Differences in Pollination Efficiency Among Three Bee Species in a Greenhouse and Their Effects on Yield and Fruit Quality of Northern Highbush ‘Bluecrop’ Blueberry

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Abstract. Different pollinators exhibit different adaptability to plants. Here, we compared the performance in visiting frequency and pollination efficiency among three bee pollinators (*Bombus terrestris*, *Apis cerana*, and *Apis mellifera*) on greenhouse-grown northern highbush ‘Bluecrop’ blueberry plants and evaluate their effects on yield and fruit quality. Our results indicated that the duration of daily flower-visiting of *B. terrestris* was 24 and 64 minutes longer than that of *A. cerana* and *A. mellifera*, respectively, and the visiting time of a single flower for *B. terrestris* was substantially shorter than the other two bee species, and pollen deposition on the stigma from single visit by *B. terrestris* was twice and three times that of *A. cerana* and *A. mellifera*, respectively. The yield of individual plants pollinated by *B. terrestris* showed an increase of 11.4% and 20.0% compared with the plants pollinated by *A. cerana* and *A. mellifera*, respectively, with the rate of Grade I fruit (>18 mm diameter) reaching 50.8%, compared with 32.9% and 22.5% for *A. cerana* and *A. mellifera* groups, respectively. Moreover, the early-to-midseason yield of plants pollinated by *B. terrestris* was higher, and the ripening time was 3 to 4 days earlier. An artificial pollination experiment demonstrated that seed set of high (≈300), medium (90–110), and low (20–30) pollination amounts were 43.0%, 42.5%, and 10.5%, respectively, and the corresponding mean weights of single fruits (related to the seed number inside) were 2.8, 2.7, and 1.2 g, respectively. The highly efficient pollination of *B. terrestris* was attributed to its behavior of buzz-pollination. Therefore, it is preferential for pollination of ‘Bluecrop’ blueberry in the greenhouse.

In nature, many plant species simultaneously share multiple pollinators (i.e., generalized pollination). Different pollinators may show various adaptations to certain plants because of their specific floral designs, resulting in variations in their pollination efficiency. Such differences exhibit a significant impact on the reproductive success of plants (Rathcke, 1992; Souza et al., 2017).

The application of pollination biology in agricultural production is mainly reflected in the pollination of cultivated plants via managed pollinator insects. Particularly in greenhouses, crop pollination by managed bees is the most common method. However, different bee species have various morphological characteristics and flower-visiting behaviors (Krenn et al., 2005; Thorp, 1979), and they

exhibit different levels of pollination efficiency (Newman et al., 2005), leading to different effects by various pollinators on the reproductive efficiency of plants (Hoehn et al., 2008; Klein et al., 2003). Therefore, the selection of appropriate bee species to pollinate certain crops is of great significance in agricultural production.

To assess whether a pollinator is suitable for a certain crop, the most effective way, nowadays, is to evaluate its flower-visiting frequency, effective visiting rate, and the pollen deposition on stigmas (Javorek et al., 2002). Usually, pollen quality and quantity required to maximize reproduction of different plants vary, and differences in pollen deposition may affect the plant reproductive efficiency (Burd, 1994; Dogterom et al., 2000; Shore and Barrett, 1984). Such differences may result in variation in fruit set, and seed number per fruit may substantially affect the ripening time of fruit and fruit quality (Sampson and Spiers, 2002).

‘Bluecrop’ blueberry is a perennial deciduous shrub belonging to the genus *Vaccinium* (Ericaceae). It has been listed as one of the top five healthy foods by the Food and Agriculture Organization owing to its high anthocyanin content and strong antioxidant capacity (Kader et al., 1996). In addition, ‘Bluecrop’ blueberry is an important new crop in some regions of China, and its planted area in open fields and greenhouses has rapidly been increasing. For example, in Northeast China, ‘Bluecrop’ blueberry is now grown in more than 10,000 ha of greenhouse, greatly impacting the development of the local agricultural economy. However, greenhouse-grown blueberries depend on pollination by bees to set fruit, and, therefore, pollination is one of the key steps in ‘Bluecrop’ blueberry production. Thus, the selection of the best pollinator can significantly enhance the yield and quality of ‘Bluecrop’ blueberry. The flower-visiting behavior of ‘Bluecrop’ blueberry pollinators is determined by the corolla characteristics, nectar-presenting strategy, and the pollen dispersal patterns of the poricidal anthers (Zhao, 2017). For example, pollination efficiency depends on different bee species, thereby affecting the yield of *Vaccinium ashei* (Cane and Payne, 1990). Because of the impact of climate and other factors in different regions, pollinator bee species for greenhouse crops may vary. The most common pollinators for the greenhouse-grown ‘Bluecrop’ blueberry in Northeast China are *B. terrestris*, *A. cerana*, and *A. mellifera*, accounting for 40%, 40%, and 20% of all pollinators used, respectively (authors, unpublished data). Although efforts have been made to study the pollination of ‘Bluecrop’ blueberry by *B. terrestris*, and *A. mellifera* and their impacts, the examination of the local species *A. cerana* remains limited. Comparative studies on the flower-visiting behavior and pollination efficiency of the three bee species during bloom, and their effects on the fruit ripening time and quality are rare. Such studies can provide a scientific basis for selecting the best pollinator bee species for greenhouse ‘Bluecrop’ blueberry production.

