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FOREST REMNANTS ENHANCE WILD POLLINATOR VISITS TO CASHEW FLOWERS AND MITIGATE POLLINATION DEFICIT IN NE BRAZIL

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Abstract—Pollination deficit could cause low yields in cashew (*Anacardium occidentale*) and it is possible that deforestation surrounding cashew plantations may prevent effective pollinators from visiting cashew flowers and contribute to this deficit. In the present work, we investigated the proximity effect of small and large forest fragments on the abundance and flower visits by feral *Apis mellifera* and wild native pollinators to cashew flowers and their interactions with yield in cashew plantations. Cashew nut yield was highest when plantations bordered a small forest fragment and were close to the large forest fragment. Yield from plantations that did not border small forest fragments but were close to the large forest fragment did not differ to yield from plantations at a greater distance to the large forest fragment. Flower visits by wild native pollinators, mainly *Trigona spinipes*, were negatively affected by distance to the large forest remnant and their numbers were directly correlated to nut yield. The number of *A. mellifera* visiting cashew flowers did not change significantly with distance to forest fragments, nor was it correlated with yield. We conclude that increasing the number of wild pollinator visits may increase yield, and proximity to large forest fragments are important for this.

Keywords: Anacardium occidentale, Apis mellifera, *crop pollination, forest fragments, native pollinators, supplementary pollination, yield increment*

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a tree belonging to the Anacardiaceae, native to Brazil but presently cultivated in many tropical countries. The fruit is a nut which has a variety of uses from its kernel in animal and human feed to industrial application of the highly-valued oil extracted from its nutshell (Agostini-Costa et al. 2005; Blomhoff et al. 2006; Tullo 2008).

The cashew nut is one of the most traded nuts in the world and important source of income to small holders in tropical countries of Central and South America, Africa and Asia. The world cashew nut production has increased from 3.8 in 2009 to 7.0 million tons in 2010, but decreased in 2011 to 4.2 million tons, from a harvested area of 4.7 million hectares. Vietnam (28.5%), Nigeria (19.3%), India (16.0%), Ivory Coast (10.7%) and Brazil (5.4%) are the world's largest producers and represent more than 80% of global cashew nut production (FAO 2013).

While the worldwide average yield for cashew nut in 2011 was 893.5 kg/ha, it barely reached one third of that figure in Brazil, only 301.9 kg/ha (FAO 2013). Previous studies have pointed out a series of reasons for Brazil's low cashew nut yield, such as lack of soil correction, irrigation and pest control and prevalence of orchards with old trees grown from seeds instead of selected, grafted and productive varieties (Aquino et al. 2004; Oliveira 2007; Rossetti & Montenegro 2012), but despite recent progress in these areas, little or no improvement has been observed in cashew yield. Notwithstanding, Reddi (1987) and Freitas et al. (2002) have raised the question of inadequate pollination playing an important role in cashew low productivity and pollination deficit in cashew plantations was demonstrated by Holanda-Neto et al. (2002).

Despite early suggestions that cashew was wind-pollinated (Haarer 1954; Aiyadurai & Koyamu 1957; Rao & Hassan 1957), some studies have shown that cashew is highly outcrossing and relies on biotic pollinators to achieve adequate pollination (Reddi 1991; Wunnachit et al. 1992; Freitas 1995). Also, flies (Roubik 1995), moths (Kevan pers. communication) and bees (Heard et al. 1990; Freitas & Paxton 1996; Bhattacharya 2004) have been implicated as the major cashew pollinators, but little information is available about the effective pollinators and honeybees have been used for pollination purposes (Phoon 1984; Mohamad & Mardan 1985; Freitas 1994). However, native wild pollinators seem to be essential for maximizing crop pollination and the honeybee alone cannot compensate for their absence in agricultural systems (Garibaldi et al. 2013; Milfont et al. 2013).

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In the case of cashew, at least one oil-collecting bee species, *Centris tarsata*, has been proven a more efficient pollinator than *A. mellifera* in an individual basis, but this bee species is scarce in commercial plantations (Freitas & Paxton 1998). According to Freitas & Pereira (2004), the lack of oil-flower species within and surrounding cashew plantations is a limiting factor for the establishment of large populations of *C. tarsata* in cropped areas. Actually, a series of works have demonstrated that the surrounding landscape around and within farms can affect pollination services provided by native wild pollinators and it is necessary to maintain high-quality habitats within their flight range to the plantation (De Marco & Coelho 2004; Ricketts et al. 2008; Kennedy et al. 2013). Such habitats can provide essential resources for the establishment and development of sustainable pollinator populations, such as nesting sites, shelter and food supplies for the period the crop is not blooming (Roubik 1992, Kremen et al. 2004; Klein et al. 2007).

