Original research article

Foraging Activities of Honeybees (*Apis mellifera* L.) and Bumblebees (*Bombus terrestris* L.) on Passionfruit (*Passiflora edulis* f. *edulis*) Flower and their Pollination Effects

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Abstract   We studied the activity and pollination effects of insect pollinators (*Apis mellifera* and *Bombus terrestris*) in passionfruit pollination to investigate the possibility of insect pollination in greenhouse-cultivated passionfruit. The climatic condition inside the greenhouse during the flowering of passionfruit, bee traffics and flower visit duration of two species were measured. In addition, the pollination effect was determined by investigating the physical characteristics of fruits according to each pollination methods. The bee traffic and foraging activity of *A. mellifera* were 3.6 and 7 times greater than those of *B. terrestris*, respectively. However, when this result was converted into a percentage to colony size, the ratio of *B. terrestris* was 19.1, and was 10.2 times higher than that of *A. mellifera*. In addition, the activity of *A. mellifera* was affected by the illumination level (R=0.593) and ultraviolet rays (R=0.647), but the activity of *B. terrestris* was less affected by light. Therefore, we considered *B. terrestris* to be more suitable for the pollination of passionfruit than *A. mellifera*. Regarding the pollination effect, there was no difference between the two insect pollinators; however, better fruit quality was observed with insect pollinators than with artificial pollination. Certain problems should be addressed when using an insect pollinator directly for the pollination of passionfruit. First, pollen robbing caused by the differences between flowering start time and bee activity start time should be considered. Second, the low pollination efficiency because of the presence of extrafloral nectaries in passionfruit should be considered. Lastly, the pollination effect caused by the colony decline because of the long duration of pollination should be factored in. If these problems are solved by controlling the activity time of the foraging bees, increasing the pollinator density by 30%, and adding or managing the bee colony, insect pollinators could be effectively used for passionfruit pollination.

INTRODUCTION

Passionfruit (*Passiflora edulis* Sims) is a perennial vine crop belonging to the family Passifloraceae (Dhawan and Sharma, 2004; Lim *et al*., 2018). It originates in southern Brazil, Paraguay, and northern Argentina and is cultivated mainly in the high altitudes of tropical and subtropical regions. Moreover, it has been introduced to five continents as an ornamental and com-mercial crop (Rendón *et al*., 2013). Passionfruit grown in Korea is mostly a hybrid of the purple passionfruit (*Passiflora edulis* f. *edulis*), and is cultivated in greenhouses for stable fruit production (RDA, 2019). Purple passionfruit blooms and sets fruit twice a year (Lederman, 1987). The spring bloom during April~May yields fruit in the summer, and the fall bloom during September~October yields fruit in the winter (Kishore *et al*., 2010). As it is not self-incompatible, the purple passion-

fruit can be self-fertilized, but owing to the unique shape of its flowers, it mainly requires pollinators, such as insects, for successful pollination (Aponte and Jáuregui, 2004; Kishore *et al*., 2010). In its country of origin, passionfruit is cultivated in open fields and several species of insect pollinators pollinate its flowers (Lederman, 1987). Among wild insect pollinators, carpenter bees (*Xylocopa* spp.) are known as the most effective passionfruit pollinators because their body is larger than that of the other bee species (Hammer, 1987; Hoffmann *et al*., 2000; Keasar, 2010). However, as a consequence of climate change, deforestation, and pesticide use, wild bee populations are rapidly decreasing, which is why many countries, including Brazil, where the passionfruit originates from, are conducting artificial pollination for the production of passionfruit (Silva, 2005; Yamamoto *et al*., 2012). In Japan and Korea, where passionfruit is cultivated in greenhouses, artificial pollination is commonly performed for the stable production of high-quality fruits (Mizuno, 2008; Lim *et al*., 2018). However, artificial pollination of passionfruit requires manpower, which is an expensive process. As flowers bloom and fall daily, there is a requirement of 3~5 individuals per ha daily during the blooming period that lasts approximately 90 days (Ish-Am, 2009). To solve this problem, studies on the artificial use of insect pollinators have been conducted in various passionfruit-cultivating countries. In Brazil, the method of attracting carpenter bees and using them for the pollination of passionfruit has been investigated in several studies (Freitas and Oliveira, 2003; Keasar, 2010; Silveira *et al*., 2012; Junqueira *et al*., 2016). Ish-Am (2009) reported a method of passionfruit pollination using honeybee (*Apis mellifera*) as a pollinator and by controlling their density to solve the problem of lowering the pollination effect because bees visit extrafloral nectaries rather than flowers. In Japan, where passionfruit is mostly cultivated in greenhouses, a study was conducted to compare the effects of Asian honeybees (*Apis cerana*), European honeybees (*A. mellifera*), and bumblebees (*Bombus terrestris*) as passionfruit pollinators (Shimabukuro *et al*., 2007; Mizuno, 2008). In Korea, a study was carried out to compare the pollination effects of two insect pollinators (*A. mellifera* and *B. terrestris*) using fan and hand pollination (Lee *et al*., 2013). However, in 94% of the area of passionfruit cultivation in Korea, artificial pollination is still used

(Yoon *et al*., 2017). In the present study, we focused on the foraging activity of insect pollinators in the cultivation environment of passionfruit, which was different from previous studies focusing only on the effect of insect pollinators. In addition, we investigated the relationship between the flowering characteristics of passionfruit flowers and the activity characteristics of two insect pollinators (*A. mellifera* and *B. terrestris*), and explained the pollination effect through changes in fruit quality related to foraging activity. Moreover, based on the obtained data, we developed a method to effectively use insect pollinators in the field.

MATERIALS AND METHODS

1. Insects and crops

The experiments were conducted from May 15 to June 30, 2018, in Imun-ri, Wanju-gun, Jeollabuk-do, Korea (35°49′24″N, 127°00′43″E). We selected the passionfruit vine (*Passiflora edulis* var. *edulis* Sims) for the study. Its bloom period was from May 6 to July 5. In order to compare their pollination effects, European honeybees (*Apis mellifera* L., Italy, three flames, over 5,000 workers) and bumblebee (*Bombus terrestris* L., 70 workers) of 13 generations reared under artificial conditions [26°C, relative humidity (RH) 80%] (Yoon *et al*., 2008) were selected and introduced to the study sites.

