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Study of Honeybee and Bumblebee Pollination for Screen-house Tomatoes in Taiwan【Research report】

台灣塑膠布設施溫網室番茄利用蜜蜂與熊蜂之授粉研究【研究報告】

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Abstract

Two pollinators, honeybee Apis mellifera and bumblebee Bombus eximius were used to evaluate the pollination effects of large-sized tomatoes in vinyl screen-houses in Taiwan. The flower pollen gathering by B. eximius was observed by using the buzzing behavior, while in honeybee it was normal foraging behavior. Three pollination trials were observed from 25 to 34 days in the cooler seasons during November 2010 and April 2013. Even though temperatures in these facilities reached up to 30°C for a total of 116 hours at midday, both bees successfully pollinated the tomatoes. The results showed that using bumblebees for pollination resulted in a lower malformation rate and a higher number of seeds compared to using honeybees and spraying traditional chemical inducers. The pros and cons of using bumblebees for commercial under the subtropical weather conditions of Taiwan were evaluated and discussed.

摘要

本研究利用二種授粉昆蟲，西洋蜜蜂及精選熊蜂，評估牠們在台灣塑膠布設施溫網室內大果番茄之授粉效果。觀察精選熊蜂訪花時使用振動行為採集花粉，而蜜蜂使用正常行為採集花粉。三個授粉試驗組於2010年11月至2013年4月間秋冬季冷涼氣候進行，每組試驗授粉25～34天，在中午設施內超過30°C的高溫總計有116小時，而西洋蜜蜂及精選熊蜂均有成功替番茄授粉。結果顯示熊蜂授粉方法較蜜蜂授粉與施用生長激素促進結果方法使番茄有較低的不良果率及較高的種子數，本文評估及討論在亞熱帶氣候的台灣，商業化利用熊蜂授粉使用的優劣性。

Key words: honeybee, bumblebee, screen-house, tomato pollination

關鍵詞: 蜜蜂、熊蜂、設施番茄、授粉。

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Study of Honeybee and Bumblebee Pollination for Screen-house Tomatoes in Taiwan

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ABSTRACT

Two pollinators, honeybee *Apis mellifera* and bumblebee *Bombus eximius* were used to evaluate the pollination effects of large-sized tomatoes in vinyl screen-houses in Taiwan. The flower pollen gathering by *B. eximius* was observed by using the buzzing behavior, while in honeybee it was normal foraging behavior. Three pollination trials were observed from 25 to 34 days in the cooler seasons during November 2010 and April 2013. Even though temperatures in these facilities reached up to 30°C for a total of 116 hours at midday, both bees successfully pollinated the tomatoes. The results showed that using bumblebees for pollination resulted in a lower malformation rate and a higher number of seeds compared to using honeybees and spraying traditional chemical inducers. The pros and cons of using bumblebees for commercial under the subtropical weather conditions of Taiwan were evaluated and discussed.

Key words: honeybee, bumblebee, screen-house, tomato pollination

Introduction

The tomato is a self-pollinated plant, of which the flower is shaped like a tubular column, and the stamens are entirely within the closed corolla. Wind or insect pollination is necessary to increase the fruiting rate. In temperate regions, bumblebees are utilized as the main pollinator for tomatoes (Velthuis and Doorn, 2006). In Korea, Japan and China, the number of pollination hives has increased rapidly in recent years (Inoue *et al*., 2008; Yoon *et al*., 2011). In the subtropical area of Taiwan, farmers used to apply plant growth regulators (usually tomatotone) rather than depend on natural crossing to ensure a good tomato fruiting rate. This is especially true during the summer in the facility houses (Kou,

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1965; Chen and Hanson, 2001).

