Scientia Horticulturae 219 (2017) 107–117

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Synergistic effects between bumblebees and honey bees in apple orchards increase cross pollination, seed number and fruit size

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ARTICLE INFO

Article history:
Received 8 December 2016
Received in revised form 1 March 2017
Accepted 2 March 2017
Available online 11 March 2017

Keywords:
Honey bee
Bumblebee
Apple (*Malus domestica*)
Insect foraging behavior

ABSTRACT

Most apple cultivars are self-sterile and completely dependent on cross-pollination from a different cultivar in order to set fruit. Various insects may be pollinators, but the main one is the honey bee [HB] (*Apis mellifera*). However, despite the advantages of the honey bee as pollinator of many plants, it is a relatively inefficient pollinator of apple flowers. The main reason for this is the tendency of HBs to visit the apple flower from the side (sideworker), thus "stealing" nectar without touching the flower's reproductive organs – stamens and stigma. In contrast, a bee that visits the flower from the top (topworker) contacts the flower's reproductive organs, which results in better pollination. Due to the low pollination efficiency, few seeds are formed, and often the resulting fruit is too small to be of commercial value. Experiments conducted in Israel over the last few years have shown for the first time that adding bumblebees [BB] (*Bombus terrestris*) into pear orchards improved cross-pollination, thus increasing the number of seeds and subsequently fruit size. The goal of the present work was to test the hypothesis that adding BBs to apple orchards may improve cross-pollination. We found that adding BBs to the HBs in the apple orchard improved pollination in all tested cultivars, especially in 'Gala', which naturally suffers from relatively few seeds in the fruit. It appears that the addition of BBs did not only increase the number of pollinating insects in the orchard that could perform cross-pollination, including in the cool mornings and in adverse weather conditions, but that it also changed HB foraging behavior, which resulted in improved cross-pollination and increased efficiency, and subsequently more seeds and larger fruit. The improved pollination was due to the greater mobility of HBs between rows of pollinated cultivar and pollenizer, and to the greater proportion of topworkers, which are more efficient pollinators.

1. Introduction

Most apple cultivars are self-incompatible (Dennis, 2003; Goldway et al., 2007; Schneider et al., 2001, 2005). Hence, for effective pollination and successful fertilization they depend entirely on the synchronization of flowering among different cultivars of trees and on the intensive activity of pollinators. The most important pollinators of apple are honey bees (HB; *Apis mellifera*) (Delaplane and Mayer, 2000; Dennis, 1979; Free, 1993). Since apple trees are usually grown in temperate zones where weather conditions during the blooming period may be unfavorable for bee flight, insufficient pollination, pollen tube growth, and fertilization are among the most common yield-limiting factors (Free, 1993; Goulson, 2010; Hoopingarner and Waller, 1993).

Honey bees often switch from apple flowers to the competing flowers, which they find more attractive and rewarding (Free, 1993). Moreover, HBs tend to restrict their mobility to one row of trees, which usually contains a single cultivar (Stern et al., 2001). In addition, the Effective Pollination Period (EPP; i.e., ovule longevity, or the time between pollination and fertilization) is very short in apple, lasting for only 1–2 days (Dennis, 1979). Thus, although the stigma remains receptive for longer periods, pollination needs to be accomplished within 1–2 days since onset of anthesis for fertilization to occur before degeneration of the ovule.

Another problem with apple pollination is that HB activity on apple flowers is not always efficient. They collect both nectar and pollen from the flower, but not necessarily at the same time (Mayer, 1984). Usually, when collecting pollen, they pollinate the flower

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http://dx.doi.org/10.1016/j.scienta.2017.03.010
0304-4238/

effectively, because they work from the top of the flower as "top-workers", but they often do not collect nectar from the top. There are gaps at the base of the stamens, especially on 'Red Delicious', that enable "sideworking" to obtain nectar without contacting the anthers and stigmas of the flower (Dennis, 1979; Roberts, 1945; Robinson, 1979). Sideworking is efficient for the honey bee, but requires time to learn (DeGrandi-Hoffman et al., 1985).

Due to these various factors, pollination of apple is especially inefficient, especially in 'Red Delicious' (Stern et al., 2001; Schneider et al., 2002). Any technique that can improve HB mobility between rows for better cross pollination and increase HB efficiency by working as topworkers would improve apple yield and especially fruit size (through more seeds in the fruit).

In previous work (Stern et al., 2001) it was shown that the sequential introduction of HB colonies into apple orchards increases bee activity on trees, bee mobility between rows, rate of topworkers, and as a result improves cross pollination, fruit size and yield. However, the fruit size remained small due to low (under-optimal) seed number (7–9 seeds fruit^{-1}).

Evidence has been accumulating in recent years showing a positive relationship between diversity and ecosystem services, like crop pollination (Klein et al., 2007, 2012). For some crops, wild bees are more effective pollinators on a per visit basis than HBs (Willmer et al., 1994; Zhang et al., 2015) and can functionally complement the dominant visitor (Albrecht et al., 2012). Interactions between floral visitors may modify their behavior through interference competition (Greenleaf and Kremen, 2006) or resource competition (Inouye, 1978). Either form of competition may augment pollination. For example, due to interference competition, interaction with non-Apis bees caused HBs to move more often between rows of sunflower (*Helianthus annuus*), thereby increasing their pollination efficiency (number of seeds production per visit) (Greenleaf and Kremen, 2006). Resource competition can also alter pollinator foraging movement (Inouye, 1978). Another form by which foraging behavior may be differentially affected by conspecific or interspecific interactions is through flower marking. For example, HBs are more likely to avoid visiting a flower recently visited by a bumblebee than by another HB (Stout and Goulson, 2001). Changes in pollinator movement are particularly important in crop species that are self-incompatible like apple and pear.