2. Treatment groups

The test plot consisted of seven net house units (155 m²/unit, mesh: 2 mm × 2 mm) with 30 passionfruit vines per net house unit. The distance between plants was 1.2 m × 2.0 m, the age of the passionfruit vine was four years, and the average vine height was 2.3 m. To investigate the effect of different pollination methods, three colonies of 5,000 (two frames) *A. mellifera* and three colonies of 100 *B. terrestris* were installed 1 m from the entrances of the six net houses on May 11, 2018. Artificial pollination was carried out in one net house during the experimental period. The bee colonies were removed from the greenhouses 46 days after installation. At each site, artificial pollination and control groups were set. For artificial pollination, we transferred the pollen from the anther directly onto the pistil

head with a brush when the flowers bloomed at 11 a.m. every day during the study period. Immediately after artificial pollination, the flower clusters were covered with a 1 mm × 1 mm mesh to prevent insect pollination. The pollination activity of insect pollinators, fruit set, and physical properties of the harvested passionfruit were investigated to compare the pollination effects.

3. Climatic conditions in passionfruit greenhouses

To investigate the climatic conditions in the greenhouses, data loggers (Illuminance UV recorder TR-74Ui; T&D Co.; Matsumoto, Nagano, Japan) were installed 5 m from the entrance of the three test plots (*A. mellifera*, *B. terrestris*, and artificial pollination) and 1.3 m above the ground. The installed data loggers recorded the temperature, relative humidity, illuminance level, and UV level inside the greenhouses at 30 min intervals from May 15 to June 30, 2018. To investigate the correlation between the climatic conditions inside the greenhouses and bee activity, a correlation analysis was conducted on the number of bees flying without pollination behavior and the number of bees visiting the flowers in relation to the climatic conditions.

4. Pollination activity and foraging characteristics

To compare the pollination activity of *A. mellifera* and *B. terrestris*, we investigated the bee traffic and bee visits on passionfruit plants. Bee traffic was defined as the number of worker bees entering and exiting the hives. To investigate it, we developed an imaging-based bee traffic measurement system (Korea Patent No. 10-1961670). Bee traffic was examined at 1 h intervals from 05:00 to 20:00 every day during the experimental period. In addition, the number of bees pollinating the passionfruit flowers per plant was observed at 1 h intervals from 05:00 to 20:00 for four days (May 15, 20, 25, and 30), that is, the number of foraging bees visiting passionfruit flowers at the same time intervals. In order to compare the preferences of honeybees and bumblebees for nectar from flowers and extrafloral nectaries (EFNs), the number of foraging workers on the flowers and petioles of passionfruit vines in the test plots was counted for 5 min every hour between 05:00 and 20:00 for four days. In addition, a regression analysis was performed to confirm how the bee traffic changed over

time after the installation of the beehive.

5. Effect of pollination method on fruit quality

The fruits were harvested 80 days after pollination (from August 16 to September 9, 2018). Fruit quality was determined by randomly selecting 120 fruits from each treatment group and analyzing non-cleaved (weight, length, and diameter) and cleaved fruit properties (weight of the pulp flesh, number of seeds, soluble solids, titratable acidity, soluble solids/titratable acidity (SS/TA) ratio, and weight of pulp flesh/fruit weight (WP/WF) ratio). The SS/TA and WP/WF ratios were expressed as percentage of fruits with a soluble solid/titratable acidity and weight of pulp flesh/fruit weight, respectively. The relationships between fruit weight, length, diameter, number of seeds, and weight of pulp flesh were analyzed to determine the commercial value of fruits pollinated by the two insect pollinators. In addition, a regression analysis was performed to investigate the changes in fruit weight in relation to the harvest date. The fruits were weighed using an electronic balance (CB-3000, AND, Seoul, Korea), and their length and diameter were measured using Vernier calipers (500-181, Mistutoyo, Kawasaki, Japan). The juice was extracted from the flesh at the median equatorial plane of the fruit using a cheesecloth, and the concentration of soluble solids in the extracted samples was measured using a digital sugar meter (PR-32α, Atago, Tokyo, Japan). For measuring the titratable acidity, the juice was extracted from five fruits from each treatment group; 5 mL of each juice sample was diluted with 35 mL of distilled water, the pH of each sample was neutralized to 8.3 using 0.1 N NaOH, and the titratable acidity was determined using citric acid.

6. Statistical analysis

A t-test was used to compare the pollination behaviors of *A. mellifera* and *B. terrestris* (bee traffic, and foraging activity, including bee flying and bee visiting the flower), as well as to compare insect preference between the flowers and petioles of passionfruit vines. The effect of the pollination method on fruit quality was analyzed using one-way ANOVA. Significant differences identified using ANOVA and Tukey's test were used for post-hoc analysis. In correlation analysis, Pearson's correla-

tion was performed after normality was verified using the Kolmogorov-Smirnov test. After performing the correlation analysis, a linear regression equation was derived using regression analysis if a significant correlation was confirmed. All statistical analyses were performed using the SPSS PASW 22.0 package for Windows (IBM, Chicago, IL, USA).

