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Utilization of Hairy Footed Flower Bee *Anthophora plumipes* (Hymenoptera: Apidae) for Pollination of Greenhouse Strawberry

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Abstract

Pollination efficiency of hairy footed flower bee *Anthophora plumipes* for greenhouse strawberry was evaluated in a small greenhouse located in Shimane University, Matsue, Japan (lat. 35˚29', long. 133˚27' and elevation 170 m). Twenty five female and six male bees were released in a greenhouse on April 15, 2014. Red clay soil blocks were provided as the nesting materials. Two hundred and fifty pots of strawberry (*Fragaria annanasa*) were allocated for the following three treatments: 1) no supplementary pollination (control), 2) hand pollination, and 3) bee pollination. Both male and female bees effectively foraged flowers throughout the day. Rate of fertilized seed by the bee pollination was significantly greater than no supplementary pollination (control) and similar to that in the hand pollinated flowers. Quality of the fruits (evaluated based on the shape and deformity) was also improved by the bee pollination. *A. plumipes* effectively pollinated strawberry flowers in the greenhouse and could be developed as an alternative pollinator for this crop.

Keywords

***Anthophora plumipes*, Greenhouse, Strawberry, Pollination, Fruit Quality**

1. Introduction

Cultivation of strawberry in greenhouses is increasing worldwide for getting better quality and higher value of the fruits. In Japan, about 90% of the total strawberry production is carried out under protected structures [1]. Strawberry flowers are hermaphrodite and self-compatible to a certain extent. Berry deformity is a severe prob-

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lem in commercial cultivation of strawberries in greenhouses. Strawberry flowers contain many carpels and it is necessary that each carpel should contain a fertilized ovule (seed) in order to produce a well-formed berry. Berries are deformed at parts in which achenes are not fertilized. Therefore, a successful pollination is required for seed formation and better quality of the fruits. Studies have shown that number of fertilized achenes and quality of strawberry fruit are directly related to the pollination [2]-[5]. Since fertilization of strawberry has direct influence on the fruit quality, it is crucial that the flower inside greenhouses needs agents for pollination.

Hand-pollination using special devices such as hand, brushes or electric brushes, is commonly practiced for the pollination of strawberry in greenhouses [6]. Although these methods improve the pollination efficiency, some devices are expensive and have risks of damaging the flowers. Alternatively, bees have been used as pollinators for quality fruit production under the greenhouse.

European honeybees (*Apis mellifera*) are commonly used as pollinators for strawberries inside the greenhouse [7]-[10] and in the open fields [11] [12]. Bumble bees are an alternative pollinator to honeybees for strawberries [7] [10] [13]. Several species of stingless bees, including *Nannotrigona testaceicornis* (Lepeletier), *Trigona minangkabau* and *Tetragonisca angustula* (Latreille) have been successfully tested for strawberry pollination inside greenhouses in Japan [14]-[17]. Some Megachilidae, such as *Osmia rufa* and *O. cornifrons* have also been found to be effective for pollinating this crop [18] [19].

Hairy footed flower bee *Anthophora plumipes* (Pallas) (Hymenoptera: Apidae) is a ground-nesting, solitary bee, occurring from Western Europe to East Asia [21]. It is one of the first bee species active during the spring [20]-[22]. In a previous study, we found that *A. plumipes* females were manageable pollinators with stable flight activities in the greenhouse [23].

In this study, pollination efficiency of *A. plumipes* bees was evaluated for developing alternative pollinators for strawberry under greenhouse conditions. This paper describes the foraging activities of *A. plumipes* bees on greenhouse strawberry and quality of the fruits pollinated by the bee.

2. Materials and Methods

2.1. Bees and Nesting Materials

Adult *A. plumipes* bees were collected from their natural habitat in a forest located in Izumo City, Shimane prefecture, Japan. The collected bees were kept in an incubator for about one month at 6˚C before liberating in the greenhouse. Twenty five female and six male bees were released in the greenhouse for the pollination experiment. Soil blocks were provided as nesting substrates of the bees in the greenhouse (**Figure 1(B)**). The blocks were constructed using red clay soil.

