

Foraging activities of *Apis mellifera* subspecies on soybean

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ABSTRACT

To make good use of honeybee for soybean pollination, the foraging activities of three honey bee races, namely: *Apis mellifera carpatica* Foti (*A.m.carpatica*), *Apis mellifera carnica* Pollmann (*A.m.carnica*), and *Apis mellifera ligustica* Spinola (*A.m.ligustica*) based on the quantity of foraging bees and soybean pollen load weight were evaluated. Results indicated that the highest peak of the number of out-going bees and in-coming foragers with pollen of *A.m.carpatica* and *A.m.ligustica* were both appeared at 9:00, respectively 56 bees and 74 bees, 40 bees and 34 bees. Foragers with pollen of three subspecies decrease over time after 12:00. The soybean pollen weights with the time from three subspecies all have a peak, peaked respectively at 12:00, 13:00, 12:00 and the weight is 0.9027g, 0.7641g and 0.8027g. The weight of soybean pollen has no significant difference ($P > 0.05$) between three subspecies. The percentage of soybean pollen weight of *A.m.carnica* was highest (60.63%), and there was a significant difference between that of other two races ($P < 0.05$). Therefore, *A.m.carnica* and *A.m.ligustica* were the best performers in relation to foraging activities on soybean during the hot summer under open fields in the region.

Keywords: *Apis mellifera*, pollen foragers, out-going foragers, soybean, pollen weight.

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INTRODUCTION

The western honey bee, *Apis mellifera* Linnaeus, naturally occurs in Europe, the Middle East, and Africa (Ruttner, 1978; Winston, 1981), now has been distributed world wide due to its pollination function and honey production (Clarke, 2002). This species has been subdivided into more than 26 recognized subspecies (or races) (Clarke, 2002) based on differences in morphology (Ruttner, 1978) and molecular characteristics (Clarke, 2001). The differences among the subspecies are not only usually discussed in terms of their agricultural output in particular environmental conditions (Seeley, 1982; Clarke, 2002; Pinto, 2004), also in their foraging behavior (Pankiw, 2003; Abrol, 2006, 2010; Ali, 2011; Mahfouz et al., 2012; Vaziritabar et al., 2015; Taha et al., 2016), defensive behavior (Seeley, 1982), and the ability to tolerate warmer or colder climates (Winston, 1983; Pankiw, 2003), etc. Even the same bee species have

different foraging behaviors with regard to different crops, such as the foraging peak of *Apis mellifera carnica* on alfalfa, pear, and almond is at 8:00-9:00 a.m., 11:30-13:30, and 11:30-13:00 respectively (Vaziritabar et al., 2015; Taha et al., 2016).

As an important pollinators, *Apis mellifera* visits over 50% of all flowers recorded, and also the most obvious soybean flower visitor (Monasterolo, 2015). The soybean (*Glycine max* L. Merrill) is the most important oil crops worldwide, contributing a quarter of the global edible oil and approximately two-thirds of protein feedstuffs for livestock (Agarwal et al., 2013). Honeybee pollination can improve the soybean yields and hybrids (Erickson, 1975; Chiari et al., 2005, 2005; Zhao et al., 2009; Yoshimura, 2011; Santos et al., 2013; Milfont et al., 2013; Gill and O'Neal, 2015; Blettler, 2018).

