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RESEARCH ARTICLE - BEES

Contribution of the Cerrado as Habitat for Sunflower Pollinating Bees

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Abstract

Agricultural landscapes sometimes include natural habitats which can support the ecosystem by enhancing the pollination of crops, thus boosting the productivity. This research was conducted between May and July 2017, in the municipality of Tangará da Serra, Mato Grosso, Brazil, to assess the Cerrado from the perspective of it being a crucial habitat to sustain the sunflower-pollinating bees (*Helianthus annuus* L.). The bees were sampled using entomological nets and pan traps, in specifically marked out plots (20 m x 150 m), in the Cerrado, and in a sunflower crop, at different distances from the Cerrado border. The assessment was done in terms of the composition and species richness, abundance of individuals and the mass (g) of the sunflower chapters exposed and isolated from the floral visitors. While species richness showed no differences between the Cerrado and sunflower crop, a difference was observed for abundance, with more numbers of individuals in the sunflower crop, most likely because of the food source supply. In the sunflower crop, the bee diversity decreased proportionally as the distance from the border increased. The seed mass of the sunflower chapters was significantly higher in the flowers open to visitors than in those of the isolated chapters open for visitation. From the results, it was evident that the bees present in the Cerrado visit the sunflower crop to gather pollen and nectar, and thus assist in cross-pollinating them and raising the productivity.

Introduction

Agricultural production is intrinsically dependent upon the ecosystem services that the insects provide absolutely free of charge (Zhang et al., 2007). Above 70% of the world's principal crops, enjoy the benefits of getting pollinated by the animals that visit the flowers (Klein et al., 2007). Globally, the value of biotic pollination-dependent crops was estimated to be US$ 235-577 billion annually (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services [IPBES], 2016). In Brazil, in 2018, this value was estimated at roughly US$ 43 billion for the biotic pollination-dependent crops (Plataforma Brasileira de Biodiversidade e Serviços Ecossistêmicos [BPBES], 2019). Bees (Hymenoptera, Apoidea) play a vital role in the pollination process, because they are completely dependent upon floral resources (Klein et al., 2007; Potts et al., 2016). However, over the last 50 years there has been a general global reduction in these insects in agricultural landscapes (Potts et al., 2010; Cameron et al., 2011). This drop in the number of pollinators may be directly linked to the considerable decline in the natural habitats, in terms of quality and quantity (Benton et al., 2003; Lindgren et al., 2018), which is due to the conversion of native vegetation to specific crops (Potts et al., 2010; Cameron et al., 2011).

Keeping this scenario, research has been developed with a focus on the conservation of natural habitats close to the cultivated areas as a good alternative for the sustenance of bees, as well as maintenance of the ecosystem by pollination of the crops (Ricketts et al., 2008; Carvalheiro et al., 2010; Lindgren et al., 2018). This concept, which several researchers still largely consider a challenge, is constructed on the principle that most of the pollinators that visit crops, particularly the bees, use natural remnants near the culture to nest and explore floral resources (Ricketts et al., 2008).

The bees in these habitats were collected using simple and replicable sampling methods that can be easily adapted to a wide range of research works (Gotelli & Colwell, 2001; Roulston et al., 2007). The common methods used most often for insect sampling are active and passive collection, termed complementary methods (Moreira et al., 2016). As pollinator diversity may be related to floristic heterogeneity in a specific region (Roulston & Goodell, 2011), it is anticipated that more diverse habitats will offer better pollinator service in adjacent agricultural areas (Garibaldi et al., 2011).

In this context, possibly the Brazilian Cerrado in the surroundings of agricultural areas is a habitat favorable to the bees and to the service of pollination of cultures. This biome is composed of a mosaic of vegetation types, from the wide-open fields to the savannas and forests; besides, it also has a high floral diversity (Oliveira-Filho & Ratter, 2002; Silva et al., 2015). Its physiognomic features may promote the presence of several species because of their propensity for interchange among the vegetation types (Almeida & Louzada, 2009; Gries et al., 2012). Despite the importance of native areas, our knowledge about the contribution of bees that live in these areas for crop pollination is still limited and, as far as we know, no study has been carried out to investigate the importance of Cerrado as a pollinator reservoir in the state of Mato Grosso, the largest sunflower producer in Brazil. Sunflower ranks prominently among the pollinator-dependent crops. It is a plant naturally possessing self-incompatibility and depends upon bees as the predominant pollinators (Free, 1993; Hevia et al., 2016). The bees forage the sunflower chapters and help by transferring the pollen from one plant to another, thus boosting the seed production. But, over time, sunflower hybrids have been selected to minimize this pollinator-dependence (Greenleaf & Kremen, 2006; Sun et al., 2012). However, this percentage of pollinator dependence on seed production varies between the hybrids or sunflower varieties (Mallinger & Prasifka, 2017). In Brazil, and specifically in the state of Mato Grosso, the country's largest sunflower producer, many farmers believe that some sunflower varieties, such as Helio250, have a low dependence on pollinators.

