

# STINGLESS BEES FURTHER IMPROVE APPLE POLLINATION AND PRODUCTION

Blandina Felipe Viana<sup>1\*</sup>, Jeferson Gabriel da Encarnação Coutinho<sup>1</sup>, Lucas Alejandro Garibaldi<sup>2</sup>, Guido Laercio Bragança Gastagnino<sup>3</sup>, Katia Peres Gramacho<sup>4</sup>, Fabiana Oliveira da Silva<sup>1</sup>

<sup>1</sup>Universidade Federal da Bahia, Programa de Pós-graduação em Ecologia e Biomonitoramento, Instituto de Biologia, Av. Ademar de Barros, 500, Ondina, CEP 40170110, Salvador, BA, Brasil

<sup>2</sup>Sede Andina, Universidad Nacional de Río Negro (UNRN) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mitre 630, CP 8400, San Carlos de Bariloche, Río Negro, Argentina

<sup>3</sup>Universidade Federal da Bahia, Escola de Medicina Veterinária, Departamento de produção animal, Av. Ademar de Barros, 500, Ondina, CEP 40170110, Salvador, BA, Brasil

<sup>4</sup>Universidade Tiradentes, Centro de Ciências Biológicas e da Saúde. Av. Murilo Dantas, Farolandia, CEP 49032-490, Aracaju, SE, Brasil

**Abstract**—The use of Africanised honeybee (*Apis mellifera scutellata* Lepeletier) hives to increase pollination success in apple orchards is a widespread practice. However, this study is the first to investigate the number of honeybee hives ha<sup>-1</sup> required to increase the production of fruits and seeds as well as the potential contribution of the stingless bee Mandaçaia (*Melipona quadrifasciata anthidioides* Lepeletier). We performed tests in a 43-ha apple orchard located in the municipality of Ibicoara (13°24'50.7"S and 41°17'7.4"W) in Chapada Diamantina, State of Bahia, Brazil. In 2011, fruits from the Eva variety set six seeds on average, and neither a greater number of hives (from 7 to 11 hives ha<sup>-1</sup>) nor a greater number of pollen collectors at the honeybee hives displayed general effects on the seed number. Without wild pollinators, seven Africanised honeybee hives ha<sup>-1</sup> with pollen collectors is currently the best option for apple producers because no further increase in the seed number was observed with higher hive densities. In 2012, supplementation with both stingless bees (12 hives ha<sup>-1</sup>) and Africanised honeybees (7 hives ha<sup>-1</sup>) provided higher seed and fruit production than supplementation with honeybees (7 hives ha<sup>-1</sup>) alone. Therefore, the stingless bee can improve the performance of honeybee as a pollinator of apple flowers, since the presence of both of these bees results in increases in apple fruit and seed number.

**Keywords:** Pollination deficit, *Apis mellifera scutellata* Lep., *Melipona quadrifasciata anthidioides* Lep., *Malus domestica*, Brazil.

## INTRODUCTION

The intensification of crop production causes the expansion of agricultural boundaries toward natural areas. Reports in the literature indicate that the intensive use of soil by conventional agriculture is one of the main factors causing the decrease in richness and abundance of pollinators throughout the world (Tilman et al. 2002; Taki et al. 2011; Klein et al. 2012; Viana et al. 2012). The current decline in managed honeybee stocks (Potts et al. 2010a; Potts et al. 2010b) and the loss of native pollinators (Cameron et al. 2011) threaten crop production by increasing pollination deficits (Kremen et al. 2002; Garibaldi et al. 2013; Kennedy et al. 2013).

To compensate for the pollination deficit, pollinator management has focused on supplementing crop fields with bee colonies to improve fruit and seed production inside farms. These managed colonies are mainly social bees of the

genus *Apis* (Kevan 1997; Delaplane & Mayer 2000). In many Brazilian crops, the management of honeybee (*Apis mellifera scutellata* Lepeletier) hives is a widely used and efficient practice for pollination. Nevertheless, this practice is under-utilised with wild solitary (Roubik 1989; Freitas & Oliveira-Filho 2001; Bosch & Kemp 2002) and social bees (Venturieri 2008), largely due to the current lack of knowledge of the basic aspects of the biology of native species, methods of husbandry and taxonomic impediments (Imperatriz-Fonseca et al. 2012).

Despite the widespread use of honeybees for crop pollination, aspects regarding the appropriate number of hives and the potential positive interaction with wild bees are poorly understood. Some authors have suggested that the recommended density of hives depends on the attractiveness of the focal crop to pollinators (Paranhos et al. 1998; Delaplane & Mayer 2000; Finta 2004). Furthermore, the wild bee abundance in the crop is linked to a combination of the surrounding landscape (Taki et al. 2011; Kennedy et al. 2013) and the on-farm practices friendly to pollinators (Kremen & Miles 2012; Kremen et al. 2012). Other important factors that may influence the foraging activity of wild bees in crops include the interspecific interactions and

Received 11 September 2013, accepted 2 September 2014

\*Corresponding author; blande.viana@gmail.com, Phone 55 (71) 3283-5951

weather conditions during the flowering season (Brittain et al. 2012; Brittain et al. 2013).

The bee species *A. mellifera scutellata* Lepeletier is more effective in pollinating apples when collecting pollen because these bees exhibit side-walking behaviour when collecting nectar (Silva FO, pers. comm.), which is favoured by the external floral morphology and has been previously recorded in apple flowers (Degrandi-Hoffman et al. 1987). However, there is a need for consistent information regarding the usage of devices, such as pollen collectors, in honeybee hives to improve pollen collection and transfer among flowers in crops (Manning et al. 2010). Loper et al. (1985) reported that the collectors remove close to 16% of the pollen collected by the bees, and this increased the collection of pollen by a factor of 1.8 in almond groves. Because of a reduction in the quantity of pollen grains stored in the colony, pollen collectors may increase pollen gathering by bees (Nelson et al. 1987; Delaplane & Mayer 2000).

