IMPORTANCE OF BEE POLLINATION FOR COTTON PRODUCTION IN CONVENTIONAL AND ORGANIC FARMS IN BRAZIL

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Abstract—This study aimed to evaluate the importance of wild bee and feral honeybee visits for cotton production on conventional and organic farms. Experiments were conducted in Brazil, on a conventional cotton farm in Mato Grosso state in the Amazon biome and on an organic farm in Paraíba state in the Caatinga biome. On the conventional farm, bee assemblage and cotton production were measured near to and far from natural vegetation. Bee richness, fibre fraction, seed number and yield (Kg/ha) were higher by 57.14, 1.95, 17.77 and 18.44% respectively in plots near natural vegetation, but bee abundance did not vary with distance to natural vegetation. On the organic farm, because the cropping area is surrounded by natural vegetation, pollination deficit was evaluated using an exclusion experiment where cotton production of flowers bagged to prevent bee visitation (spontaneous self-pollination) was compared to production of flowers open to bee visitation (open pollination). Open pollinated flowers had higher average boll weight, fibre weight and seed number. Although cotton is not directly dependent on bee pollination, bees increased cotton production on the organic farm by more than 12% for fibre weight and over 17% for seed number. Our data confirm the importance of maintaining communities of pollinators on cotton farms, especially for organic production.

Keywords: bee community, fibre quality, seed vigour, species richness

INTRODUCTION

Information concerning pollination requirements and possible production deficits of Brazilian main crops is scarce (De Marco & Coelho 2004; Yamamoto et al. 2012, Milfont et al. 2013). Previous studies showed that even autogamous species, which are not directly dependent on pollinator activity for production (e.g. coffee, oilseed rape, soybean), undergo considerable production gains when visited by bees (De Marco & Coelho 2004; Veddele et al. 2008, Bommarco et al 2012, Milfont et al. 2013). Coffee, for example, shows increases in production varying between 14.6% (De Marco & Coelho 2004) and 50% (Ricketts et al. 2008), depending on how favourable the surrounding vegetation is to pollinators. Similarly, increases in cotton production, seed production and fibre quality have been linked with visits by Apis mellifera L. and wild bees (McGregor 1976; Tanda, 1984; Waller et al. 1985; Free 1993; Rhodes 2002).

Despite the rich bee fauna associated with cotton flowers in different regions of Brazil (Pires et al.2006; Martins et al 2008; Malerbo-Souza & Halak 2011) and the number of cotton varieties available on the market, few studies have been conducted to evaluate the role of bees in cotton pollination (Sanchez Junior & Malerbo-Souza 2004; Silva 2007; Cardoso 2008; Martins et al 2008), and farmers are generally unaware of the potential benefits of bee pollination for crop production (personal observation in our study areas). Moreover, on conventional farms, which generally are unfriendly environments for bees, the possible gains promoted by bee pollination may be lower than the potential yield losses due to heavy attacks of pests, such as the boll weevil (Fontes et al 2006, Lima et al 2013). With all this, despite the increase in seed number observed in flowers opened to bee visitation in experimental conditions (Sanchez Junior & Malerbo-Souza 2004; Silva 2007), the influence of bee pollination on commercial cotton production in the country has been largely ignored and sometimes considered negligible (personal observation in our study areas).

Cotton in Brazil is cultivated on large and small farms, using different production systems, in two production regions, which are also ecologically distinct. The level of technology and inputs used with this crop varies from very low on smallholdings in the Northeast to very high on large farms in the Midwest (Fontes et al. 2006). In this work, we evaluated the importance of wild bees and feral honeybee flower visitations on cotton production, in conventional and organic cotton systems. The following questions guided this study: a) are richness and abundance of bees higher in areas near by natural vegetation, considering the large areas of conventional farms? b) if so, do richness and abundance of
bees influence the characteristics of cotton production? and c) does the lack of bee visitation reduce cotton production on organic farms?