Thus, our work aimed to evaluate determined to assess the part played by the Cerrado as a natural habitat for the bees, which offers the ecosystem the service of sunflower pollination and the contribution of the pollinators towards the production of linoleic sunflower seeds.

This study can help to answer some significant questions, such as - whether the richness and abundance of bees declines with the distance from the natural habitat (closed); whether the distance of the natural habitat influences the sunflower yield; whether the visit of the bees to the linoleic sunflower chapters boosts the seed weight and whether the canopy richness and abundance vary with the sampling technique.

Material and Methods

Study area

The study was conducted at the Aparecida da Serra farm (14°18'36.64"S, 57°44'47.00"W), in the municipality of Tangará da Serra, Mato Grosso state, Brazil. The climate in that region is classified as rainy tropical (Aw), according to the Köppen Geiger classification, with an annual average rainfall of ~ 1,860 mm and annual temperature of around 24º C. The region experiences a rainy (October to May) and a dry season (June to September) (Dallacort et al., 2011). The study area has 4,450 ha of Cerrado *sensu stricto*, with expressive floristic diversity, that is maintained as Legal Reserve - RL. The RL represents approximately 56% of the total area of the farm and is bordered by 151.66 ha of sunflower. The Hélio: H250 (linoleic) variety of sunflower used is the one normally recommended for crop in Brazil, Bolivia and Paraguay. In Brazil, the Helio: H250 crop is cultivated under conditions of a second crop, in the production system that adopts the soybean-sunflower succession. The sunflower was cultivated following the conventional method, and included the addition of herbicides, fungicides and insecticides.

Sampling of bees

The bees were sampled from May 2017, at the height of the sunflower bloom (10 days) in eight plots (20 x 150 m), four in the Cerrado and four in the cultivated sunflower crop. The plots were any one of these distances (50, 150, 300 and 600 m) from the Cerrado border and were demarcated in the direction of the interior border of the Cerrado and the interior border of the crop. Five traps were set up, 30 m from each other. These traps were composed of two plastic trays (30 cm long x 23 cm wide), one blue and one yellow painted with UV-reflecting paint (SPRL, Spray-color) (Moreira et al., 2016), fixed to a wooden stake to 1.90 m above the soil level, being the identical height of the sunflower chapters. This methodology was adapted, following the methodology advocated by Hevia et al. (2016). A similar pattern of traps was followed in the Cerrado (Fig 1). Each tray contained the following ingredients: water 1L, liquid soap 1 teaspoon, and salt (NaCl) 1 teaspoon, to trap the insects. The traps were left open for 24 hours during the peak of the sunflower flowering season. According to Westphal et al. (2008) this methodology is popular in studies which compare the pollinator communities in different places or environments. The bees trapped in these pans were collected daily and placed in 70% alcohol, in containers.

Active collections, using the entomological nets, were also performed in all eight plots (20 x 150 m), four in the Cerrado and four in the sunflower crop, in transects installed 100 m distance from pan traps. Sampling was done in the plots corresponding to one of these distances (50, 150, 300 and 600 m) using the traps directed towards the interior border of the Cerrado and interior edge of the crop in both the environments (Cerrado and crop). Collection was performed between 7 am and 2 pm, alternately, one day in the Cerrado and one day in the crop. This time was selected as it marked the interval of the most intense floral visitation by the bees in the sunflower crop (Free 1993). To implement this technique, three collectors spent 10 minutes in each plot to collect the bees. The collection time was timed to ensure that the sampling effort was done in approximately equal lengths of time. The bees were trapped using the net, beginning at the 50 m plot and ending at the 600 m plot in about 40 minutes. After a 20-minute interval, the same route was once again traversed (in the reverse direction).

The specimens collected were identified to the lowest possible taxonomic level (genus and species) by the bee taxonomy specialists. Some of the specimens were deposited in the INPA invertebrate collection, Manaus-AM, in the didactic collection of the Mato Grosso State University-CPEDA Research Center, Tangará da Serra, Mato Grosso, Brazil.