Furthermore, pollen collectors may influence the development of the hive by reducing the construction of brood combs (Loper et al. 1985), causing the death of individual bees, decreasing pollen storage inside hives (Manning et al. 2010) and changing the behaviour and intensity of bee foraging, especially in weak hives (with less than four brood boxes) that contain fewer workers than strong hives (Hoopingartner & Waller 1993; Delaplane & Meyer 2000; Dag et al. 2003). Based on these results, the same authors also recommend that the number of brood boxes in honeybee hives used for crop pollination should be greater than four. These findings underline the importance of proper management with a pollen collector to promote adequate pollination conditions.

In Chapada Diamantina (Bahia state, Brazil), Eva and Princess ("polliniser") apple varieties are raised together in the studied orchard. In 2010, the seed number was significantly lower in naturally pollinated (mean = 4 seeds/fruit) than in manually crossed fruits (mean = 8 seeds/fruit) and was taken as indicative of pollination deficit in the studied apple orchard, although the orchard was supplemented with 5 honeybee hives ha<sup>-1</sup> (Silva et al. unpublished data). In the same year, the productivity was 10 tons ha<sup>-1</sup>, which is less than that observed in the SE region of the country, where productivity may reach levels of up to 50 ton ha<sup>-1</sup> (Petri et al. 2011; BRASIL 2012).

The productivity data for apple trees in the region prompted us to investigate whether a higher number of honeybee and pollen collectors at the hive would diminish the pollination deficit. However, the efficiency of pollen collectors in *A. mellifera scutellata* hives managed for crop pollination has never been experimentally tested. Based on previous observations, we expected that pollen collectors could improve foraging for pollen among apple trees and, in turn, successful pollination.

We also investigated whether Africanised honeybee hives (*A. mellifera scutellata*) together with managed hives of the stingless bee *Melipona quadrfasciata anthidioides* Lepeletier "Mandaçaia" (Meliponini) would reduce the pollination deficit in the apple orchard. Our study is expected to provide

information that will support apple producers in successfully managing pollinating bees to improve yield. Moreover, the generation of empirical data on the effectiveness of native bees as pollinators may contribute to an improvement of on-farming friendly practices within apple orchards and their surroundings that will conserve natural populations of wild pollinators.

## MATERIALS AND METHODS

### Study area

Our two-year study was performed in an apple orchard belonging to the Bagisa Company for Agriculture and Commerce, South America (13°24'5.7"S and 41°17'7.4"W), located in a region of irrigated agricultural land between the municipalities of Ibicara and Mucugê, State of Bahia, Brazil (Figure 1). The area is bordered by the Chapada Diamantina National Park, the dominant natural vegetation in the region is of the savannah arboreal type, and the area includes elements of high-altitude grasslands growing at an altitude of almost 1,100 m. The mean annual temperature is 21 °C, varying between 26 °C and 16 °C. The rainy season is from November to March, and the mean annual precipitation is 757 mm (data obtained from the Bagisa farm weather station).

### The apple orchard and design

The 43-ha orchard consists of 36 parcels of 1.2 ha each, which are organised into three blocks located 10 m apart from each other (Figure 2). The apple trees were planted in 2006 and 2007 (18 parcels each), and the trees planted each year correspond to 50% of the orchard area. Each parcel contained 14 rows, and the distance between two consecutive rows is 4 m. In every row, the apple trees are spaced 1.5 m apart from each other and are arranged at a ratio of five producing trees per one "polliniser" tree (5 Eva:1 Princess). However, in some rows, this proportion varies because the farmer used 7 to 8 Eva trees to one Princess such that the latter represents 10-12% of the trees inside the orchard. The features that make Princess trees a suitable "polliniser" for Eva trees are pollen compatibility, coincidence in flowering period, number of anthers/flowers, number of pollen grains/anthers, and higher pollen germination level compared with other polliniser varieties (Albuquerque-Junior et al. 2010).

The farmer applies synthetic hormones during June; the trees bloom between July and the beginning of August, and the blooming lasts for approximately ten to fifteen days. Hormone application was performed every eight to ten days in six parcels at a time such that two parcels in each block are sequentially in bloom. Therefore, according to the location and number of parcels in bloom, we established rectangular experimental areas 50 m × 25 m in size aligned along the rows and placed at a distance of 10 m from the edge of the parcel of the orchard between rows 5 to 12 (Figure 2). The dataset on flower density and number of seeds and fruits used in this study was gathered from four plots consisting of two trees of each variety marked within the experimental areas following the method described by Vaissière et al.