Chen and Hsieh (1996) evaluated the imported bumblebee *Bombus terrestris* as the pollinator in place of labor-costing artificial treatment for facility tomatoes; thereafter the import on commercial sale bumblebee pollinator into Taiwan was stopped. For local farmers, honeybees are easily obtained and released into facilities for pollination purposes because there are about 110,000 honeybee hives existed in Taiwan (Anonymous, 2012). In addition, Sabara and Winston (2003) mentioned that pollination using the honeybee (*Apis mellifera*) was able to enhance the tomato quality in Canada. Chiang *et al*. (2009) developed a method for rearing 2 indigenous bumblebee species, *B. eximius* and *B. sonani*. Li *et al*. (2010) evaluated the possibility of using the bumblebee *B. eximius*, for the pollination of facility tomatoes. However, to date there has been no report in Taiwan proposing which insect pollinator as a substitute for using a plant growth regulator for tomatoes. Therefore, the main purpose of this study was to find out how honeybees and bumblebees react to the change in temperature and foraging, and compare those pollinated fruit qualities that reflect their pollination effects in the common seen vinyl screen-houses in the subtropical area of Taiwan.

Materials and Methods

The tomato pollination experiments were conducted in PVC vinyl screen-houses from November 2010 until April 2013. The tomato fruits were large size, including Taoyuan Asveg No. 20, Hualien Asveg No. 18, and Known-You 933 of green shoulder hybrid varieties. The pollination trials were started one or one half month later after transplanting, depending on the first layer of tomato flowering. Pollination activities and temperature in the screen-houses were measured during three observation periods: period A (34 days), from December 1, 2010 to January 3, 2011; period B (25 days), from February 14 to March 9, 2012; period C (30 days), from January 9 to February 7, 2013. The temperatures were measured by thermal couple sensors and recorded at one-hour intervals by means of HOBO® UTBI-001 data loggers. Thereafter, the average temperature, maximum and minimum temperatures at every three days retrieved from loggers were statistical calculated.

The natural crossing flowers, treated by artificial vibrator and emasculation flowers were marked and bagged under bee-free conditions in the screen-houses. Afterward, each colony of honeybee *A. mellifera* and bumblebee *B. eximius* was released for pollination in separated screen-house. Each colony was under normal conditions, i.e., 4 frames for honeybees with 8,000 to 10,000 workers, and a wooden hive for bumblebee with 60 to100 workers, respectively. Blankets were spread over the pollination hives to avoid direct sunlight in the screen-houses. The activities of the honeybees and bumblebees within their hives were observed when the temperature ranged between 25°C to 35°C. The flower visiting behavior of both bees were investigated via visual inspecting, direct checking and repeat recording on the single flower-visiting process. The flowers that were visited by either a honeybee or bumblebee were marked right away. A number of the 4-CPA (tomatotone) treated flowers were also processed during the bee-pollination periods. All observations were stopped as soon as the bumblebee colony diminished their foraging activities. To compare the difference in fruit quality between the different treatments, the fruit malformation rate, fruit weight, and number of seeds were kept track. The descriptive statistic, t-test, Mann-Whitney U-test and one way ANOVA statistical analyses were conducted using the MS-EXCEL and SPSS software.

Results

Hives activities of bees under different temperatures in screen-houses

The bumblebee workers exhibited normal activities within their nests under ambient temperatures between 25 and 29°C, including nest maintenance and larva incubation. When the ambient temperature exceeded 30°C, workers became impatient and started fanning at the nest entrance and at areas higher up in nest comb. The comb tended to deform, when the temperature reached 35°C in the nest, and the workers left the combs. As to honeybees, they also exhibited a fanning behavior under high ambient temperatures (30-35°C), but their nest activities did not change as drastically as observed in nests of the bumblebees.

The flower visiting activities of a bumblebee colony lasted from 25 to 34 days depending on hive conditions in the screen-houses. The foraging activities of honeybees were consecutive seen in the meantime, and the foragers were not diminished compared with those of the bumblebees. The honeybee foragers did not seem comfortable in the enclosed space of a screen-house, and a number them frequently aggregated on top of the screen-house, with most of them dying off. When comparing the foraging activities of both bees under changing temperatures, it was found that both showed a drastic decrease in their number of foragers when the screen-house temperature exceeded 30°C. During the period A, the average 3-days temperature ranged from 13.3 to 21.9°C, with a minimum of 3.2°C and a maximum of 39°C. During the period B, the average 3-days temperature ranged from 11.9 to 20.7°C, with a minimum of 9.4 and a maximum of 46.1°C (Fig. 1). The accumulated number of hours with a temperature ranging between 0-10°C and 30-40°C were 129 hours and 74 hours, respectively for period A; temperature ranging between 30-40°C and 40-50°C were recorded for an accumulated 30 hours and 12 hours respectively in period B; and temperature ranging between 0-10°C and 10-30°C were 42 hours and 678 hours, respectively in period C (Fig. 2).