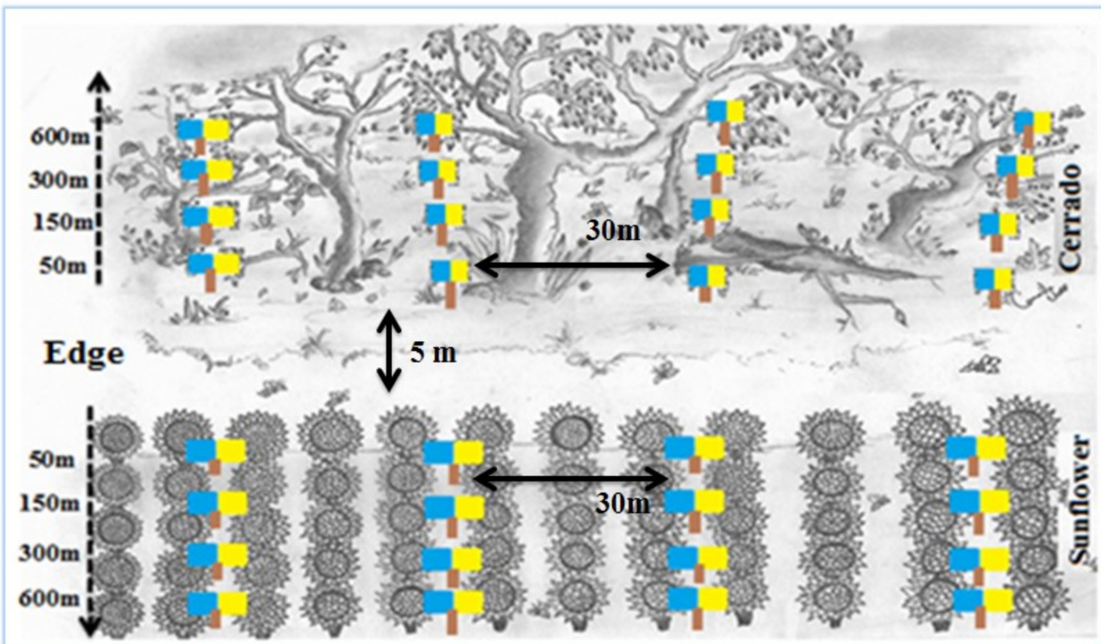

Fig 1. Schematic representation of bee sampling using blue and yellow pan traps, set up at 50, 150, 300 and 600m from the border to the interior of the Cerrado and from the edge to the interior of the sunflower crops.

Exclusion Experiment

An exclusion experiment was done to study the contribution made by the flower visitors to self-sustain the sunflower pollination. First, 20 plants were selected at 4 points viz., 50, 150, 300 and 600 m away from the Cerrado border. At each point, 20 flower buds were isolated using white voile tissue (1 mm mesh) during the reproductive phase (R) of the sunflower, in the pre-flowering (R3-R4) stage. When the flowering ended (Phase: R6-R7) the bags were removed to facilitate full seed development (Ball et al., 1992). At about 40 days after flowering, when the seeds achieved full maturity (Phase: R9), the sunflower chapters were collected and manually processed. Next, in order to remove the moisture, the seeds were placed in a greenhouse at 60º C for 48 hours. The seeds of each chapter were then weighed in a precision analytical balance, to four decimal places.

Data analysis

The normality of the data was tested, after which different statistical tests were applied. Rarefaction analysis based on individuals was done to compare the patterns of species richness and sample effort in the Cerrado and the crop. The two environments were compared based on a visual evaluation of the 95% CI (confidence interval) overlap of the rarefaction curves, implemented in EstimateS 7.5 (Colwell, 2009). The Student's t test was used to compare (i) total species richness and (ii) total abundance of the individuals of the Cerrado and that of the crop. The species composition of each environment was evaluated using the Principal Component Analysis (PCoA), and the Bray-Curtis similarity index. The difference in species composition between the Cerrado and that of the sunflower crop was tested using the permutative multivariate variance analysis (PERMANOVA) (Anderson, 2001).

The Generalized Linear Model-GLM (with Poisson error distribution) was used to estimate the effect exerted by distance and habitat type (explanatory variables) on the richness and abundance of the bees (response variables) using the vegan package (Oksanen et al., 2019). Subsequently, the analysis of variance (ANOVA) followed by Tukey's post-hoc test were done to compare the differences in richness and abundance between the points (distance) of each habitat using the agricolae package (Mendiburu, 2019). The ANOVA followed by the Tukey post hoc test were also performed to compare the seed weight of the chapters under the open and closed conditions for the pollinators. To evaluate the relationship between weight and distance, linear regression (lm), followed by ANOVA were used. The efficiency of the collection methods was assessed with the Kruskal-Wallis (KW) test, since the data failed to meet the normality assumptions. The analyses were performed using the R 3.2.4 package (The Development Core Team, 2017).

Results

In this study, 901 individuals belonging to 31 genera and 54 species of bees were collected (Table 1). Among these, 680 individuals belonging to 25 genera and 46 species were collected from the sunflower crop and 221 individuals belonging to 28 genera and 50 species from the Cerrado. Species such as *Nannotrigona melanocera* (Schwarz), *Oxytrigona flaveola* (Friese), *Pseudaugochlora flammula* (Almeida), were recorded for the first time in the Cerrado Matogrossense. *Apis mellifera* L. was abundant in the sunflower crop and Cerrado (331 and 63 individuals, respectively) and represented more than 40% of the total number of bees collected. Among the wild bees collected in the crop, *Melipona quinquefasciata* (Lepeletier) (n = 54) was the species with the highest abundance, followed by *Geotrigona* gr.*mombuca* (Smith) (n = 36). Of the 54 species collected in the area under study, 37 were found in both the sunflower crop and the Cerrado.