In the present work we investigated the proximity effect of small and large forest fragments on the abundance and flower visits by *A. mellifera* and native pollinators to cashew flowers, and their interactions with yield, in cashew plantations.

MATERIALS AND METHODS

The work was carried out in cashew commercial plantations belonging to small growers in the county of Horizonte (05° 08' 72"S, 37° 59' 14"W and 30 m above sea level), state of Ceará, Brazil. The weather in this region is tropical sub-humid, hot with rains from January to May, but peaking in the autumn, between March and May, and little rain between July and December. Yearly averages for rains and temperature are 780.2 mm and 27.0°C, respectively (IPECE 2012).

Experimental design

The experiment was set up in ten cashew sites cultivated with the dwarf cashew variety CCP76. Trees were 10 years old and spaced 7.5 x 7.5 m, with a total number of 178 trees per hectare. Each site measured 2 ha (200 x 100 m) and was part of larger plantations of 30-40 ha. Five of these sites, named areas 1 to 5, were bordered by small forest fragments (average of 5ha) and five, named areas 6 to 10, were not bordered by any forest fragment (Figure 1). However, five of these areas (4, 5, 8, 9 and 10) were located within 1 km to a large forest fragment (105 ha) and the other five (1, 2, 3, 6 and 7) situated further than 2.5 km to that forest remnant (Figure 1). The average distance between all areas was 3 km and the minimum distance between two areas was 1 km because most native flower visitors show flight range shorter than 1 km (Ballivián 2008).

All areas were submitted to the main standard agricultural practices for cashew crops such as pruning, soil clearing and weed control prior or during the blooming season.