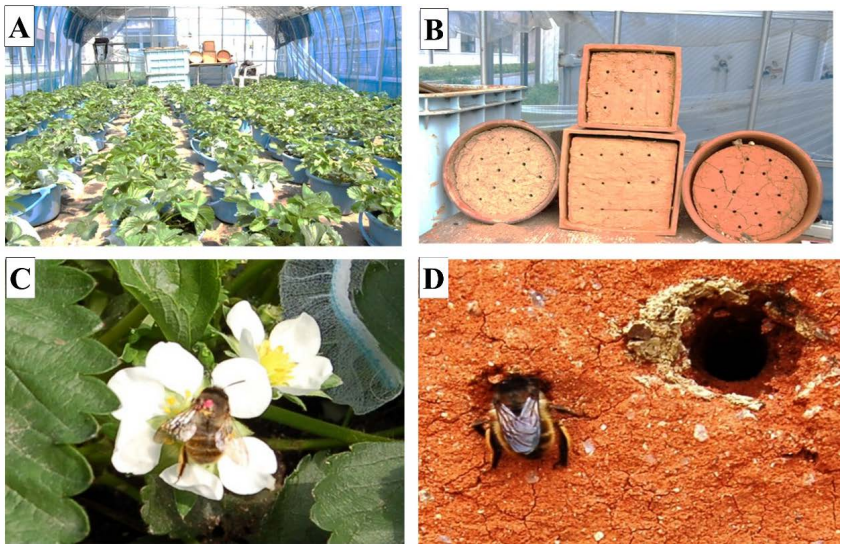

Figure 1. Photographs showing strawberry plants in a greenhouse (A), soil blocks for nesting (B), a *A. plumipes* bee visiting strawberry flower (C), and a *A. plumipes* bee digging its nest in soil block (D).

2.2. Greenhouse and Treatments

A small greenhouse (8.1 m long, 4.5 m wide, and 2.9 m high) located at Shimane University, Matsue, Japan (lat. 35˚29', long. 133˚27', and elevation 170 m) was used in this study. Two hundred and fifty strawberry (*Fragaria ananassa*) (cv. Hokowase) seedlings at 5-leaf stage were planted in the plastic pots (each pot was 35 cm wide and 40 cm deep) in October 2013 (**Figure 1(A)**). Twenty, 50, and 180 pots of strawberry were allocated for control (no supplementary pollination), hand-pollination (using a brush), and bee pollination, respectively. Plants receiving hand-pollination and no supplementary pollination (control) were covered with nets before releasing the bees. The bees were released on April 15, 2014. The flowers bloomed before liberating the bees were hand-picked.

During the experiment, temperature inside the greenhouse ranged from 10.7˚C to 29.6˚C, relative humidity was from 21% to 77%, and solar radiation levels were from 1082 to 1830 lux.

2.3. Fertilizer, Disease and Insect Management

Pots were filled with soil mixed with composted cow manure (2%, w/w). Ammonium sulfate (3 g/pot) was top dressed just before the flower initiation. No pesticide was applied for controlling disease and insects. Insects were hand-picked when observed.

2.4. Observation of Foraging Activities

Foraging and related activities of the bees after liberation in the greenhouse were observed during the flowering period. Number of flowers visited per unit of time and handling time (time spent per flower) by male and female bees were also recorded.

2.5. Measurement of Fruit Quality Parameters

Strawberries were harvested every two to three days from May 8 to May 31, 2014 when fruits were red. Number of fertilized and non-fertilized achenes were recorded to evaluate the pollination efficiency. After separating the seeds from the pulp, it was taken in a beaker filled with water. Submerged and floated seeds in the water were recorded as fertilized and non-fertilized achenes, respectively.

Fruit length and width were measured using Vernier calipers. Five-level deformity ranking was made based on the measurement of shapes and visual observations as follows: quite few unfertilized achenes and not deformed (A), some unfertilized achenes but not deformed (B), many unfertilized achenes and slightly deformed (C), quite unfertilized achenes but slightly deformed (D), and many unfertilized achenes and highly deformed (E).

2.6. Data Analysis

Significance of three pollination treatments was evaluated by analyzing the data using ANOVA (Minitab version 14). Treatment means were compared using the least significant difference test at $p < 0.05$.

3. Results

3.1. Foraging Behavior of *A. plumipes*

The bees started foraging nectar from the strawberry flowers soon after the liberation. Pollen collection was observed after four days from the liberation in the greenhouse. While visiting the flowers (**Figure 1(C)**), the bees always landed on the top of the flower with a circular movement either from right to left or left to right. The bees landed at the corner of flower disc for collecting nectar and walked to the whole pistils for collecting pollen. Both females and males successfully visited strawberry flowers (**Table 1**). The average time (±SD) spent on the flower (handling time) by the male bees was 8.7 sec (±4.9) (n = 132) and that of the females was 11.0 sec (±6.9) (n = 340). Male bees visited an average of 10.2 (±3.0) (n = 28) flowers per minute, while females visited 6.5 (±3.0) (n = 68) flowers per min.

