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Original Article

EFFECT OF HONEY BEE (*APIS MELLIFERA* L.) DENSITY ON POLLINATION AND FRUIT SET OF AVOCADO (*PERSEA AMERICANA* MILL.) CV. HASS.

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Abstract

The objective of this research was to determine the effect of honeybee density on pollination and fruit set of the Hass avocado. The research was carried out in the municipality of Popayán, Colombia. Three avocado orchards were selected, each one with an area of one hectare and trees with an age of six years. The treatments were: 1) four hives/ha, 2) six hives/ha, and 3) control without hives. Treatments of six and four hives/ha presented significant differences with respect to the control, with a honeybee density per tree of 7.72, 6.04 and 2.72, pollination rate of 60, 55 and 50%, pollination efficiency of 7.57, 6.04 and 5.98 grains of pollen per stigma, 6.11, 4.13 and 3.54% fruit set initial, 0.058, 0.048 and 0.028% fruit set final, 231, 212 and 137 of fruits per tree, 46.2, 38.2 and 21.6 kg fruit per tree, respectively. The results obtained show an increase of honeybee density per tree, pollination rate, pollination efficiency, % fruit set, % fruit set final, number of fruits per tree and total fruit weight per tree when six and four bee hives/ha are introduced in the avocado crop.

Keywords: ***Apis mellifera*, avocado, honeybee, pollination efficiency, pollination rate**

INTRODUCTION

Pollination is a key process for both ecosystems and agroecosystems, whose efficiency is directly affected by the richness of pollinators (Cepeda, Gómez, & Nicholls, 2014). At the global level, 75% of primary crop species and 35% of crop production rely on some level of animal pollination (Klein et al., 2007). Economically, the value of insect pollination services to crop agriculture has been estimated at €153 billion per annum globally (Gallai et al., 2009). In recent years, decreases in pollinator populations have been reported (Dewenter et al., 2005; Geslin et al., 2016). Decreased abundance and diversity of such pollinators as wild bees, hoverflies and butterflies at local and regional levels have been documented, (Potts et al., 2005; Aizen & Harder 2009; Biesmeijer et al., 2006; Carvalheiro et al., 2013). Furthermore, a significant decrease has been reported in the number of honey bee colonies on a regional scale in Europe and North America. These reductions of bee populations are mainly due to the loss and homogenization of habitats, pesticides, parasites and pathogens, invasive species and climate change (Potts et al., 2005; Brown et al., 2016).

It is estimated that 73% of crops are pollinated by bees (Zych & Jakubiec, 2006). They are probably the group of insects best adapted to the floral visit, which is why they have become an essential group for pollination and therefore for the sexual reproduction of most flowering plants, including many of agricultural interest (Michener, 2000; Parra, 2005). In addition, the honey bee (*Apis mellifera* L.) is the most versatile, ubiquitous, and commonly used managed pollinator (Free, 1970; Klein et al., 2007).

Inadequate pollination has been suggested as an important factor in the limiting of the avocado (*Persea americana* Mill.) yield. This species presents the phenomenon of dichogamy proterogyny synchrony, i.e. sequential development of reproductive functions. Avocado cultivars are classified into complementary flowering groups A and B, based o their daily flowering pattern. The type A flowers open in the female form in the morning and close in the afternoon but then open again in the masculine form in the afternoon of the following day. The type B flowers open in the female form in the afternoon; they then close and reopen the following morning in the male form (Sedgley, 1979; Ish-Am, 2004). The adoption of dichogamy as a breeding strategy implies that for an effective transfer of pollen, insects should visit the flowers in both sexual states. Fruit set is minimal or absent when insect pollinators such as bees and flies are excluded through caging (Malerbo-Souza et al., 2000).

Pollen-carrying vectors play a key role in the pollination and increase in the genetic variability of avocados because a considerable number of fruits are produced only through cross pollination (Bergh, 1977; Gazit & Degani, 2002). Avocado flowers are visited by a variety of insects including bees, flies, wasps, beetles and thrips (Vithanage, 1990). In Central America the avocado is pollinated by stingless bees (Meliponinae) and wasps (Vespidae) (Ish-Am & Eisikowitch, 1993), in Colombia Vásquez et al. (2011) established that ants, bees (*A. mellifera* and trigone), flies and wasps are the most frequent floral visitors.