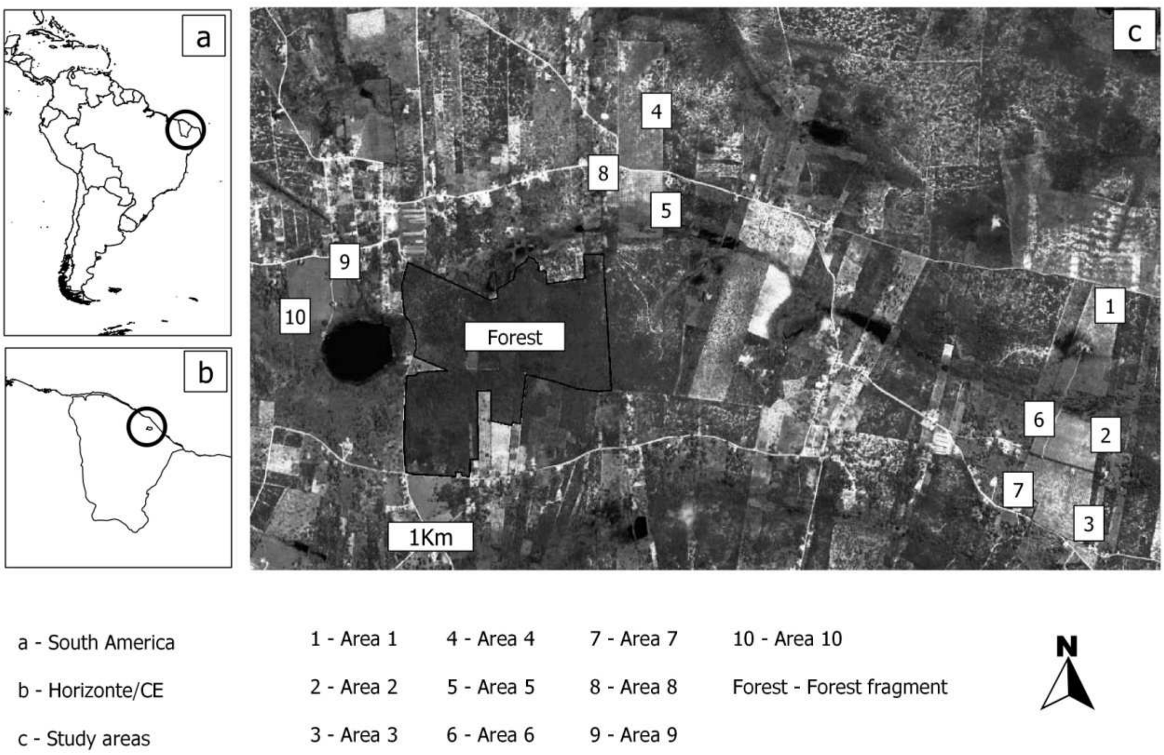

FIGURE 1. Location of the study areas: a – South America map showing Brazil and highlighting the state of Ceará; b – the county of Horizonte (circled) in the state of Ceará; c - satellite image showing the 105 ha large forest fragment and the five nearby (<1 km) and five distant (>2.5 km) cashew plantation areas.

Monitoring and identifying floral visitors

Floral visitors of cashew were observed and collected for identification in all 10 areas studied following the methodology proposed by Vaissière *et al.* (2011). A small area measuring 25 m x 50 m was demarked in the middle of each cashew area and an observer walked along the border lines stopping at every 25 m and collecting with a net only the insects he/she could see at that moment visiting any cashew flower of the tree immediately in front of him/her. After five minutes, the observer moved on other 25 m and collected in the next tree and repeated this procedure other four times until reaching again the first tree, totalling six trees sampled. Insect sampling was carried out six times per day (7 h, 9 h, 11 h, 13 h, 15 h and 17 h) on two consecutive days, every fortnight during the cashew blooming season, resulting in a total of 16 field trips and 192 collection bouts in each of the 10 areas.

Insects observed and then captured in cashew flowers were sacrificed in killing jars with methyl acetate. Then, they were kept refrigerated before being pinned at the Bee Laboratory of the Universidade Federal do Ceará. Later, they were tagged and sent away for identification by specialists in the Universidade Federal da Bahia.

Nut yield

Nut yield was obtained for all ten cashew areas adapting the methodology proposed by Vaissière *et al.* (2011). In each area, ten trees were randomly selected throughout the 2ha, then they were marked and all fruits produced during that season were collected and the nuts counted and weighed. Yield per tree for each area was obtained as the mean value of the ten trees per area.

The proximity effect of small and large forest fragments to cashew fields on nut production was tested using a two-way ANOVA for unbalanced data (Langsrud 2003) with small forest fragments (SFF) bordering cashew plantations (presence or absence) and distance to the large forest fragment (LFF, <1 km or >2.5 km) and their interactions as factors. Means were compared *a posteriori* by a 5% Tukey test. Nut yields were log10-transformed to fit normality. Residual normality (Shapiro-Wilk) and homogeneity of variance (Levene test) were tested.

Effects of distance to forest fragment on floral visits and nut yield

Because the distance does not affect yield directly, but may have some influence on the number of visits cashew flowers receive and this factor may play a role in crop productivity, linear models based on F test were carried out to identify the influence of distance to the large forest fragment (LFF) and the presence of small forest fragments (SFF) on flower visits. Two separate analyses were carried out where the number of visits (by *A. mellifera* or native pollinators) was the dependent variable while distance to LFF (<1 km and >2.5 km) and SFF (presence or absence) were independent variables. Interactions were considered in the analyses. First, we generated a complete model (number of visits = LFF + SFF + interaction). A post-hoc Tukey test was carried out to identify differences between classes in each factor. Residual normality (Shapiro-Wilk) and homogeneity of variance (Levene test) were tested.

The effect of floral visits on nut yield was investigated by linear regression. In this case, the dependent variable was nut production (log10-transformed) and independent variables were the number of visits by *A. mellifera* and native pollinators. Diagnosis tests were carried out to test the assumptions of residual normality and homogeneity of variance.

The relationship between the number, richness and diversity (Shannon's H) of flower visitors and nut yield was quantified by calculating Pearson's correlation coefficient (R).

Since the stingless bee *Trigona spinipes* was abundant visiting the flowers and to test if this species is driving the results obtained for the native pollinators, we carried out linear regression analyses on nut yield using only visit data from other native floral visitors, excluding *T. spinipes*, and separately, using only *T. spinipes* data.

