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Using Bee Attractants to Improve Honeybee Foraging on Dangshan Pear (*Pyrus communis* L.)

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ABSTRACT

The fruit set rate and yield of pear are commonly low due to insufficient pollination, as the species is unattractive to honeybees. To improve honeybee foraging behavior for the pollination of Dangshan pear (*Pyrus bretschneideri* cv. *dangshansuli*), nine methods were used to attract bees. A control treatment of colonies was fed with normal sugar syrup, while six other treatments were fed using sugar syrup mixed with Pear syrup, Gallic acid, Arginine (Arg), Lysine (Lys), Methionine (Met), or 8-Br-cGMP; plates containing Juvenile Hormone analog ZR-512, Brood Pheromone (BP), and Queen Mandibular Gland Pheromone (QMP) were placed inside the hives of another three treatments. Pollination efficacy was compared using the pollen load weight and quantity of foraging bees. The peak time of pear pollen gathering was 10:00–11:00 regardless of treatment. The pear pollen load weight per day was increased by all nine treatments. Pear pollen load weight per day was 49.11 g in the control. The QMP treatment yielded the heaviest pear pollen load weight per day (77.56 g), followed by the 8-Br-cGMP (64.45 g) and BP treatments (64.20 g). The percentages of pear pollen weight and quantity in the total pollen per day were both highest in the BP treatment (80.23%, 87.27%), followed by those in the QMP (79.32%, 86.74%) and Lys treatments (76.25%, 85.81%). In conclusion, BP was the most effective treatment for improving honeybee pollination behavior in the pear orchard, while other treatments, including Arg, Lys, 8-Br-cGMP, ZR-512, and QMP, could also be useful.

Keywords: *Apis mellifera ligustica* Spin., Inducer, Pollen quantity, Pollen weight, Pear pollination

INTRODUCTION

Honeybees, as important pollinators, have been shown to increase yield and improve quality in fruits and vegetables (Vidal *et al.*, 2010; Rai and Srivastav, 2012; Sushil *et al.*, 2013). However, plants such as pear, lowbush, and blueberry experience low yields because of insufficient honeybee pollination (Vickery, 1991). Although honeybees are naturally attracted by some plants, bee attractants can be used to induce pollination of otherwise unattractive target plants by honeybees. For example, Queen Mandibular Gland Pheromone (QMP), Juvenile Hormone (JH), Brood Pheromone (BP), the worker Nasanov Pheromone, and phenolic compounds have been proven as attractive to honeybees (Isilaaya and Yablonski, 1976; Currie *et al.*, 1992a, b; Winston and Slessor, 1993; Ambrose *et al.*, 1995; Pankiw, 2004; Liu *et al.*, 2006; Pateel and Sattigi, 2007; Ellis and Delaplane, 2009; Sivaram *et al.*, 2013).

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Because the species experiences a low fruit setting percentage due to self-incompatibility (Hiratsuka and Zhang, 2002; Liu *et al*, 2005), pollination is essential for Dangshan pear (*Pyrus bretschneideri* cv. *dangshansuli*). Along with the development of modern agriculture in China, natural pollination has been replaced by artificial treatments to ensure fruit setting (Wu *et al.*, 2011), which has caused the acute reduction of pollinators. Thus, to decrease labor costs, it is important to introduce honeybees as a pollinator for Dangshan pear. Typically, two approaches may be employed to induce bees to a target plant: pheromones or attractants (e.g., brood pheromone, juvenile hormone, and phenolic compounds), which may be sprayed on crops or fruit trees or used within colonies; and the modification of food sources inside colonies to regulate honeybee gustation. The aim of the present study was to examine and evaluate the usefulness of these methods for improving honeybee foraging and pollination efficiency in Dangshan pear.

MATERIALS AND METHODS

Geographical and Botanical Information

The fieldwork was conducted in the region around Hongzhiyi Salt Lake in Yuncheng County, Shanxi Province, People's Republic of China (N 34° 48′–35° 30′, E 110° 12′–111° 41′), during March 23–27, 2013. This region is characterized by low elevation (370 m) and a mild continental temperate monsoon climate with an annual average rainfall of 559 mm, annual average sunshine duration of 2,247 hours, annual average temperature of 13.6°C, and annual frost-free period of 208 days. The plantation area of Dangshan pear (*Pyrus bretschneideri* cv. *Dangshansuli*) in this region is approximately 4,936 acres, with an annual output of 90 million kg.