RESULTS

1. Climatic conditions inside the greenhouses during the passionflower blooming period
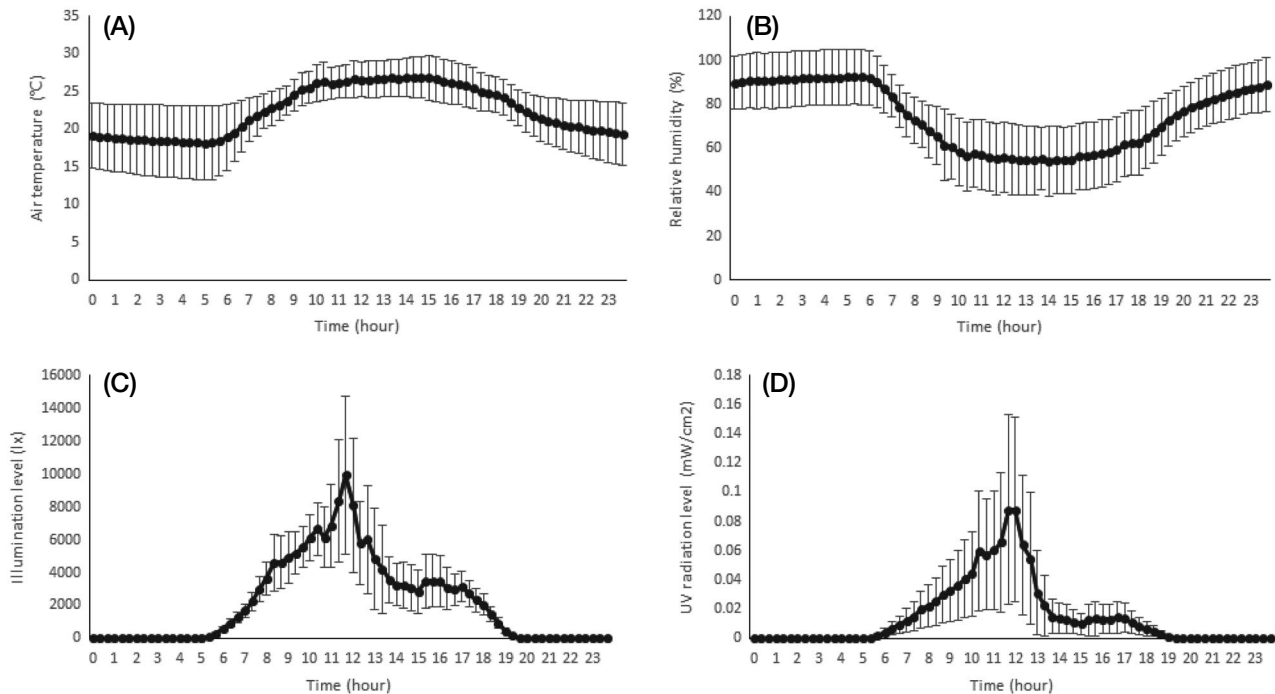
The climatic conditions during the bee activity time (from 05:00 to 20:00) throughout the 46 days of the passionfruit blooming period (May 15 to June 30, 2018) were as follows: average temperature of $24.1 \pm 1.6°C$,

relative humidity of $66.4 \pm 10.3\%$, illuminance level of 6094.4 ± 2295.9 lx, and UV level of 0.077 ± 0.048 mW/cm^2 (Table 1). Daily changes in the climatic conditions were as follows: the temperature began to rise at 6:00 in the morning, was relatively unchanged from 10:00 to 17:00, and then sharply decreased after 16:00. The highest temperature was recorded at 14:00 ($26.8 \pm 2.7°C$), and the lowest at 4:00 ($18.2 \pm 4.7°C$) (Fig. 1A). The relative humidity remained 80~90% from 21:00 to 07:00, began to decrease at 7:30, and then increased again after 15:30. The highest humidity ($92.4 \pm 4.9\%$) was recorded at 5:30 and the lowest humidity ($54.3 \pm 16.0\%$) at 14:00. In general, the relative humidity tended to be inversely related to temperature (Fig. 1B). Illuminance began to increase from 5:00 and continued increasing until 12:00, after which it began to decrease, sharply decreasing after 13:00. The highest illuminance level (17703.1 ±

Table 1. Climatic conditions during the bee activity time throughout passionfruit blooming period in the greenhouse

Diurnal* time	Temperature (°C)	Relative humidity (%)	Illuminance level (lux)	UV level (mW/cm^2)
Mean	24.1 ± 1.6	66.4 ± 10.3	6094.4 ± 2295.9	0.077 ± 0.048

*bee activity time, 05:00~20:00

Fig. 1. Climatic conditions during the survey period (May 17~June 17, 2017) in the passionfruit greenhouse: (A) temperature, (B) relative humidity, (C) illuminance level, and (D) UV level.

Table 2. Sequence of flower blooming over time in the daytime

Sequence of flower blooming	Blooming start	Petal opening	Anther bending	Stigma bending	Petal complete blomming
Time (hh:mm)	10:20 ± 25 AM	10:38 ± 17 AM	10:58 ± 17 AM	11:10 ± 29 AM	12:34 ± 22 PM

Table 3. Bee traffic of insect pollinators per hour throughout passionfruit blooming period

Insect pollinators	N	Incoming[y] (Rate[z])	Outgoing (Rate)	Total traffic (Rate)
Apis mellifera	14	27.0 ± 5.9* (0.5 ± 0.1)	25.4 ± 5.7* (0.5 ± 0.1)	52.4 ± 11.6* (1.0 ± 0.2)
Bombus terrestris	14	5.5 ± 0.8 (7.9 ± 1.1*)	9.1 ± 1.3 (13.0 ± 1.9*)	14.6 ± 2.0 (20.9 ± 2.9*)

[y]Number of observations
[z]Percentage of foraging bees related to colony size
* indicates a significant difference at p < 0.05 between treatment plots.

13000 lx) was recorded at 12:00 (Fig. 1C). The pattern of the UV level almost coincided with the pattern of the illuminance level. The highest UV level (0.244 ± 0.200 mW/cm^2) was recorded at 11:00. The passionfruit plants started to bloom at 10:20 ± 25, and within 40 min from when the petals opened, the stigmas spread downward and descended. Blooming was completed within 2 h from the beginning of blooming, and pollen had already appeared during the beginning of flowering.

2. Pollination activity of different insect pollinators

The bee traffic per hour showed significant differences between species (Table 3). Number of incoming individuals (t-test: $t_{26} = 3.584$, $p = 0.001$), outgoing individuals ($t_{26} = 2.770$, $p = 0.010$), and total traffic ($t_{26} = 3.216$, $p = 0.003$) of *A. mellifera* were 4.9-fold, 2.7-fold, and 3.6-fold higher, respectively, than those of *B. terrestris*. However, colony size varied between the two bee species. Thus, if the previous results were converted to percentages, the rates of incoming individuals, outgoing individuals, and total traffic rates of *B. terrestris* were 14.6-fold ($t_{26} = -6.446$, $p = 0.0001$), 25.6-fold ($t_{26} = -6.557$, $p = 0.0001$), and 19.1-fold ($t_{26} = -13.171$, $p = 0.0001$) higher, respectively, than those of *A. mellifera*, and these differences were significant (Table 3). The bee traffic of *A. mellifera* and *B. terrestris* peaked at 15:00 and 08:00, respectively (Fig. 2A). Regarding foraging activity, the number of bees visiting the flowers ($t_{30} = 2.473$, $p = 0.019$) and the total foraging activity
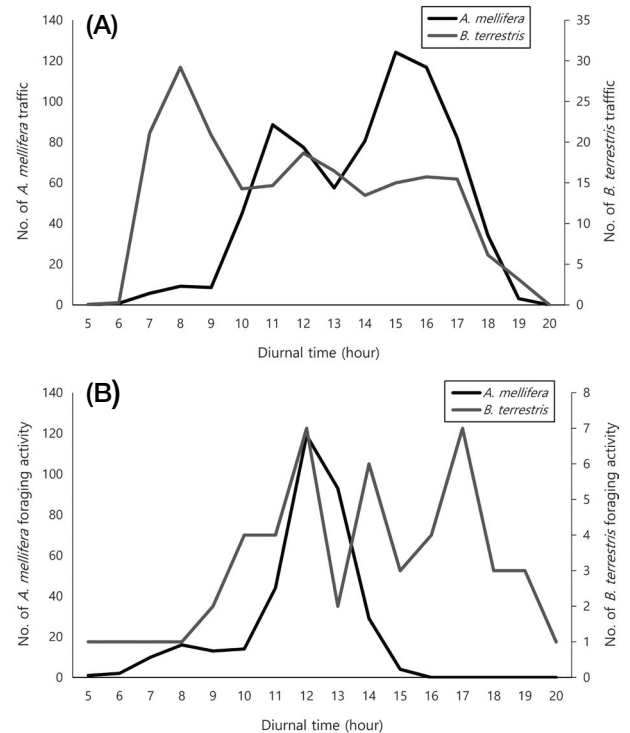

