Effects of pollination by *Melipona quadrifasciata* (Hymenoptera: Apidae) on tomatoes in protected culture

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Abstract

To evaluate the pollination efficiency of a stingless bee, *Melipona quadrifasciata*, individuals originating from tropical and subtropical regions of South America were examined on tomatoes (cv. Momotaro 8) cultivated in a greenhouse in summer and autumn. We compared differences in the rates of foraged flowers, pollen weights foraged from anthers, rates of fruit set of tomatoes, seed number of tomato fruits and yields of tomato fruit weight between *M. quadrifasciata* and the bumblebee, *Bombus terrestris*. When the amount of tomato pollen was sufficient, there were no differences in pollination efficiency between the stingless bee and the bumblebee; however, the rates of foraged flowers, the rate of fruit set and the yields were significantly reduced in the stingless bee compared with the bumblebee when fertile tomato pollen decreased markedly during the hottest period of the summer. During this period, the rate of flowers foraged by bumblebees did not decrease, but the rates of fruit set, the seed number and the yields of tomato fruits decreased significantly in comparison with the other periods. It is known that tomato pollen production is inhibited at high temperatures; thus, we consider that the high temperatures in the greenhouse (average daily temperature higher than 28°C) caused the extreme decrease in pollen production. These results indicate that *M. quadrifasciata* can be used as a suitable pollinator of tomatoes if sufficient amounts of fertile pollen are provided.

Key words: Pollination; stingless bee; bumblebee; tomato; protected culture

INTRODUCTION

Artificial treatment for fruit set is necessary for the commercial production of tomatoes in greenhouses. A European bumblebee species, *Bombus terrestris*, exhibits buzz-pollinating behavior that is effective for the pollination of tomato with united poricidal anthers (Pinchinat et al., 1979; Banda and Paxton, 1991; Matsuura, 1993); therefore, the bumblebee has generally been used as a pollinator of greenhouse tomatoes in Japan. However, because the exotic bumblebee might disturb natural ecosystems should it be inadvertently introduced, its use in agricultural production has been restricted since the establishment of the Invasive Alien Species Act in 2006. Recently, use of a Japanese native bumblebee species, *B. ignites*, as a pollinator of greenhouse tomatoes was initiated (Asada and Ono, 1996; Mitsuhata and Wada, 2005); however, even if a mass-produced native bumblebee species is used to replace *B. terrestris*, some potential ecological risks can not be ignored. The following factors are thought to be ecological risks of using bumblebees. First, competition might occur between native and exotic bumblebee species. Second, pollination by native bumblebee species might be disturbed by exotic bumblebee species. Third, there is a possibility that reproductive disturbance through interspecific crosses between native and exotic bumblebee species might occur. Fourth, the risk of migration and expansion of alien parasites is increased by the import of bumblebees. Fifth, the genetic diversity of wild *B. ignites* might be disturbed by mass-produced *B. ignites* with low genetic variation (Yoneda et al., 2008).

Stingless bee species of the genus *Melipona* are distributed in tropical and subtropical regions of...
Central and South America (Sakagami, 1982; Michener, 2000), and have long been used in apiculture (Sakagami, 1958). Recently, as stingless bees exhibit buzz-pollinating behavior (Roubik, 1989; Heard, 1999), their use as alternative pollinators for tomato production in greenhouses has been promoted, mainly in Central and South America (Slaa et al., 2000, 2006; Cauich et al., 2004; Del Sarto et al., 2005). However, stingless bees have scarcely been investigated in temperate regions for use as pollinators (Maeta et al., 1992; Amano et al., 2000; Hikawa and Miyanaga, 2006, 2007). *Melipona* species might not establish populations in Japan because most do not have sufficient ability to thermoregulate their nest (Sakagami, 1958; Sung et al., 2008) and can not overwinter in the temperate zone (Slaa et al., 2006). In fact, the stingless bee species, *M. quadrifasciata*, used in this study, cannot overwinter in an open field in Hioki City (31°40′N; 130°19′E), Kagoshima, Japan (Hirano, personal communication); therefore, the stingless bee poses few ecological risks, except for the migration of alien parasites by imported bees, as shown by Yoneda et al. (2008). These characteristics of stingless bees make them very suitable for introduction into Japan.