The buff-tailed bumblebee (BB; *Bombus terrestris* L.) is another potential vector for pollination in apple (Goulson, 2010), although it is most commonly used in vegetable greenhouses to pollinate tomato, strawberry, and pepper (Dimou et al., 2008; Kearns and Inouye, 1997; Pressman et al., 1999; Van den Eijnde et al., 1991; Velthuis and Van Doorn, 2006). BB possess several attributes that may be effective for outdoor pollination of field crops and orchards. As with HBs, BBs collect nectar and pollen from numerous sources (Heinrich, 2004). However, their capacity to carry nectar and pollen is greater than that of HBs (Free and Williams, 1972) as their body is about twice the size of the HB body. By carrying greater loads per foraging bout, a BB would make more floral visits, thus increasing the probability of switching between rows within a bout, which could make it a more efficient pollinator (Heinrich, 2004). BBs are very active, generally visiting many more flowers than HBs (Goodell and Thomson, 1997; Willmer et al., 1994). For example, the level of cross-pollen in 'Conference' pear trees pollinated by BBs was twice that in their HB-pollinated counterparts (Jacquemart et al., 2006). We found in apples that BBs visited about 40 flowers min^{-1}, whereas HBs visited only about ten flowers min^{-1} (R.A. Stern, unpublished data). In addition, the range of activity of BBs is usually restricted to a few hundred m from the hive, increasing the chance of pollination within the orchard, whereas HBs are active over thousands of m from the hive (Wolf and Moritz, 2008).

BBs are active at temperatures below 14 °C, which is the limiting temperature for HB activity (Vicens and Bosch, 2000). Thus, BBs can commence foraging earlier in the day than HBs. Furthermore, in contrast to HBs, BBs can forage under harsh winter condition. They have even been observed to forage in wind and rain (Corbet et al., 1993; Goulson, 2010; Lundberg, 1980; Tuell and Isaacs, 2010).

BBs do not signal the location of floral resources as HBs do, and therefore, unlike HBs, they do not recruit nest mates out of the orchard to competing wild flowers in surrounding meadows (Heinrich, 2004).

The use of BBs as commercial pollinators has mainly been applied in greenhouses and, to a minor extent, in open fields of alfalfa (*Medicago sativa*) and red clover (*Trifolium pretense*). Recently we reported the first commercial use of BBs in pear orchards (Zisovich et al., 2012). Observations of BBs in apple orchards have been reported (Goodell and Tomson, 1997), but not their commercial application. The aim of the present study was to evaluate the effects of adding hives of *Bombus terrestris* to HB colonies in apple orchards on cross-pollination, seed number per fruit and fruit size.

2. Materials and methods

2.1. Orchard design

The experiments were conducted in four commercial apple (*Malus domestica*) orchards located in the Upper Galilee (Baram – 700 m asl) and in the Golan Heights (Ortal, Elrom and Ramat Magshimim – 1000 m asl) in the north part of Israel (the main experiments were done in Baram). The cultivars tested were 'Gala', 'Red Delicious', 'Golden Delicious' and 'Pink lady' grown on MM 106 rootstocks. The trees in each orchard were uniform in age (ca. 10 years) and size, and had similar crop loads in the year prior to the experiments. Two rows of the same cultivar were planted at spacing of 2.5 m between trees and 4.5 m between rows (900 trees ha^{-1}) with an adjacent 2 rows of another cultivar on each side as a pollenizer, throughout the entire orchard. The rows were always aligned in the North-South direction. There was a good overlap between the flowering times of the cultivars in each year. As in previous experiments with HBs (Stern et al., 2001, 2004) and BBs (Zisovich et al., 2012), the treatments were kept in the same orchard, which was divided into two areas: HB + BB [+BB] *vs.* HB only [−BB] = control, with a distance of *ca.* 1000 m between the two areas. Thus, treatment effects could be compared while maintaining other parameters such as cultivars, rootstocks, agricultural practices, climate conditions, competing flora, etc., as similar as possible.

2.2. Bees treatments

HB and BB colonies were distributed homogenously throughout the orchard. HB colonies (Kibbutz Dan Apiary, Upper Galilee, Israel) were introduced into each orchard according to commercial recommendations developed in Israel, following Stern et al. (2001). The first introduction was at 2.5 colonies ha^{-1} at 10% full bloom (FB) and the second introduction was an additional 2.5 colonies ha^{-1} at FB, for a final total of 5.0 HB colonies ha^{-1}. A HB colony reaches up to 30,000–40,000 bees, whereas a BB colony reaches only 150–300 bees.

The BB hives (BioBee Ltd., Kibbutz Sde-Eliyahu, Emek-Hamaayanot, Israel) were introduced into the orchard at a density of ten hives ha^{-1} at the start of bloom (i.e. 1–2 days before the introduction of HB). From our experience, it takes BB colonies a few days to acclimate to the orchard and to start foraging on the trees. Since BBs are less flower constant (Goulson, 2010), they are probably able to strengthen the colony by foraging on competing plants outside