**MATERIALS AND METHODS**

**Areas of study**

The study took place in 2011 in two cotton producing regions in Brazil (Fig. 1, Tab. 1, Fig. S1 and S2 in Supplementary Information). The experiments were conducted on one intensively managed conventional farm (4,393 ha) in Sinop (11°52’89”S, 55°36’01”W), Mato Grosso state (MT), and one organic intercropping farm (8 ha) in Remígio (6°53’83”S, 35°49’07”W), Paraíba state (PB). The vegetation in Sinop is the typical southern Amazon tropical rainforest (IBGE 2012), while in Remígio it is characterized by shrubby-arboreal trees, partly corresponding to the arboreal savannah steppe (*Caatinga* sensu IBGE (2012)).

**Bee assemblage and evaluation of pollination deficit**

We used two experimental approaches to evaluate how the pollination promoted by bees influenced cotton production. On the conventional farm, we used a protocol proposed by the FAO (Food and Agriculture Organization of the United Nations) (Vaissière et al. 2011). The protocol assumes that richness and abundance of bee species are high near patches of natural vegetation. Thus, crop production and bee population were evaluated near to and, approximately, 1.5 km away from natural vegetation. On the organic farm, because all farms were located less than one km from patches of natural vegetation, an exclusion experiment (flowers where bee visits were not allowed) was carried out, following an adaptation of the methodology proposed by Dafni (1992). Bees visiting the cotton flowers, on both farms, were native species and the exotic honey bee (*Apis mellifera*). Honey bees were feral africanized hybrids, since no apiary existed in the vicinities of the sample crops. Bees were identified in the Laboratory of Systematics and Ecology of Bees of the Universidade Federal de Minas Gerais (UFMG), with the aid of a stereoscopic microscope, taxonomic keys (Silveira et al. 2002) and by comparing the specimens to a reference collection. The specimens were deposited in the Taxonomic Collections of the Universidade Federal de Minas Gerais (UFMG). The classification adopted for the bees is that of Michener (2000) with the modifications proposed by Silveira et al. (2002).

**Pollination deficit on the conventional farm**

Ten plots (25 m × 50 m) were delimited, five of them located near natural vegetation (153 m ± 7.34 SD and the other five away from the natural vegetation (1,576 m ± 342.10 SD). In each plot, bee observations were carried out on one day at the peak of the flowering season (between February and April) to reduce the effect of flower abundance variation on bee abundance and species richness. Data were collected between 9:00 and 13:00 h, when bees are most active on flowers (Cardoso 2008). Bee richness was assessed by collecting bees found inside cotton flowers in six subplots (2 m × 25 m), for five minutes at each of four intervals (9:00-10:00, 10:00-11:00, 11:00-12:00 and 12:00-13:00 h), totalling two hours of sampling per plot and 20 hours considering all 10 plots (Vaissière et al. 2011). The relative abundance of each species in each plot was estimated by dividing the number of individuals in the sample by the number of sampling hours (two hours). Use of these frequencies allows for comparison of bee abundance in samples obtained from different sites by different collectors expending different sampling efforts (Silveira and Godinez, 1996). Even though the study was conducted during the flowering peak, the flower density was quantified near to and far from natural vegetation to check if the available resource for bees was similar in the experimental plots. In each plot all

**Figure I.** Location in Brazil of the municipalities of Sinop (conventional farm) and Remígio (organic farm) in the states of Mato Grosso (MT) and Paraíba (PB) respectively.
open flowers were counted in four rows of 50 m, equidistant five meters from each other, and the number of flowers per linear meter was calculated.

To assess fibre production and quality, all cotton bolls were harvested in 10 one-linear-meters of crop randomly chosen in each plot. To choose these, first a line was randomly picked from about 30 lines in each plot and then the position on the 50 m line was randomly picked. Fibre weight (g), fibre fraction (%) and number of seeds were recorded for each linear meter. Fibre quality (i.e. micronaire - (HIV), mean fibre fineness – Fine mTex; fibre maturity – Mat Ratio; immature fibre content – IFC) were measured for each cotton boll. Because smallholders generally save seeds for the next crop season, germination (%), days for radicle protrusion and days for normal seedling emergence were evaluated in laboratory conditions. The seeds were de-linted, using H2SO4 (98%) (Godoy & Abrahão 1977), then washed with a detergent solution, rinsed under running tap water and dried at 23 - 26°C for 24h. After this, germination tests were conducted with four replicates of 50 seeds each per treatment, on paper substrate and incubated in germination chambers at 25°C. The percentage of germination (ISTA, 2009) was recorded daily over a seven-day period, and mean days for radicle protrusion and normal seedling emergence were calculated for the two treatments (Santana & Ranal 2004).