In this study, we compared the pollination efficiencies of the stingless bee, *M. quadrifasciata* and the European bumblebee, *B. terrestris*, and evaluated the practical value of *M. quadrifasciata* for use with tomatoes cultivated in side-opening greenhouses in summer and autumn, which is the major cropping system for tomatoes in Japan.

**MATERIALS AND METHODS**

**Pollinators and hive management.** *M. quadrifasciata* colonies were imported from Brazil in May 2006. The number of workers in a hive was about 300. As shown in Fig. 1, the hive was placed in a cabinet made of plywood and furnished with a hand-made air conditioning system, using a simple air conditioner for bumblebees (Hayama Electric Works Co., Ltd., Tsu, Japan) and electronic heat mats (Showa Seiki Kogyo Co., Ltd., Kikugawa, Japan). The temperature in the hive in the cabinet was kept at 25–30°C. *M. quadrifasciata* was supplied with 30 ml of 50% honey solution, 2 g fresh pollen, 1 g propolis and 10 ml water every 4–5 days throughout the study.

*B. terrestris* was a commercial strain produced as CATS MARUHANABACHI® (supplied by Agrisect Inc., Toride, Japan). The bee was supplied with 2 g dry pollen every day and fed a continuous supply of 50% sugar solution, which was provided from the hive of a congener.

**Experiment.** The experiments were conducted in two cages (each 4×22 m, height 3 m) made of 2–4 mm mesh polyethylene screen (Wide Raschel®; Nihon Widecloth Co., Ltd., Kashiwara, Japan) in a plastic greenhouse with a side opening (9×25 m, maximum height 3.5 m) at Okayama Prefectural Agricultural Experiment Station (Akaiwa, Okayama, 34°47′N; 134°01′E). On 16 May 2006, 26 plastic containers (23×64 cm, depth 19 cm) were planted with two tomato plants (cv. Momotaro 8) at the 5-leaf-stage. Thirteen containers per row were arranged in two parallel rows at intervals of 2.3 m in each cage. As the control,
flower clusters of 26 tomato plants per cage were covered with polyethylene screen (2–4 mm mesh) to prevent flower foraging by the pollinators until every flower had withered.

The release period for both pollinator species was 3 June to 6 October, 2006. To avoid injury to tomato styles by overvisiting, the release time of *B. terrestris* was limited to between 9:00 and 10:30 once every 2–3 days, but the release of *M. quadrifasciata* was not restricted throughout the experimental period. Although the hive of *B. terrestris* was replaced on 3 August, the original hive of *M. quadrifasciata* was maintained because this pollinator’s colony did not decline.

The number of *M. quadrifasciata*-foraging workers was directly counted at 8:30 every 5 days from 12 June to 25 September. The rates of foraged flowers by workers of *M. quadrifasciata* and *B. terrestris* were calculated from the bite marks on each of 20 flowers at the petal-deployment stage in the center of each row every 7 days.

To measure the rate of foraged pollen from anthers, half the number of flower buds in each flower cluster in both cages was covered with polyethylene screen (1×1 mm mesh) just before flowering and these tomatoes were prevented from foraging by pollinators for 3 days. Three days later, 40 individual anthers, taking four anthers per flower, were collected from covered and uncovered flowers in both cages. Collected pollen was dried and weighed by the method of Hikawa and Miyanaga (2006). The mean foraged pollen weight per anther was calculated by subtracting the pollen weight of non-covered flowers from that of covered flowers. The rate of pollen foraged from anthers was shown as foraged pollen weight per non-foraged pollen weight.