Foraging behaviors on tomato flowers

The typical schematic of honeybee and bumblebee foraging behaviors are shown in figure 3. When honeybees and bumblebees approach flowers, they usually tested and then collected the nectar from preferred flowers. When collecting nectar, honeybees usually grasped the tomato corolla with their legs in an upside down position (Fig. 4A). The ventral side of their body was often far away from the corolla opening. They seldom opened their mandible to press the corolla, instead they tried to suck nectar from the corolla opening by their tongues, and subsequently the pollen grains stuck to their mouth and dropped onto their body hairs. The pollens were then gradually arranged around their legs. Honeybees frequently visited and moved around the same flower (Fig. 3), but any pollen collected on their hind legs were not readily apparent (Fig. 4A). The duration of visiting a flower was between 6 and 222 seconds, and the average was 47.4 ± 43.8 seconds (n = 33). After a honeybee left a flower, a scar on the corolla tips was visible (Fig. 4B).

The bumblebees tended to move the ventral side of their body toward the tip of flower (Fig. 4C), then opened and pressed their mandible several times on the side of corolla, while simultaneously vibrating their wings and produce a buzzing sound. This sonication allows the pollen grains within a few seconds to loosen, and then drop onto the ventral side of the bumblebee's body, from which they are then gathered by their legs. The bumblebees repeated this same behavior on different flowers, until there was sufficient pollen collected on their hind legs (Fig. 4C). They would not visit the same flower again for a while (Fig. 3). The duration of a flower visit

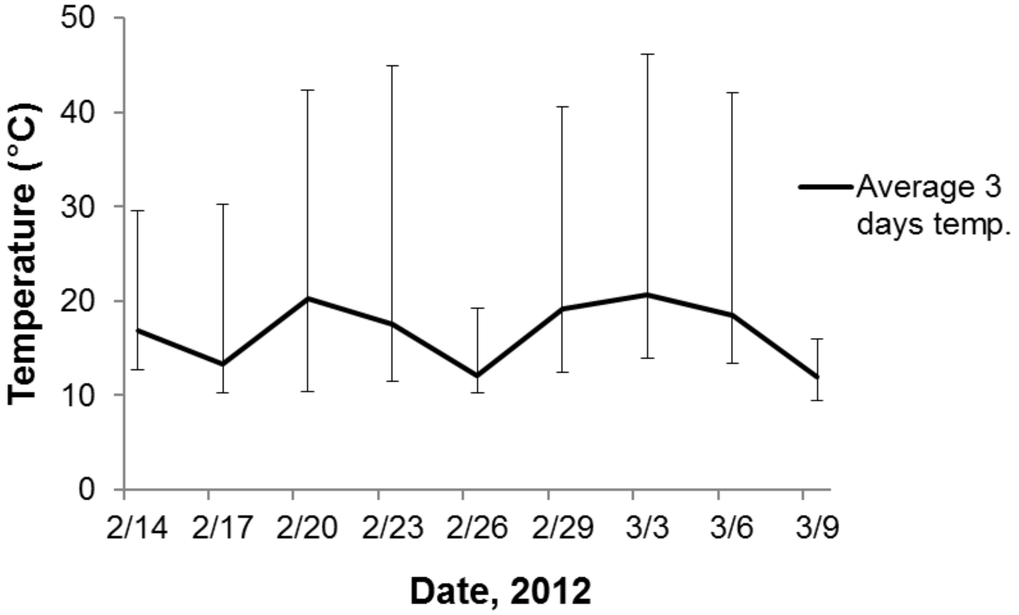

Fig. 1. Temperature fluctuations (average for 3 days) in the screen-house from February 14 to March 9, 2012; the thin vertical lines on the chart indicate the range of temperature.