The honey bee *A. mellifera* is considered the main pollinating agent of the avocado (Free, 1970; Nieto, 1984; Davenport, 1986; Ish-Am & Eisikowitch, 1993; Avilán & Rodríguez, 1995; Castañeda et al., 1999; Peña, 2003; Can-Alonso et al., 2005; Goodwin, 2012). However, despite this and in comparison with other fruit trees, bees do not work efficiently in avocado flowers as they get more attracted by flowers of wild plants grown in orchards (Ish-Am & Eisikowitch, 1993). Furthermore, it has even been suggested that bees do not frequently visit avocado flowers due to repellent properties and high concentrations of minerals and perseitol alcohol in their nectar (Afik et al., 2006; Pérez-Balam et al., 2012; Afik et al., 2014).

An avocado tree produces about one million flowers and 10000 to 40000 female flowers open each day in Israel (Lahav & Zamet, 1999). A good seasonal crop of 400 to 600 fruits per tree requires the pollination and fertilization of about the same number of flowers, which may be accomplished with only two or three forager honey bees. However, in practice a measurable initial fruit set under field condition demands the work of at least five to ten honey bees per tree throughout the female bloom (Ish-Am, 2004).

In avocado orchards, it is common practice to introduce colonies of bees to promote pollination (Pérez-Balam et al., 2012). In New Zealand, four to ten hives/ha is recommended (Evans, Goodwin, & Mcbrydie, 2010), while in Israel, it the optimal number of hives/ha has been found to be eight, placed at distances not less than 100 m between them (Bergh, 1977). Goodwin (2012) recommended the introduction of hives when the crop presented between 5 and 10 % of flowering, guaranteeing its persistence in the flowers.

Bee-pollinator number density is the most important of the several factors that contribute and change to increase productivity. Research has shown that pollination by honey bee increases the fruit set in avocados (Ish-Am & Eisikowitch, 1993; Goodwin, 2012). Even though measuring pollinator performance is difficult, it assesses pollinator behavior and estimates stigmatic pollen deposition (Freitas & Paxton, 1998; Ne'eman et al., 2010; Pérez-Balam et al., 2012). This study deals with honey bee visitation activity per tree evaluated per unit time, quantity of pollen deposited on the stigma, initial and final fruit set, and the weight and number of fruits per tree. Considering the limitations of pollination in avocado, the objective of this research was to determine the effect of honey bee density on pollination and fruit set of avocado cv. Hass.

MATERIAL AND METHODS

Field trial

The research was conducted in the municipality of Popayán, department of Cauca, Colombia, during 2015 in three avocado orchards (*Persea americana* Mill.) cv. Hass with an area of one hectare for each. The trees had a distance of 6x6 m between one another, an average height of four meters and age of six years old. The first orchard was located geographically at 02°27'44.0"N, 76° 34' 03.8"W, the second orchard 02° 27'17.1"N, 76° 34' 05.5"W and the third orchard 02° 27' 32.4"N, 76° 34' 03.7"W, and all were separated by a distance of one km. The average annual precipitation in the research area was 1941 mm, a mean annual temperature of 19°C and altitude of 1735 m above sea level. Around the orchards there were large intensive monocultures systems planted with eucalyptus (*Eucalyptus grandis* W. Hill ex Maiden.) and pine (*Pinus tecunumanii* Eguiluz & J.P. Perry.), as well as small avocado orchards.

The treatments were 1) four hives/ha, 2) six hives/ha and 3) control without hives. The honey bee hives (*A. m. scutellata*) used were of the Langstroth type and located in the center of each orchard. Within each orchard five sites were selected, covering a range of distance to the hives: 50, 100, 150, 200 and 250 m. At each site four trees were sampled, with a total of 20 trees per orchard. These sites were selected to quantify the effect of the distances from the hives to the selected trees.

Density of bees per tree (BPT)

The BPT was recorded during the flowering period of the second half of 2015; between 1 and 30 of September, the average temperature for this period was 20.3°C and relative humidity on average 68.7%. The BPT was quantified by a person walking around a tree and counting with a manual counter the presence of the bees per minute (Free & Spencer, 1963; Mayer, Johansen, & Burgett, 1986; Ish-Am & Eisikowitch, 1998; Ish-Am & Lahav, 2011). The BPT was documented on every observation day in each treatment, and recorded every hour from 8.00 to 16.00. Four recordings (one per week) were performed per treatment during the flowering phase in one tree for each distance.