From 26 June to 25 September, we sampled 40 anthers, taking four anthers per flower, every 7 days. To count the germinated pollen number, a medium for pollen culture was prepared by the method of Fujishita (1981) and poured to a depth of 5 mm in Petri dishes (diameter 9 cm, depth 1 cm). Collected pollen was sown in 10 parts of the medium using one side of a cover glass (18×18 mm, thickness 0.12–0.17 mm) and maintained at 25°C under dark conditions in an incubator. This procedure was replicated twice. After 24 hours, we counted the numbers of germinated pollen and total pollen within 10 microscope fields of view (diameter 1.8 mm) per dish. The fertile pollen weight was estimated by the following equation,

\[ \text{Fertile pollen weight} = \text{pollen weight} \times \text{number of germinated pollen} / \text{total number of pollen}. \]

Thirteen clusters, from which two or three buds were flowering, were chosen at random from both cages every 7 days from 12 June to 25 September, and the numbers of fruit set were counted when the first fruit in the cluster had grown to the size of a ping-pong ball. We harvested the tomato fruits at 3- to 4-day intervals, counted the numbers of fruit, and weighed each fruit. The number of seeds was counted for about 5 fruits from each cage, excluding deformed fruits.

**Data analysis.** Data were analyzed using ANOVA. The data of foraged flowers, foraged pollen and fruit set were arcsine square root transformed prior to analysis. Means were compared using the Tukey-Kramer HSD test at *p*<0.05.

**RESULTS**

**Fertile pollen weights and foraged flower rates**

The fertile pollen weight per anther began decreasing from 45 µg on 26 June to less than 1 µg on 7 August, which we called the decreasing period (DP), and remained at less than 1 µg until 4 September, which we called the lacking period (LP). The fertile pollen weight increased again to 39 µg in 25 September, which we called the increasing period (IP) (Fig. 2). The highest negative correlation between fertile tomato pollen weight and average daily temperature occurred during the 5-day period from the 10th to the 6th day before flowering (Hikawa and Ishikura, 2008). The average daily temperature during the 5-day period from the 10th to the 6th day before flowering was 25.1–27.8°C during the DP, 28.6–29.7°C during the LP and 24.1–25.4°C during the IP (Fig. 2). The numbers of *M. quadrifasciata*-foraging workers during these three periods were significantly different, being 2.1 during DP, 1.0 during LP and 2.0 during IP (*p*=0.05) (Table 1). The number of foraging workers during LP was significantly fewer than during DP and IP (*p*=0.05) (Table 1). The rates of flowers foraged by *M. quadrifasciata* and *B. terrestris* were 94.4 and 99.4% during DP (*p*>0.05),...
60.0 and 100% (p<0.05) during LP, and equal at 100% during IP, respectively (Table 2). The rates of foraged pollen from anthers by *M. quadrifasciata* and *B. terrestris* were 78.9 and 76.4% (p>0.05) during DP, 58.7 and 61.6% (p>0.05) during LP; and 73.3 and 75.1% (p>0.05) during IP, respectively (Table 2).

**Pollination efficiencies**

The rate of fruit set, the seed number per fruit, the weight per fruit and the fruit yield per cluster in cages of both *M. quadrifasciata* and *B. terrestris* during each period were significantly greater than those values for the control (p<0.05) (Table 3). Fruit yields per cluster are shown by fruit weight. Seed number is recognized as a direct indicator of fertilization by pollination. There is a high positive correlation between fruit weight and seed number in tomatoes (Dempsey and Boynton, 1965). These four values in both cages with released pollinators during LP were significantly lower than during DP and IP (p<0.05) (Table 3). The seed number per fruit and the fruit weight per fruit during each period were not significantly different between *M. quadrifasciata* and *B. terrestris* (p>0.05) (Table 3), however, the rate of fruit set and the fruit yield per cluster in the cage with released *M. quadrifasciata* during LP were significantly lower than those of *B. terrestris* for the same period (p<0.05) (Table 3).