The species accumulation curve for the Cerrado revealed no inclination to stabilize, suggesting that more species could be collected in this area. For the sunflower crop, the curve appeared to stabilize from 500 individuals (Fig 2). The mean richness was significantly equivalent between both the environments (t = -1.148; p = 0.09; Fig 3A) however, the number of individuals differed, with greater abundance being observed in the sunflower crop (t = -4.87; p = 0.0001; Fig 3B). The results of the PERMANOVA test showed a significant difference in the species composition identified in the Cerrado habitat from that found in the sunflower crop (F = 3.96; p = 0.001), as shown by PCoA (Fig 4).

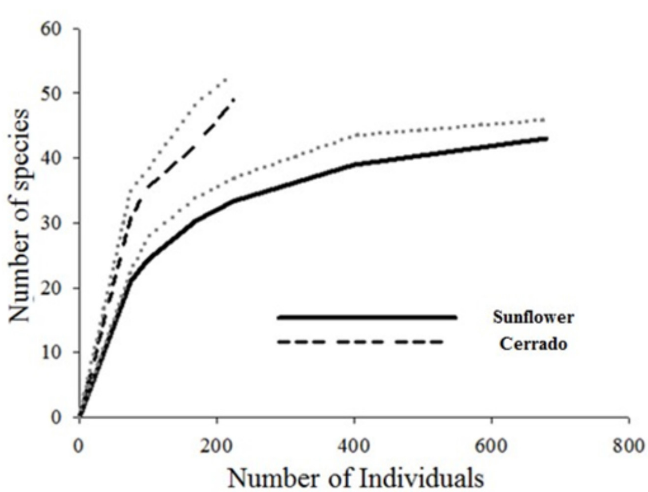

Fig 2. Accumulation curves of the species based on the individuals for the bee community in the Cerrado and sunflower crop. The dotted lines are 95% CI, which indicate no significant difference between the two environments.

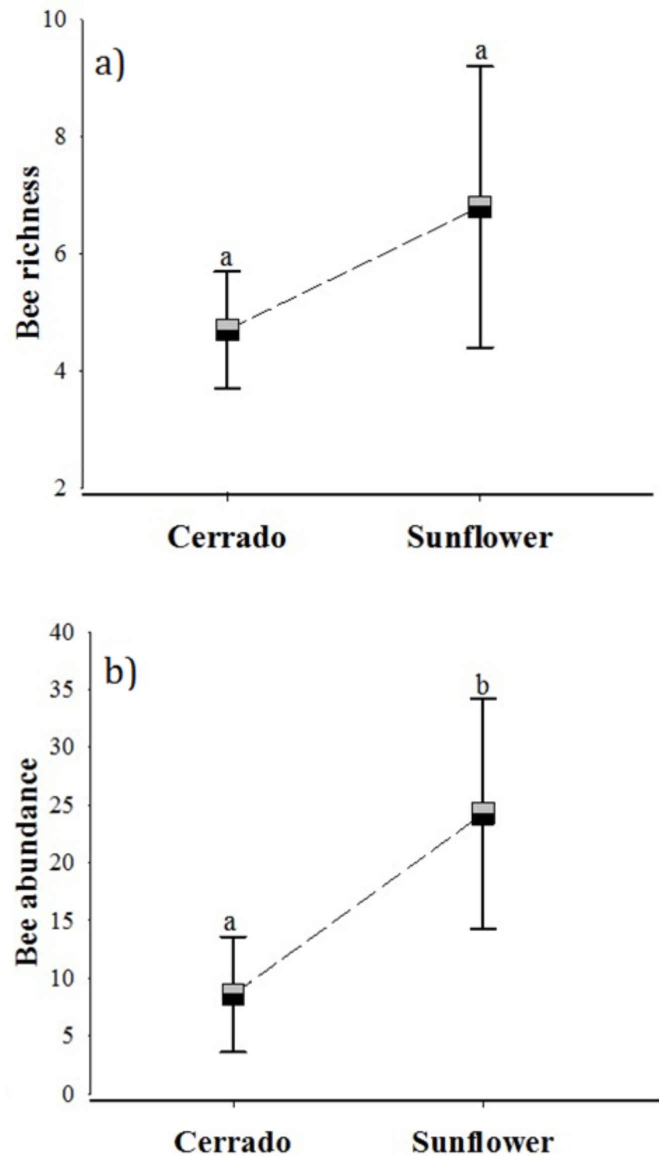

Fig 3. Average and standard deviation of the wealth (A) and abundance (B) of bees in the Cerrado and sunflower crop. Means followed by different letters indicate significant difference (Test t; p <0.05).