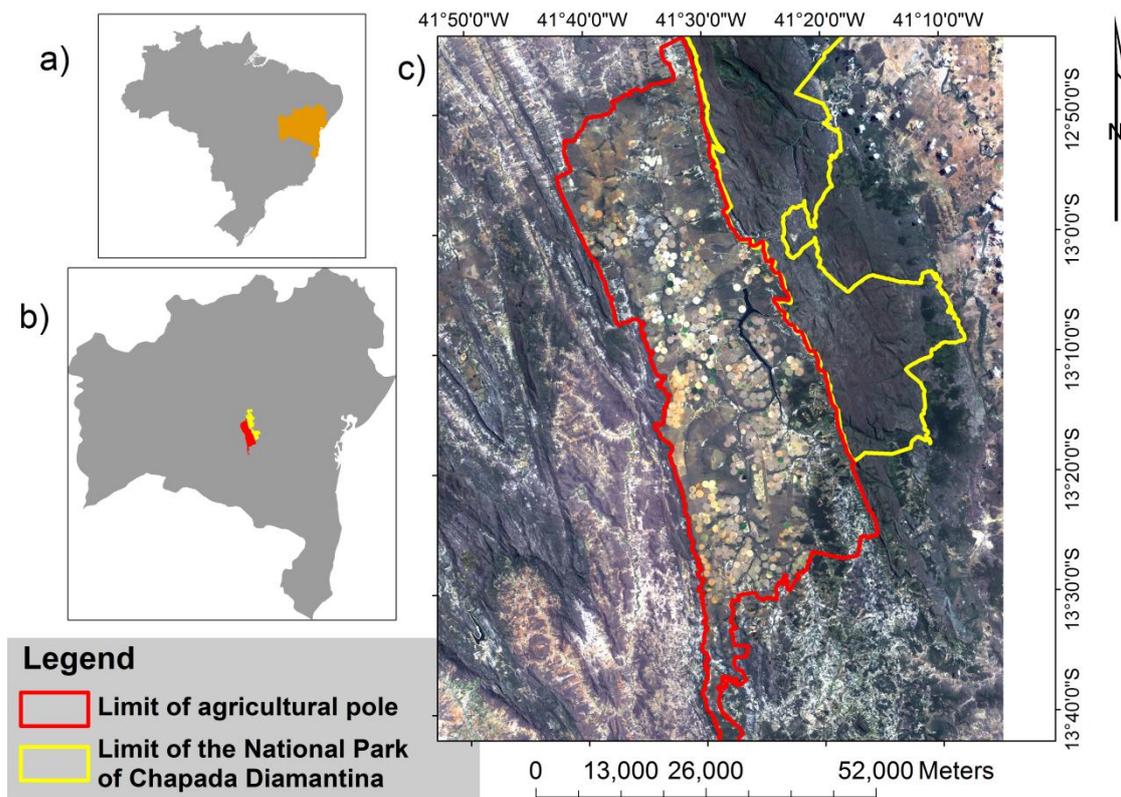


FIGURE 1. Location of the studied apple orchard in the region of Chapada Diamantina. Bahia State is located in the northeast of Brazil (a), and Chapada Diamantina is in the central part of Bahia State (b). Right panel, high-resolution (pixels of 5 m per side) satellite image (SPOT) obtained in September of 2008 (c); the studied apple orchard is located in the pole of irrigated agriculture between the municipalities of Ibicoara and Mucugê (190,000 ha or approximately 470,000 acres) and is bordered by Chapada Diamantina National Park.

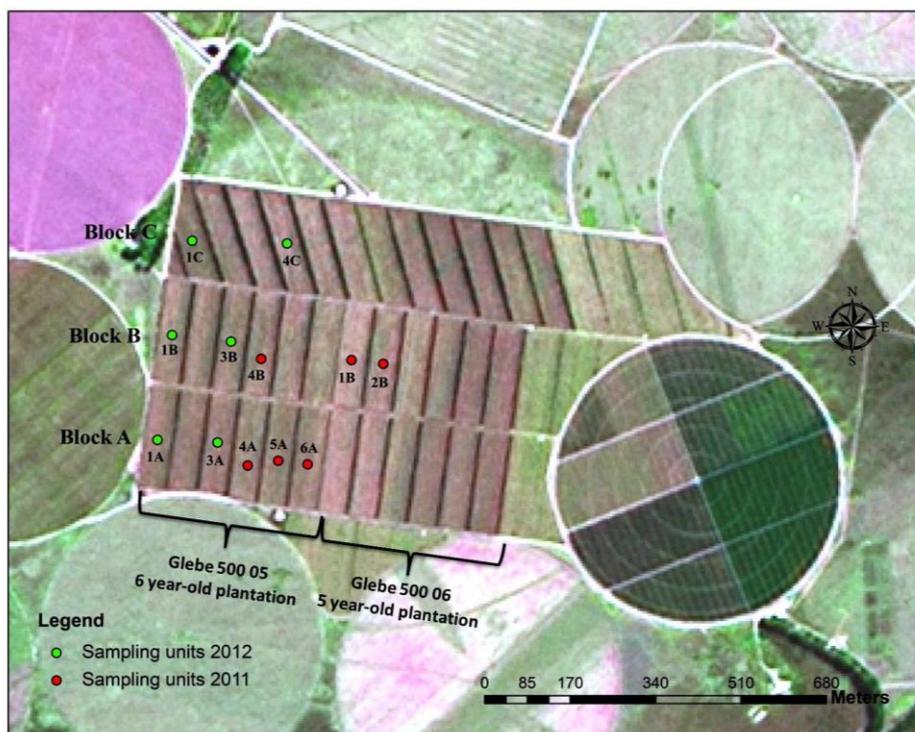


FIGURE 2. High-resolution (pixels of 5 m per side) satellite image (SPOT) obtained in September of 2008 showing the 43-ha study area, which constitutes an apple orchard in the Bagisa Company farm (X: 238,519.993 UTM; Y: 8,531,325.085 UTM) in the municipality of Ibicoara (State of Bahia, Brazil). The apple orchard is divided into three blocks (A, B and C) comprising 36 parcels (1.2 ha or 60 × 200 m each); each block has 12 parcels. The orchard area is divided into two 18-parcel grids (Glebe 500 05 and Glebe 500 06) according to the age of trees.

TABLE 1. Bee colony and management tested in the apple orchard in 2011 and 2012 using hives of *Apis mellifera scutellata* Lepeletier and *Melipona quadrifasciata anthidioides* Lepeletier to improve crop pollination in Chapada Diamantina, State of Bahia, Brazil.

Bee colony density and management tested	Parcel ID	Number of blooming parcels
2011		
7 <i>A. mellifera</i> hives ha <sup>-1</sup> without pollen collector	4A, 4B	12
7 <i>A. mellifera</i> hives ha <sup>-1</sup> with pollen collector	4A, 4B	12
9 <i>A. mellifera</i> hives ha <sup>-1</sup> without pollen collector	5A, 1B	18
9 <i>A. mellifera</i> hives ha <sup>-1</sup> with pollen collector	5A, 1B	18
11 <i>A. mellifera</i> hives ha <sup>-1</sup> without pollen collector	6A, 2B	24
11 <i>A. mellifera</i> hives ha <sup>-1</sup> with pollen collector	6A, 2B	24
2012		
7 <i>A. mellifera</i> hives ha <sup>-1</sup> with pollen collector + 12 native bee hives ha <sup>-1</sup>	1A, 1B, 1C	3
7 <i>A. mellifera</i> hives ha <sup>-1</sup> with pollen collector	3A, 3B, 4C	6

(2011). Because of the spatial arrangement of the varieties, we were able to mark adjacent Eva trees but not adjacent Princess trees, which were marked in adjacent rows instead.