**DISCUSSION**

The production of fertile tomato pollen is known to decrease under high temperatures during the summer season (Ikeda and Tadauchi, 1995; Pressman et al., 2002). Hikawa and Ishikura (2008) showed that the correlations of the weight of fertile tomato pollen with an average daily temperature are higher than those of maximum and minimum temperatures. Also, the highest negative correlation between fertile tomato pollen weight and average daily temperature occurs during the 5-day period from the 10th to the 6th day before flowering in the

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**Table 1. Number of *M. quadrifasciata*-foraging workers during the three periods**

<table>
<thead>
<tr>
<th>Period</th>
<th>Number of foraging workers (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>2.1±0.7 a b</td>
</tr>
<tr>
<td>LP</td>
<td>1.0±0.6 b</td>
</tr>
<tr>
<td>IP</td>
<td>2.0±0.7 a</td>
</tr>
</tbody>
</table>

*a* See Fig. 2 for explanations of DP, LP and IP.  
*b* Means followed by the same letter in the same column were not significantly different at p=0.05 (Tukey-Kramer HSD test).

**Table 2. Rate of foraged flowers and pollen from anthers by *M. quadrifasciata* and *B. terrestris* during the three periods assigned by the fluctuation of fertile pollen weight**

<table>
<thead>
<tr>
<th>Pollinator</th>
<th>Rate of foraged flowers (%)</th>
<th>Rate of foraged pollen from anthers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DP</td>
<td>LP</td>
</tr>
<tr>
<td><em>M. quadrifasciata</em></td>
<td>94.4 a</td>
<td>60.0 b</td>
</tr>
<tr>
<td><em>B. terrestris</em></td>
<td>99.4 a</td>
<td>100 a</td>
</tr>
</tbody>
</table>

*a* See Fig. 1 for explanations of DP, LP and IP.  
*b* Means followed by the same letter in the same column and row were not significantly different at p=0.05 (Tukey-Kramer HSD test).
summer season. A similar result was reported by Sato et al. (2002) who showed that inhibition occurred around the 10th day before flowering. Also, Peet at al. (1997) estimated that the production of fertile tomato pollen decreased markedly with an average daily temperature over 30°C. As shown in Fig. 2, the fertile pollen weight per anther decreased markedly to less than 1 mg from early August to early September (LP), when the average daily temperature during the 5-day period from the 10th to the 6th day before flowering exceeded 28°C. The rate of flowers foraged by *M. quadrifasciata* declined to 60% (Table 2). During this period, the foraging activity of workers and the rate of foraged flowers decreased for *M. quadrifasciata* compared with *B. terrestris* (Table 2). For this reason, the rate of fruit set in the cage with released *M. quadrifasciata* decreased, and consequently, fruit yield was reduced compared with these values for *B. terrestris* (Table 3). Although the rate of foraged flowers in the cage with *B. terrestris* was 100% during LP, the rate of fruit set and fruit yield were reduced compared with in other periods (Table 3). These results suggest that the use of *M. quadrifasciata* may be difficult even if the rate of foraged flowers rises in the pollen-lacking season.

However, Del Sarto et al. (2005) reported in Brazil that the rate of tomato flowers foraged by *M. quadrifasciata* in a greenhouse reached 95% under the average daily temperature of over 30°C. There are three possible explanations for the differences with the present study. First, the variety of tomato in the study by Del Sarto et al. had a high temperature tolerance for pollen production (Del Sarto et al., 2005), because the seed mass was sufficient throughout their experimental period. Second, as the density of released workers in the greenhouse in their study (2,051 workers per 100 m²) was higher than that of our study (341 workers per 100 m²), the rate of foraged flowers by workers was higher. Third, surplus amounts of dry pollen were always given to the stingless bees in our study, but if they had not fed on an alternative diet, the workers might have been motivated to explore the tomato pollen.

As mentioned above, the use of *M. quadrifasciata* is difficult during the season when fertile tomato pollen is lacking; however, if fertile pollen is sustained throughout summer, *M. quadrifasciata* will be a very useful tomato pollinator in summer.