**Statistical analysis**

We used R (R Core Team 2013) to carry out the statistical analysis. Non-parametric Mann Whitney or Wilcoxon tests were used to compare yield, fibre quality, bee richness and bee abundance in plots near and far from natural vegetation located on the conventional farm. We used simple regression analysis to investigate the influence of bee richness on cotton production and fibre quality. For the data obtained in the experiment conducted on the organic farm, we used the Student’s t-test (or the Mann-Whitney test whenever normality and homoscedasticity were not met) to compare cotton production (boll and fibre weight, seed number and fibre fraction) fibre quality and seed quality (%) germination) in the two treatments.

**RESULTS**

**Bee assemblage**

Bee species richness was lower in the conventional farm (five species in 20 h of sampling) than the organic farm (18 species in 18 h of sampling). The relative abundance of each species (individuals/hour) was also lower on the conventional farm than on the organic farm (Tab. 2); for instance, 3.6 individuals per hour of *Apis mellifera* were...
TABLE 2. Relative abundance (bee/hour) of bee species collected on conventional and organic cotton farms. On the conventional farm, bees were collected at different distances from the nearest natural vegetation patch (FV= far from vegetation, NV= near vegetation). The study was conducted in 2011 in Brazil.

<table>
<thead>
<tr>
<th>Distance to natural vegetation (m)</th>
<th>Conventional</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV1</td>
<td>1,950</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>FV2</td>
<td>1,870</td>
<td></td>
</tr>
<tr>
<td>FV3</td>
<td>1,600</td>
<td></td>
</tr>
<tr>
<td>FV4</td>
<td>1,260</td>
<td></td>
</tr>
<tr>
<td>FV5</td>
<td>1,200</td>
<td></td>
</tr>
<tr>
<td>NV1</td>
<td>147</td>
<td>145</td>
</tr>
<tr>
<td>NV2</td>
<td>152</td>
<td>158</td>
</tr>
<tr>
<td>NV3</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>NV4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NV5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TAXA**

**APIIDAE**

Alpidosceles cf. imitatrix (Schrottky, 1909) 0.5

*Apis mellifera* Linnaeus, 1758 0.5 0.5 11 2.5 0.5 2 4 1.5 30.22

*Bombus* (Thoracobombus) brevivilus Franklin, 1913 0.05

*Cephalotrigona cf. capitata* (Smith, 1854) 0.5

*Ceratina* (Crewella) sp1 0.05

*Ceratina* (Crewella) sp9 0.22

*Diadasia* sp 0.33

*Diadasina* sp 0.05

*Exomalopsis* (*Exomalopsis*) analis Spinola, 1853 1.89

*Melipona* (Melikerria) interrupta Latreille, 1811 1 0.5

*Melitomella murihirta* (Cockerell, 1912) 0.05

*Trigona spinipes* (Fabricius, 1793) 1.17

**HALICTIDAE**

Augochlora (*Augochlora*) sp6 0.05

Augochlora (*Augochlora*) sp10 0.05

Augochlora (*Augochlora*) sp12 0.5 0.11

Augochlora (*Augochlora*) sp6 0.05

Augochlora (*Augochlora*) sp9 0.05

*Diacticus* sp 0.33

*Paroxystoglossa* sp 0.05

Pseudaugochlora pandora (Smith, 1853) 0.15

*Rhinochorynura* sp 0.05

collected on the conventional farm and 30.2 on the organic farm.