In the present study, we used greenhouse-grown 'Bluecrop' blueberry in Northern China, and aimed to address two questions: 1) what are the differences in the flower-visiting behavior and pollination efficiency of *B. terrestris*, *A. cerana*, and *A. mellifera*? 2) What are the effects of different pollinators on the ripening time, yield, and quality of 'Bluecrop' blueberry fruits? We also examined the effects of pollination intensity on the ripening time and fruit quality of 'Bluecrop' blueberry. Furthermore, we discuss appropriate choices and the deployment pattern of pollinator bee species for the greenhouse-grown 'Bluecrop' blueberry.

Materials and Methods

Experimental site. The experiment was performed from January to June 2017 at a 'Bluecrop' blueberry farm of 120 plastic solar greenhouses at Meilin Agricultural Technology Co., Ltd., located in Wulongbei Town, Dandong, Liaoning Province, China (lat. 40°14'16"N, long. 124°20'14"E). Each greenhouse was 100 m long, 9 m wide, and 3.1 m tall, with an orientation to the southwest. The structure of greenhouse was an arched circular steel frame, and a thermal insulation quilt was installed outside the plastic film in winter with insect-proof nets at the air vent. The experiment was performed in three adjacent greenhouses. Only one 6-year-old 'Bluecrop' blueberry variety of 273 individuals (39 rows × 7 plants) was used in each greenhouse with a row spacing of 1.80 m and the space between individuals was 1 m. Planting soil and fertilization was the same in each greenhouse.

Plant and bee materials. The blueberry cultivar used in the experiment was Bluecrop. It was introduced into China in 2002 and has become one of the most popular cultivars grown in the northeastern part of the country. Six-year-old plants were used in the experiment with heights ranging from 1.6 to 1.9 m. The flowers opened before leaf emergence, and each raceme produced 15 to 50 flowers. Single flowers bloomed for 5 to 6 d, and the entire flowering period lasted for half a month. Opened flowers showed a drum- or bell-shaped corolla, with a fused base and four- or five-lobed white petals. Ten to 12 anthers and stamens are embedded in the corolla base around the style, shorter than the style, with a

thin, bicellular, poricidal anther on each. One pistil with wet stigma was higher than or equal to the corolla. There were ≈21,000 pollen grains and 100 ovules in a single flower (Zhao, 2017). In the greenhouse, bloom started in mid to late January with peak flowering at the beginning of February. Fruit harvest period was from early April until early May.

The pollinator bee species in the experiments included *B. terrestris* (200–300 per box), *A. cerana* (1200–2000 per box), and *A. mellifera* (1200–2000 per box), and they were all provided by the Bee Research Institute, Agricultural College, Eastern Liaoning University (Fig. 1). Based on our experience, this number of bees per box can pollinate 1000 m² of greenhouse-grown 'Bluecrop' blueberry.

Flower-visiting behavior and pollination efficiency. At an early flowering stage of 'Bluecrop' blueberry in mid-January, we selected nine greenhouses, with three adjacent greenhouses as a group, and total of three groups to conduct the experiment. The three greenhouses of each group were used for the different bee species to pollinate for the 'Bluecrop' blueberry plants, so there were three replicates. The beehive was placed in the middle of the greenhouse against the wall with a distance to the ground of 50 cm and a south-facing entrance. During bloom, the lowest temperature and highest relative humidity of experimental greenhouses were observed and recorded for 7 consecutive days when each bee species started flower-visiting. The daily visiting time of the different bee species were from start flower-visiting in morning to end flower-visiting in afternoon. On three sunny days, the number of flowers visited by a single bee of each species in 1 min was recorded from 10 AM every day, which was designated as the flower-visiting frequency (a total of 30 bees were followed). Meanwhile, a stopwatch was used to record the time required for a single bee to visit a flower (a total of 20 bees of each species was recorded).