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**Table 3. Rate of fruit set, seed number, fruit weight and fruit yield among treatments of *M. quadrifasciata* and *B. terrestris* in the three periods assigned by the fluctuation of fertile pollen weight.**

<table>
<thead>
<tr>
<th>Pollination</th>
<th>Rate of fruit set (%)</th>
<th>Seed number/fruit</th>
<th>Fruit weight (g/fruit)</th>
<th>Fruit yield (g/cluster)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. quadrifasciata</em></td>
<td>DP: 61.4b&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0d</td>
<td>79.9a</td>
<td>32.8b</td>
</tr>
<tr>
<td></td>
<td>LP: 54.2bc</td>
<td>12.3b</td>
<td>72.5a</td>
<td>27.8c</td>
</tr>
<tr>
<td></td>
<td>IP: 51.3bc</td>
<td>12.1bc</td>
<td>71.5a</td>
<td>27.8c</td>
</tr>
<tr>
<td><em>B. terrestris</em></td>
<td>DP: 62.8b</td>
<td>14.6b</td>
<td>58.2a</td>
<td>22.2a</td>
</tr>
<tr>
<td></td>
<td>LP: 46.5bc</td>
<td>13.4bc</td>
<td>58.2a</td>
<td>22.2a</td>
</tr>
<tr>
<td></td>
<td>IP: 46.5bc</td>
<td>13.4bc</td>
<td>58.2a</td>
<td>22.2a</td>
</tr>
<tr>
<td>Control</td>
<td>DP: 0.1e</td>
<td>0.1e</td>
<td>0.1e</td>
<td>0.1e</td>
</tr>
<tr>
<td></td>
<td>LP: 0.1e</td>
<td>0.1e</td>
<td>0.1e</td>
<td>0.1e</td>
</tr>
<tr>
<td></td>
<td>IP: 0.1e</td>
<td>0.1e</td>
<td>0.1e</td>
<td>0.1e</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Fig. 2 for explanations of DP, LP and IP.
<sup>b</sup> Means followed by the same letter in the same column and row were not significantly different at p = 0.05 (Tukey-Kramer HSD test).

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and autumn cultivation in Japan. The fertile pollen weight of tomato (cv. Momotaro 8), which is a major cultivation variety for summer and autumn cultivation in greenhouses, rises to more than 1.6 µg in greenhouses with an average daily temperature below 28°C (Hikawa and Ishikura, 2008). Moreover, the foraging efficiency of *M. quadrifasciata* and the rate of fruit set of tomatoes released in late summer to early winter excelled to values seen for *B. terrestris* at average daily temperatures of 14–23°C (Hikawa and Miyanaga, 2007). In addition, the seed number, yield and quality of tomato pollinated by *M. quadrifasciata* were equal to those of *B. terrestris* (Hikawa and Miyanaga, 2007). In the present study, *M. quadrifasciata* could set tomato fruits at 60–80% under average daily temperatures of 24–28°C (Table 2). Although a limitation of this study is the lack of replication, these results supported that *M. quadrifasciata* promises to be a useful tomato pollinator at an average daily temperature of less than 28°C. Our experimental field is located in warm lowlands in southwestern Japan and is not well suited to tomato cultivation in summer and autumn. Irrespective of the pollinator, such cultivation is typically undertaken in the cool highlands of southwestern Japan because fruit cracking increases at high temperatures (Frazier and Bowers, 1947). *M. quadrifasciata* could be applied to summer and autumn cultivation in areas where the average daily temperature is less than 28°C, and these areas include most areas typically used for tomato production in the summer and autumn. Judging from the findings of Hikawa and Miyanaga (2007) and this study, we conclude that *M. quadrifasciata* could be used as a pollinator in areas well suited to year-round tomato production.

*M. quadrifasciata* presents a minimal ecological risk because the possibility of the stingless bee overwintering is low in temperate zones, and native stingless bees do not inhabit Japan. Additionally, stingless bees with high eusociality can not establish themselves if their colony disperses with nest materials. *M. quadrifasciata* is also safe for growers because they do not have a stinger. Moreover, as the colony of *M. quadrifasciata* is perennial, it is not necessary to replace the hive as is necessary for the bumblebee, which has an annual colony; therefore, the stingless bee may be a more economical pollinator than bumblebees. Thus, *M. quadrifasciata* is a profitable candidate as a tomato pollinator in Japan. In particular, *M. quadrifasciata* has potential as a valuable alternative pollinator in Hokkaido where it can not be used because *B. ignites* is not distributed there. For the practical use of *M. quadrifasciata* in the future, it is necessary to develop a mass production method for this excellent pollinator.

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