Fig. 2. Daily bee traffic and foraging activity of *Apis mellifera* and *Bombus terrestris* in the passionfruit greenhouse.

($t_{30} = 2.100$, $p = 0.044$) over time showed statistically significant differences between the two species (Table 4). The number of flying bees (t-test: $t_{16} = 2.473$, $p = 0.019$), number of flower visits ($t_{16} = 1.955$, $p = 0.080$), and total foraging activity ($t_{16} = 2.100$, $p = 0.044$) of *A. mellifera* were 10.6-fold, 7.7-fold, and 7.0-fold greater than those

of *B. terrestris*, respectively. Converted to percentages, the rates of flying bees, flower visits, and total foraging activity of *B. terrestris* was 6.7-fold ($t_{16} = -2.858$, $p = 0.008$), 9.3-fold ($t_{26} = -3.785$, $p = 0.001$), and 10.2-fold ($t_{26} = -0.4383$, $p = 0.0001$) higher, respectively, than those in *A. mellifera*. The total foraging activity of *A. mellifera* was the highest at 12:00, followed by 13:00, 11:00, and 14:00. In contrast, *B. terrestris* showed the highest activity at 10:00, 17:00, and 14:00 (Fig. 2B). Meanwhile, regarding the bee traffic, *A. mellifera* showed a pattern where its activity was concentrated during the daytime (11:00~16:00) and then rapidly decreased, whereas *B. terrestris* activity was concentrated in the morning (07:00~09:00) and then gradually decreased. Regarding the foraging activity, *A. mellifera* was mainly active from 11:00 to 14:00, whereas *B. terrestris* showed a relatively even activity from 9:00 to 19:00. Both *A. mellifera* and *B. terrestris* had a higher preference for flowers than for the petioles on the passionfruit vines (Fig. 3). The percentage of honeybee flower visits per hour (66.4 ± 10.24 bees) was 2-fold higher than that of petiole visits per hour (33.6 ± 10.24 bees) ($t_{28} = 2.268$, $p = 0.031$). Likewise, *B. terrestris* visited the flowers 1.9-fold more often than they did the petioles ($t_{36} = 2.299$, $p = 0.027$).

3. Relationship between bee activity and climatic conditions

There was a significant correlation between the climatic conditions in the greenhouses and bee activity (Table 5). The foraging activity of *A. mellifera* was correlated with temperature ($R = 0.499$), illuminance (0.593), and UV (0.647). In particular, illuminance and UV showed highly significant correlations with foraging activity ($p < 0.01$). On the other hand, there was no cor-
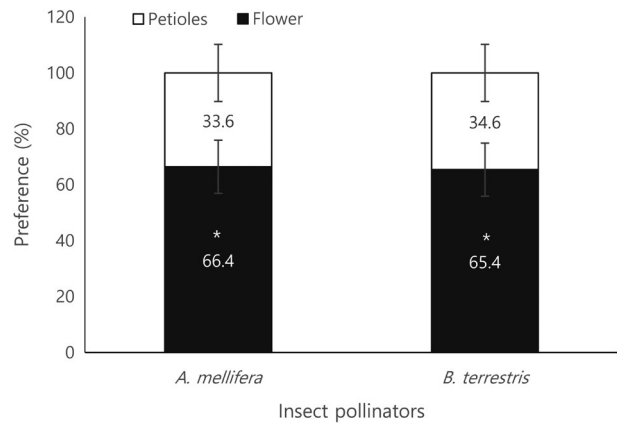
Fig. 3. Comparison of the rate of bees visiting the passionfruit tree between *Apis mellifera* and *Bombus terrestris*. * indicates a significant difference at $p < 0.05$ between treatment plots.

Table 4. Foraging activity of honeybees per hour Bee traffic of insect pollinators per hour throughout passionfruit blooming period

| Insects pollinators	N	Flying[y] (Rate[z])	Visits to flowers (Rate)	Total foraging activity (Rate)
Apis mellifera	16	$9.3 \pm 3.4*$ (0.2 ± 0.1)	12.5 ± 5.5 (0.3 ± 0.1)	$21.8 \pm 8.9*$ (0.4 ± 0.2)
Bombus terrestris	16	0.9 ± 0.3 $(1.3 \pm 0.4*)$	1.6 ± 0.3 $(2.3 \pm 0.5*)$	3.1 ± 0.5 $(4.5 \pm 0.7*)$

[y] Number of observations
[z] Percentage of foraging bees related to colony size
* indicates a significant difference at $p < 0.05$ between treatment plots.