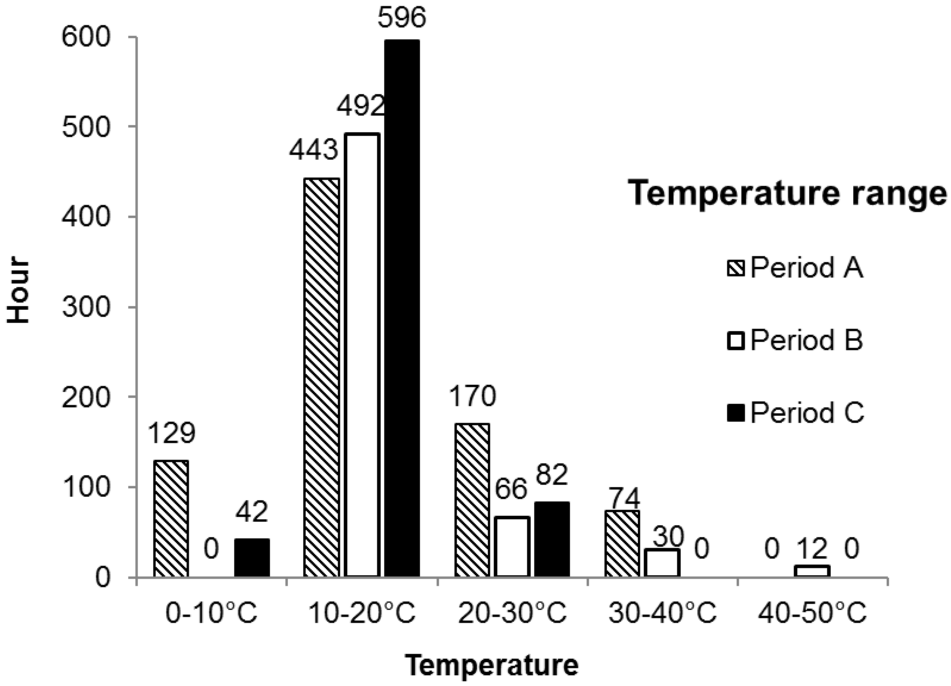

Fig. 2. Cumulative hours of different temperature ranges during pollination periods; period A: 34 days, period B: 25 days, period C: 30 days.

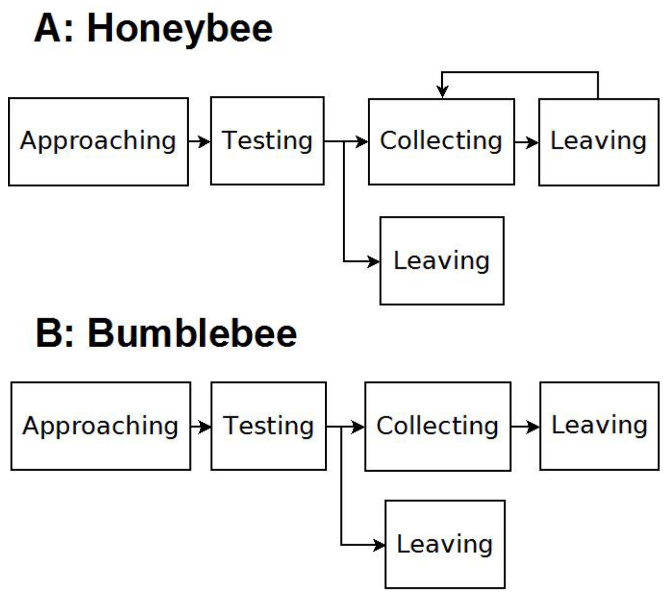

Fig. 3. Typical schematic of the foraging behavior of honeybees and bumblebees on tomato flowers.