Pollination rate and efficiency (PR and PE)

A sample of stigmas per tree was collected in the female flowering stage, three hours after the opening of the flower in the female phase, between 12.00 and 14.00, which was the time lapse overlap of the female and male phases. The average temperature for this period was 21.3°C and relative humidity on average 67%. The stigmas were collected with a clamp and placed on a microscope slide covered with 2% (w/v) gel of carboxymethyl cellulose [in a ratio of 1:2:7 (v/v/v) ethanol: glycerol mixture: water, with the addition of aniline blue, to create a light blue solution] and the number of pollen grains per stigma was recorded under a microscope optical (Ish-Am & Eisikowitch, 1991a; Ish-Am & Eisikowitch, 1991b; Ish-Am & Lahav, 2011). A total of 600 stigmas samples were collected, 200 per treatment, 40 stigmas per tree, 4 recordings per treatment (one per week) during the female flowering phase. The pollination rate (percentage of stigmas pollinated \ total stigmas collected) and pollination efficiency (average number of pollen grains in the stigma, only for pollinated stigmas) was quantified for each treatment.

Percentage of fruit set initial (PFSi)

In order to determine the PFSi, the number of flowers (flowering) and the number of fruits (four weeks after the end of flowering) were recorded on five inflorescences per randomly selected tree, in twenty trees per treatment. The PFSi was calculated as follows: PFSi= Number of fruits set / Number of flowers * 100.

Percentage of fruit set final (PFSf)

In order to determine the PFSf, the number of flowers (flowering) and the number of final formed fruits (40 weeks after the end of flowering) were recorded on five inflorescences per randomly selected tree, in twenty trees per treatment. To determine the PSFf, the following equation was used: PSFf = Number of fruits formed / Number of flowers * 100.

Weight and total number of fruits per tree (WFTT and NFTT)

The WFTT and NFTT were recorded 40 weeks after the end of flowering and all the fruits of each tree (20 trees per treatment) were harvested, counted and weighed.

Statistical design

For the statistical analysis a split-plot design was applied. Treatments with different densities of honey bee hives (four, six hives/ha and control without hives) constituted the main plot. The distances from the apiary to the trees selected as the sampling unit (50, 100, 150, 200 and 250m) constituted the sub-plots, for each sowing distance, four trees were evaluated for the variables number of bees per tree, pollination rate, pollination efficiency, % of fruit set initial, % of fruit set final, number of total fruits per tree and total fruit weight per tree. A generalized linear model was applied. The Duncan test was used for the comparison of means for each factor. Subsequently, a Pearson correlation analysis was performed on the evaluated variables. All statistical analyzes were carried out using the SAS® statistical package (Statistical Analysis System Version 9.4).

RESULTS

Number of bees per tree (BPT)

The BPT differed significantly among the treatments, the orchard with six and four hives/ha had a higher BPT in comparison to the control (Tab. 1). By introducing six and four hives/ha, BPT increased by 4.93 and 3.26, respectively, compared to an orchard without hives. The BPT were much higher in distances of 50, 100 and 150 m compared to distances of 200 and 250 m (Tab. 2). The BPT was much higher at 14.00, 13.00, 12.00 and 11.00 with means of 7.46, 6.23, 5.76 and 5.83, respectively, compared with recordings at 10.00, 9.00, 8.00, 15.00 and 16.00 with means 5.35, 4.93, 4.60, 5.26 and 4.20, respectively.

Pollination rate (PR)

The PR differed significantly between treatments with a higher PR in the treatment of six hives/ha, compared to the treatment of four hives/ha and the control (Tab. 1). By introducing six and four hives/ha, PR increased by 12 and 4%, respectively, compared to an orchard without hives. The PR was not significantly different in the evaluated distances (50, 100, 150, 200 and 250 m) (Tab. 2).

Pollination efficiency (PE)

The PE differed significantly between treatments, presenting a higher PE in treatments of six and four hives/ha compared to the control (Tab. 1). By introducing six and four hives/ha, PE increased by 2.29 and 1.32 pollen grains in the stigma, respectively, compared to an orchard without hives. The PE differed significantly between treatments at the evaluated distances (50, 100, 150, 200 and 250 m), being higher at distances of 50 and 100 compared with distances of 150, 200 and 250 m (Tab. 2).

Percentage of fruit set initial (PFSi)

The PFSi differed significantly between treatments, with a higher PFSi in the treatment of six hives/ha compared to the treatment of four hives/ha and the control (Tab. 1). By introducing six and four hives/ha, the PFS increased by 2.57 and 0.59%, respectively. The PFSi differed significantly between treatments at the evaluated distances (50, 100, 150, 200 and 250 m). The highest PFSi was present at distances of 50 and 100 m compared with distances of 150, 200 and 250 m (Tab. 2).