**Pollination deficit on the conventional farm**

Bee species richness was significantly greater in plots near natural vegetation than in those far from it (*Z* = 2.08; *P* = 0.04, Fig. 2A). In plots far from natural vegetation, only the exotic species *A. mellifera* was collected on cotton flowers, while in plots near natural vegetation, another four native species were collected beside *A. mellifera* (Tab. 2). The bee abundance did not differ (*Z* = 0.04; *P* = 0.96) between plots near and far from natural vegetation (Fig. 2B). The flowers’ availability for bees (mean number per linear meter of cotton plants) did not differ between plots near to (1.9 ± 0.78 SD) and far from natural vegetation (1.7 ± 0.46 SD; *W* = 9; *P* = 0.55). Fibre fraction (*Z* = 2.10; *P* = 0.03), number of seeds per boll (*Z* = 2.75; *P* < 0.01) and yield (*Z* = 2.85; *P* < 0.01) were significantly greater in plots near natural vegetation (Fig. 3). The other parameters of production and fibre quality did not vary with distance from the natural vegetation (Tab. S1 in supplementary information). Yield was positively influenced by bee species richness (*P* = 0.02, *R*² = 0.49; Fig. 4).

**Pollination deficit on the organic farm**

Bee visitation (OP treatment) positively influenced the following cotton production parameters: boll weight (Fig. 5A; *t* = -2.22; *P* = 0.03), fibre weight (Fig. 5B; *t* = -2.28; *P* = 0.02) and seed number (Fig. 5C; *t* = -3.59; *P* < 0.01). The boll weight, fibre weight and seed number were greater in flowers that received bee visits (OP – open pollination) than in flowers where only spontaneous self-pollination occurs (SS). However, bee visitation did not affect fibre fraction (*t* = -0.88; *P* = 0.38), short fibre content (*t* = -1.86; *P* = 0.06), mean fibre fineness (*t* = -0.66; *P* = 0.50), fibre maturity (*t* = -0.03; *P* = 0.97) and immature fibre content (*t* = -0.81; *P* = 0.42) (Tab. S2 in supplementary information). The only fibre quality parameter that was different between treatments was the length by weight (UQL), which was lower in flowers where only spontaneous
Figure 3. Different cotton production parameters measured in plots located near to (NV) and far from (FV) natural vegetation in the 2010/2011 crop season on a conventional farm in Sinop – Mato Grosso state, Brazil. Different letters in the box indicate significant differences between the plots based on Mann-Whitney U tests: (A) Fibre fraction (B) Number of seeds; and (C) Yield. N_{tot} = 30.

Figure 2. Richness (left) and abundance (right) of bees in plots near to (NV) and far from natural vegetation (FV) on a conventional cotton farm located in Sinop (MT), Brazil. Different letters above the bars indicate significant differences between the plots based on Mann-Whitney U tests: (A) Species Richness (B) Abundance. N_{tot} = 30. Thick black lines represent the median. Upper and lower bounds of the boxes are the 25th and 75th percentiles. The dotted lines represent the lowest and highest data still within 1.5 interquartile ranges of the upper or lower quartile. Outliers (open circles) are any points outside this range.
Figure 4. Relationship between cotton yield and bees species richness measured on a conventional farm in Sinop, MT, Brazil (y = 328.0x + 2556.9). Open circles represent plots near natural vegetation and black circles plots far from natural vegetation.

Figure 5. Boll weight, lint weight, number of seeds per boll, seed vigor (% of germination) and length by weight (UQL) measured in pollination experiments conducted on an organic farm in Remigio - PB, Brazil. SS = spontaneous self-pollination (tagged flowers) and OP = open pollination (open flowers) treatment. Different letters in the box represent significant differences. (A) Boll weight (B) Fibre weight (C) Seed number (D) UQL (E) Germination. Ns = 74 and Np = 64.
self-pollination (SS) occurred ($t = 2.43; P = 0.01$, Fig. 5D). The SS treatment had higher mean of germination percentage ($t = 3.00; P = 0.02$; Fig. 5E), but in both treatments the germination rate was higher than 90%, a value above the expected standard (ISTA 2009). Number of days for radicle protrusion (SS = 2.03 ± 0.16 SD; OP = 0.16 ± 0.67 SD) and normal seedling emergence (6 days for SS and OP) were equal for both treatments.