To investigate the pollination efficiency of a single flower visit for each bee species, we selected 10 full flowering plants in the center of each greenhouse, tagged one inflorescence on each plant after removing the opened flowers, and covered it with a net to prevent bee visiting. Three days later, all the flowers were opened on the tagged inflorescence, and the nets were removed at 10:00 AM. The stigma was collected using forceps as soon as it was visited by a single

bee and stored in a 1.5-mL centrifuge tube. A total of 50 flowers were collected for each bee species. The collected stigmas were rolled on a Vaseline-covered slide from left to right, and we determined the pollen number using a microscope (NOVEL XSZ-N107; Shanghai Caikon Optical Instrument Co., Ltd., Shanghai, China), as described previously by Kearns and Inouye (1993). The observed number was designated as the pollen deposition per stigma per visit. The effective visiting rate was calculated as the ratio of stigma number with pollen deposition to the total number of stigmas collected. The effective visits per unit time and effective pollination amount per unit time of a single bee were determined using the following formulas:

Number of effective visits per unit time = visit frequency (flowers visited/min.) × effective visiting rate (stigmas with pollen/total stigmas).

Effective pollination amount per unit time = flower-visiting frequency × effective visiting rate × pollen deposition on stigma per visit.

Effect of different bee species on fruit ripening time and yield. To explore the effects of the three bee species on the ripening time, commodity-grade, and yield, 10 plants with similar individual size at the center of each greenhouse were labeled and open-pollinated. The flower number on each branch was also recorded to calculate fruit set. When the fruit started to ripen in early April, the fruits on the branches were collected every 3 to 4 d until early May, due to the gradual maturation of 'Bluecrop' blueberry. The harvest date and quantity of fruits were recorded. The harvesting period was ≈20 d, and therefore, we harvested six times, and the fruit numbers on each branch were individually recorded. The mature fruits were weighed to determine the total yield of the labeled plants. Among those, 30 fruits were randomly selected to measure their diameters using a vernier caliper (MNT-150T with a resolution of 0.01 mm; MNT, Shanghai Zhangqing Trading Co., Ltd., China), and were classified according to their diameters based on the 'Bluecrop' blueberry commercial standard (Grade I: > 18 mm; Grade II: 15–18 mm, and Grade III: < 15 mm). Finally, the number of each category was counted and their ratios were calculated.

Fig. 1. Different bees provide pollination services for northern highbush 'Bluecrop' blueberry in greenhouses. (A) *Bombus terrestris*; (B) *Apis cerana*; and (C) *Apis mellifera*.

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The effect of pollination intensity on the seed set and single fruit weight. To assess the effect of pollination intensity on the ‘Bluecrop’ blueberry fruit, artificial pollination was performed. Right before the full flowering period, 10 plants with the same individual size were selected, and three inflorescences were labeled and then bagged on each plant after removing the already opened flowers. When more than 10 flowers opened on each inflorescence, unopened buds were removed and three different pollination treatments (low, mid, and high pollen amount) were performed on each of the inflorescences. The pollen grains were collected and mixed from 10 flowers of the same plant and 10 flowers of an adjacent plant at the ratio of 1:1. After removing the bag, the anthers were then removed using forceps, and artificial pollination was performed. For the low-pollen treatment, human hair was used for pollination (a pilot experiment showed that 20–30 pollen grains are deposited on the stigma). Toothpicks (90–110 pollen grains deposition on each stigma) and cotton swabs (≈ 300 pollen grains) were used for the mid and high pollen treatments, respectively. The bags were put back on until the fruit ripened. The single fruit weight was determined using an electronic balance, and then the fruits were dissected to evaluate the number of mature (dark brown) and immature (white) seeds according to the standards of Isaacs and Kirk (2010). The seed set was calculated as the ratio of mature seed number to the total seed number.

Statistical analyses. The significance between treatments was determined using one-way analysis of variance. When the difference was significant, least significant difference tests were used to compare the differences within each treatment. All data were tested for normality and homogeneity of variance to ensure the validity of various statistical methods. Arcsine transformation was performed for fruit and seed setting rate to conform to the normal distribution. The significance level was set as 0.05 and all the statistical analyses were conducted using SPSS software version 20.0 (IBM Corp., Armonk, NY).