Table 1. Richness and abundance of bees found at distances of 50, 150, 300 and 600m from the Cerrado habitats and sunflower crops in Tangará da Serra-MT.

		Cerrado Habitat				Sunflower Culture				
		Distance (m)								
	Species	-600	-300	-150	-50	50	150	300	600	Total
Collect Pollen and Nectar	*Apis mellifera* (Linnaeus, 1758)	8	4	20	31	102	89	45	95	394
	Augochlora sp.1				2	1	1			4
	Augochlora sp. 2				2	1				3
	Augochloropsis sp. 1			3	2	2				7
	Augochloropsis sp. 2				1	3				4
	Augochloropsis sp. 3					2				2
	Augochloropsis sp. 4		1			3				4
	Eulaema cingulata (Fabricius, 1804)		1	3	4					8
	Eulaema nigrita (Lepeletier, 1841)				1		6	4		11
	Exomalopsis sp.			1			3			4
	Exomalopsis analis Spinola, 1853				2		7			9
	Exomalopsis fulvofasciata Smith, 1879		2	2	2		12			18
	Geotrigona gr.*mombuca* (Smith, 1863)	2	2	5	5	15	21			50
	Lasioglossum (Dialictus) sp. 1				1	1	4			6
	Lasioglossum (Dialictus) sp. 2		5	1	2		5	1		14
	Lasioglossum (Dialictus) sp. 3			1		3				4
	Lasioglossum (Dialictus) sp. 4				1					1
	Megachile sp. 1				2	7	3	1		13
	Megachile sp. 2		1				2			3
	Megachile sp. 3					3	5			8
	Megachile sp. 4					2				2
	Melipona quinquefasciata Lepeletier, 1836		3	3	3	25	29			63
	Melissodes nigroaenea (Smith, 1854)			1	4	4	6			15
	Melissoptila sp.				2					2
	Nannotrigona melanocera (Schwarz,1938)		1	1	1	9				12
	Oxaea sp. 1			1	1		7			9
	Oxaea sp. 2		4	2			2			8
	Oxytrigona flaveola (Friese, 1900)			2	1		9			12
	Pereirapis sp.			1						1
	Paratrigona lineata (Lepeletier, 1836)	2	4	2	1	15	3			27
	Plebeia sp.		1	4						5
	Pseudaugochlora flammula Almeida, 2008	1								1
	Thectochlora alaris (Vachal, 1904)		1	1			1			3
	Trigona guianae Cockerell, 1910		1	7		6	17			31
	Trigonisca sp.			2	4	1	1			8
	Xylocopa sp.	1	2			3	8	1	13	28
Collect Pollen and Oil	*Centris aenea* Lepeletier, 1841		2				5	1		8
	Centris cf. *fuscata* Lepeletier, 1841		1	3						4
	Centris cf. *spilopoda* Moure, 1969	1	2	3			9		2	17
	Centris flavifrons (Fabricius, 1775)	1	2	1						4
	Centris lutea Friese,1899				2					2
	Centris nitens Lepeletier, 1841						2			2
	Centris scopipes Friese, 1899				1		6	1	2	10
	Centris tarsata Smith, 1874		1		2		4	5		12
	Epicharis cf. *analis* Lepeletier, 1841				1			7	4	12
	Epicharis cockerelli Friese, 1900			1	2	3	5			11
	Epicharis inhering Friese, 1899	1	1	1	1					4
	Epicharis cf. *analis* Lepeletier, 1841				1			7	4	12
	Abundance of Individuals	17	42	73	90	214	283	66	116	901
	Species Richness	8	21	26	31	23	33	8	5	54

The factor distance from the border was seen to influence the richness and abundance of the bees in the Cerrado (R^2 = 0.53; p = 0.0001) and sunflower crop (R^2 = 0.40; p = 0.0013). In the sunflower crop, the number of species was significantly higher at 150 m (Tukey: p = 0.0012; Fig 5A) and abundance was greater at 50 and 150 m (Tukey: p = 0.0018; Fig 5B). The points located at the extremes (300 and 600m) revealed a lower degree of richness and abundance of individuals in both the Cerrado and sunflower crop (Fig 5).

Methods of Bee Sampling

The number of bees captured by the passive method was notably more than the number of species captured by the active method (F = 12.23; p = 0.0012; Fig 6A). For abundance, the passive method was revealed to be more effective than the active one, in bee sampling (F = 14.26; p = 0.0016; Fig 6B).