Bee Colonies

One week before the experiments, 30 colonies of *Apis mellifera ligustica* Spin. were inspected and matched to ensure similar levels of adult bees, brood, and food frames. All colonies were obtained from the Yiming Apiculture Cooperative. The queens had the same genetic background, as they were all sisters from the same queen.

Reagents

Analytical pure Gallic acid, Arginine, Lysine, and Methionine were obtained from Tianjin Hongda Reagent LTD, China. ZR-512 and 8-Br-cGMP were purchased from Sigma-Aldrich Co. LLC, USA. BP and QMP were obtained from Mann Lake LTD, USA.

The concentrations of reagents used were as follow. For fresh Pear syrup, pear flowers were dipped into 1:1 sugar syrup (Water/Sucrose= wt/wt) for 12 hours at 25–30°C (Wu and Chen, 1984). For the other treatments, 1 mM Gallic acid (Liu, 2006), 1 mM Arginine (Arg), 1 mM Lysine (Lys), 2 mM Methionine (Met), or 500 µM 8-Br-cGMP (Ben-Shahar *et al*., 2002) were all dissolved into 1:1 sucrose syrup.

Glass plates coated with 800 larval equivalents of BP (solution with acetonitrile) (Pankiw, 2004) and 200 mg L^{-1} ZR-512 (solution with acetone) (Isilaaya and Yablonski, 1976) were used for those two treatments. QMP was bought ready to use from Mann Lake LTD.

Experimental Design

The 30 colonies were equally divided into 10 groups. The control treatment was only fed with 1:1 sugar syrup; six treatments were fed with 1:1 sugar syrup mixed with Pear syrup, Gallic acid, Arg, Lys, Met, or 8-Br-cGMP; and plates containing ZR-512, BP, or QMP were hung inside the hives of the three remaining treatments (which were fed on 1:1: sugar syrup).

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After 20% of the pears had blossomed, the colonies were placed in a central location 20 m from the pear orchard and fed every evening until the end of the blooming period, which lasted five days. During the experiment, pollen traps were installed at the hive entrances at 08:00, as the outside temperature was too low for honeybees to work before that time. Pollen was then collected once an hour from 09:00 to 18:00. Pear and non-pear pollen loads were sorted by color and then dried, counted, and weighed.

The number of bees that foraged on pear flowers was obtained by dividing the number of pear pollen loads by two, as each foraging bee carried two pollen loads. The pear flower visitation rate was assessed using the quantity and weight of pear pollen as percentages of total pollen in each period.

Statistical Analyses

All response variables were analyzed statistically using analysis of variance (ANOVA), Least Significant Difference (LSD), and Duncan's multiple-range test using SPSS (version 19.0). A P value< 0.05 was considered statistically significant.

RESULTS

Pear Pollen Load Weight

The dynamics of pear pollen load weight for the different treatment groups are shown in Figure 1. For all treatments, the weight of pear pollen load greatly increased from 09:00 to 12:00 and then decreased sharply from 12:00 to 13:00, after which point the import of pollen was maintained at approximately 5 g per hour. However, the nine treatments differed at the peak time (10:00–11:00). The most effective treatments were QMP and 8-Br-cGMP, which significantly increased pollen import by 5.91 g and 3.96 g, respectively, over that of the control (9.24 g), followed by BP, which increased pollen import by 3.87 g.

As shown in Figure 2, pear pollen load weight per day was higher in all experimental groups than in the control (49.11 g). QMP treatment achieved the highest quantity (77.56 g), followed by 8-Br-cGMP (64.45 g) and BP (64.20 g) treatment; these groups were significantly different from the control.