RESULTS

Monitoring and identifying floral visitors

A diversity of floral visitors was observed on cashew flowers throughout the blooming season in the ten study areas (Figure 2). Out of the 1558 flower visits recorded, 94.6% were made by 14 bee species (Table 1). *Apis mellifera* and *Trigona spinipes* were the most frequent species and stood out in relation to the other floral visitors with a total of 1353 observations, representing 86.8% of all visits. However, Centridini was represented by six species and became the most diverse group in the areas, though with only 20 individuals in total (Table 1).

Wasps were the second most frequent group of flower visitors, but represented only 3.4% of all records and most visits were made by *Brachygastra lecheguana*. Other insects visiting cashew flowers comprised other Hymenoptera (ant), Coleoptera, Lepidoptera, Odonata and a hummingbird species (Table 1).

All floral visitors collected only nectar from cashew flowers, except Coleoptera which also fed on pollen from the staminoids inside the flower corolla and Odanata that used the flowers only as perching spots between hunting flights. The latter were included in the analysis as potential pollinators because the long fertile stamen and the stigma of the cashew flower protrude from the corolla and make contact with any insects landing on the flower.

Nut yield

Nut yield varied between the ten studied areas from a mean of 780 to 3890 g per tree (Table 2). The presence of small forest fragments near cashew fields did not affect nut yield ($F_{1,6}$ = 0.0120, P = 0.917), but the distance to the large forest fragment ($F_{1,6}$ = 16.239, P = 0.006) and the interaction between this large fragment and the small ones ($F_{1,6}$ = 13.225, P=0.011) showed significant effect on yield (Figure 3). Cashew trees in areas closer than 1 km to the