Results

The flower-visiting behavior and pollination efficiency. The three bee species exhibited substantial variation in their adaptability to the environment. *B. terrestris* started to visit flowers at 11.5°C and 86.7% relative humidity. *A. cerana* initiated the flower-visiting at 13.8°C and 85.0% relative humidity, whereas *A. mellifera* only started visiting flowers when the temperature and relative

humidity reached 16.0°C and 82.9%, respectively. In addition, the duration of daily flower-visiting of *B. terrestris* was 435.33 min, *A. cerana* was 411.10 min, and *A. mellifera* was 371.36 min; the difference was statistically significant ($F_{2, 18} = 39.788, P < 0.001$).

The flower-visiting frequency of *B. terrestris* was 60% higher than that of *A. cerana* and *A. mellifera* ($F_{2, 267} = 85.727, P < 0.001$). The visiting time of a single flower for *B. terrestris* was substantially shorter than the other two bee species, and the difference was significant among the three species ($F_{2, 177} = 58.251, P < 0.001$). Regarding the individual pollination efficiency, a significant difference was observed in the number of pollen grains deposited on a stigma per visit among the three species ($F_{2, 325} = 104.271, P < 0.001$). Similarly, the difference was also significant in the effective visiting rate of the three species ($F_{2, 12} = 1398.908, P < 0.001$) with *B. terrestris* visiting 10.2 flowers each minute, significantly higher than the 6.8 and 6.1 flowers by *A. cerana* and *A. mellifera* (Table 1).

Effect of different bee species on the yield per plant and fruit ripening time. The fruit set of ‘Bluecrop’ blueberry was $\approx 92\%$ (88.3% to 93.4%) with the pollination by the three different bee species, and no significant difference was observed among the species; however, the mean yield per plant exhibited a significant difference ($F_{2, 27} = 6.322, P < 0.01$). The mean yield per plant of *B. terrestris* pollination was higher, compared with the pollination by *A. cerana* and *A. mellifera*, showing a difference of 11.4% and 20.0%, respectively (Fig. 2). Moreover, the first four harvests accounted for 81.3% of the total yield in the plants pollinated by *B. terrestris*, whereas this ratio was 66.9% and 61.3% in the plants pollinated by *A. cerana* and *A. mellifera*, respectively, suggesting a higher early-to-mid yield of plants with *B. terrestris* pollination. That is, the fruit ripening time was 3 to 4 d earlier than the plants pollinated by the other two bee species (Fig. 2).

Furthermore, the fruit commodity-grade showed significant differences among pollination by the three bee species. The fruit of Grade I was 50.8% in the *B. terrestris*-pollinated plants, substantially higher than the 32.9% and 22.5% in the *A. cerana*- and *A. mellifera*-pollinated plants, respectively (Fig. 3).

Effect of pollination intensity on the seed set and fruit weight. The artificial pollination experiment showed that fruit set was 100% regardless of the pollen deposition level, but seed set was significantly different ($F_{2, 87} = 517.505, P < 0.001$). Seed set was higher with medium and high levels of pollination

than with a low level of pollination (Fig. 4A). In addition, the mean weight of a single fruit in the high-level pollination plants was slightly higher than in the midlevel pollination plants, but without a statistical significance. However, it was significantly lower in the plants with low pollination than the other two pollination treatments ($F_{2, 87} = 264.531, P < 0.001$, Fig. 4B).

