joshi and Joshi, 2010), but the foraging activity for collecting nectar and pollen can be affected by the spawning of queen bees inside a bee colony (Free *et al.*, 1985), number of larvae (Amdam *et al.*, 2009), and pheromone effects (Sagili, 2015), as well as by climatic conditions such as temperature and UV level.

In general, the foraging behavior of bees is affected most by temperature compared with the other climatic conditions. It has been reported that both the activity of bumble bees in onion greenhouses (Lee and Yoon, 2017) and the activity of honeybees during apple or pear flowering season are affected most by temperature (Yoon *et al.*, 2013; Lee *et al.*, 2016). Similarly, in our research the greatest influencing factor was temperature. The foraging temperature of honeybees is 22–25°C, and the activity of bees is limited under 10°C (Heinrich, 1996). However, the lowest temperature for bee activity observed in this study was 12.1°C, and the largest number of foraging bees was observed when temperature was in the range of 25–35°C,

both of which are somewhat higher than previously reported temperatures (Fig. 4A). In particular, since the number of high-flying bees that did not participate in pollination showed a positive correlation with the temperature inside the greenhouse, a second-order regression equation was derived (Table 6, Fig. 5).

In the above section, we confirmed the difference between the temperature derived from the foraging activity regression equation and the temperature derived from the regression equation of high-flying bees (Fig. 6). Consequently, 50% or more of all foraging bees were high-flying bees at 28°C, and this number was 100% at 36°C. Assuming that among the foraging bees, the bees that do not fly high are those that actually perform pollination, the number of pollinating bees was calculated by entering the temperature variable in each regression equation (Fig. 7). As a result, it was predicted that the number of pollinating bees would be highest ( $13.5 \pm 0.2$ ) in the range of 21~25°C. The recommended number of honeybees required for the fruit setting of watermelons is 1 per 100 flowers in the cultivation environment under field conditions (21~29°C) (Boyhan *et al.*, 2000). During the flowering term of each flower cluster in this study, generally one female flower and five male flowers bloomed. Therefore, approximately 900 watermelon flowers bloomed and fell per test plot each day of the 10-day survey period, meaning at least 10 pollinating honeybees are required per day for the pollination of this number of flowers. To improve the pollinating effect of honeybees and secure the maximum number of pollinating bees, the temperature range inside the greenhouse during the flowering season must be set to 21~25°C. In addition, the temperature inside the greenhouse must be controlled to between 29°C and 17°C so that the number of pollinating honeybees does not fall below 10. Furthermore, besides temperature, the UV level was correlated with landing on flowers at a significance level of 0.1. It has been reported that bees can detect the UV level range alongside the illuminance level of visible light wavelengths, and bumble bees show higher activity in an environment with

high UV levels (Costa *et al.*, 2002). Therefore, the films of greenhouses should have a high UV transmittance and a UV protection coating should be avoided when using a shading agent to decrease the temperature inside the greenhouse.

In South Korea, where the greenhouse area for watermelon cultivation is 4-5-fold larger than the field cultivation area, hand pollination through human labor is required because natural pollination by insects is difficult to achieve. However, pollination by honeybees is increasing because of the higher labor costs of hand pollination (Yoon *et al.*, 2017; Statistics Korea, 2018). Because the environmental conditions of greenhouses in winter are inappropriate for bee activity, development of techniques to effectively use honeybees during the short flowering season is required. Our study confirms the effects of climatic conditions on bee activity in greenhouses during the winter and proposes the optimal temperatures for the most efficient pollination. However, for the foraging behavior of honeybees, factors inside the bee colony - temperature inside the colony, spawning of queen bees, and number of broods - are as important as external factors - flowering situation and climatic conditions (Cooper and Schaffer, 1985; Weidenmuller and Tautz 2002; Abou-Shaara, 2014). This study has limitations in clarifying the changes to pollinating activity of honeybees, because environmental changes inside the bee colonies were not considered. Therefore, in future research, the internal environment of bee colonies and the life therein need to be investigated in accordance with the external environment of the colony in the greenhouse. Furthermore, because the daily temperature difference inside a greenhouse is large during winter, changes in bee activity the day after overnight variation in internal colony temperature need to be researched as well.

## ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institute of Agricultural Sciences, Rural Development

Administration, Republic of Korea (Project No.: PJ012470032018).