Percentage of fruit set final (PFSf)

The PFSf differed significantly between treatments, being in the treatment of six hives/ha compared to the treatment of four hives/ha and the control (Tab. 1). By introducing six and four hives/ha, the PFSf increased by 0.03 and 0.02%, respectively, compared to an orchard without hives. The PFSf did not significantly differ at the distances evaluated (50, 100, 150, 200 and 250 m) (Tab. 2).

Number of total fruits per tree (NFTT)

The NFTT differed significantly between treatments, higher in treatments of six and four hives/ha compared to the control treatment (Tab. 1). By introducing six and four hives/ha, the NFTT increased by 93.4 and 74.4 fruits per tree, respectively, compared to an orchard without hives. The NFTT did not significantly differ at the distances evaluated (50, 100, 150, 200 and 250 m) (Tab. 2).

Total fruit weight per tree (WFTT)

The WFTT differed significantly between treatments, higher in treatments of six and four hives/ha compared to the control treatment (Tab. 1). The WFTT increased by 26.4 and 17.5 Kg, respectively, compared to an orchard without hives. The WFTT did not significantly differ at the distances evaluated (50, 100, 150, 200 and 250 m) (Tab. 2).

Table 1.
Averages and significance of differences between the treatments number of hives

Variables	Number of hives/ha			GLM
	0	4	6	Pr > F
Density of bees per tree (BPT)	2.78 c	6.04 b	7.72 a	<.0001
Pollination rate (PR)	50.0 b	55.5 b	66.5 a	0.004
Pollination efficiency (PE)	5.98 c	7.34 b	8.27 a	<.0001
Percentage of fruit set initial (PFSi)	3.54 b	4.13 b	6.11 a	0.0004
Percentage of fruit set final (PFSf)	0.028 b	0.048 ab	0.058 a	0.0159
Total number of fruits per tree (NFTT)	137.9 b	212.3a	231.3 a	<.0001
Weight of fruits per tree (WFTT)	21.6 c	38.2 b	46.2 a	<.0001

* Mean with the same letter in the rows are not significantly different.

Table 2.
Averages and significance of differences between the treatments' distance to apiary

Variables	Distance to the apiary					GLM
	50m	100m	150m	200m	250m	Pr > F
Density of bees per tree (BPT)	6.48 a	6.13 a	6.2 a	4.62 b	4.05 b	<.0001
Pollination rate (PR)	53.0 a	55.8 a	55.0 a	52.5 a	58.3 a	0.9509
Pollination efficiency (PE)	9.32 a	8.04 b	6.81 c	5.95 c	5.84 c	<.0001
Percentage of fruit set initial (PFSi)	5.15 ab	5.82 a	3.55 b	4.64 ab	3.80 b	0.0401
Percentage of fruit set final (PFSf)	0.050 a	0.045 a	0.049 a	0.040 a	0.030 a	0.8300
Total number of fruits per tree (NFTT)	210.7 a	198.8 a	192.5 a	184.5 a	182.5 a	0.4871
Weight of fruits per tree (WFTT)	37.9 a	33.3 a	33.7 a	35.4 a	34.7 a	0.6365

* Mean with the same letter in the rows are not significantly different.

Correlation and linear regression between the evaluated variables

A correlation analysis was performed between the BTP and the evaluated variables. For the variables that were significant, we performed a linear regression analysis. For the variables BPT and PE, a mean positive correlation was presented (0.50465), when the linear regression was performed (r^2 = 0.2547, P <0.0001). Therefore, only 25% of the BPT explained only 25% of PE. For the variables BPT and WFTT, a mean positive correlation was presented (0.50100), when linear regression was performed (r^2= 0.2510, P <0.0001). Therefore, only 25% of the BPT explained only 25% of WFTT.

DISCUSSION

In Central America, where the avocado is native, it is pollinated by a wide variety of insects, mainly melipona bees and wasps (Ish-Am et al., 1999; Can-Alonso et al., 2005). However, outside of its native region the main pollinator is the honey bee (*A. mellifera*), whose importance is evident in the strong positive correlation that exists between the activity of the bees and the yield of the crop (Vithanage, 1990; Ish-Am & Eisikowitch, 1995; Ish-Am & Eisikowitch, 1998; Gazit & Degani, 2002). In this study, a mean positive correlation found between honey bee activity and pollination efficiency and total fruit weight per tree emphasizes the dependency of avocado pollination and yield on honey bees and the importance of high pollination efficiency for fruit set.