### Flower density

The flower density may influence the attractiveness of and bee visitation to apple flowers. Therefore, following the method described by Vaissière et al. (2011), we recorded the flower number from eight sampling trees that were grouped in four plots marked in the inner rows of each experimental area. To estimate the flower production per tree, two opposite branches (minimal base width of 3 cm) were selected to record the number of open flowers. Then, the average number of flowers from these two branches was multiplied by the total number of branches in the tree.

In each parcel, the flower density of both varieties ( $n = 48$  Eva and  $n = 48$  Princess) was analysed only once per season during the bee colony density experiment to obtain a total of 96 data points each year. In 2011, the trees were sampled from six parcels, resulting in a total of 32 trees for each bee colony density tested (Table 1), whereas in 2012, the flower counting was performed with 48 trees in three parcels with stingless bee hives and Africanised honeybee hives and six parcels with Africanised honeybee hives only (Table 1). Then, we estimated the number of flowers in the parcel by multiplying the total number of trees ( $n = 2800$  trees per parcel) by the mean flower density of individual trees.

### Bee colony density

For the tests performed in 2011 and 2012, the number of Africanised honeybee and stingless bee colonies was increased sequentially to control the density level in relation to the number of blooming parcels. In both years, the hives were placed only in blooming parcels. Therefore, the number of honeybee hives needed for the tested densities of 7, 9 and 11 hives ha<sup>-1</sup> were, respectively, 84, 162 and 264 (Table 1). To obtain an independent dataset and avoid biasing the results of the experiments, each set of two sampling areas had different levels of bee colony density with no superposition in time.

The honeybee hives were transported into the orchard at night during the peak of flowering and 24 hours prior to the start of the controlled density test. Within the parcels, the hives were organised at a distance of 10 m from the edge, separated from each other by a distance of 8 m in the treatments with 7 and 9 hives ha<sup>-1</sup> and by a distance of 4 m in the treatments with 11 hives ha<sup>-1</sup>, and left there until the end of flowering.

The hives were placed on a metal support to avoid attacks by predators at a height of approximately 0.5 m above the ground. The pollen collector model used in this study consisted of a screen of plastic material containing orifices measuring 4.5 mm in diameter that removed part of the pollen transported in the bee's corbicula as it enters the hive. The pollen was collected underneath in a receptacle that did not allow its recovery by bees or ants. The hives were inspected by one of the authors in the apiary to equalise the bee colony strength based on the number of brood boxes (between rows 3 and 4) and health state (ex. presence of a new and active queen, foraging activity and no signals of diseases).

Because we had a limited number of stingless bee hives available for the tests ( $n = 36$  colonies), we performed the density tests in three blooming parcels. To achieve the desired bee colony density, we used 21 honey bee hives (7 hives ha<sup>-1</sup> with pollen collectors) and 36 stingless bee colonies (12 colonies ha<sup>-1</sup>) (Table 1). Later on, when six parcels were in bloom, we removed the stingless bee colonies to test the Africanised honeybee colony density only using 42 hives (7 hives ha<sup>-1</sup> with pollen collectors). Prior to the start of the density experiment, the stingless bees were left in the orchard for five days to forage before the Africanised honeybee hives were introduced into the orchard. The stingless bee hives were left in the orchard for nine consecutive days during the peak flowering period.

The Africanised honeybee hives were placed in alternating rows at a distance of 10 m from the edge of the parcel, whereas the stingless bee (*M. quadrifasciata anthidioides*) hives at 12 hives ha<sup>-1</sup> were placed in the centre of the parcel in groups of four colonies per row, and the distance between the colonies within the row was 3 m. The

stingless bee hives were maintained over wood supports 0.8 m above the ground.

The stingless bee hives were obtained from a meliponary located 410 km from the orchard. Each colony had approximately 300-400 workers bees and was sensitive to changes in the weather conditions (Nogueira-Neto 2007). Because of the transportation distance and the different weather conditions between their original place and the orchard area, the colonies were allowed to acclimate for 30 days. During that period, the colonies were maintained in a bee yard located in the study region and foraged for pollen and nectar on native plants and crops, such as eggplant, giloh and pumpkin.

### Number of seeds and fruits

Following the method described by Vaissière et al. (2011), we marked and bagged two buds from two different inflorescences in each apple tree from the pollination tests in 2011 and 2012. For each variety, we marked a total of 24 buds in the centre of the inflorescence ("king blossom"). In 2011, opened flowers were unbagged and exposed for visitation by the potential pollinators for 24 h when the pollen collectors were not at the hives (two flowers/tree/parcel) and when the pollen collectors were present (two flowers/tree/parcel). Therefore, eight trees of the Eva variety and eight trees of the Princess variety were marked in each parcel. In total, 48 apple trees were used ( $n = 24$  trees for each type of colony density tested) for each apple variety (total = 96 trees). In 2012, three flowers per marked tree (i.e., 288 flowers in total) were randomly chosen to record the number of seeds in the fruits formed. As in 2011, the flowers were also bagged, and the seeds and fruits were counted thirty days later.

In 2011, only the data from the Eva trees were statistically evaluated, whereas in 2012, data from both varieties were analysed. The number of seeds was estimated for the two years of the study, whereas the fruits were counted only in 2012 due to the great loss of samples caused by their accidental removal during pruning by the farmer.