**DISCUSSION**

Our data indicate that bee visitation is positively correlated with cotton yield on the conventional farm and with boll weight, lint weight and number of seeds per boll on the organic farm. Of the studies carried out in Brazil to check the contribution of bee pollination to cotton varieties (Sanchez Junior & Malerbo-Souza 2004; Silva 2007; Cardoso 2008; Martins et al 2008), only two found an increase in seed production (Sanchez Junior & Malerbo-Souza 2004; Silva 2007). One possible explanation for the small increment obtained in the production parameters would be the high genetic homogeneity verified in most commercial varieties of *G. hirsutum* (Lacape et al 2007), for example, the DeltaOpal var. tested by Cardoso (2008). Besides the differences between the variety of genotypes tested, the contrasting results may also be due to differences in bee fauna in the different localities, differences in crop pest management or a combination of both. Silva (2008) tested the same variety we evaluated on the organic farm, but the bee richness in the area was low, as noted by the authors, and insecticide was applied, even in periods after bee visits to the flowers.

Our results also suggest that the contribution of a set of different pollinator species can be more advantageous for cotton production than that of just one species. Increases in yield provided by bee species richness have been found in coffee (De Marco & Coelho 2004), almond trees (Klein et al 2012; Brittain et al 2013), passion fruit (Yamamoto et al 2012) and many different crops (Garibaldi et al 2013).

Under conventional farming, our data show that bee species richness but not bee abundance was important for yield increase. This might be explained by two factors: 1) only honeybees were present away from natural vegetation, and honeybees, individually, are not particularly efficient as cotton-flower pollinators (Free 1993; Martins et al 2008; Cardoso 2008). Since they were present in relatively low abundances throughout the conventional cotton fields, yields where these bees were alone can be expected to be lower; 2) several species were present near natural vegetation and, thus, yield there could have benefitted from functional complementarity of different species, with different body sizes and foraging behaviours (Blüthgen & Klein 2011; Brittain et al 2013). In cotton flowers, different behaviours carried out by different flower visitors can increase cross-pollination and self-pollination (Silva 2007; Cardoso 2008; Pires 2009), and would explain our observation of production increase. Occasional observations of foraging behaviour showed that small species such as *Exomalopsis analis* (Fig. 6A) mainly carried out self-pollination on cotton flowers. *Apis mellifera* collects nectar on most visits (Cardoso 2008) but leaves the flowers with pollen adhered all over its body (Fig. 6B). Due to its high abundance in the areas and its body size (medium size), it possibly contributes to both self-pollination and cross-pollination. The large size of *Bombus brevivillus* made this bee species come into contact with all the reproductive structures (anthers and stigma) when it visited the flower, and cross-pollination took place (V.C. Pires 2011, unpublished data). Functional complementarity of different species has been documented on almond trees, where the foraging behaviour of *Apis mellifera* changed with the presence of wild species, increasing the pollination effectiveness (Brittain et al. 2013).
It is necessary to investigate for different crops how functional complementarity among bee species generates increases in production. Based on this knowledge, new forms of management of pollinators and agroecosystems could be proposed, aiming to increase pollination services.

Our data also suggest that increasing distance from natural vegetation does not influence honeybee abundance, but contributes to a significant decrease in wild bees on cotton flowers. Decline of bee-visitation rates with distance to native vegetation appears to be slower for honeybees than for native bees (Ricketts et al. 2008), so our most distant plots were probably not far enough to show it. Wild bee species primarily nest in native vegetation (Michener 2000) and are smaller than honeybees, so they have lower flight capacities (Greenleaf et al. 2007).

It seems that a bee-friendly environment, including natural-vegetation strips, diversification of cultivated crops and organic management practices, is important for maintaining higher bee populations and a richer bee assemblage on cotton flowers, as compared to the conventional farm (see also Kremen et al. 2007; Ricketts et al. 2008; Pinheiro & Freitas 2010). That a bee-friendly environment, and not other regional factors, such as climate, is responsible for the richer bee assemblages recorded on cotton flowers is further supported by the fact that the Caatinga domain (where we recorded our greatest bee-richnesses in cotton fields) is known for housing the poorest local bee faunas in Brazil (Michener 1979; Zanella 2000; Zanella & Martins 2003).