Apis mellifera carpatica Foti (*A.m. carpatica*), *Apis*

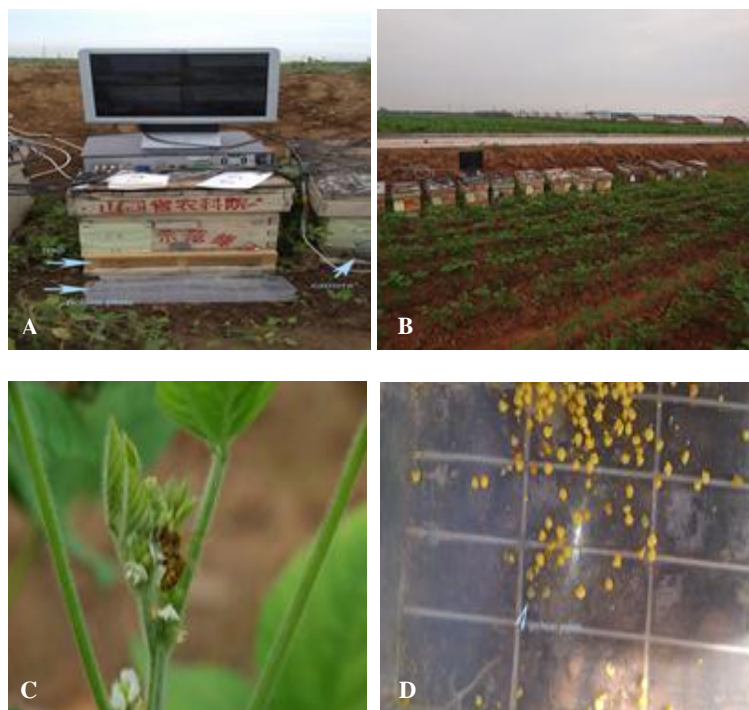


Plate 1A: Pollen traps used to collect pollen from colony, **1B:** Pollen trap installed at the entrance of the beehive **1C:** Pollens collecting by honeybee, **1D:** Soybean pollen sorted out for the total pollen by the color.

mellifera carnica Pollmann (*A.m.carnica*), and *Apis mellifera ligustica* Spinola (*A.m.ligustica*) are widely kept, even used to hybrid in China. Therefore, the foraging activities of these honeybee races based on the quantity of foraging bee and soybean pollen load weight were studied during soybean blooming under open conditions to provide fundamental materials for soybean pollination.

MATERIALS AND METHODS

Study Sites and plant materials

The experiment was carried out in a 1.33 ha open field of soybeans during flower bloom in the Dongyang test site of Academy of Agricultural Sciences, Shanxi Province in China. Some soybean CMS lines and their corresponding maintainer lines were used for the study.

Honeybee species

Apis mellifera carpatica Foti (*A.m.carpatica*), *Apis mellifera carnica* Pollmann (*A.m.carnica*), and *Apis mellifera ligustica* Spinola (*A.m.ligustica*) were provided for the test species by Apiculture Science Institute of Jilin Province in China and bred by Honeybee Pollination Demonstration apiary in Academy of Agricultural Sciences, Shanxi Province in China.

Nine bee colonies from three species were prepared for this study, namely three colonies per species. Three combs per colony were used as an experimental unit. These colonies were consistently adjusted 1 week before the test.

The foraging activity

To compare the pollen foraging effort of three honey bee races, used pollen traps (Plate 1A) was used to collect pollen from each colony. According to the early-stage test and observation, the soybean pollen quantity before 8:00 was very little. Pollen trap was installed at the entrance of the beehive (Plate 1B) and pollen plate was placed at 8:00. Pollens were collected every hour from 9:00 to 17:00 (Plate 1C), and then placed in bags and numbered to be brought into the laboratory. Soybean pollen was sorted out for the total pollen by the color (Plate 1D). All pollens were dried and weighed.

The foraging activity was estimated from 8am-6pm by a camera at the entrance of the beehive. The foragers were estimated by counting all out-going workers. The number of out-going bees and foraging bees in-coming with pollen (pollen foragers) was recorded for 10 min per hour.

Statistical analysis

All recorded data were inputted into Microsoft Excel 2010, and the daily pollen weight of soybean and the