Table 5. Correlations between climatic conditions and bee foraging activity

Foraging activity ×	Air temperature	Relative humidity	Illuminance level	UV level
	R	R	R	R
Apis mellifera	$0.499*^{z}$	-0.481	$0.593**$	$0.647**$
Bombus terrestris	$0.665**$	$-0.681**$	0.432	0.402

[z] Pearson's correlation coefficients were calculated with foraging activity and greenhouse environmental parameters as controlling variables. As each set of values was used to calculate two correlations, ** and * indicate significant correlations at the $p < 0.05$ and $p < 0.1$ level, respectively (two-tailed test).
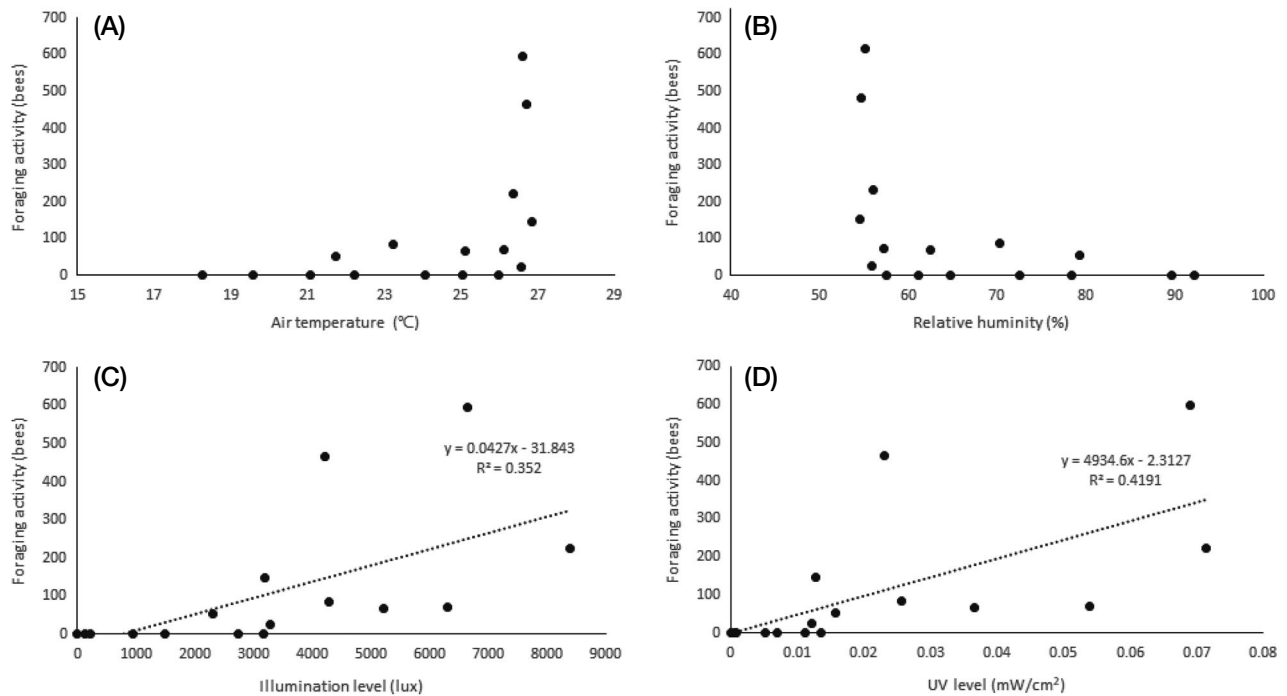
Fig. 4. Relationship between foraging activity of *Apis mellifera* and the following climatic conditions: (A) temperature, (B) relative humidity, (C) illuminance level, and (D) UV level. There was no significant regression model in (A) and (B).

relation between foraging activity and relative humidity. A regression equation was derived from the regression analysis only for illuminance (ANOVA $F_{1, 14} = 7.604$, $p = 0.015$, $R^2 = 0.352$, $DW = 1.015$) and ultraviolet radiation ($F_{1, 14} = 10.099$, $p = 0.007$, $R^2 = 0.419$, $DW = 0.951$; Fig. 4). In the case of *B. terrestris*, temperature (0.665) and relative humidity (-0.681) were significantly correlated with foraging activity, whereas no correlation was observed between foraging activity and illuminance as well as between foraging activity and UV levels. Using regression analysis, significant regression equations were derived between the foraging activity of *B. terrestris* and temperature ($F_{1, 14} = 6.757$, $p = 0.021$, $R^2 = 0.326$, $DW = 1.961$) as well as between foraging activity and relative humidity ($F_{1, 14} = 7.635$, $p = 0.017$, $R^2 = 0.345$, $DW = 2.008$; Fig. 5).

4. Effect of pollination methods on fruit quality

There was no significant difference in the weight and size of passionfruit fruits among the different pollination methods (Table 6). In contrast, the pulp weight, soluble solids, titratable acidity, and pulp flesh weight/fruit weight ratio were significantly different among different pollination methods (Table 7). The fruit pulp flesh weight of plants pollinated by insect pollinators (38.7~39.4 g) was 8~9% higher than that of plants pollinated by artificial pollination (one-way ANOVA test: $F_{2, 274} = 3.230$, $p = 0.041$). The soluble solid content of fruits pollinated by insect pollinators (14.6~14.7°Brix) was 1.1°Brix higher than that of fruits pollinated by artificial pollination ($F_{2, 274} = 17.168$, $p = 0.0001$). The fruit titratable acidity of plants pollinated by insect pollinators (2.31~2.35%) was 0.16~0.20% higher than that of plants pollinated by artificial pollination ($F_{2, 274} = 6.423$, $p = 0.002$). The pulp flesh weight/fruit weight ratio of plants pollinated by insect pollinators (0.54~0.55) was 6~8% higher than that of plants pollinated by artificial pollination ($F_{2, 274} = 5.597$, $p = 0.004$). There were no significant differences in these parameters when the plants pollinated by the two insect pollinators were compared.