Discussion

Reasons for different pollination performance by three bee species. Our research showed that bumblebees have better pollination performance than the other two honeybees for ‘Bluecrop’ blueberry (*Vaccinium* spp.), this is because the plant often grows in low-temperature and high-humidity climates and has long-term adaptive coevolution with bumblebees. Therefore, in the greenhouse, the pollination efficacy of *B. terrestris* was better than that of *A. cerana* and *A. mellifera*, showing not only a longer daily flower-visiting time but also a higher flower-visiting efficiency, which has been substantiated in previous studies (Dogterom, 1999). The three bee species showed different flower-visiting behaviors and pollination efficiencies depending on the unique nectar-presenting strategy of ‘Bluecrop’ blueberry, which is mainly determined by the physical characteristics of the pollinator and the flower characteristics of ‘Bluecrop’ blueberry (Sampson et al., 2013). The nectar of ‘Bluecrop’ blueberry is located at the base of the pistil, and the corolla entrance is narrow. Thus, the head of all three pollinators cannot enter the corolla when collecting nectar, and the nectar must be taken by a long proboscis through the style. The proboscis of *B. terrestris* is longer than that of *A. cerana* and *A. mellifera*, and, therefore, it is easier for them to obtain nectar when the nectar volumes of flowers are equal. Therefore, a shorter single flower-visiting time and higher flower-visiting were observed in *B. terrestris*. In addition, the anther of ‘Bluecrop’ blueberry sheds pollen through poricidal anthers and *B. terrestris* can disperse the pollen from a ‘Bluecrop’ blueberry anther to their abdomen via buzz-pollination, and therefore, most of the visits can be effective (Free, 1993; Sampson, 1993). In contrast, the contact-pollination of *A. cerana* and *A. mellifera* often fails in extraction of pollen from the poricidal anther of ‘Bluecrop’ blueberry during flower visitation, exhibiting a low pollination efficiency (Buchmann, 1983; Dedej and Delaplane, 2005). Moreover, MacKenzie (1997) substantiated that the low

Table 1. Foraging behavior and pollination efficiency of the three different pollinators for ‘Bluecrop’ blueberry in the greenhouse. Values within columns with different superscript letters differ significantly, $P < 0.05$. Mean ± 1 SE.

	Work duration every day (min) (n = 7)	Handing time per flower (sec) (n = 60)	Effective visiting rate (%) (n = 5)	No. of visits flower per min (n = 90)	No. of pollen deposition on stigma per visit
<i>Bombus terrestris</i>	435.33 $\pm$ 5.80 a	2.67 $\pm$ 0.14 c	92.00 $\pm$ 1.37 a	11.07 $\pm$ 0.23 a	34.71 $\pm$ 0.87 (n = 230) a
<i>Apis cerana</i>	411.10 $\pm$ 5.56 b	5.63 $\pm$ 0.32 b	24.14 $\pm$ 0.96 b	6.83 $\pm$ 0.32 b	16.31 $\pm$ 0.95 (n = 60) b
<i>Apis mellifera</i>	371.36 $\pm$ 3.75 c	6.58 $\pm$ 0.30 a	15.75 $\pm$ 0.98 c	6.13 $\pm$ 0.31 b	11.55 $\pm$ 0.97 (n = 38) c
	$F = 39.788, P < 0.001$	$F = 58.251, P < 0.001$	$F = 1398.908, P < 0.001$	$F = 85.727, P < 0.001$	$F = 104.271, P < 0.001$

yield of 'Bluecrop' blueberry is often caused by their inability to buzz pollinate, and the consequent low pollination rate. *A. mellifera* shows poor adaptability to the greenhouse environment and low pollination efficiency to 'Bluecrop' blueberry, which is mainly caused by the small pollen deposition of each flower visit, leading to the low rate of high-grade fruit

Although commercial *A. mellifera* has been used as the first choice for 'Bluecrop' blueberry pollination (Stubbs et al., 1994), studies have demonstrated that only 2% to 10% of *A. mellifera* can collect pollen from the poricidal anthers of the lowbush 'Bluecrop' blueberry during flower visitation (Stubbs and

Drummond, 2001). During the nectar-feeding process, only a small number of pollen grains attach to its abdomen. Therefore, as documented by our data, *A. mellifera* is not a suitable bee species to pollinate the 'Bluecrop' blueberry (Dogterom, 1999; Javorek et al., 2002; Mohr and Kevan, 1987; Parker et al., 1987; Rogers et al., 2013). However, even though *A. mellifera* is inefficient on a per-bee basis, if the colony stocking rate is high enough, that pollination can be effective (Aras et al., 1996).

Although the pollination efficiency of *A. cerana* was inefficient, its adaptability to the greenhouse was better than that of *A. mellifera*, and therefore, numerically it might

compensate for the deficiency of pollination efficiency, which somewhat increases the probability of cross-pollination to acquire a better fruit uniformity. If *A. cerana* serves as a pollinator together with *B. terrestris*, the effect may be better than each of them alone due to the flower-visiting loyalty of *A. cerana* at the end of the flowering time. However, the escape behavior of *A. mellifera* was very serious in the early stage of their release and some individuals died from colliding with the greenhouse, resulting in a decrease of the population number, and this source of mortality was rarer in *B. terrestris* and *A. cerana*.