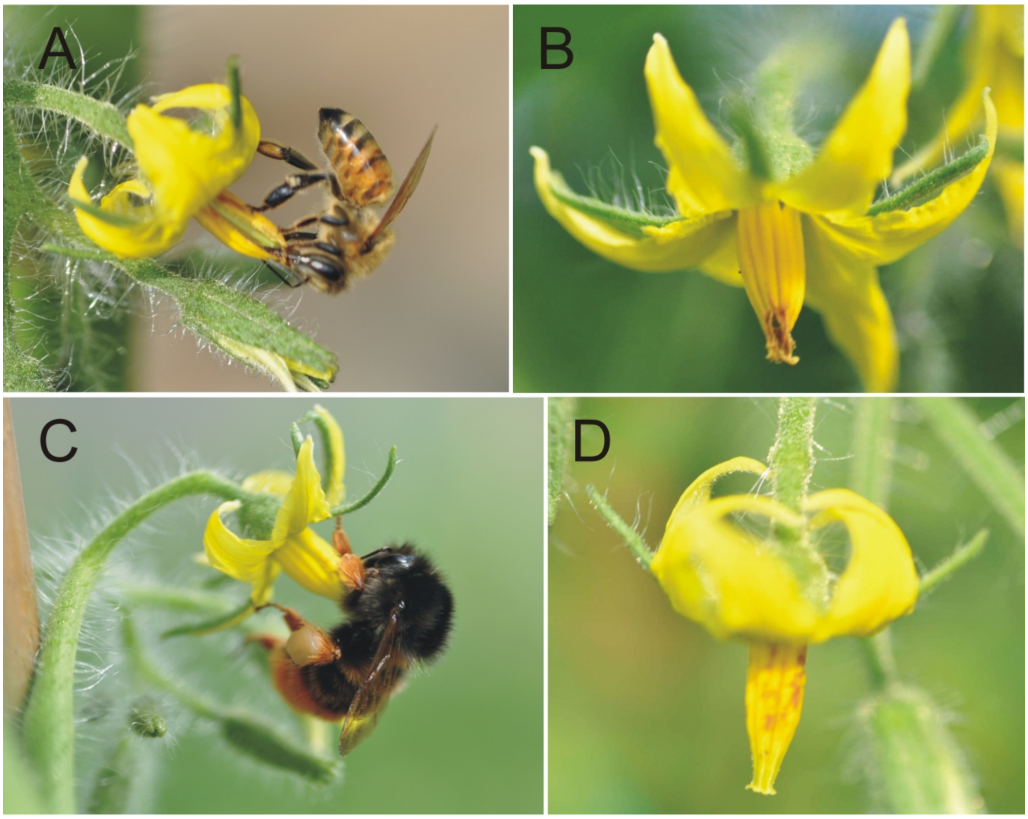

Fig. 4. Foragers on tomato flowers. A: honeybee; B: bite scar by a honeybee visiting a flower; C: bumblebee; D: bite scar by a bumblebee visiting a flower.