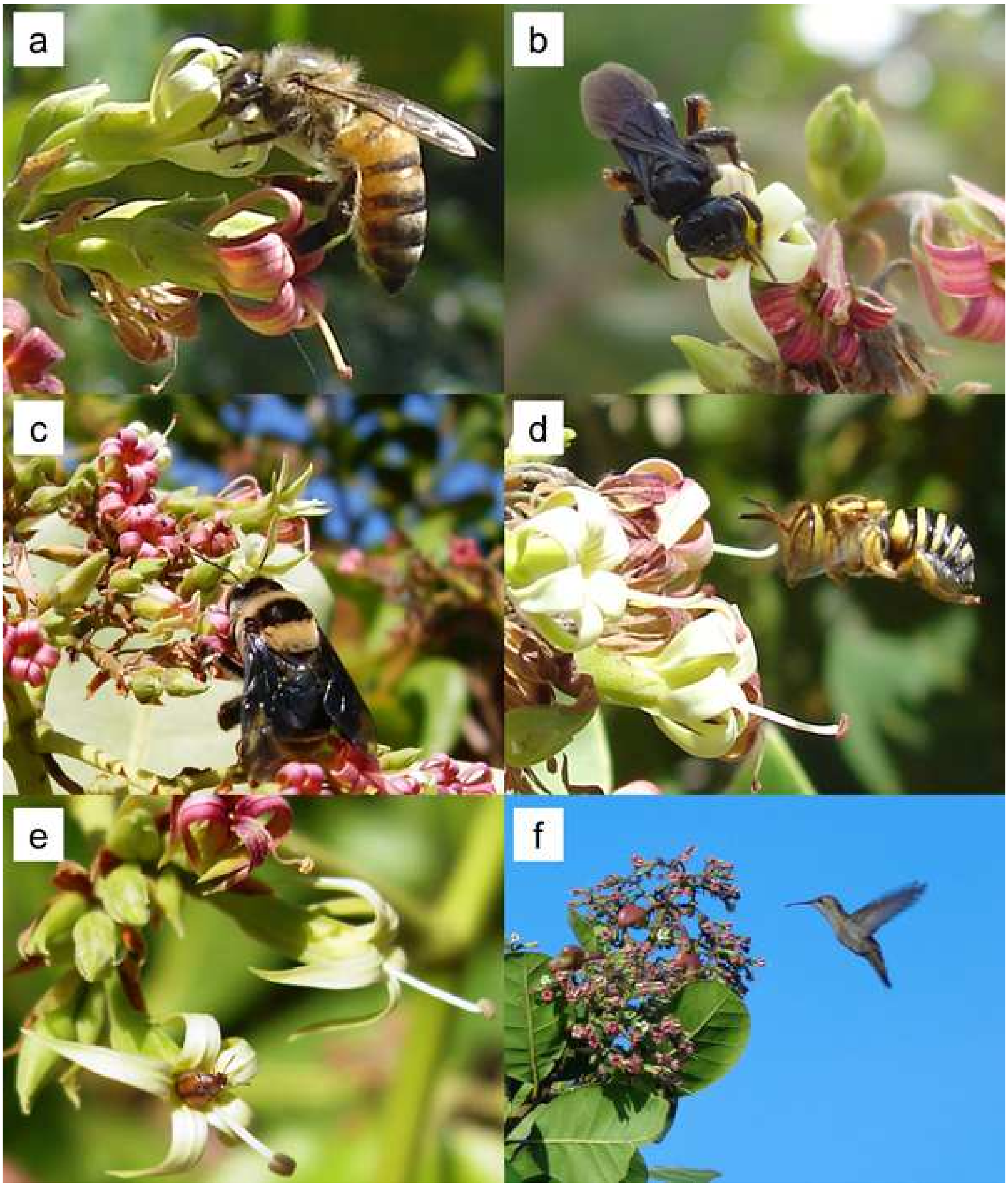

FIGURE 2. Plate showing some of the floral visitors recorded in cashew flowers: a – the exotic honeybee *Apis mellifera* and b to f – native visitors (b - the stingless bee *Trigona spinipes*; c – the oil-collecting bee *Centris flavifrons*; d – a solitary Anthidiini bee; e – a beetle and f – a hummingbird).

large forest fragment produced greater nut yield than those located over 2.5 km away (mean yield $_{<1\ km}$ = 2279.29 g/tree, mean yield $_{>2.5\ km}$ = 1164.66 g/tree; P = 0.006). Also, trees from plantations bordering small forest patches and <1 km to the large forest fragment produced significantly greater nut yield than those bordering small forest patches but more than 2.5 km away from the large forest fragment (mean yield $_{/<1\ km}$ = 2886.69 g/tree, mean yield $_{>2.5\ km}$ = 799.46 g/tree; P = 0.006). Trees from areas without bordering small forest patches and close to the large forest fragment did not differ ($P > 0.05$) in nut yield from those bordering small forest fragments located close or far away to the large fragment (Figure 3).

Effects of distance to large forest fragment on floral visits and nut yield

There was variation in the number of wild native pollinators among areas studied (Table 2). Distance to the large fragment and presence of small forest patches surrounding cashew areas did not affect visits of *A. mellifera* to cashew flowers (LFF – $F_{1/8}$ =3.558, P-value = 0.096; SFF – $F_{1/8}$ = 1.663, P = 0.233).

TABLE 1. Number of individuals belonging to native and exotic flower visiting species recorded in cashew (*Anacardium occidentale*) flowers in ten plantations of the state of Ceará, Brazil.

Floral visitors	Species	Areas A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	Abundance	(%)	Total
Native	*Trigona spinipes* Fabricius, 1793	54	51	32	65	74	20	22	47	72	91	528	34	733
	Scaptotrigona sp.	7	3	1	9	7	0	2	1	7	23	60	3.9	
	Melipona subnitida Ducke 1910	0	1	0	3	5	0	0	0	4	6	19	1.2	
	Centris (*Centris*) *byrsonimae* Mahlmann & Oliveira, 2012	0	0	2	0	0	0	1	1	0	0	4	0.3	
	Centris (*Ptilotopus*) aff. *Sponsa* Smith, 1854	0	1	0	0	0	0	2	0	0	0	3	0.2	
	Centris (*Hemisiella*) *tarsata* Smith, 1874	0	0	1	0	0	2	0	0	0	0	3	0.2	
	Centris (*Heterocentris*) *analis* Fabricius, 1804	1	1	1	0	1	0	3	0	1	0	8	0.5	
	Centris (*Trachina*) *fuscata* Lepeletier, 1841	0	0	0	0	0	0	1	0	0	0	1	0.1	
	Centris (*Centris*) *flavifrons* Fabricius, 1775	0	1	0	0	0	0	0	0	0	0	1	0.1	
	Xylocopa (*Neoxylocopa*) *cearensis* Ducke, 1910	0	2	1	0	0	0	2	2	0	0	7	0.5	
	Xylocopa (*Neoxylocopa*) *grisescens* Lepeletier, 1841	0	1	2	0	0	0	0	1	0	0	4	0.3	
	Euglossa sp.	0	0	0	0	0	0	0	1	0	0	1	0.1	
	Anthidiini	1	5	0	0	0	0	0	0	3	1	10	0.6	
	Brachygastra lecheguana Latreille, 1824	5	6	2	4	1	6	3	1	9	5	42	2.7	
	Wasp species 1	0	3	0	0	0	2	0	0	0	0	5	0.3	
	Wasp species 2	0	3	0	1	0	1	0	1	0	0	6	0.4	
	Ant	0	1	0	0	0	4	0	0	3	0	8	0.5	
	Hummingbird	1	0	0	1	0	0	3	0	1	0	6	0.4	
	Butterfly species 1	1	0	0	0	0	1	0	0	0	1	3	0.2	
	Butterfly species 2	0	1	0	0	0	0	0	0	1	1	3	0.2	
	Butterfly species 3	0	0	0	0	0	2	0	0	0	0	2	0.1	
	Dragonfly species 1	1	1	0	1	1	0	0	0	1	0	5	0.3	
	Dragonfly species 2	0	2	0	0	0	0	0	0	0	0	2	0.1	
	Beetle	1	0	0	1	0	0	0	0	0	0	2	0.1	
Exotic	*Apis mellifera* Linnaeus 1758	85	98	92	95	84	123	70	53	58	67	825	53	825
Total		157	181	134	180	173	161	109	108	160	195	1558	100	1558