DISCUSSION

We investigated the activity characteristics and pollination effects of two insect pollinators (*A. mellifera* and
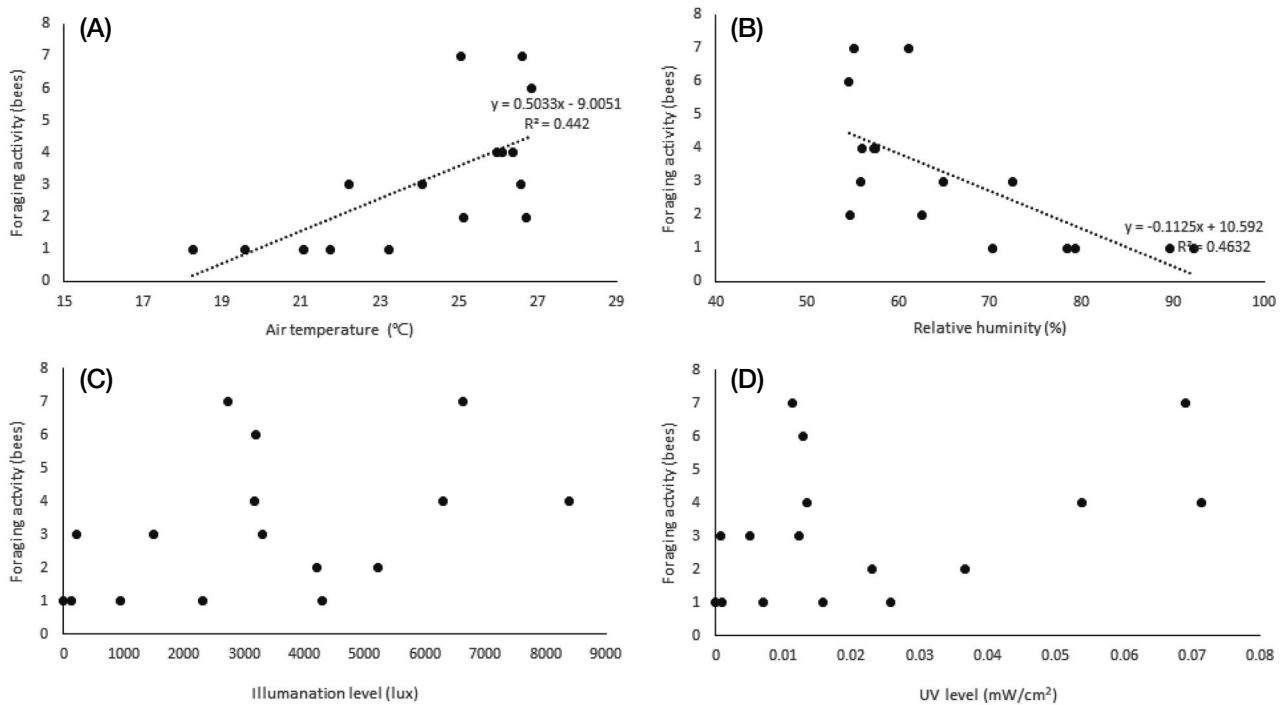
Fig. 5. Relationship between foraging activity of *Bombus terrestris* and the following climatic conditions: (A) temperature, (B) relative humidity, (C) illuminance level, and (D) UV level. There was no significant regression model in (C) and (D).

Table 6. Effects of different pollination methods on the physical properties of non-cleaved passionfruit

Pollination method	N[y]	Weight (g)	Length (cm)	Diameter (cm)
Artificial pollination	40	71.2 ± 5.3[z]	20.8 ± 1.8	18.8 ± 0.5
Apis mellifera	120	71.4 ± 10.3	20.4 ± 0.9	18.6 ± 0.9
Bombus terrestris	120	71.1 ± 9.5	21.9 ± 17.1	18.5 ± 0.7

[y]Number of fruits
[z]mean ± SD

B. terrestris) to investigate the possibility of insect pollination in greenhouse-cultivated passionfruit. Increasing bee foraging activity is important for obtaining effective pollination by bees (8). Climatic conditions are important factors determining the foraging activity of insect pollinators (9). As the temperature range in the passionfruit greenhouse during bee activity was 23~25°C, foraging activity of *A. mellifera* and *B. terrestris* was in accordance with their usual foraging activity. However, as the average illumination level was less than 10,000 lux, the foraging activity of *B. terrestris*, which is less affected by illuminance than *A. mellifera*, was not significantly affected by illumination, but *A. mellifera* may have been affected by illumination (see Figs. 4 and 5). In Korea, passionfruit vines are mostly trained to a "T"-shape

(RDA, 2019). Passionfruit is a vine, which is why it is difficult to secure enough light for bees to forage in their "T"-shaped vines in greenhouses. Therefore, it would be more effective to use *B. terrestris* for pollination, as it is relatively less affected by illumination or ultraviolet rays than *A. mellifera*. Our results showed that the pollination effect by insect pollinators was the same as or greater than that of artificial pollination (Tables 6 and 7). There was no significant difference in fruit weight among the pollination methods; however, the amount of edible flesh was significantly higher in plants that were pollinated by insects than those pollinated artificially. In addition, the pulp flesh weight/fruit weight ratio, which can determine the amount of pulp in a fruit, was significantly higher in plants that were pollinated by insects

Table 7. Effects of different pollination methods on the physical properties of cleaved passionfruit

Pollination method	N	Weight of pulp flesh	Number of seeds	Soluble solid (Brix)	Titratable acidity (%)	SS/TA[y]	WP/WF[x]
Artificial pollination	40	36.3 ± 4.9[bz]	202.2 ± 15.4	13.6 ± 1.4[b]	2.05 ± 0.43[b]	7.02 ± 2.02	0.51 ± 0.07[b]
Apis mellifera	120	39.4 ± 7.3[a]	193.5 ± 30.9	14.7 ± 1.0[a]	2.35 ± 0.42[a]	6.45 ± 1.29	0.55 ± 0.06[a]
Bombus terrestris	120	38.7 ± 6.6[ab]	189.5 ± 33.4	14.6 ± 0.9[a]	2.31 ± 0.51[a]	6.68 ± 1.80	0.54 ± 0.07[a]

[z]mean ± SD, Different letters indicate significant differences among pollination methods based on the results of one-way ANOVA and Tukey's HSD (p < 0.05).
[y]Soluble solid/Titratable acidity
[x]Weight of pulp flesh/Fruit weight

Table 8. Correlations between weight of pulp flesh and fruit weight and length, diameter, number of seeds, soluble solids, and WP/WF ratio

| Pearson's correlation	Weight of pulp flesh ×					
	Weight	Length	Diameter	Number of seeds	Soluble solids	WP/WF ratio
R	0.737**	0.421**	0.136*	0.546**	0.389**	0.650**

Each set of values was used to calculate the two correlations. * and ** indicate significant correlations at p < 0.05 and p < 0.01, respectively (two-tailed test).

than those pollinated artificially. Pulp flesh weight was correlated with sugar and acidity, which are factors determining fruit taste. Furthermore, it was confirmed that fruit weight related to fruit yield had a high correlation with pulp flesh weight (Table 8). Thus, we concluded that insect pollination was more advantageous in yielding better fruit quality than artificial pollination. The pollination effect among insect pollinators was investigated at the same level. Considering the colony size and the number of foraging bees in the present study, *B. terrestris* showed a higher pollination efficiency than *A. mellifera*. This was because, in general, compared to *A. mellifera*, *B. terrestris* is more adaptable to confined spaces, such as net houses (Katayama, 1987; Dimou *et al.*, 2008). Therefore, future studies should compare the pollination effect of different insect pollinators in a greenhouse without a net house installed.