The application of various pollinators may affect the ripening time of 'Bluecrop' blueberry, which has been previously ignored in the growing of field 'Bluecrop' blueberry. However, an early-to-midseason yield greatly influences the overall profit, as the price of early-to-midseason fruit is higher than that of the late fruit in greenhouse production. The plants pollinated by *B. terrestris* exhibited significantly higher early-to-midseason yield than plants pollinated by *A. cerana* and *A. mellifera*, probably because the ripening time of most fruit was 3 to 4 d earlier in the *B. terrestris* group than that in the other two groups. However, the artificial pollination experiment indicated that the ripening time of the high amount pollination was only 2 d earlier than that of the low amount pollination. Thus, we suspect that the high level of pollination is only part of the reason for the early ripening of the plants pollinated by *B. terrestris*. Further investigations are needed on whether the stimulation to the corolla by *B. terrestris* is different from that by *A. cerana* and *A. mellifera*.

Pollination intensity affects seed set and single fruit weight. The artificial pollination experiment showed that the pollination level within a certain range significantly affects the seed set, yield, and fruit quality of 'Bluecrop' blueberry. The pollination of various 'Bluecrop' blueberry cultivars also showed that the number of pollen grains deposited on stigmas was often positively correlated with the seed number, single fruit weight, and fruit grade of 'Bluecrop' blueberry (Burd, 1994; Dogterom, 1999; Dogterom et al., 2000; Hoffman et al., 2018). For example, a positive relationship between the seed number per fruit and fruit grade was observed in northern highbush 'Bluecrop' blueberry, showing that the more seeds per fruit, the larger the fruit was (Dogterom et al., 2000; Shore and Barrett, 1984), which is because fruit enlargement is stimulated by auxin secreted by fertilized ovules or developing seeds. However, no impact on fruit yield and quality was observed with a continuous increase of pollination amount out of a certain range, implying that a certain amount of pollen can satisfy the maximum 'Bluecrop' blueberry reproduction, which is consistent with previous findings (Dogterom et al., 2000). A single 'Bluecrop' blueberry flower has 100 ovules on average, and usually, one ovule needs one to five pollens to be

Fig. 2. Yield per plant of 'Bluecrop' blueberry harvested six times in the greenhouse under different bee species pollination conditions. Asterisks indicate significance of * $P < 0.05$, ** $P < 0.01$. Different letters show significant differences ($P < 0.05$). Values are mean $\pm$ 1 se.

Fig. 3. Effects of pollination provided by different bee species on the fruit size (diameter) of 'Bluecrop' blueberry growing in the greenhouse. Grade I: >18 mm, Grade II: $15\text{--}18$ mm, Grade III: <15 mm.

Fig. 4. Effects of artificial pollination with different pollen load on seed set (A) and fruit weight (B) of 'Bluecrop' blueberry growing in the greenhouse. Low: 20–30 pollen grains, Middle: 90–110 pollen grains, High: $\approx$ 300 pollen grains. Different letters in the same picture show significant differences, $P < 0.05$. Mean $\pm$ 1 SE.

successfully pollinated and produce seed (Bertin, 1990). 'Bluecrop' blueberry has tetrad pollen (Parker et al., 1987), and therefore it needs 100 pollen grains or 400 microspores to fertilize 100 ovules in a single flower theoretically. The pollination experiment by Parrie and Lang (1992) demonstrated that although the 'Bluecrop' blueberry stigma can hold a maximum of 300 pollen grains, the seed set, single fruit weight, and fruit grade were not further improved if the pollen number exceeded 125. The seed per fruit of 'Bluecrop' blueberry can range from 26 to 63 (Dogterom et al., 2000; Macfarlane, 1992; MacKenzie, 1997), indicating that seed set is a relatively stable trait, if the pollen number reaches its the maximal pollen requirement (i.e., 100), then further increases of pollen amount will not affect seed set.

Overall, our results indicated that all the three bee species can as pollinators to 'Bluecrop' blueberry crop in greenhouse, the difference lies in that pollination by bumblebees can obtain higher yield and better quality compared with the other two bee species, which was supported by the results of artificial pollination experiments and previous studies (Klein et al., 2003; MacKenzie, 1997; Sampson and Spiers, 2002). Thus, we believe that *B. terrestris* can be the best pollinator for the greenhouse-grown 'Bluecrop' blueberry in the region.

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