In addition, our data underline the potential economic importance of maintaining pollinator communities for crop pollination. On the conventional farm, the yield could potentially be increased 27.23% in relation to the average production reached in the all-cotton area by the presence of only four species of bees (honeybee and three wild species) in the field. This could result in a gain of approximately US$580/hectare, considering the prices of the 2011/2012 crop season (CONAB, 2012). For smallholder farms, where cotton is usually grown with low inputs, the gains in production related to the application of chemical insecticides are compensated by the increases resulting from the pollination services provided by bees (Silva 2007). If pollinators on the small farm are lost, a reduction of 14% in boll weight (52 kg/hectare) would generate a loss of approximately US$35/hectare of cotton (CONAB, 2012). This loss would be still greater if we consider the prices paid on the fair-trade and organic markets. The company VERT, for example, paid US$ 3.11/ kg of cotton in 2013, a price 65% higher than the market price according to the NYBOT index (VERT 2013). Moreover, the 17% loss in seed number may result in a reduction in oil and cake production. These products are used in animal food and could also be converted into monetary value.

The Brazilian government is striving to implement actions that favour beneficial insects in agricultural crops (Rocha 2012) as a way of reducing pesticide use, along with pest monitoring and restriction of aerial applications in areas near natural vegetation (Diário Oficial da União 2012). However, even basic measures recommended for pollinator protection, such as avoiding insecticide applications during daylight and during flowering periods, are not adopted in conventional cotton planting. One reason for this is that conventional farms are very extensive (thousands of hectares) and farmers claim they are unable to apply insecticides across the whole property between late afternoon and sunrise, when bees are not in activity in the cotton fields. Moreover, one of the key cotton pests in Brazil, the boll weevil (Anthonomus grandis), feeds and reproduces on cotton buds and bolls and occurs throughout the flowering season. In consequence, large producers apply chemical insecticides throughout the flowering period (Fonseca et al. 2011, Lima et al. 2013). In small organic plantations, farmers can use a combination of pest control methods that promote natural enemies on the crop and do not negatively affect pollinators. Farmers in the semiarid zone of Brazil (the Caatinga biome) adopt wider spacing between plants, which facilitates both the collection of buds infested with the boll weevil and manual weeding.

Although the international literature emphasises the importance of bees for cotton pollination (McGregor 1976; Tanda 1984; Waller et al. 1985; Free 1993; Rhodes 2002; Kumar et al. 2011), the use/conservation of bees as a way to improve production is not considered in cotton monoculture in Brazil. Three main actions are needed to draw the attention of Brazilian cotton producers to the advantages of wild-pollinator management: a) carrying out the assessment of pollination deficit in different regions of cotton production and for other varieties commonly used by producers, since the possible benefits of bee pollination vary depending on the cotton variety and crop management; b) promoting broad publicity among conventional farmers about the benefits pollinators can bring to cotton production, encouraging actions that favour bees such as conserving natural-vegetation tracts near plantations and using integrated pest management to reduce insecticide applications; and c) promoting greater commercial value for cotton produced under pollinator-friendly conditions. It should be stressed that protecting pollinators would have a broader conservation effect, benefiting other wild organisms and human beings as well.

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APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I. Figure S1 - Study sites located on the conventional cotton farm in Sinop municipality, Mato Grosso state, Brazil.

APPENDIX II. Figure S2 - Study site located on the organic farm in Remígio municipality, Paraíba state, Brazil.

APPENDIX III. Tab. S1 - Different production parameters measured in plots of cotton crop located near to and far from natural vegetation in the 2010/2011 season on a conventional farm in Sinop – Mato Grosso state, Brazil.

APPENDIX IV. Tab. S2 - Different production parameters measured in fruit originated from spontaneous self-pollination (SS) and open pollination (OP) on an organic cotton farm.

APPENDIX IV. Tab. S3 - Characteristics of commercial cotton varieties cultivated in the experimental areas.

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