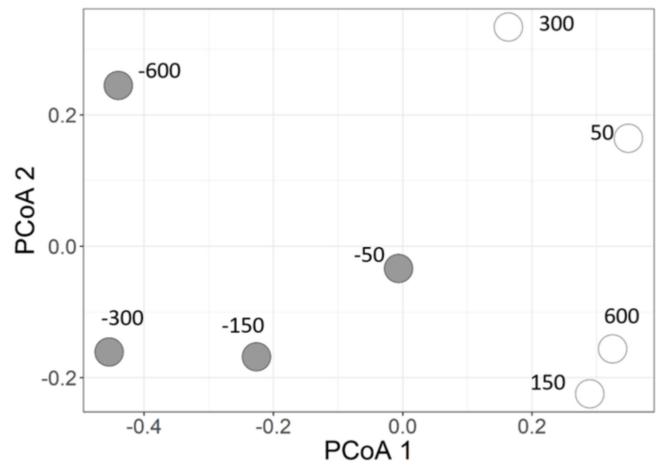

Fig 4. Principal Component Coordination Order (PCoA) of the bees sampled in the Cerrado (closed symbols) and sunflower (open symbols) at different distances, based on the Bray-Curtis similarity.

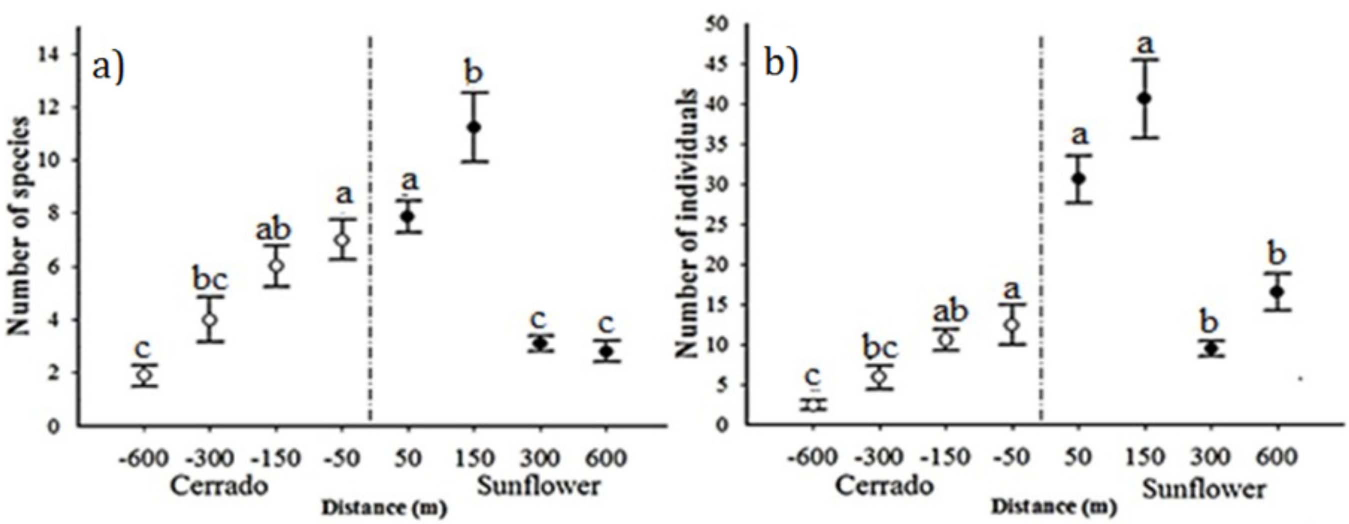

Fig 5. Average and standard deviations of the wealth (A) and abundance of bees (B) in the Cerrado and sunflower crops at different distances from the border. Means followed by different letters in the same habitat indicate significant difference by the Tukey test (p <0.05).