### Statistical analysis

For the 2011 data, we estimated a general linear mixed-effects model (GLMM) of the influences of supplementation with honey bee hives (quantitative predictor: 7, 9, and 11 hives  $\text{ha}^{-1}$ ), pollen collector (categorical predictor with two levels: yes, no), and their interactions as fixed effects on the number of seeds per fruit of the Eva variety (Gaussian error distribution). For the 2012 data, we estimated a GLMM of the influences of supplementation with 12 *Melipona quadrifasciata* hives  $\text{ha}^{-1}$  (categorical predictor with two levels: yes, no), apple variety (Eva or Princess), and their interactions as fixed effects on the number of seeds per fruit and the number of fruits per tree (Gaussian error distribution). All of the parcels during 2012 were supplemented with 7 *A. mellifera* hives  $\text{ha}^{-1}$  as pollen collectors. For the two models (2011 and 2012), we included the number of flowers per ha as a fixed-effect covariate and the parcel as a random effect (random intercept model) to account for the fact that individual trees were

spatially nested within parcels (treatments were applied to spatially and temporally segregated parcels, see Table I). The significance was tested through analysis of variance (ANOVA; assumptions were valid in all cases). The models were estimated using the lme function of the nlme package (Pinheiro, Bates, DebRoy, Sarkar & Team, 2012) in the R software (R Development Core Team, 2011).

## RESULTS

In 2011, apple trees from the Eva variety set six seeds per fruit on average (seed production). A greater number of hives (from 7 to 11 hives  $\text{ha}^{-1}$ ;  $F_{1,3} = 0.83$ ,  $P = 0.43$ ) or the addition of pollen collectors ( $F_{1,52} = 1.7$ ,  $P = 0.19$ ) produced no general effects on the seed production (Figure 3). However, we found a marginally significant interaction between the effects of the Africanised honeybee number and the pollen collectors ( $F_{1,52} = 3.6$ ,  $P = 0.064$ ) associated with a higher seed production (eight seeds on average) of trees without pollen collectors in plots with 11 hives  $\text{ha}^{-1}$  (Figure 3). There was no further significant increase in the seed production that could be related to pollen collectors beyond the density of 7 hives  $\text{ha}^{-1}$  because the mean number of seeds under the treatment of 7 hives  $\text{ha}^{-1}$  + pollen collectors was similar to that obtained with the treatment with 11 hives  $\text{ha}^{-1}$ . The number of flowers per ha did not affect the seed production ( $F_{1,3} = 1.3$ ,  $P = 0.34$ ).

In 2012, supplementation with the stingless bee (*Melipona quadrifasciata anthidioides*) and Africanised honeybees (7 hives  $\text{ha}^{-1}$ ) produced a higher seed production and higher fruit production than supplementation with Africanised honeybees (7 hives  $\text{ha}^{-1}$ ) alone (Figure 4). However, the magnitude of this increase depended on the crop variety (*M. quadrifasciata* × variety interaction: seed production  $F_{1,86} = 7.9$ ,  $P = 0.0062$ ; fruit production  $F_{1,86} = 6.2$ ,  $P = 0.015$ ). For the Eva (production) variety, the addition of *M. quadrifasciata* increased the seed production and fruit production by 67% (from three to five seeds) and 44% (from 64 to 92 fruits), respectively. In contrast, for the Princess (polliniser) variety, the addition of *M.*

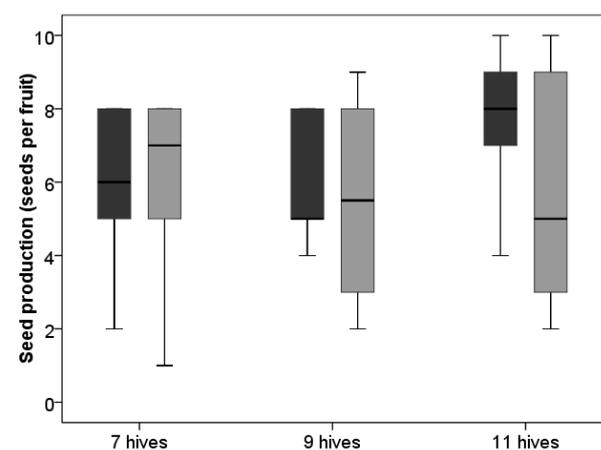


FIGURE 3. Number of seeds per fruit (seed production) from Eva trees in 2011 in relation to the number of Africanised honeybee hives  $\text{ha}^{-1}$  without pollen collectors (dark grey bar) and with pollen collectors (light grey bar).

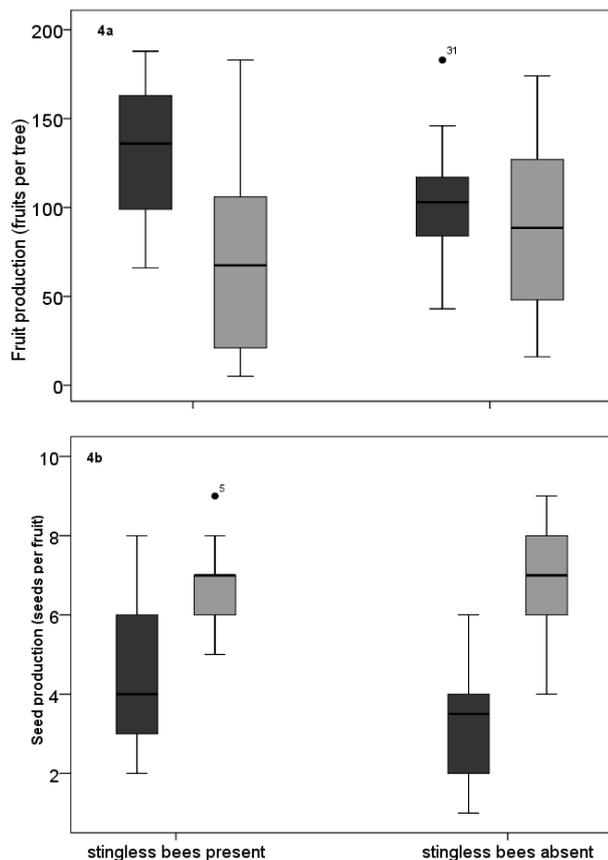


FIGURE 4. Fruit (4a) and seed production (4b) in Eva (dark grey bar) and Princess (light grey bar) apple trees ( $n = 24$  trees for each tested density). The dataset obtained in 2012 was from three parcels densely populated with both stingless bees ( $n = 12$  colonies  $\text{ha}^{-1}$ ) and Africanised honeybees ( $7$  hives  $\text{ha}^{-1}$  with pollen collectors) and three parcels populated with Africanised honeybees only ( $7$  hives  $\text{ha}^{-1}$  with pollen collectors).