Percentage of Pear Pollen Weight in Total Pollen Load Weight per Day

The percentages of pear pollen load weight in the total pollen weight per day for the different treatments are illustrated in Figure 3. The proportion of pear pollen in the total pollen of the control was 68.10%. Among all treatments, BP achieved the highest proportion (80.23%), followed by QMP (79.32%) and Lys (76.25%), and had significant differences from the control. The average proportion of pear pollen in the total pollen was elevated by all treatments, except

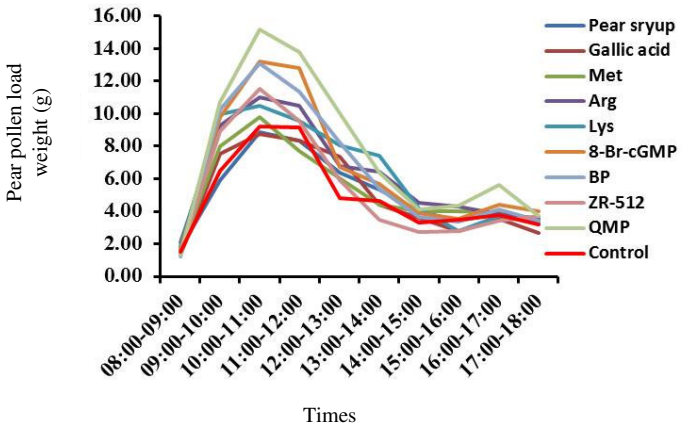

Figure 1. Dynamic change of pear pollen load weight.

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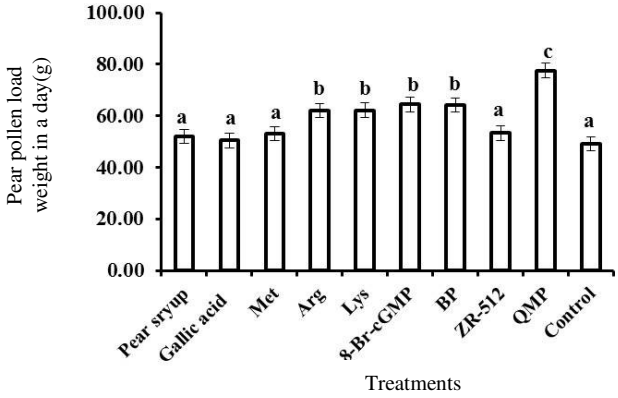

Figure 2. The weight of pear pollen load in a day.

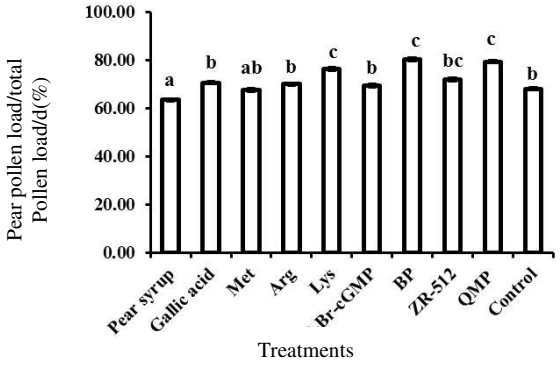

Figure 3. Percentage of pear pollen weight in the total pollen load weight per day.

Pear syrup and Met.

Percentage of Pear Pollen Quantity in the Total Pollen Load Weight per Day

The percentages of bees on pear flowers among the total foraging bees per day are shown in Figure 4. In the control, this percentage was 82.73%. The BP treatment had the highest percentage value (87.27%), followed by the QMP (86.74%) and Lys (85.81%) treatments. However, no significant differences were observed between the nine treatments and the control.

DISCUSSION

Honeybees exhibit biological tendencies toward some plant pollens but not others (Hill *et al.*, 2001). For example, bees tend to prefer bright flowers with rich odors or pollen abundant in proteins (Boelter and Wilson, 1984; Liu *et al.*, 2006).However, the pear tree is among the plants bees dislike (Vickery, 1991). We attempted to enhance honeybee visitation of pear using chemical attractants. In this study, we compared the effects of nine treatments on honeybees foraging behavior toward pear flowers by measuring the quantity and weight of pollen loads. The results suggested that substances such as pheromones could promote honeybee foraging activity.