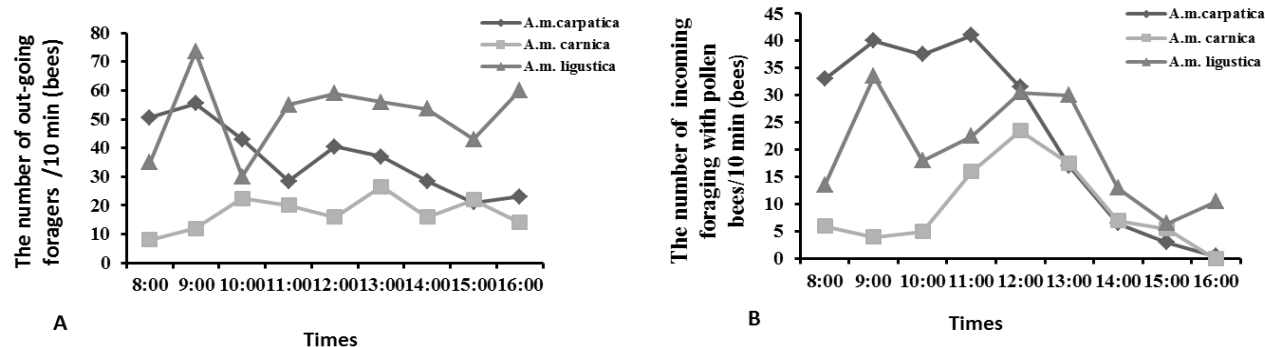


Figure 1: Number of outgoing foragers and incoming foragers with pollen of different bee species. **Figure 1A:** Dynamic changes of number of outgoing foragers (numbers of bees per ten minutes) from different bee species with the time; **Figure 1B:** Number of incoming foragers with pollen (numbers of bees per ten minutes) from different bee species with the time. *A.m.carpatica*, *Apis mellifera carpatica* Foti. *A.m.carnica*, *Apis mellifera carnica* Pollmann. *A.m.ligustica*, *Apis mellifera ligustica* Spinola.

quantity of the proportion of soybean pollen weight was calculated. SPSS version 17.0 statistical software was used for variance analyses. LSD method was used for multiple comparisons. Microsoft Excel 2010 was used for graphical analysis. Results were expressed as Mean \pm SD.

RESULTS

Out-going foragers and in-coming foragers with pollen

Observation on the out-going foragers of *A.m.carpatica*, *Apis mellifera carnica* and *A.m.ligustica* were presented in (Figure 1A). The out-going forager number of three subspecies with the time was shown with a double peak. The highest peak of *A.m.carpatica* and *A.m.ligustica* appeared at 9:00, the number of bees reaches 56 and 74 respectively. That of *A. m. carnica* peaked at 13:00, 27 bees.

In-coming foragers with pollen were different with time among the different species (Figure 1B). The number of in-coming foragers of three subspecies with the time was presented with two peaks. The highest peak of *A.m.carpatica* and *A.m.ligustica* appeared at 9:00, the number of bees reaches 40 and 34 respectively. That of *Apis mellifera carnica* peaked at 12:00, 24 bees. After 12:00, the number of three bee species with pollen decreased over time.

Soybean pollen weights

Figure 2A showed the dynamic changes of soybean pollen weights collected by different bee species in different periods. Soybean pollen weight increased from 09:00, reached the peak at 12:00-13:00, and then started to decrease. No significant difference was observed between each group at 09:00-10:00 ($P>0.05$). The soybean pollen weights of *A.m.carnica* and *A.m.ligustica*

reached the peak at 12:00 with 0.7641g and 0.8027g, respectively; whereas those of *A.m.carpatica* reached the peak at 13:00 with 0.9027g.

The daily total soybean pollen weights of the different bees in Figure 2B showed that the soybean pollen weights of *A.m.carpatica*, *A.m.carnica*, and *A.m.ligustica* were 3.22g, 2.78g, 2.84g respectively. The three showed no significant differences.

Percentage of daily soybean pollen weight in total pollen weights

The percentage of soybean pollen weight of *A.m.carnica* and *A.m.ligustica* were significantly higher than those of *A.m.carpatica* ($P<0.05$), of which soybean weight percentage of *A.m.carnica* was highest, at 60.63% (Figure 3).

DISCUSSION

As the effective pollination insects of soybean, the foraging behaviors of honeybee are influenced by several factors, including bees species, bee colonies, target crops, weather, and so on (Pankiw and Page Jr, 1999; Fenster et al., 2004).