*quadrifasciata* did not affect the seed production (seven seeds per fruit on average) or fruit production (80 fruits per tree on average). In general, the trees in plots with a higher flower number per ha set more seeds ( $F_{1,86} = 9.6$ ,  $P = 0.0026$ ), but no differences were found in fruit production ( $F_{1,86} = 0.33$ ,  $P = 0.57$ ).

## DISCUSSION

The pollination deficit in the study orchard was detected by supplementation experiments, in which increased seed production and fruit production were obtained with the addition of pollinators. Our study also highlighted the finding that the productivity of the apple crop in Chapada Diamantina relies on supplementation with Africanised honeybee hives, with densities much greater than those traditionally used for this crop, which range from 1 to 5 hives  $\text{ha}^{-1}$  (Degrandi–Hoffman et al. 1987; Delaney & Tarpay 2008; Khan & Khan 2004; Paranhos et al. 1998). In the Minas Gerais State, orchards with the Eva variety require only 0.5 hives  $\text{ha}^{-1}$  for efficient pollination (Farmer's Communication, Mr. José Lázaro, owner of the Corredor Farm (X: 0597793 UTM; Y: 7627415 UTM), Piedade do Rio Grande, MG). One explanation for this difference is the colony strength used in our experiments. Most investigators

use strong hives with high numbers of worker bees in which the number of brood boxes varies between four and six (Hoopingarner & Waller 1993) and between six and 10 (Delaplane & Meyer 2000; Dag et al. 2003). We used intermediate or weak honeybee hives bearing three to four brood combs, which could explain the differences in the densities found. The careful observation of the optimal hive criteria for pollination purposes in the region is only in its infancy. Therefore, their management should be improved by incorporating them into the beekeeper's current practices.

Nevertheless, the required number of colonies in the orchard remains high. Our data indicate that the efficiency of supplementing the apple orchard with additional managed bee hives levels off, suggesting a threshold in the bee density of 7 hives  $\text{ha}^{-1}$ . Higher densities did not result in improved pollination. Furthermore, it may be impossible to manage higher densities due to the high aggressiveness of Africanised honeybees (Dietz 1982).

We found that, without wild pollinators, pollination is not improved by adding pollen collectors to Africanised honeybee hives (Rashad & Parker 1958; Thomson & Goodell 2002; Manning et al. 2010). Two possible explanations are that the honeybee hive density is more important than pollen collector in promoting pollen flow within an apple orchard and that the densities of honey bees were too high at seven hives per ha, which resulted in the pollen collectors having no detectable effect.

A suitable alternative for increasing pollination success in apple orchards and complementing the use of Africanised honey bees is the addition of hives of the native stingless bee *Melipona quadrifasciata anthidioides*. According to our data, when we added the stingless bee hives, the seed and fruit production in the Eva variety was higher than that obtained with supplementation with Africanised honey bees alone. Other authors have already noted the link between the presence of native bees and increased pollination due to interspecific interactions through an increase in between-row movements by *A. mellifera* subspecies, which favour fruit production in dioecious and self-incompatible crops (Greenleaf & Kremen 2006; Carvalheiro et al. 2011; Brittain et al. 2013). This possibly explains why flower density turned out to be an important co-variable favouring successful pollination when diversity of visitors was high in the studied orchard. Our data also agree with records from important crops worldwide, which show that the pollination services performed by native species cannot be substituted by managed exotic species, mainly *A. mellifera* (Garratt et al. 2014; Garibaldi et al. 2013). Fründ et al. (2013) highlighted the importance of bee functional diversity for the reproduction of plant communities and the need to identify complementarity traits for accurately predicting pollination services by bee communities and assessing whether this arises via facilitation or competitive interactions or a species' behaviour in isolation (Cardinale et al. 2002; Casula et al. 2006; Ashton et al. 2010).

Apple productivity in the apple orchard in Chapada Diamantina increased from 10 ton  $\text{ha}^{-1}$  in 2010 to 22 ton  $\text{ha}^{-1}$  and 27 ton  $\text{ha}^{-1}$  in 2011 and 2012, respectively (BRASIL

2012), which is significant increase in a regional context but represents only 50% of the productivity of the Eva variety at the Corredor Farm in Piedade do Rio Grande (State of Minas Gerais, Brazil), which produced 40 ton ha<sup>-1</sup> (BRASIL 2012). This evidence indicates that the age of apple trees is insufficient to explain the annual change in productivity both in Chapada Diamantina and among apple orchards because both share similarities in the age of the trees, size of the planted area, conventional management system and climatic conditions. The lower apple productivity in the Chapada Diamantina region could be explained by the low percentage of natural and semi-natural habitats surrounding the apple orchard (23% measured in a radius of 2 km from the centre of the orchard) (Coutinho JGE, personal communication). We observed that very few wild bees visited the apple flowers. *Melipona quadrifasciata anthidioides* and other stingless bees are no longer found naturally in the region (Silva FO, personal communication). The loss of native insect species in landscapes dominated by agricultural matrices will likely influence both natural vegetation and agricultural crops isolated from natural areas (Ricketts et al. 2008; Watson et al. 2011). Consequently, fruiting decreases considerably in locations with a lower abundance of wild insects visiting the target crop flowers (Klein et al. 2007; Carvalheiro et al. 2011; Garibaldi et al. 2013).