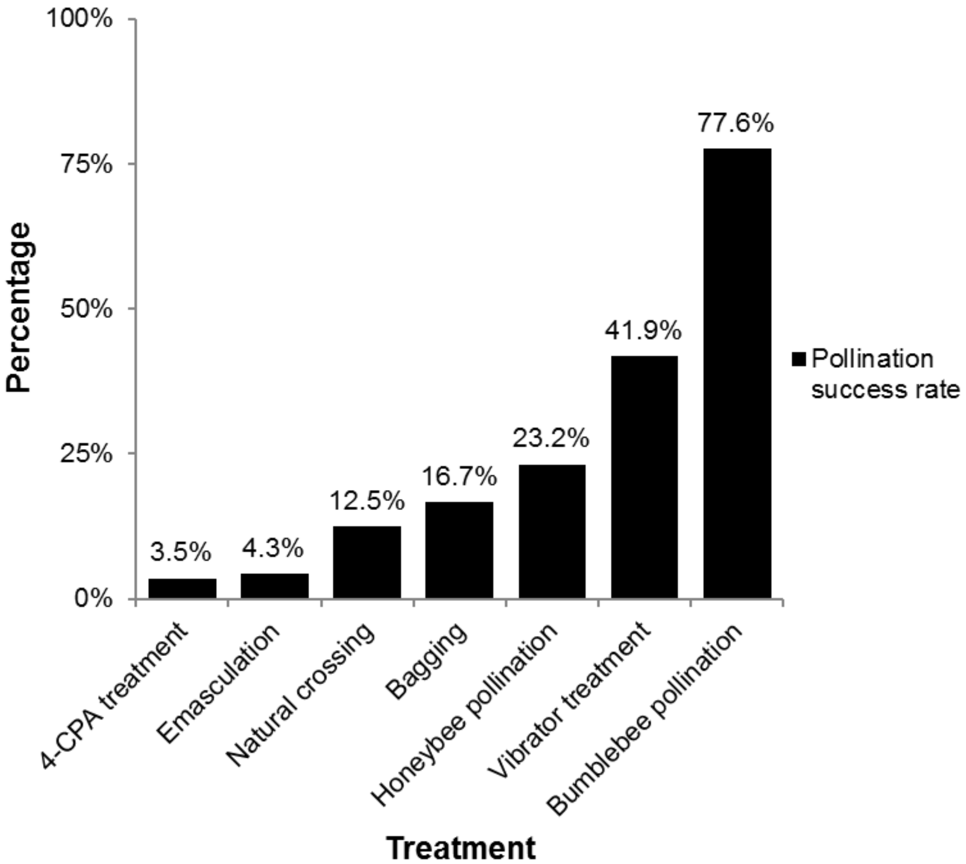

Fig. 5. Pollination success rate for 7 treatments of Hualien Asveg No. 18 tomato.

ranged between 4 and 67 seconds, while the average being 16.4 ± 13.0 seconds (n = 42). The duration of a flower honeybee visiting a flower was significantly longer than bumblebee (Mann-Whitney U-test, $p < 0.05$). After a bumblebee left a tomato flower, a scar on the side of corolla was seen (Fig. 4D).

Differences in fruit quality between those treated with 4-CPA and those that were bees pollinated

For Hualien Asveg No. 18, the percentage of fruit included seeds was 77.6% (n = 98) for bumblebee pollination, 41.9% (n = 43) for artificial vibrator treatment, 23.2% (n = 56) for honeybee pollination, 16.7% (n = 72) for bagging treatment, 12.5% (n = 112) for natural crossing, 4.3% (n = 47) for emasculation, and 3.5% (n = 119) for the 4-CPA treatment (Fig. 5). For Taoyuan Asveg No. 20, fruit malformation was 2.2% (n = 45) for bumblebee pollination, 12.8% (n = 47) for honeybee pollination, 37.5% (n = 16) for natural crossing, and 57.7% (n = 26) for the 4-CPA treatment (Fig. 6). The average number of seeds per Taoyuan Asveg No. 20 tomato using bumblebee pollination, honeybee pollination, natural crossing, 4-CPA, and honeybee pollination +4-CPA treatments was 195.2, 177.3, 173.9, 89.2, and 96.3 seeds, respectively. There was no comparative difference in the number of seeds for bumblebee pollinated, honeybee pollinated, and naturally crossed tomatoes (One-way

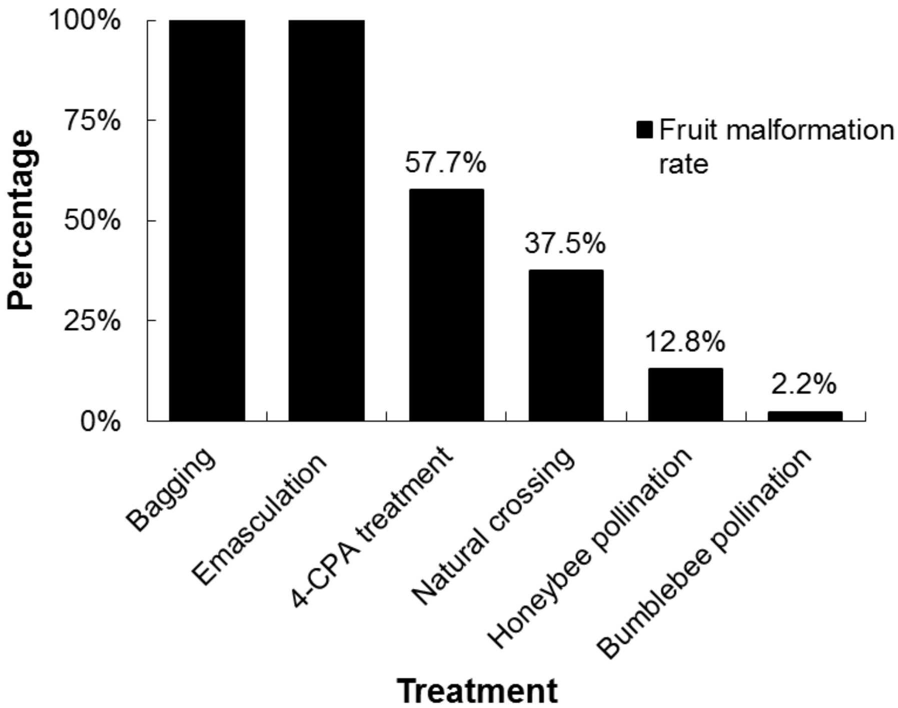

Fig. 6. Percentage of fruit malformation for 6 treatments of Taoyuan Asveg No. 20 tomato.