## LITERATURE CITED

- Abou-Shaara, H. F. 2014. The foraging behaviour of honey bees, *Apis mellifera*: a review. *Vet. Med.* 59: 1-10.
- Amdam, G. V., O. Rueppell, M. K. Fondrk, R. E. Page and C. M. Nelson. 2009. The nurse's load: early-life exposure to broodrearing affects behavior and lifespan in honey bees (*Apis mellifera*). *Exp. Gerontol.* 44: 447-452.
- Boyhan, G. E., D. M. Granberry and W. T. Kelley. 2000. Commercial watermelon production. Bulletin 996. UGA Cooperative Extension Service.
- Choi, S. Y. 1987. Diurnal foraging activity of honey bees in the pear blossoms. *J. Apic.* 2: 108-116.
- Clarke, D. and D. Robert. 2018. Predictive modelling of honey bee foraging activity using local weather conditions. *Apidologie.* 49: 1-11.
- Cooper, P. D., W. M. Schaffer and S. L. Buchmann. 1985. Temperature regulation of honey bees (*Apis mellifera*) foraging in the Sonoran desert. *J. Exp. Biol.* 114: 1-15.
- Costa, H. S., K. L. Robb and C. A. Wilen. 2002. Field trials measuring the effects of ultraviolet-absorbing greenhouse plastic films on insect populations. *J. Econ.*
- Free, J. B. 1968. Dandelion as a competitor to fruit trees for bee visit. *J. Appl. Ecol.* 5: 168-178.
- Free, J. B., A. W. Ferguson and J. R. Simpkins. 1985. Influence of virgin queen honeybees (*Apis mellifera*) on queen rearing and foraging. *Physiol. Entomol.* 10: 271-274.
- Fulop, A. and R. Menzel. 2000. Risk-indifferent foraging behavior in honeybees. *Anim. Behav.* 60: 657-666.
- Graham, J. M., 1993. The hive and the honeybee. Hamilton, Dadant and Sons, Illinois, USA.
- Heinrich, B. 1979. Keeping a cool head: honeybee thermo-regulation. *Science.* 205(4412): 1269-1271.
- Heinrich, B. 1996. How the honey bee regulates its body temperature. *Beeworld.* 77: 130-137.
- Joshi, N. C. and P. C. Joshi. 2010. Foraging behaviour of *Apis* spp. on apple flowers in a subtropical environment. *NY Sci. J.* 3: 71-76.
- Kevan, P. G. and H. G. Baker. 1983. Insects as flower visitors and pollinators. *Annu. Rev. Entomol.* 28: 407-453.
- Ko, H. K., W. M. Lee, J. J. Noh, K. S. Park, D. K. Park, K. D. Ko, J. M. Lee and Y. C. Huh. 2012. Growth and development of watermelon plants grafted onto *Citrullus* rootstocks with resistance to *Fusarium wilt* at two temperature regimes. *J. Bio. Environ. Con.* 21: 33-38.
- Konzmann, S. and L. Lunau. 2014. Divergent rules for pollen and nectar foraging bumblebees - a laboratory study with artificial flowers offering diluted nectar substitute and pollen surrogate. *PLoS one.* 9, e91900.
- Lee, K. Y. and H. J. Yoon. 2017. The pollination properties and pollination efficiency of bumblebee (*Bombus terrestris* L.) relation to colony ventilation under high temperature condition in a greenhouse. *J. Apic.* 32: 205-221.
- Lee, K. Y., S. H. Yim, H. J. Seo, S. Y. Kim and H. J. Yoon. 2016. Comparison of pollination activities between honeybee (*Apis mellifera* L.) and bumblebee (*Bombus terrestris* L.) during the flowering period of Asian Pear (*Pyrus pyrifolia* N.) under variable weather Conditions. *J. Apic.* 31: 247-261.
- Lee, M. L., M. Y. Lee, H. S. Sim, Y. S. Choi, H. K. Kim, G. H. Byoun, I. S. Kim and C. R. Kwon. 2014. Characteristics of superior triple crossed honeybee (*Apis mellifera* L.) - honey collection, hibernation, hygienic behavior. *J. Apic.* 29: 257-262.
- Lee, S. B., Y. S. Kim, M. L. Lee, M. Y. Lee and H. J. Yoon. 2006. The Foraging activity and the effect of honeybee, *Apis mellifera* L. (Hymenoptera : Apidae) in the watermelon houses. *J. Apic.* 21: 49-54.
- Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25: 345-353.
- Puskadiza, Z., E. Stefanić, A. Mijić, Z. Zdunić, N. Parad-iković, T. Florijancić and A. Opacak. 2007. Influence of weather conditions on honey bee visits (*Apis mellifera carnica*) during sunflower (*Helianthus annuus* L.) blooming period. *Poljoprivreda*, 13(1): 230-233.
- Rural Development Administration (RDA). 2014. Watermelon cultivation, RDA press, Jeonju, Korea.
- Sagili, R. R., C. R. Breece, R. Simmons and J. H. Borden. 2015. Potential of honeybee brood pheromone to enhance foraging and yield in hybrid carrot seed. *HortTechnology.* 25: 98-104.
- Sedgley, M. and M. S. Buttrose. 1978. Some effects of light intensity, daylength and temperature on flowering and pollen tube growth in the watermelon (*Citrullus lanatus*). *Am. J. Bot.* 42: 609-616.
- Statistics Korea. 2018. Crop production statistics. <http://kosis.kr>.
- Vicens, N. and J. Bosch. 2000. Weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). *Environ. Entomol.* 29: 413-420.
- Walters, S. A. 2005. Honey bee pollination requirements for triploid watermelon. *HortScience*, 40(5): 1268-1270.
- Weidenmuller, A and J. Tautz. 2002. In-hive behavior of pollen foragers (*Apis mellifera*) in honey bee colonies under conditions of high and low pollen need. *Ethology.* 108: 205-221.
- Wien, H. C. 1997. The Cucurbits: cucumber, melon, squash

- and pumpkin. In: The Physiology of Vegetable Crops. H.C. Wien (ed.). Cambridge University Press, UK. pp. 345-377.
- Yoon, H. J., K. Y. Lee, M. A. Kim, I. G. Park and P. D. Kang. 2013. Characteristics on pollinating activity of *Bombus terrestris* and *Osmia cornifrons* under different weather conditions at apple orchard. *J. Apic.* 28: 163-171.
- Yoon, H. J., K. Y. Lee, H. S. Lee, M. Y. Lee, Y. S. Choi, M. L. Lee and G. H. Kim. 2017. Survey of insect pollinators use for horticultural crops in Korea, 2016. *J. Apic.* 32: 223-235.
- Young, H. J., D. W. Dunning and K. W. Von Hasseln. 2007. Foraging behavior affects pollen removal and deposition in *Impatiens capensis* (Balsaminaceae). *Am. J. Bot.* 94: 1267-1271.