Despite these results, there are several problems with using bees for direct passionfruit pollination. First, pollen robbing by insect pollinators occurs because of the difference between the start time of passionfruit flowering and the start time of insect pollinator activity. Pollen appears on passionfruit flowers immediately after blooming, but fertilization does not occur immediately, as the flower can be fertilized only when the stigma is spread downward and descended (McGregor, 1976; Kishore *et al.*, 2010). In our study, passionfruit flowering started after 10:00, pollen was observed from the begin-

ning of flowering, and the stigma spread downward and descended only 1 h after flowering (Table 2). However, a problem in pollination was related to the active time of the bees. As shown in Fig. 2, both *A. mellifera* and *B. terrestris* started to be active at 06:00. Therefore, pollen collection by bees was observed even before the flowers completely opened, meaning that during this time, bee visits to flowers could not result in pollination as the pollen could not yet reach the stigma. This type of bee behavior was described by Hammer (1987) and Yamamoto *et al.* (2012) as pollen robbing. To prevent this phenomenon, a method of controlling the density of the foraging bees, such as adjusting the entrance door to delay the bee activity start time, is required. Ish-Am (2009) also reported the problem of pollen robbing by bees in the pollination of passionfruit, and as a countermeasure, suggested a method of opening and closing the entrance of the beehives according to the flowering time of passionfruit. Passionfruit flowers are known to produce the highest amount of fruit when their stigmas are completely descended to increase the response of stigmas to pollen (Kishore *et al.*, 2010). The second problem with using bees as passionfruit pollinators is that the efficiency of insect pollination decreases because of the presence of EFNs in passionfruit (Izaguirre *et al.*, 2013). For this reason, bee foraging time is not entirely spent on collecting pollen or nectar from flowers, but also on visiting places irrelevant to pollination, which is why

the labor force is dispersed. Our results also confirmed that 33~34% of foraging bees were directed to EFNs, such as those in petioles, instead of flowers (Fig. 3). Accordingly, the insect pollinator density per tree needs to be increased by more than 30%. In addition, there was no significant difference between the preference of the two insect pollinators for EFNs and flowers. However, as it is generally known that *A. mellifera* prefers nectar collection more than *B. terrestris* does (Pomeroy and Fisher, 2002), *B. terrestris* is considered to be more effective than *A. mellifera* for passionfruit pollination. Lastly, because of their relatively long pollination period, it is necessary to manage bee pollinators during passionfruit pollination. In greenhouses, passionfruit blooms in May, and has a flowering period of two months (Ish-Am, 2009; RDA, 2019). Nevertheless, the effective pollination period of *B. terrestris* is less than two months. This is because, in greenhouses, *B. terrestris* colonies usually develop for about one month and decrease thereafter (Blacquière *et al*., 2007). In the case of *A. mellifera*, colony size continues to decrease if management (such as feeding) is not carried out to maintain the oviposition of queen bees (DeGrandi-Hoffman *et al*., 2008). Decreasing colony size may cause problems with the production or quality of passionfruit. As shown in Fig. 6, unlike the weight of fruit obtained by artificial pollination, the weight of fruit obtained by insect pollination according to harvest period showed an increasing or decreasing pattern over time. Interestingly, this pattern was similar to the bee traffic pattern according to the period installed bee hive (Fig. 7). Based on the correlation analysis (Table 9), a negative correlation was confirmed for both *A. mellifera* (p=0.005) and *B. terrestris* (p=0.006) in daily bee traffic and beehive installed period. Moreover, the daily bee traffic of *A. mellifera* showed a significant correlation only with the weight of fruits obtained after pollination by *A. mellifera* (p=0.027), and the daily bee traffic amount of *B. terrestris* was correlated only with the weight of fruits obtained after pollination by *B. terrestris* (p=0.021). In other words, stable foraging activity of insect pollinators is required for the stable pollination of passionfruit. Thus, for stable passionfruit production, when using *B. terrestris* as a pollinator, a new colony must be installed when the foraging activity of the first colony decreases. In the case of *A. mellifera*, it is necessary to maintain the ovi-
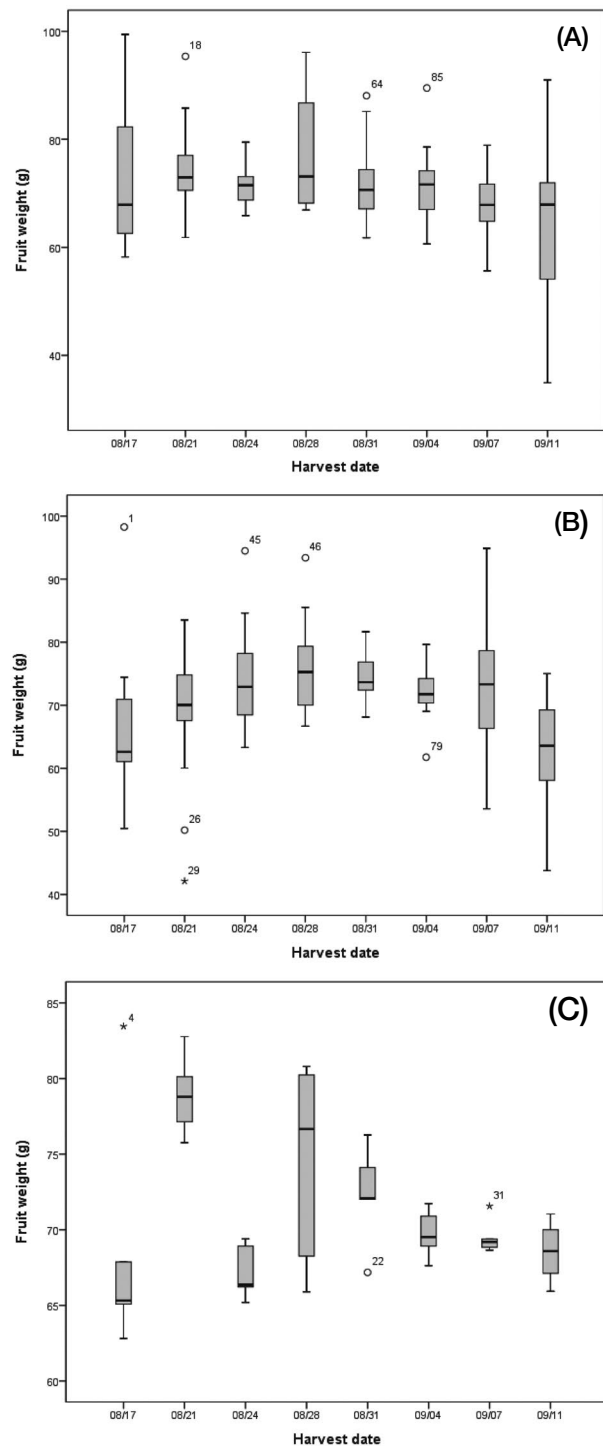
Fig. 6. The weight of fruits (at harvest) pollinated by (A) *Apis mellifera*, (B) *Bombus terrestris*, and (C) artificial pollination. Gray boxes indicate the interquartile range; bold line in the boxes indicates the median; stars and empty cycles are the outliers.