Vithanage (1990) studied the introduction of hives into avocado orchards and found a significant increase in production, on average 227.2 fruits/tree in orchards without hives and 788.2 in orchards with hives, an increase of 247%. Vásquez et al. (2011) used direct pollination with *A. mellifera* in four varieties of avocado. They incorporated an average of 3.6 hives/ha and found that production increased between 21% and 96%. These results differed with those found in this research where the average number of fruits/tree was 212 and 231 and the increase in production of 54 and 68% with the introduction of four and six hives/ha. However, these studies agreed that the activity of honey bees increase the number of fruits. The total weight of the fruits per tree was divided by the total number of fruits per tree, the results were 150, 180 and 200 grams for the 0, 4 and 6 hives/ha, respectively. These results indicate that the number of bees per tree had a major effect on the individual fruit weight.

Avocado is characterized as having a low fruit set, and the values worldwide vary from 0.2 to 0.001% (Salazar & Lovatt, 1998; Lahav & Zamet, 1999; Gazit & Degani, 2002; Cossio-Vargas et al., 2007; Evans, Goodwin, & Mcbrydie, 2010). The Hass avocado has been described to have a fruit set of 0.14% in years of high production and 0.07% in years of low production (Garner & Lovatt, 2008). Cossio-Vargas et al. (2007) found in Chile in the Hass avocado an initial and final fruit set of 0.04% and 0.01%. Romero (2012) found in Colombia that the Lorena avocado in a year of high production in of 108,000 flowers, had only 168 fruits.

In this study, it was determined that the % of fruit set final was 0.05 and 0.04% when six and four hives/ha were introduced, respectively. These proportions were within the values mentioned for other producing regions. This low fruit set is due to a huge fall of flowers and small fruits during the months following the flowering stage (Lahav & Zamet, 1999), mostly because of a lack of fertilization (Sedgley, 1987). Although the avocado flower ovary holds only one ovule, it was demonstrated in the cv. Hass that when only a single pollen grain reaches the stigma the fertilization probability is very low. Actually, twenty pollen grains or more must reach the stigma for a high fertilization probability, so a cooperative effort of many pollen grains is needed for breaking through the style and into the ovary (Ish-Am, 2004; Arpaia & Hofshi, 2004). In this research, the number of pollen grains in the stigma increased in orchards where four and six hives/ha were introduced, with seven and eight pollen grains in the stigma on average, respectively. However, some collected stigmas contain more than twenty pollen grains per stigma that are necessary to reach a high rate of fertilization (Shoval, 1987).

Ish-Am (2004) reported that five to ten BPT were necessary to achieve a reasonable pollination. However, the optimal density is at least ten to twenty BPT, and six to ten grains of pollen per stigma and 60 to 80% of pollinated stigmas are obtained. In this investigation with the introduction of four and six hives/ha, six and eight BPT were recorded; based on this assumption a BTP must be present to have a reasonable pollination. However, these data refer to orchards located in Israel, where there are no original native avocado pollinators and few pollinator species and *A. mellifera* is considered the main pollinator. In Colombia, Carabalí et al. (2017) found numerous families of insect pollinators of the avocado, mostly belonging to the orders Diptera, Hymenoptera, Coleoptera and Hemiptera. With this wide diversity of pollinators in Colombia, it is possible that the number of honey bees required is lower than in Israel, as these species contribute to pollination. This information is corroborated by the high pollination rates, greater than 50%, that occurred in all three treatments.

The BPT number was higher in the distance range of 0 to 150 m which indicates that the distance that exists from the hives to the trees influences the number of bees visiting the flowers of the trees. This coincides with Ish-Am (2004) who determined that the mobility of bees during foraging was limited and worker bees operated within an area of one to three trees. At distances between 200 and 250 m, the BPT was smaller, and these bees may have been scouts that had a broader flight range whose objective was to collect information from the available food sources and were able to transport the avocado pollen up to several hundreds meters away from the origin. Based on these results it is recommended to install the hives in the orchard, each spaced at 150 m. This is in agreement with recommendations by Bergh (1977) to place honey bee hives in distant groups up to no more than 160 m.

Honey bee activity was highest in the time period between 11.00 and 14.00, when the male and female states of the flowers more likely overlapped, the period that the bees move freely between pistillate and staminate flowers and collect pollen and nectar,. These results were similar to those found by Cautin (1996) who determined that the period of the greatest activity of honey bees in avocado orchards was from 11.00 to 14.00. The results obtained in the orchards affirm that when introducing a different number of hives/ha (six and four) compared to an orchard without hives, there are an increase of bee density per tree, pollination rate, pollination efficiency, % fruit set initial, % fruit set final, number of fruits per tree and total fruit weight per tree.

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