Adjusting bee food composition is known to be effective for inducing bees to forage on a target plant (Liu *et al.*, 2006). In this study, honeybee foraging tendencies and their effects on pear pollination could be enhanced to different degrees through the feeding of Pear syrup, Gallic acid, Arg, Lys, and 8-Br-cGMP. Pear pollen load weight per day was increased by 2.87 g over that of the control through feeding with Pear syrup. The

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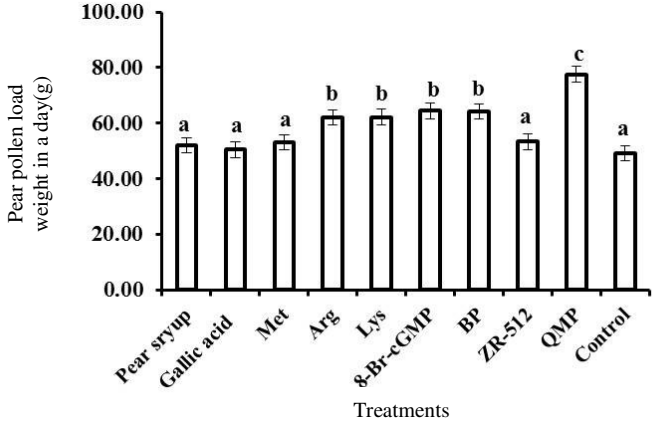

Figure 4. Foraging bees on pear flower in the percentage of foraging bees per day.

use of bees for pear pollination has previously been studied, and Pear syrup attractant was used to increase bee visits. The data of that two-year study showed that pear fruit-set rate and yield under bee pollination were averaged 30.65% and 86 kg, and under artificial pollination 31.2% and 74 kg, respectively (Wu and Chen, 1984). The use of bees and Pear syrup for pear pollination was therefore considered feasible, as is consistent with the results of the present study. However, the weight and quantity percentages of pear pollen in the total pollen load were lower than those in the control for the Pear syrup treatment in the present study. During the experiment, the colonies were inspected, and unconsumed Pear syrup was found in the hives. This observation may be associated with the dislike of honeybees for the pear flower, especially competitive flowers are in bloom,such as oilseed. Gallic acid, associated with secondary metabolism, increases honeybee colony food intake to stimulate foraging behavior (Liu, 2006). Therefore, we used Gallic acid as an inducer on pear. The data showed that pear pollen weight was increased and that bees visited more pear flowers under Gallic acid treatment. Phenolic compounds can regulate bee pollen foraging and have previously been used for cauliflower hybrid seed production (Liu *et al,* 2006; Liu and Li, 2009). Pollen amino acid composition also affects honeybee foraging preferences (Cook *et al.*, 2003). However, the previous literature contains few reports of amino acids being used as bee attractants. The present study represents the first use of three amino acids to improve bee foraging behavior, as based on the amino acid detection results of our previous pear and oilseed pollen studies. Pear pollen weight per day increased by the Met, Arg, and Lys treatments. Pear flowers produce a pungent odor, which may be associated with sulfur. Because Met contains sulfur, this amino acid was used as an inducer. However, the percentage of pear pollen in the total pollen load was lower under the Met treatment than in the control, as was the percentage of the foraging bees on pear flowers. We therefore hypothesized that bees disliked the pear flower odor. The weight and quantity of pear pollen load per day were increased by Arg and Lys treatments, indicating that these amino acids could be used as attractants. Arg is a component of the ornithine cycle and has extremely important physiological functions; additionally, Arg and Lys both promote body development and immune function. Treatment with 8-Br-cGMP also increased honeybee foraging. Ben-Shahar *et al.* (2002) found that treating colonies with 8-Br-cGMP induced precocious foraging while studying the effects of cGMP or cAMP treatments on

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honeybee foraging behavior, consistent with our results.

Pheromones have also been used to attract honeybees (Pankiw and Page, 2003; Pankiw, 2004). In this study, pear pollen load weight was significantly increased by BP treatment, and the percentage of pear pollen load in the total pollen load was improved by 17.81%. The pear pollen load percentage per day was highest under BP treatment, suggesting an increased frequency of bee visits to pear bloom compared with the other treatments. BP components can increase the activity of the hypopharyngeal glands, which are associated with honeybee division of labor, thereby impacting honeybee behavior (Mohammedi *et al.*, 1996; le Conte *et al.*, 2001; Pankiw, 2004). Additionally, the pollen load weight and foraging bee percentage for target crop have been shown to significantly increase in BP-treated colonies compared to control colonies (Pankiw, 2004, 2007; Tsuruda and Page, 2009). However, the use of BP has rarely been examined for pear pollination. In this study, we found that QMP was also effective at improving honeybee behavior. QMP has been sprayed on fruit trees in great quantities to attract honeybees, increasing yields in cranberry and blueberry (Currie *et al.*, 1992a, b). JH plays an important role in timing the onset of foraging behavior in honey bees (Jassim *et al.*, 2000), and we used a JH analog (ZR-512) to change bee behavior. Pear pollen load weight was increased by ZR-512 in the present study.