TABLE 2. Decreasing average cashew tree yield (g) per studied area, their respective distance to large forest fragment, proximity to small forest fragment and number of *Apis mellifera* and native pollinators visiting flowers in each cashew area. A1 to A10 – Area 1 to area 10, s.e.m. – standard error of mean.

Area	Bordered by small forest fragment	Distance to large forest fragment (m)	Number of floral visitors		Average tree yield (g) ± s.e.m.
			honeybee	native pollinator	
A10	No	620	67	128	3890.9 ± 1704.71
A9	No	432	58	102	3420.2 ± 1008.93
A8	No	465	53	55	1808.0 ± 1029.37
A4	Yes	860	95	85	1788.6 ± 660.06
A2	Yes	2909	98	83	1695.5 ± 751.29
A3	Yes	2920	92	42	1544.3 ± 727.61
A5	Yes	446	84	89	1429.0 ± 519.08
A1	Yes	2974	85	72	1280.9 ± 552.56
A6	No	2569	123	38	819.3 ± 317.71
A7	No	2513	70	39	780.0 ± 288.07

However, distance to the large forest fragment influenced the frequency of flower visits by native pollinators but not the presence or absence of small forest patches surrounding cashew areas (LFF - $F_{1/8}$ = 5.940, P = 0.041; SFF – $F_{1/8}$ = 0.008, P-value = 0.931).Trees from areas close to the large fragment were more visited by native flower visitors than those from distant areas (Figure 4). Only the visits made by native insects affected nut yield (native visitors – R^2 = 0.715, $F_{1,8}$ = 20.06, P = 0.002; *Apis mellifera* – R^2 = 0.288, $F_{1,8}$ = 3.24, P = 0.109), the more visits a tree received by wild native pollinators, the greater its nut yield (Figure 5). The relationship between yield and native pollinator visits was driven by the most common native visitor, the stingless bee *Trigona spinipes*. Visitation by other native floral visitors did not affect nut yield (R^2 = 0.361, $F_{1,8}$ = 4.51, P = 0.066),while *T. spinipes* visits were significantly related, to yield (R^2 = 0.698, $F_{1,8}$ = 18.51, P = 0.002).

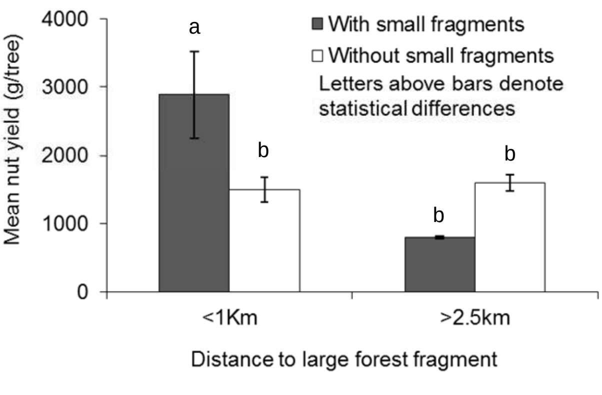

FIGURE 3. Effect of the distance (m) between cashew (*Anacardium occidentale*) plantations and a large (> 100ha) forest fragment on the mean cashew nut yield (g/tree) in the state of Ceará, Brazil.