By highlighting the importance of native bees as pollinators of apples, this study adds to arguments for the adoption of friendly practices to conserve native pollinators and the spread of initiatives to value local biodiversity and develop native bee management techniques for pollination. The lack of appropriate techniques for the management of native bees for pollination in Brazil is partly due to the Brazilian legislation that regulates the management of native species and affects the widespread commercial use of native bees for pollination (Imperatriz-Fonseca et al. 2013). In this context, both farmers and beekeepers require technical support and information to become aware of the economic, social and environmental benefits of incorporating these practices. These practices should include the conservation and/or restoration of natural and semi-natural areas within crop areas, the promotion of diversity, including polycultures, which provide a variety of floral and nesting resources for pollinators, and the more prudent use of toxic agricultural chemicals.

#### ACKNOWLEDGEMENTS

The authors are grateful for the financial aid provided by the National Council for Scientific and Technological Development, CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq), the Global Environmental Facility (GEF), the Food and Agriculture Organization of the United Nations (FAO), the United Nations Environmental Programme (UNEP) and the Brazilian Biodiversity Fund (Fundo Brasileiro para a Biodiversidade, FUNBIO). The authors thank the Bagisa Company for Agriculture and Commerce (Companhia Bagisa S/A Agropecuária e Comércio) for the logistical support and access to the orchards, Professor Dr. Pedro Luis Bernardo da Rocha for his contribution to the experimental design, and Marina Siqueira de Castro (Bahian Company of Agricultural Development/State University of Feira de Santana, EBDA/UEFS) and her team for their help with transporting and managing the colonies of stingless

bees used in the experiments. Blandina F. Viana acknowledges the research productivity grant from the CNPq. Fabiana O. da Silva acknowledges the Brazilian National Agency for the Support and Evaluation of Graduate Education (CAPES) for Post Doc grants, and Antonio da Costa Diakos acknowledges the Master's grant awarded by the GEF/FAO/UNEP/FUNBIO.

#### REFERENCES

- Albuquerque-Junior CL, Denardi F, Dantas ACM, Nodari RO (2010) Número de anteras por flor, grãos de pólen por antera e capacidade germinativa do pólen de diferentes cultivares de macieiras. *Revista Brasileira de Fruticultura* 32: 1255-1260.
- Ashton IW, Miller AE, Bowman WD, Suding KN (2010) Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* 91:3252-3260.
- Bosch J, Kemp WP (2002) Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera; Megachilidae) and fruit trees. *Bulletin of Entomological Research* 92:3-16.
- BRASIL. Ministério do Planejamento, Orçamento e Gestão. Instituto Brasileiro de Geografia e Estatística. Diretoria de Pesquisas, Coordenação de Agropecuária. Levantamento Sistemático da Produção Agrícola. Rio de Janeiro 10:1-80 [online] URL: <[http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/lspa/lspa\\_201110.pdf](http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/lspa/lspa_201110.pdf)> (accessed on: jan. 2014).
- Brittain C, Williams N, Kremen C, Klein AM (2013) Synergistic effects of non-*Apis* bees and honeybees for pollination services. *Proceedings of the Royal Society B: Biological Sciences* 280: 20122767.
- Brittain C, Kremen C, Klein AM (2012) Biodiversity buffers pollination from changes in environmental conditions. *Global Change Biology* 19:540-547.
- Cameron S, Lozier J, Strange J, Koch J, Cordes N, Solter L, Griswold T (2011) Patterns of widespread decline in North American bumble bees. *Proceedings of national academy of sciences (PNAS)* 108: 662-667.
- Cardinale BJ, Palmer MA, Collins SL (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature* 415: 426-429.
- Carvalheiro LG, Veldtman R, Shenkute AG, Tesfay GB, Pirk CWW, Donaldson JS, Nicolson SW (2011) Natural and within-farmland biodiversity enhances crop productivity. *Ecology Letters* 14: 251-259.
- Casula P, Wilby A, Thomas MB (2006) Understanding biodiversity effects on prey in multi-enemy systems. *Ecology Letters*, 9:995-1004.
- Dag A, Doron I, Stern RA (2003) Recommendation for pollination of apple, pear and plum. Newsletter – The extension service, Galilee-Golan region (in hebrew), 2 pp.
- Degrandi-Hoffman G, Hoopingarner R, Pulcer R (1987) Redapoll: pollination and fruit-set prediction model for "delicious" apples. *Environmental Entomology* 16:309-318.
- Delaney DA, Tarpy DR (2008) The role honeybees in apple pollination. Department of Entomology, North Carolina State University, NCnc State apiculture program. Beekeeping note 3.03. (Rewrite of former Beekeeping Note #7A), NC State University, Cooperative Extension Service.
- Delaplane KS, Mayer DF (2000) Crop pollination by bees. Cab publishing, Walling Oxon Ox 108 de UK, UK University Press, Cambridge.