Observations on foragers of out-going and in-coming with pollen showed the peak time of out-going foragers and foragers with pollen of *A.m.carpatica* and *A.m.ligustica* were all earlier than that of *A.m.carnica*. It was suggested that *A.m.carpatica* and *A.m.ligustica* were more active at 8:00-9:00am. Taha et al. (2016) conducted the foraging behavior of *Apis mellifera carnica* Pollmann (*A.m.carnica* P.) and *A. florea* F. on alfalfa flowers and the results revealed that Carniolan bees were more active in gathering pollen at 8:00-0:00 h. During soybean flowering period, the corresponding temperature of 9:00, 12:00 and 13:00 is 24.50°C, 28.77°C and 30.18°C respectively. Alqarni (2006) compared the foraging activity of *Apis*

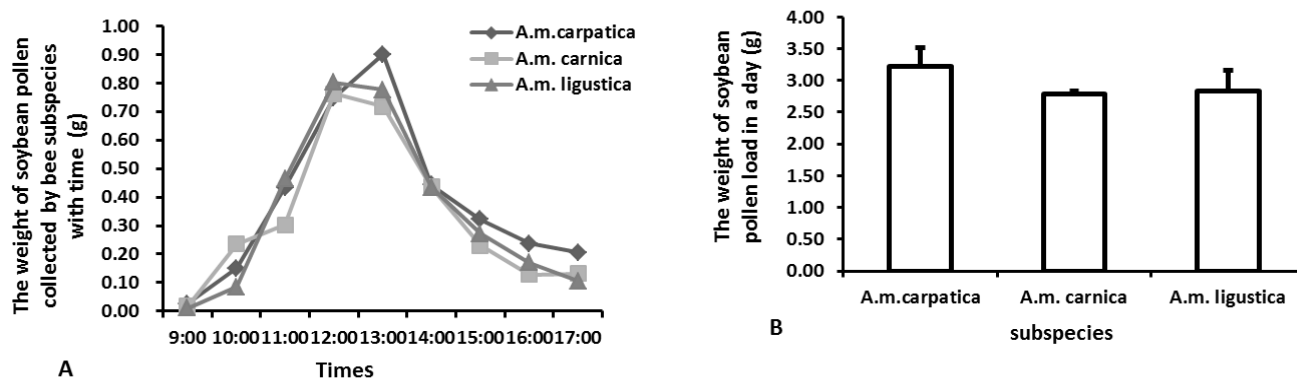


Figure 2: Daily soybean pollen weights collected by different bee species. **Figure 2A:** Dynamic changes in soybean pollen weights collected by different bee species in different periods; **Figure 2B:** Daily total soybean pollen weights of different bees. *A.m.carpatica*, *Apis mellifera carpatica* Foti. *A.m.carnica*, *Apis mellifera carnica* Pollmann. *A.m.ligustica*, *Apis mellifera ligustica* Spinola.