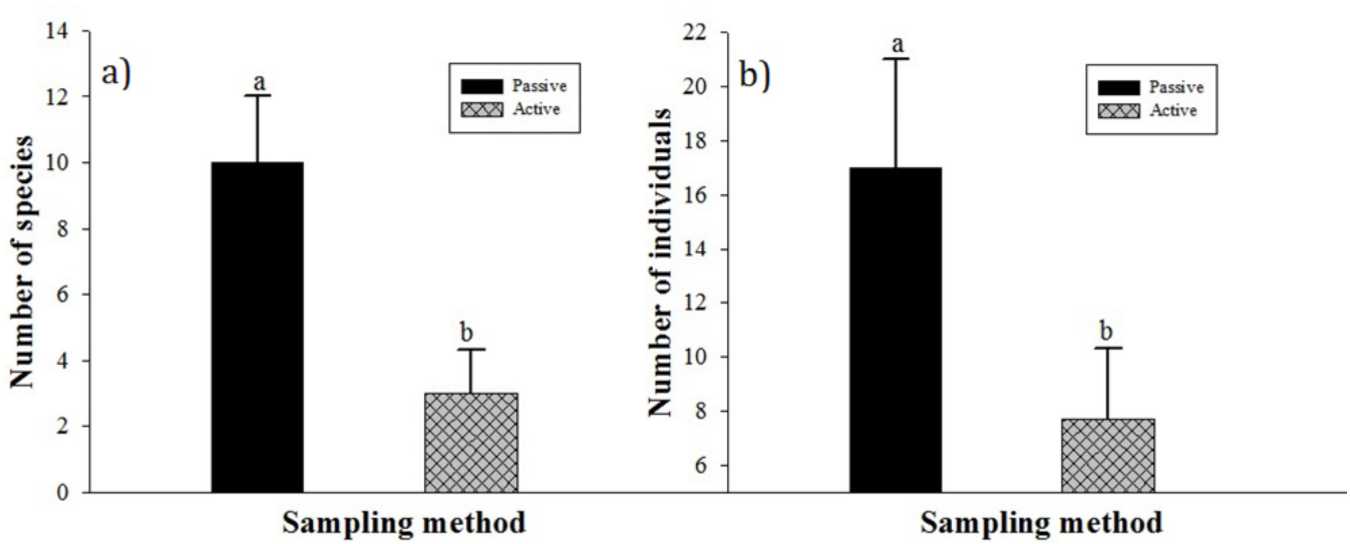

Fig 6. Number of species (A) and individuals (B) sampled using the active collection method (entomological net) and passive traps (pan traps). Means followed by different letters show significant difference (KW p < 0.05).

However, while the specimens belonging to family Megachilidae were captured solely by the passive method, the species *Centris* cf. *fuscata* (Lepeletier) were captured only by the active method. The active collection involved 210 hours of sampling effort.

Exclusion Experiment

The lack of pollinators in the sunflower chapters directly affected the crop yield. The exclusion experiment revealed that the total mass of the seeds was significantly higher in 49% of the open chapters for floral visitors, compared to the isolated chapters (Tukey: F = 30.77; p = 0.0019; Fig 7A). The distance from the sunflower crop to the Cerrado border also affected the seed weight of this crop ($R^2 = 0.25$; p = 0.001), which was significantly higher at the point installed at 150 m from the Cerrado (Tukey: F = 4.999, p = 0.0007; Fig 7B).

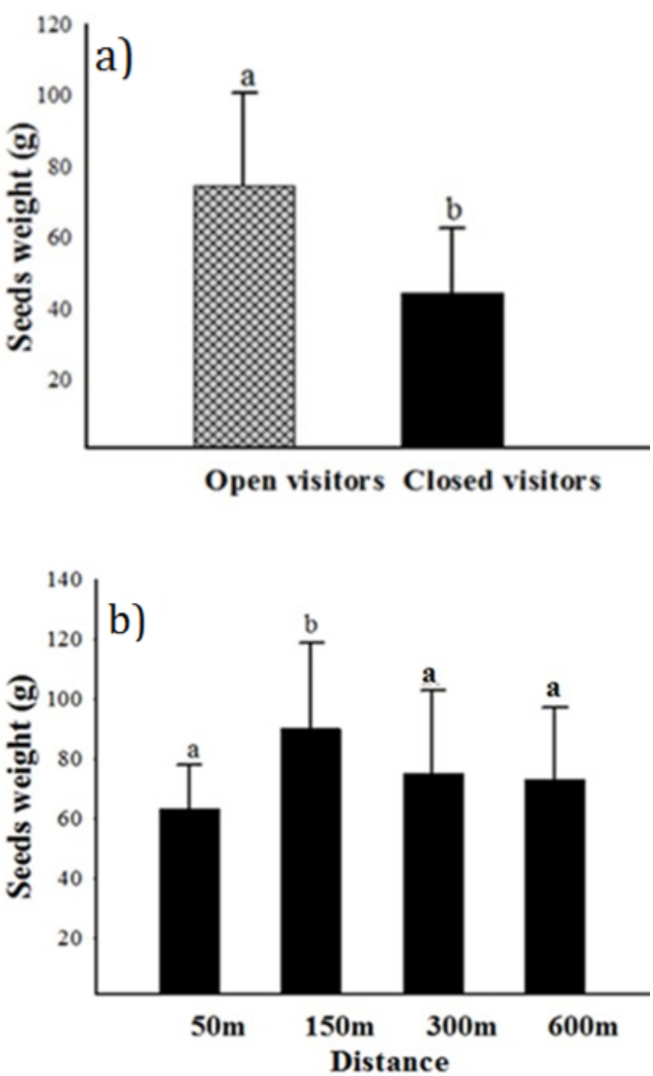

Fig 7. Weight of the sunflower seeds with and without the bee visitors (A) at the different distances of 50, 150, 300 and 600 m (B) from the Cerrado border. Means followed by different letters indicate significant difference by the Tukey test (p <0.05).