- Dietz A (1982) Honeybees. In: Henry H (Ed) Social Insects. Elsevier Inc., London, pp 323-354.
- Finta K (2004) Insect pollination of apple orchards. University of West Hungary, Faculty of Agricultural and Food Science.
- Freitas BM, Oliveira-Filho JH (2001) Criação racional de mamangavas para polinização em áreas agrícolas. Banco do Nordeste, Fortaleza.
- Fründ J, Dorman CF, Holzschuh A, Tschamtker T (2013) Bee diversity effects on pollination depend on functional complementarity and niche shifts. *Ecology* 94(9): 2042-2054.
- Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F, Boreux V, Cariveau D, Chacoff NP, Dudenhöffer JH, Freitas BM, Ghazoul J, Greenleaf S, Sousa JH, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Ricketts TH, Rundlöf M, Seymour CL, Schüpp C, Szentgyörgyi H, Taki H, Tschamtker T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM (2013) Wild pollinators enhance fruit set of crops regardless of honeybee abundance. *Science* 6127: 1608-1611.
- Garratt MPD, Truslove CL, Coston DJ, Evans RL, Moss ED, Dodson C, Jenner N, Biesmeijer JC, Potts SG (2014) Pollination deficits in UK apple orchards. *Journal of Pollination Ecology* 12: 9-14.
- Greenleaf SS, Kremen C (2006) Wild bees enhance honeybees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences* 103: 13890-13895.
- Hoopengarner RA, Waller GD (1993) Crop pollination. In: Graham JM (ed) The hive and the honeybee, Dadant and Sons, Carthage, Illinois, p.1044-1080.
- Imperatriz-Fonseca VL, Canhos DAL, Alves DA, Saraiva AM (2012) Polinizadores no Brasil: Contribuição e perspectivas para a biodiversidade, uso sustentável, conservação e serviços ambientais. São Paulo: Editora da Universidade de São Paulo.
- Kennedy CM, Lonsdorf E, Neel MC, Williams NM, Ricketts TH, Winfree R, Bommarco R, Brittain C, Burley AL, Cariveau D, Carvalheiro LG, Chacoff NP, Cunningham SA, Danforth BN, Dudenhöffer JH, Elle E, Gaines HR, Garibaldi LA, Gratton C, Holzschuh A, Isaacs R, Javorek SK, Jha S, Klein AM, Krewenka K, Mandelik Y, Mayfield MM, Morandin L, Neame LA, Otieno M, Park M, Potts SG, Rundlöf M, Saez A, Steffan-Dewenter I, Taki H, Viana BF, Westphal C, Wilson JK, Greenleaf SS, Kremen C (2013) A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology Letters* 16:584-599.
- Kevan PG (1997) Honeybees for better apples and much higher yields: study shows pollination services pay dividends. *Canadian Fruitgrower* 14:16.
- Khan M, Khan M (2004) The role of honeybees *Apis mellifera* L. (Hymenoptera, Apidae) in pollination of apple. *Journal of Biological Science* 3:359-362.
- Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tschamtker T (2007) Importance of pollinators in changing landscapes for world crops. *Royal Society of Biology* 274:303-313.
- Klein AM, Brittain C, Hendrix SD, Thorp R, Williams N, Kremen C (2012) Wild pollination services to California almond rely on semi-natural habitat. *Journal of Applied Ecology* 49: 723- 732.
- Kremen C, Miles A (2012) Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. *Ecology and Society* 17: 40.
- Kremen C, Iles A, Bacon C (2012) Diversified farming systems: an agroecological, systems-based alternative to modern industrial agriculture. *Ecology and Society* 17: 44.
- Kremen C, Williams NM, Thorp RW (2002) Crop pollination from native bees at risk from agricultural intensification. *Proceedings of national academy of sciences* 99:16812-16816.
- Loper GM, Thorp RW, Berdel R (1985) Improving honeybee pollination efficiency in almonds. *California Agriculture* 39:19-20.
- Manning R, Sakai H, Eaton L (2010) Methods and modifications to enhance the abundance of pollen on forager honeybees (*Apis mellifera* L.) exiting from beehives: implications for contract pollination services. *Australian Journal of Entomology* 49:278-285.
- Nelson DL, McKenna D, Zumwalt E (1987) The effect of continuous pollen trapping on sealed brood, honey production and gross income in northern Alberta. *Apicultural Research*. 24:107-114.
- Nogueira-Neto P. (1997) Vida e criação de abelhas nativas sem ferrão. São Paulo. São Paulo, Editora Nogueirapis.
- Paranhos BAJ, Walder JMM, Marchini LC (1998). Densidade de colmeias de abelhas africanizadas, *Apis mellifera* L. 1758 (Hymenoptera: Apidae), para polinizar maçã cv. Anna. *Scientia Agricola* 55:355-359.
- Petri J, Leite GB, Couto M, Francescato P (2011) Avanços da cultura da macieira no Brasil. *Revista Brasileira de Fruticultura* 33: 48-56.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RDC (2012) nlme: Linear and nonlinear mixed effects models.
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010a) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution* 25: 345-353.
- Potts SG, Roberts SPM, Dean R, Marris G, Brown M, Jones R, Settele J (2010b) Declines of managed honeybees and beekeepers in Europe? *Journal of Apicultural Research* 49: 15-22.
- R Development Core Team. (2011) R: A language and environment for statistical computing. Vienna, Austria. ISBN 3-900051-07-0: R Foundation for Statistical Computing. Retrieved from <http://www.r-project.org/>
- Rashad AE, Parker RL (1958) Pollen as a limiting factor in brood rearing and honey production. *Transactions of the Kansas Academy of Science* 61:237-248.
- Ricketts HT, Regetz J, Steffan-Dewenter I, Cunningham SA, Kremen C, Bogdanski A, Gemmill-Herren B (2008) Landscape effects on crop pollination services: are there general patterns? *Ecology Letters* 11:499-515.
- Roubik DW (1989) Foraging behavior of competing africanized honeybees and stingless bees. *Ecology* 61: 836-845.
- Taki H, Yamaura Y, Okabe K, Maeto K (2011) Plantation vs. natural forest: Matrix quality determines pollinator abundance in crop fields. *Scientific Reports* 1:132.
- Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practice. *Nature* 418:671-677.
- Thomson JD, Goodell K (2002) Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology* 38: 1032-1044.
- Vaissière BE, Freitas BM, Gemmill-Herren, B (2011) Protocol to detect and assess pollination deficits in crops: a handbook for its

- use. Food and Agriculture Organization of the United Nations (FAO/IFAD), Rome.
- Venturieri, GC (2008) Criação de abelhas indígenas sem ferrão. 2.ed. Belém: Embrapa Amazônia Oriental, 60p.
- Viana, BF, Boscolo, D, Neto EM, Lopes LE, Lopes AV, Ferreira PA, Pigozzo CM, Primo LM (2012) How well do we understand landscape effects on pollinators and pollination services? *Journal of Pollination Ecology* 7(5): 31-41.