No correlation was found between the number of *A. mellifera* and native pollinators (Pearson's R = -0.35141; P-value = 0.31939) per area. Also, species richness (Pearson's R = -0.03556; P-value = 0.92231) and diversity (Pearson's R = 0.50977; P-value = 0.13227) were not correlated to fruit yield.

DISCUSSION

The identity of effective cashew pollinators has produced a controversial dispute in favor of bees or moths. However, moths have not been reported as potential cashew pollinators in previous studies in NE Brazil and pollen viability and stigma receptive drops to near zero in the evening of the day the flower opens (Holanda-Neto et al. 2002; Freitas & Paxton 1998). In the present study Lepidoptera were rare visiting cashew flowers while bees represented over 94% of floral visitors and visited flowers all-day long, including when the flowers were most receptive for pollination, and probably were the most effective pollinators.

Honeybees, stingless bees and the Centridini bee *C. tarsata* had been implicated previously as important cashew pollinators (Free 1993; Freitas & Paxton 1998; Aidoo 2009) and all of them were present in the cashew plantations. However, while the exotic honeybees comprised almost 53% of the floral visitors, the native stingless bees represented approximately 39%, but the presence of *C. tarsata* was only 0.19%.

Apparently, cashew flowers are attractive to Centridini bees because other five *Centris* species were recorded as floral visitors, but also in low numbers, like *C. tarsata*. According to Freitas &Pereira (2004), the lack of oil-flower species within and surrounding cashew plantations may prevent the establishment of large populations of *C. tarsata* in cropped areas, and Magalhães & Freitas (2013) demonstrated that the lack of adequate nesting areas can impede population growth of Centridini bees within an agricultural setting. Other recent

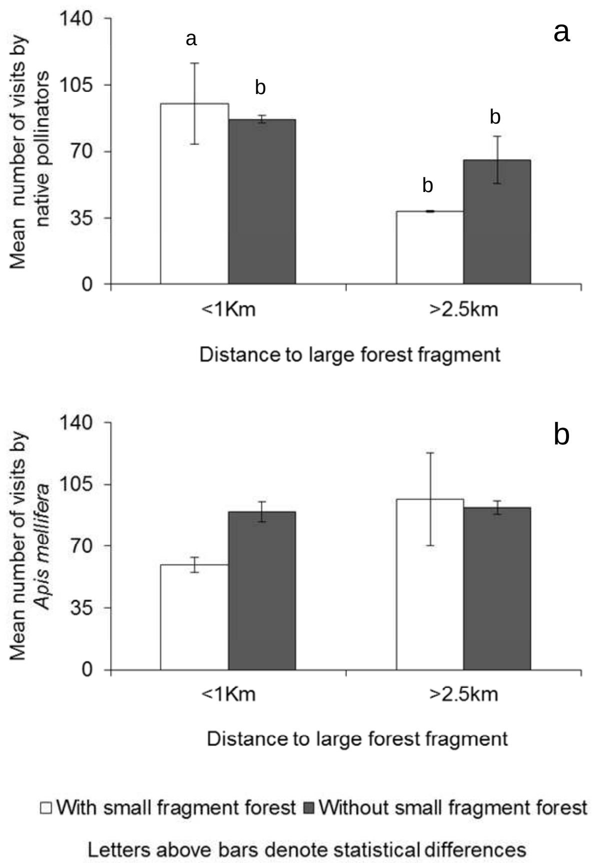

FIGURE 4. Effect of the distance (m) between cashew (*Anacardium occidentale*) plantations and a large (> 100ha) forest fragment on the mean number of honeybees (*Apis mellifera*) and native wild pollinators visiting flowers in the state of Ceará, Brazil.

works have shown that the landscape around and within farms can affect the richness of species, abundance and pollination services provided by wild pollinators (Kremen et al. 2004, Viana et al. 2012, Kennedy et al. 2013). This may also have influenced the low occurrence of other flower visitors like wasps, butterflies and hummingbirds in our study areas. Nevertheless, although individually these low frequent flower visitors may contribute little for cashew pollination, the overall sum of their services can be significant (Schemske & Horvitz 1984; Cavalcante et al. 2012).