position of queen bees so that the activity of the foraging bees does not decrease. If the above-described problems
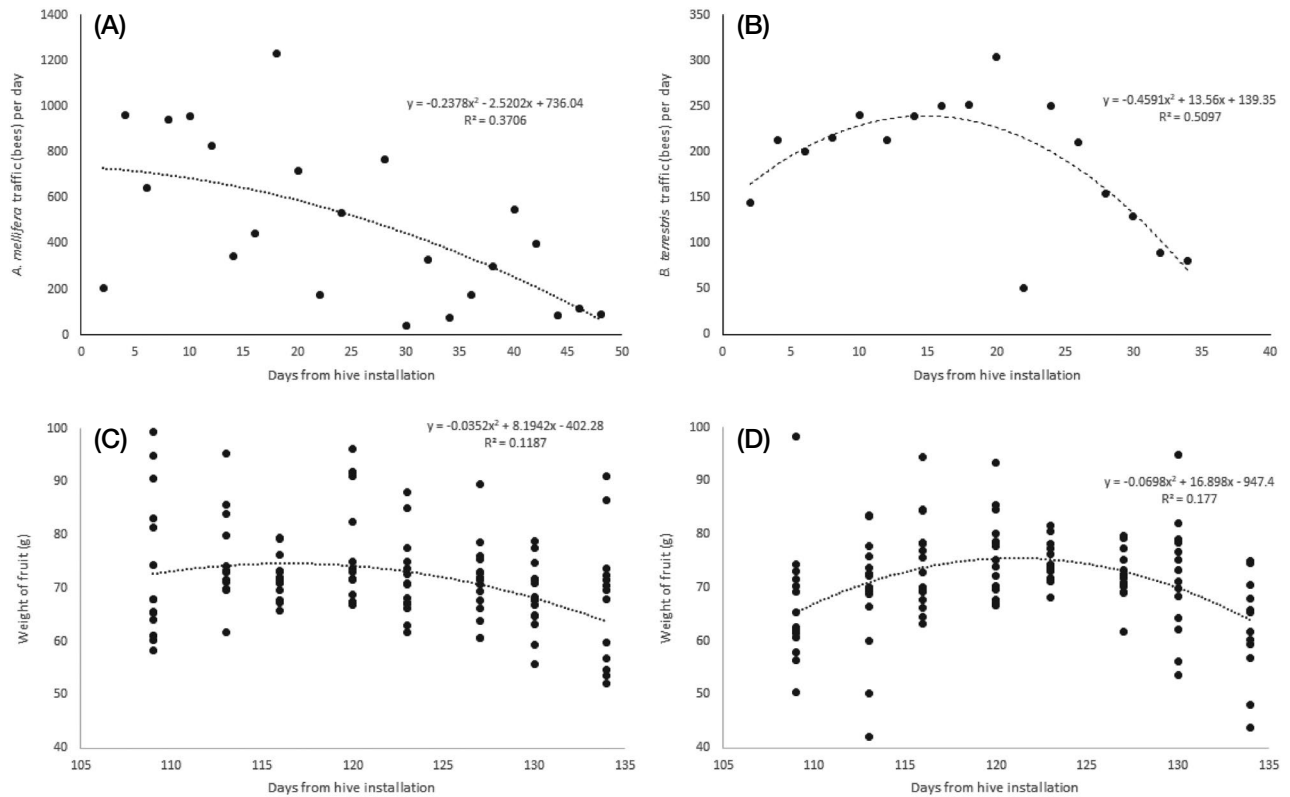
Fig. 7. Comparison of the bee traffic pattern and fruit weight pattern according to the day from beehive installation: (A) bee traffic of *A. mellifera*, (B) bee traffic of *B. terrestris*, (C) fruit weight pollinated by *A. mellifea*, and (D) fruit weight pollinated by *B. terrestris*.

Table 9. Correlations between days from hive installation and the weight of fruits pollinated by *Apis mellifera* and *Bombus terrestris*

Bee traffic per day ×	Days from hive installation	Fruit weight	
		A. mellifera	*B. terrestris*
A. mellifera	−0.615**	0.507*	N/S^z
B. terrestris	−0.865**	N/S	0.760*

^{z}No significance
Each set of values was used to calculate the two correlations. * and ** indicate significant correlations at $p < 0.05$ and $p < 0.01$, respectively (two-tailed test).

are recognized when using insect pollinators, a sufficient pollination effect can be obtained in passionfruit.

CONCLUSION

In Korea, passionfruit cultivators mostly depend on artificial pollination to obtain stable fruit sets. This type of pollination requires a high amount of labor during the flowering period, as each flower opens for only one day. Our results suggested that artificial pollination can be sufficiently replaced by insect pollination. In addition, our results confirmed that the management of insect pollinators is necessary during insect pollination, as simply placing beehives in the greenhouse is not enough for high yields. Our results provided an alternative pollination method that can be used to reduce the amount of labor needed for passionfruit cultivation. However, our study was limited as it only investigated the behavioral characteristics and effects of insect pollinators in confined spaces such as net houses. Therefore, in order to clearly determine the effects of various pollination methods on passionfruit cultivation, further studies are needed to compare the predicted yield and economic feasibility by examining the fruit set obtained using each pollination method. In addition, to effectively use insect pollinators in the field cultivation of passionfruit, it is essential to study the colony size of bees and the pollination method, while considering the total greenhouse area and the number of plants. Moreover, research on the control of foraging bee density to prevent pollen robbing by insect pollinators should also be conducted.

ACKNOWLEDGEMENTS

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