ANOVA and Bonferroni-Holm Posthoc test, $p < 0.05$), but there was a significant differences in the number of seeds that were 4-CPA treatment and those that were honeybee pollination+4-CPA treatment. The number of seeds treated by 4-CPA and honeybee pollination+4-CPA treatments was not significantly different (One-way ANOVA and Bonferroni-Holm Posthoc test, $p < 0.05$) (Table 1).

For Known-You 933, fruits with a commercial sale value were selected and compared (Table 2). The average number of seeds per bumblebee pollinated, honeybee pollinated, and 4-CPA treated tomatoes was 126.5, 58, and 37.1, respectively. There was a significantly different higher number of seeds in bumblebee pollinated tomatoes than in honeybee pollinated and 4-CPA treated tomatoes (One-way ANOVA and Bonferroni-Holm Posthoc test, $p < 0.05$), but there was no significantly difference between honeybee pollination and 4-CPA treatment. The ratios of fruit weight/number of seeds for the three treatments were 1.68, 3.54 and 5.32, respectively.

Discussion

In this study, the experimental trials were carried out under the cool weather conditions of the winter and spring seasons. The diurnal temperature range was usually large, with an average temperature below 25°C. In addition, the temperature in a vinyl constructed screen-house can change drastically, often exceeding 30°C at noon during warm days. Bumblebees and honeybees are well known to have a superior thermal regulating mechanism in their nest (Heinrich, 1996). We observed that both bees exhibited hive activities while in their nests, and that

Table 1. The number of seeds, standard deviation, and the range of the tomato (Taoyuan Asveg No. 20) under the five treatments

Treatment	Seed number*	SD	Range
Bumblebee pollination	195.2 a	55.8	64-319
Honeybee pollination	177.3 a	59.9	29-308
Natural crossing	173.9 a	53.4	59-260
Honeybee pollination+4-CPA treatment	96.3 b	84.7	0-204
4-CPA treatment	89.2 b	71.4	0-272

* Mean values followed by the different letters for a given variable are significantly different by Bonferroni-Holm posthoc test ($p < 0.05$)

Table 2. The fruit weight, number of seeds, standard deviation, and the range of the tomato (Known-You 933) under the three treatments

Treatment	Fruit weight (n, ranges (g))*	Number of seeds*	Weight/ number of seeds ratio
Bumblebee pollination	212.5 ± 59.7 a (n = 36, 100.3-349.0)	126.5 ± 72.9 a	1.68
Honeybee pollination	205.0 ± 81.0 a (n = 64, 105.0-487.1)	58.0 ± 48.1 b	3.54
4-CPA treatment	197.5 ± 69.8 a (n = 48, 107.9-336.2)	37.1 ± 34.2 b	5.32

* Mean values followed by different letters for a given variable are significantly different by Bonferroni-Holm posthoc test ($p < 0.05$)

they decreased the number of foragers when the screen-house temperature was around 30°C or higher. During periods A-C the temperature in the screen-houses exceeded 30°C for 116 hours, those periods were not consecutive during nights. Furthermore, in the screen-houses the bumblebee pollinating boxes were used only for 25-34 days, which was shorter than for the honeybee hives. This was done because we assumed that a considerable period with high temperatures might impact the nest activities of the bumblebees and reduce the longevity of the colony.