Despite all studied areas having similar characteristics, such as same cashew variety, tree age, agricultural practices, soil, climate, etc., yield varied greatly from an average 780.0 to 3890.9 g nuts/tree, which is typical of cashew plantations in NE Brazil (Pinheiro et al. 2004; CONAB 2011). Considering the high biotic pollination-dependence of cashew trees for fruit set (Reddi 1991; Wunnachit et al. 1992; Freitas 1995) and the fact that cashew areas with greater number of wild pollinators also produced higher yields, one can assume that pollination deficit is the main driving factor for such yield discrepancy among those areas. Moreover, based on the direct correlation found between wild native pollinators and nut yield, but not with *A. mellifera*, it is possible to state that wild native pollinators can mitigate pollination deficit in cashew plantations, independently of the presence and abundance of honeybees. Similar conclusions were reached by Garibaldi et al. (2013) in a work involving 41 agricultural systems, but not cashew.

This direct correlation between the number of wild pollinator visits to cashew flowers and nut yield, which varied significantly from areas close to the large forest fragment to those far from it, also suggests that large remnants are important in providing effective cashew pollinators. These observations support the claims of Roubik (2002) and Kremen et al. (2002; 2004) that conserving the native vegetation in the surroundings of cultivated areas is essential to keep stable populations of pollinators, such as the bees of the present study, for providing food, nesting, and other resources indispensable for their survival. The considerable number of stingless bee flower visits observed may be a consequence of that. Indeed, visits by the stingless bee *T. spinipes* were responsible for the significant yield increment promoted by wild pollinators and although this species adapts well to disturbed environments, it relies on a variety of natural resources, including plant resins and fibers, to build its nest, and may become a pest to crops by biting flower buds and young fruits instead of being a beneficial pollinator when it cannot find its ordinary resources (Silveira et al. 2010; Santos et al. 2012).

Honeybees have been pointed out as efficient cashew pollinators (Reddi 1991; Free 1993; Freitas & Paxton 1998) and in the present work they were present and visited cashew flowers, but the number of flower visits by *A. mellifera* did not vary significantly among the areas nor show any correlation to nut yield. This result is not surprising because no colony was introduced to the areas and the honeybees present were from feral colonies established in the vicinity, resulting in much lower honeybee density than when this bee is introduced for pollination purposes (Mohamad & Mardan 1985; Freitas 1994). In such a condition, honeybees probably set a minimum number of flowers upon which native pollinators increased nut yield as the number of their flower visits increased in areas close to the large fragment(Hoehn et al. 2008). In Ghana, nut yields of 1,250 kg/ha were related to great diversity and abundance of native bee pollinators favoured by the cashew agro-ecosystem adopted (Aidoo 2009). In our study, with most pollination

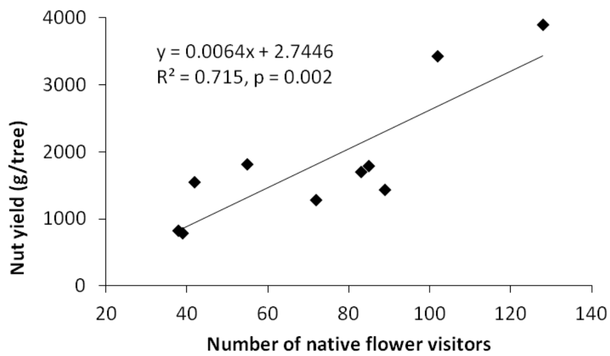

FIGURE 5. Effect of the number of flower visits by native pollinators on cashew (*Anacardium occidentale*) nut yield (g/tree) in ten plantations of the state of Ceará, Brazil.

relying on *A. mellifera* and *T. spinipes*, the greatest yield harvest was equivalent only to 692.4 kg/ha, below the world average of 893.5 kg/ha, in a clear demonstration that pollination levels can potentially increase both for increments in wild pollinator richness and diversity providing them with good-quality refuges near the cashew plantations and by the introduction of managed honeybee colonies to increase flower visitation rate. Complimentary pollination between honeybees and wild pollinators enhancing crop yield has been reported recently (Garibaldi et al. 2013; Milfont et al. 2013) and this may also be the case for cashew. This deserves further research.

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