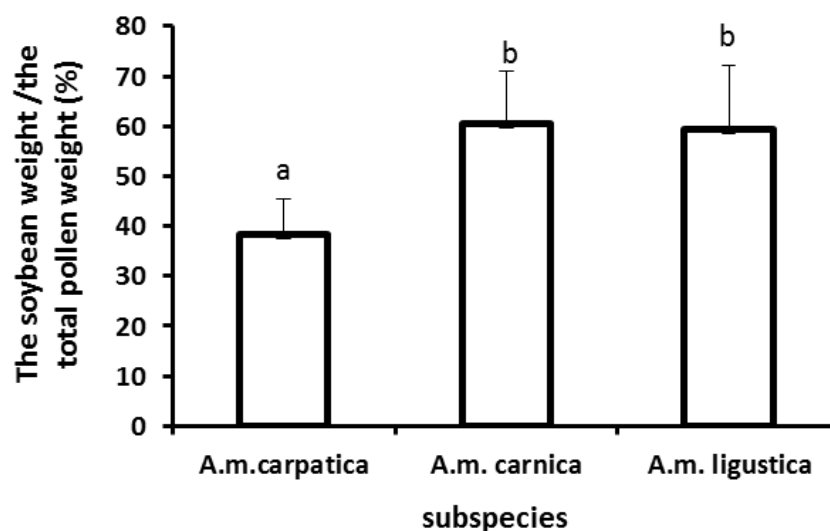


Figure 3: Weight percentage of soybean pollen in total pollen. Different lowercase letters within the histogram indicated significantly different at 5%. *A.m.carpatica*, *Apis mellifera carpatica* Foti. *A.m.carnica*, *Apis mellifera carnica* Pollmann. *A.m.ligustica*, *Apis mellifera ligustica* Spinola.

mellifera jementica Ruttner, Italian (*Apis mellifera ligustica* Spinola) and Carniolan (*Apis mellifera carnica* Pollmann) honey bees in tolerating air temperature during summer season under Riyadh condition; results revealed that the foraging workers from Yemeni colonies were higher than those of Carniolan and Italian. These indicate that *A.m.carnica* is more resistance to high temperature than *A.m.carpatica* and *A.m.ligustica*. *Apis mellifera carpatica* Foti belongs to the black bees, which are sensitive to external climate and nectar source conditions, have strong collection capacity, good at utilizing scattered nectar sources, and not heat-resistant. Vaziratabar et al. (2015) compared the foraging behavior of *Apis cerana* F and *Apis mellifera carnica* for pear and apricot pollination and found that the peak of foraging

activity for *Apis cerana* F occurred earlier and was longer than for *Apis mellifera carnica*. *Apis mellifera carnica* which prefer to collect pollen in the morning and forage nectar in the afternoon. In this study, the time of in-coming foragers of *Apis mellifera carnica* reached the peak at 10:00 and 13:00, which did not agree with the above. This can be attributed to the local habitat and the soybean flowering habit. It infers that the foraging behavior of the same kind of bee species on different crops was also different. The number of foragers with pollen of *A.m.carpatica* and *A.m.ligustica* was higher than that of *A. m. carnica* in this study, which suggested *A.m.carpatica* and *A.m.ligustica* have stronger foraging pollen force than *A. m. carnica*. Parajuli and Thapa (2012) compared the foraging behavior of landraces of

(*Apis mellifera* L. from March to July under Terai condition of Nepal, and found that *A.m. buckfast* had the highest out-going foragers/5 min significantly higher than *A.m. carnica*, *A.m. Russian* and *A.m. ligustica* in turn. The average number of in-coming foragers with pollen of *A. m. buckfast* was the highest, followed by *A. m. ligustica*, *A. m. russian* and *A. m. carnica*.

The weight of soybean pollen indicted that the foraging force of *A.m.carpatica* and *A. m.ligustica* were stronger than *A. m. carnica*. While soybean pollen weight percentage of *A. m. carnica* was significantly higher than that of *A.m.carpatica* and *A. m.ligustica*. The weight of soybean pollen showed that the amount of soybean pollen collected is more for 11:00-15:00, 27-34°C temperature at this time. The climate condition of soybean flowering can be consistent with burning hot summer weather of *A. m. carnica* and *A. m.ligustica* origin, to show the better performance of economic characters and foraging pollen, however, the thermolabile nature of *A.m.carpatica* makes less visit to soybean flowers. This shows different species has different foraging behavior and adaptability to climate on soybean flowers.

The differences between the foraging activity of honeybee subspecies may be related partly to the variations in their morphological characteristics (Abou-Shaara, 2014). Also, it can be associated with the flowering season and habits of target crops, as well as the different subspecies of its ability to adapt (Alqarni, 2006).

In this study, *A.m.carnica* and *A.m.ligustica* have better foraging force and the ability to adapt during soybean flower blooming under open conditions. Therefore, this study recommends that soybean can be pollinated by *A.m.carnica* and *A.m.ligustica*. Moreover, during the soybean blossoming period, the honey bee density can be increased appropriately to be more beneficial to soybean pollination when there are relatively good nectar and pollen plant (example, corn, pumpkin).

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