We tested the bumblebee species *B. eximius*, as well as the commercially used species *B. terrestris*, because they are superior in collecting pollen from tomato flowers in the screen-house compared to the honeybee *A. mellifera*. When bumblebees visit the flower of the tomato plant they use their mandible to frequently press the lateral side of the corolla, while at the same time buzzing to collect pollen. The duration of the flower manipulation process by a bumblebee is relatively short, and the flower is not revisited repeatedly. On the other hand, honeybees show a normal foraging behavior when collecting pollen and nectar from the tomato flowers. Their tongues seemed difficult to contact the nectary inside the corolla, led them sucked nectars difficulty from the corolla opening. Thus, the honeybee spends a longer time visiting a flower, in consequence, and the pollen transferred onto the stigmas is considerable less resulting in fruits with a higher malformation rate and a lower number of seeds than tomatoes pollinated by bumblebees. Although honeybees seem to be pollinators to produce facility tomatoes with a higher number of seeds, their foraging behavior results in incomplete pollination task, increasing the risk of pollination failure (Wilcock and Neiland, 2002). The buzzing behavior of bumblebees is very effective when visiting tomato flowers, and results in tomatoes with a higher number of seeds, a lower fruit malformation rate, and considerable better fruit textures compared to tomatoes from plants pollinated by honeybees or received CPA treatment. Based on these observations, we considered that pollination by bumblebees of facility tomatoes in the subtropical area of Taiwan has merits for enhancing both tomato quality and quantity, similar to what is already common practice in many temperate regions (Velthuis and Doorn, 2006).

Most bumblebees are known to have the ability to tolerate low temperatures in higher latitude regions (Williams, 1991). We considered that bumblebee pollination for facility tomatoes can be effective in the subtropical area of Taiwan if we ensure suitable conditions. We considered the fact that temperature barriers would be the most obstacles for releasing bumblebees in a simple constructed facility. High temperatures are lethal to the activities of bumblebees, inside and outside their nest, and it greatly decreases their pollination efficiency. Thus, reducing temperature fluctuations in their hive would increase the colony's longevity and reduce damage to the hive. Tomato production in the winter months could result in the bumblebee colonies suffering from high noon temperatures on warm days. Nevertheless, a few simple measures could protect the pollination hives from extreme temperatures. Properly designed retractable shade nets, or carefully chosen sites that avoid direct sunlight or solar radiation would go a long way to solving the temperature issues for the bumblebees. In addition, the pollination hives should consist of large colonies, or ensure a continuous supply of healthy colonies to support long-term production. For the production tomatoes during the hot summer months it might be more practical to apply a plant growth regulator or use honeybee pollination to increase the fruiting rate. Nevertheless, when using bumblebee pollination when the weather becomes hotter, applying a cooling technique in the screen-house, or adjusting the temperature in the hive would extend the length of time bumblebees could be used effectively.

Although the indigenous bumblebee *B. eximius* could be kept and pollinate tomatoes under experimental conditions, there is still a long gap to be done before they could be used for large-scale commercial production (Sung, personal communication). Sung *et al*. (2011) indicated that the bumblebees in Taiwan are common to mountainous areas above 1,000 m, and these wild populations are confined to steep forested terrains and nearly unable to capture. Asada (2004) indicated that to create a year-round mass production system of bumblebees required not only the appropriate rearing techniques and facilities, but also necessitated having a large stock of wild bumblebees. In addition, parasites had to be excluded under check at all times in any breeding program. However, it seems more likely to use imported commercial species such as *B. terrestris*, to achieve the bumblebee pollination requirements for the near future (Sung, personal communication). Therefore, in order to avoid the problem of imported species leading to the ecological problems in Taiwan, it is becoming critical that advanced measures such as quarantine and regulatory controls are established and implemented as soon as possible.

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台灣塑膠布設施溫網室番茄利用蜜蜂與熊蜂之授粉研究

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摘　要

本研究利用二種授粉昆蟲，西洋蜜蜂及精選熊蜂，評估牠們在台灣塑膠布設施溫網室內大果番茄之授粉效果。觀察精選熊蜂訪花時使用振動行為採集花粉，而蜜蜂使用正常行為採集花粉。三個授粉試驗組於 2010 年 11 月至 2013 年 4 月間秋冬季冷涼氣候進行，每組試驗授粉 25～34 天，在中午設施內超過 30°C 的高溫總計有 116 小時，而西洋蜜蜂及精選熊蜂均有成功替番茄授粉。結果顯示熊蜂授粉方法較蜜蜂授粉與施用生長激素促進結果方法使番茄有較低的不良果率及較高的種子數，本文評估及討論在亞熱帶氣候的台灣，商業化利用熊蜂授粉使用的優劣性。

關鍵詞：蜜蜂、熊蜂、設施番茄、授粉。

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