The pear pollen load weight results are consistent with those for the amount of bees foraging on pear flowers per day: BP treatment had the greatest effect, followed by QMP and Lys. Differences in the improvement of foraging may be attributed to differences in plants and environments. The percentage of pear pollen load weight in each period of the day, however, does not agree with the percentage of bees foraging on pear flowers. This result may be associated with pear pollen size and weight.

In conclusion, we compared nine methods for increasing honeybee foraging behavior and pear pollination, finding that all treatments improved these variables. The application of pheromone-based bee attractants, especially BP, to pear was more effective at increasing bee visitation. Arg and Lys were first used as bee-attractants on pear tree pollination, and can improve the enthusiasm of bees gathering pear pollen in order to provide a new way.

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کاربرد مواد جلب کننده زنبور برای بهبود غذایابی زنبور عسل روی گلابی دانگشن (*Pyrus communis* L.)

و. ه. ما، ی. ک. شاوو، ه. ت. ژاوو، س. ه. تیان، ج. منگ، س. س. یانگ، ی. ل. دو، و ی. س. جیانگ

چکیده

به طور کلی، گلابی مورد پسند زنبور عسل نیست و به این علت گرده افشانی آن ناکافی بوده و درنتیجه نرخ میوه دهی و عملکرد آن عموما کم است. در این پژوهش، به منظور بهبود رفتار غذایابی زنبور عسل و گرده افشانی گلابی دانگشن (*Pyrus communis* L.)، ۹ روش برای جلب زنبور به کار بسته شد. این روش ها عبارت بودند از : یک کلنی شاهد شامل زنبور هایی که با شربت قند تغذیه می شدند در حالی که شش تیمار دیگر با شربت قند مخلوط با شربت گلابی(Pear syrup)، اسید گالیک آرژنین (Arg)، لایسین (Lys)، متیونین (Met)، یا 8-Br-cGMP تغذیه شدند. افزون بر این، درون کندوی سه تیمار دیگر بشقاب های حاوی Juvenile Hormone analog ZR-512، فرومون های غدد همزادان (Brood gland pheromone, BP) و فرومون غدد آرواره ای ملکه (QMP) قرار داده شد. در ادامه، کفایت گرده افشانی در روش های مزبور با مقایسه وزن بارگرده و مقدار غذایابی زنبورها انجام شد. زمان اوج جمع آوری گرده ها در همه تیمارها بین ساعت ۱۰ تا ۱۱ صبح بود. وزن بار گرده های گلابی در همه ۹ تیمار نسبت به شاهد افزایش نشان داد و مقدار روزانه آن در تیمار شاهد ۴۹/۱۱ گرم بود. تیمار QMPبیشترین وزن بار گرده گلابی را داشت(۷۷/۵۶ گرم) و بعد از آن تیمار 8-Br-cGMP (با ۶۴/۴۵ گرم) و تیمار BP (۶۴/۲۰ گرم) قرار داشت. در صد وزن گرده گلابی و مقدار آن در کل گرده های جمع آوری شده روزانه در تیمار BP بیشترین بود(به ترتیب ۸۰/۲۳٪ و۸۷/۲۷٪) و بعد از آن تیمارQMP (۷۹/۳۲٪ و۸۶/۷۴٪) و تیمار Lys(۷۶/۲۵٪ و۸۵/۸۱٪) قرار داشتند. نتیجه این که برای بهبود رفتار گرده افشانی زنبور عسل در باغ گلابی، تیمار BPموثر ترین روش بود در حالی که روش های دیگر شامل Arg، Lys، و 8-Br-cGMP، ZR-512 وQMP نیز مفید بودند.

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