3.2. Effect of Pollination on Seed Fertilization

Figure 2 shows the rate of seed fertilization for each treatments. The rate of fertilization in bee-pollinated flowers

Table 1. Hourly flower handling and visiting patterns of males and females of *A. plumipes* bees on strawberry flowers in a greenhouse.

Time	Handling time (sec/flower) ±SD (number of observations)		Number of flower visited/min ±SD (number of observations)	
	Male	Female	Male	Female
8:00-9:00	6.3 ± 3.0 (8)	12.7 ± 8.4 (6)	13.4 ± 3.0 (9)	5.5 ± 4 (6)
9:00-10:00	7.7 ± 4 (15)	12.6 ± 8.7 (27)	11.1 ± 4.0 (4)	7.1 ± 4 (17)
10:00-11:00	9.0 ± 5.6 (35)	13.2 ± 8.3 (29)	10.5 ± 2.0 (9)	7.5 ± 2.0 (6)
11:00-12:00	7.1 ± 3.7 (25)	9.4 ± 5.3 (68)	11.3 ± 5.0 (3)	8.7 ± 1.0 (9)
12:00-13:00	7.7 ± 4.8 (39)	8.0 ± 2.6 (43)	6.2 ± 1.0 (2)	7.3 ± 5.0 (9)
13:00-14:00	8.7 ± 4.5 (4)	10.77 ± 6.4 (47)	8.5 ± 0.0 (1)	4.9 ± 5.0 (4)
14:00-15:00	7.5 ± 3.9 (2)	11.0 ± 8.9 (61)	ND	5.2 ± 2.0 (8)
15:00-16:00	15.6 ± 9.3 (4)	10.5 ± 6.3 (59)	ND	5.5 ± 1.0 (9)
Mean	8.7 ± 4.9 (132)	11.0 ± 6.9 (340)	10.2 ± 3 (28)	6.5 ± 3 (68)

ND, Not detected.

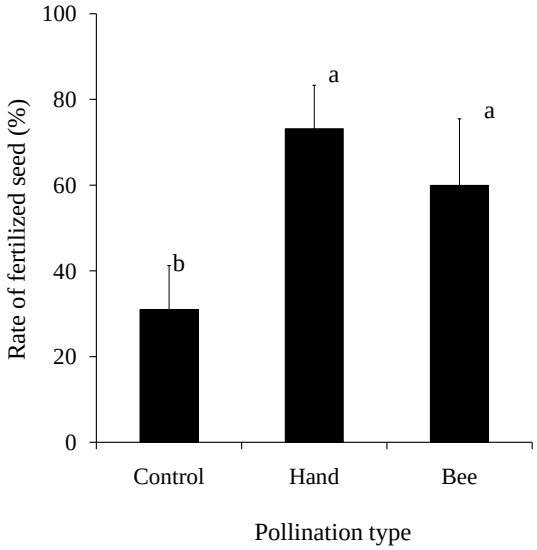

Figure 2. Rate of fertilized seeds in control, hand-pollinated, and *A. plumipes* bee pollinated strawberry flowers. Values with the same letter are not significant ($p < 0.05$, least significant difference test).

was 59.9%, which was significantly higher than that in the control (31.0%). No significant difference in seed fertilization rate was observed between the bee- and hand-pollination indicating the *A. plumipes* bees can effectively pollinate the strawberry flowers in the greenhouse.

3.3. Fruit Quality

The shape of the fruits varied among the three pollination treatments (**Figure 3**). The length/width ratio of almost all bee- and hand-pollinated fruits (>95%) was from 1.0 to 1.5, while the length/width ratio in non-pollinated (control) fruits was 40% only. As the length/width ratio of strawberry fruits from 1.0 to 1.5 is highly preferred by the Japanese consumers, pollination seems necessary for not only increasing the fruit production, but also for getting higher profit.

Less deformed fruits were observed in bee pollinated and hand-pollinated fruits (**Figure 4**). On a 5-scale deformity ranking basis, most of the fruits in non-pollinated control were medium to highly deformed, while a large proportion of the fruits were normally formed with bee-pollination.