Discussion

In this study, the intense richness of the wild bee species observed reiterates that the Cerrado bordering the agricultural tracts acts as a vital habitat for insects, because this ecosystem contains great structural diversity and supports one of the richest flora in the world (Mendonça et al., 2008). To survive and forage in the agricultural terrains, wild bees must be able to occupy supportive habitats that provide suitable nesting areas and adequate food sources (Westrich, 1996) the whole year through. The Cerrado, being composed of a vegetation mosaic (Oliveira-Filho & Ratter, 2002; Silva et al., 2015) meets these requirements that according to Ricketts et al. (2008) and Garibaldi et al. (2016) are essential for maintaining bees and pollinating ecosystem service in adjacent crops.

This study shows a higher abundance of bees in the sunflower crop, which offers additional support for the hypothesis that crops having mass flowering, (like the sunflower), may temporarily decrease the number of bees in the adjacent forests (Montero-Castaño et al., 2016). The reason for this is likely due to the abundance of pollen and nectar the sunflower crop offers which attracts several bee species, inducing them to leave their natural habitat and forage in the cultivated areas. It is noteworthy that sunflower is a monoculture of temporary resource, which does not provide all the nutritional requirements of the bees (Naug, 2009); therefore, the maintenance of these bees in the cultivated environment is conditioned by the quality and quantity of the natural habitats that surround the crop (Montero-Castaño et al., 2016).

Results found in this search followed the pattern revealed in other studies on the sunflower pollinators, indicating that the exotic bee *A. mellifera* was the most abundant floral visitor (Carvalheiro et al., 2011; Pisanty et al., 2014; Sardiñas & Kremen, 2015; Hevia et al., 2016). Besides mass flowering, colony size and food demand are some other factors that could explain the predominance of these insects in the crop. *A. mellifera* requires large quantities of stored food to meet the needs of its extensive colonies (Rollin et al., 2013).

In consonance with previous studies (Ricketts et al., 2008; Carvalheiro et al., 2010, 2011) the distance negatively affected the richness and abundance of bees in the Cerrado, but in sunflower crop effect was not negative. Probably this result is associated with the irregular development of sunflower plants in the first 50 m of the crop. At this distance, many chapters were destroyed due to herbivory and, according to Jacobsen and Raguso (2018) flowers destroyed by the herbivores reduces the floral display, which the pollinating insects ignore, as they represent poor food resources. As they received fewer floral visitors, the seed weight of the sunflower chapters was also affected and unexpectedly larger in the point installed at 150 m distance from the Cerrado. In this point, the plants displayed uniformity in the developmental pattern and the sunflower chapters were morphologically more attractive; this resulted in a higher visitation rate and, therefore, greater seed yield.

In the Cerrado, as well as in the sunflower crops, the points found at the extremes revealed lower levels of both richness and abundance of bees. Native bees prefer to forage plants near their nests, and in this study we found nests of these bees on the edge of the Cerrado, justifying the greater richness and abundance at the points closest to it. Le Feon et al. (2013) report that the borders of native areas may offer one or more vital habitats for a variety of wild bee species in the agricultural terrains, mostly when related to a mass flowering crop, such as sunflower.

More numbers of bees could be collected in the Cerrado and in the sunflower crops using the passive trapping method with pan traps than with the active entomological net. This could be due to the attraction (Toler et al., 2005; Wilson et al., 2008) and bee preference for particular colors (Gumbert & Kunze, 2001; Heneberg & Bogusch, 2014). In melitophilia, the colors yellow and blue (Chittka & Thomson, 2004) indicate the presence and quality of the resources, like nectar and pollen, for the bees (Chittka & Thomson, 2004). This is the plausible reason for the findings in this study for the effective use of pan traps. The active collection, including the sampling of less numbers of species even, must be done alongside the passive method, as species like *C. fuscata* could be trapped using the entomological net alone.

The experiments conducted including and lacking the involvement of the floral visitors demonstrated that the high productivity of the mass of linoleic sunflower seeds is pollinator-dependent. In this study the 49% increase in the weight of the chapters open to pollinators compared to that of the closed ones, highlights the dependence of the sunflower by the pollinators. Mallinger et al. (2018) documented a similar result revealing a 45% increase in the seed produced from the chapters open to the bee visitors. Both results may encourage greater endeavor in wild bee conservation, especially of those species recognized for enhancing crop quality and the commercial value (Klatt et al., 2013).

From this study, it is evident that the conservation of the Cerrado abutting onto a sunflower crop enhanced the crop productivity by the action of the pollinating bees. As these bees collected the pollen and nectar, they promoted cross-pollination and thus boosted the yield of the linoleic cultivar H250. This proves that the maintenance of the Brazilian Cerrado directly contributes to bee conservation, as these insects are critical to the sunflower production, including that of the linoleic cultivar.

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