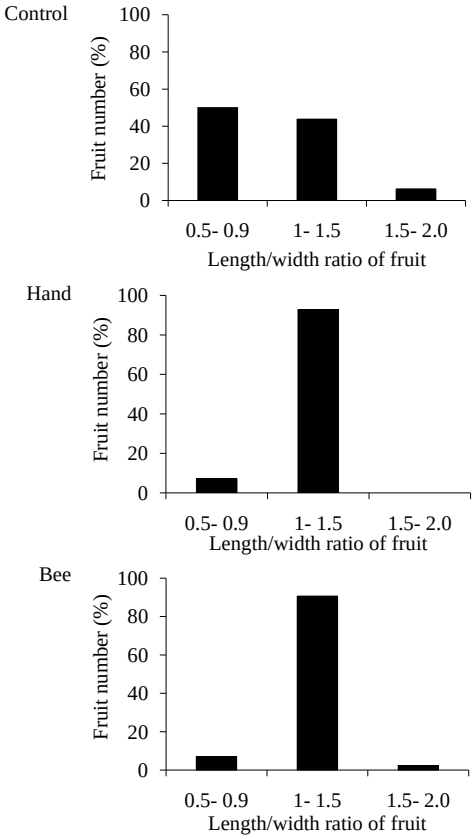

Figure 3. Proportion of fruits with different shapes (length/width ratio) in control, hand-pollinated, and *A. plumipes* bee pollinated strawberry flowers.

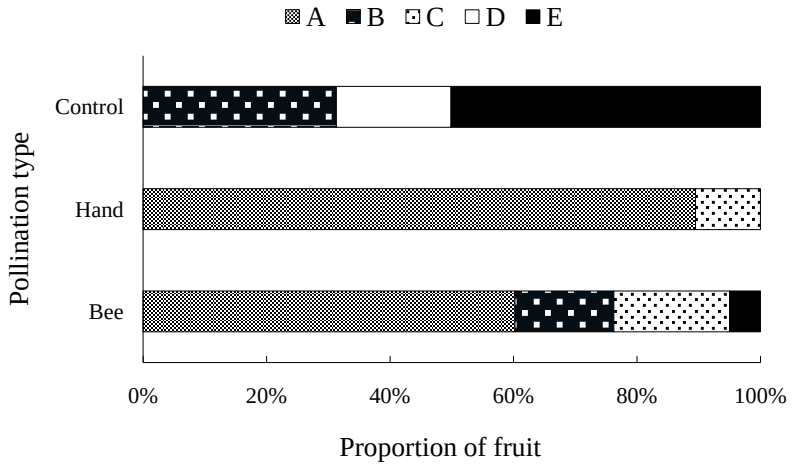

Figure 4. Relative proportion of the fruits of different deformity levels in no supplementary pollination (control), hand pollination and *A. plumipes* bees pollinated strawberry. Deformity levels: quite few unfertilized achenes and not deformed (A), some unfertilized achenes but not deformed (B), many unfertilized achenes and slightly deformed (C), quite unfertilized achenes but slightly deformed fruits (D), and many unfertilized achenes and highly deformed (E).

4. Discussion

A. plumipes pollinated strawberry flowers in the greenhouse as effectively as hand pollination. Both female and

male bees visited the flowers efficiently suggesting this bee as a potential alternative pollinator for greenhouse strawberry. The seed fertility and quality were significantly higher in bee- and hand-pollinated flowers compared with the flowers which received no supplementary pollination. These results are similar to those reported for strawberry pollination by honeybees under greenhouse conditions [24].

A. plumipes bees showed a high rate of adaptability in closed condition and started foraging and nesting activities soon after liberation [23], while no such adaptability to the closed condition is reported in honey bees, bumble bees, stingless bees, carpenter bees and *Osmia* spp. [15] [16] [19] [25]. Results indicated that when the weather condition is adverse, *A. plumipes* could be a suitable pollinator for strawberry and other flowering plants.

Although, the handling time and foraging duration of *A. plumipes* was longer than that of *Osmia cornifrons* in a similar study [19], the longer handling time in this study might be due to a small number of flowers during the active foraging period.

Flowers pollinated by bees make a better quality of fruit than self-pollinated and open-pollinated [5] [15] [16] [19]. Also in this study, a higher quality of fruits by the pollination of bees was found. Higher rate of the fertilized seed and shape of the fruits pollinated by the bees suggested that use of *A. plumipes* could be a good alternative for making higher profit from the greenhouse strawberries.

5. Conclusion

A. plumipes bees could be used as an alternative pollinator for greenhouse strawberry. For the sustainability of greenhouse strawberry production using *A. plumipes* as a pollinator, study on the reproductive ability of this bee under greenhouse condition is necessary when strawberry is the only floral resource.

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