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C F Martins, K M M de Siqueira, L H P Kiill, I I S Sá & C M L Aguiar

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Density and Distribution of *Xylocopa* Nests (Hymenoptera: Apidae) in Caatinga Areas in the Surroundings of Passion Fruit Crops

CF Martins[1], KMM de Siqueira[2], LHP Kiill[3], IIS Sá[3], CML Aguiar[4]

[1]Depto de Sistemática e Ecologia, Univ Federal da Paraíba, João Pessoa, PB, Brasil
[2]Univ do Estado da Bahia, Juazeiro, BA, Brasil
[3]Embrapa SemiÁrido, Petrolina, PE, Brasil
[4]Depto de Ciências Biológicas, Univ Estadual de Feira de Santana, Feira de Santana, BA, Brasil

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Correspondence
CF Martins, Depto de Sistemática e Ecologia, Univ Federal da Paraíba, João Pessoa, PB, 58051-900, Brasil; cmartins@dse.ufpb.br

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Abstract

Due to their importance as pollinators of many plant species, this study aimed to know the nest density, spatial distribution, and nesting substrates used by *Xylocopa* species in the Caatinga, a xerophilous vegetation of Northeastern Brazil. Three areas of Caatinga in the surroundings of passion fruit crops were sampled. The bee species found in these areas were *Xylocopa grisescens* Lepeletier and *Xylocopa frontalis* (Olivier). All nests were in *Commiphora leptophloeos* (Burseraceae) trees (n=113). Phytosociological analysis showed that this tree species presented the highest absolute density (212.5 individuals/ha) and index of importance value (52.7). The distribution pattern of the *C. leptophloeos* was aggregated. The nests were located in dead and dried branches with an average diameter of 5.3±2.0 cm (n=43). The mean number of nests/tree was 3.1±2.8 (n=113). The less disturbed area showed 6.7 nests/ha and 4.2 nests/tree. In the disturbed areas, 0.9 nests/ha and 2.4 to 2.7 nests/tree were observed. The availability of substrate for nesting in the studied areas and its importance as a limiting factor for nesting are discussed.

Introduction

The availability of substrates for nesting is a factor that influences the organization of bee communities, but the effect of nesting resources on the local abundance of bee species has rarely been investigated (Potts *et al* 2005). Bees can be grouped into distinct guilds (mason bees, miners, carpenters, and social nesters) accordingly to their nesting requirements (Potts *et al* 2005). Most of the *Xylocopa* species, except in the subgenus *Proxylocopa*, are carpenters, excavating their nests in dead and dry trees or branches, in solid wood with no cracks, oriented vertically or horizontally, and often with parallel branches and a single entry (Hurd 1955, Gerling *et al* 1989, Michener 2000). The length and number of galleries and cells is highly variable and related to female founder productivity and nest age (Camillo & Garófalo 1982).

Niche breadth of different bee species in relation to the plant species used as a nesting substrate can vary from much broad to somewhat narrow, and the selection of substrates can vary greatly from one location to another, as plant species characteristics can affect substrate selection for nesting females (Anzenberger 1977), and carpenter bees in general do not show an affinity for nesting in specific plant taxa (Bernardino & Gaglianone 2008). Some plant species used as a nesting substrate by *Xylocopa* species in Brazil were *Eucalyptus* sp. and *Phoebe porosa* for *Xylocopa frontalis* (Olivier) and *Xylocopa grisescens* Lepeletier (Camillo & Garófalo 1982, Camillo 2003); *Agaristha resoluta* for *Xylocopa cearensis* Ducke, *X. frontalis* and *Xylocopa subcyanea* Pérez (Silva & Viana 2002); *Vellozia* spp. for *Xylocopa artifex* Smith (Silveira 2002); *Encholirium spectabile* for *Xylocopa abbreviata* Hurd & Moure (Ramalho *et al* 2004); *Pera glabrata*, *Sideroxylon obtusifolium*, and *Eugenia ovalifolia* for *Xylocopa ordinaria* Smith (Bernardino & Gaglianone 2008); and *Spathodea campanulata*, *Ligustrum lucidum*, and *Ficus* sp. for *X. grisescens* and *Xylocopa suspecta* Moure & Camargo (Chaves-Alves *et al* 2011).

However, most studies about the plant species used as nesting substrate are punctual, and there are few specific studies on their nesting ecology.

Xylocopa bees are the principal effective pollinators of passion fruit flowers (Siqueira *et al* 2009, Yamamoto *et al* 2012), and they also pollinate other crops, such as tomatoes and melons (Keasar 2010). However, pollinator limitation in crop areas has increased production costs because hand pollination has been increasingly used. The introduction of artificial substrates for nesting in cultivated areas was an efficient strategy to increase local populations of carpenter bees (Camillo 2003, Oliveira Filho & Freitas 2003, Junqueira *et al* 2012, 2013). Despite its economic importance, there are few studies about the distribution and nest density of these bees in natural areas. Nesting substrate identification, distribution, and nest density of *Xylocopa* species in natural areas close to crops are important to guarantee pollination services and knowledge to manage bees and nests. This study aimed to survey nest location, substrate used, density, and spatial distribution of the natural nests of *Xylocopa* and some aspects of foraging in Caatinga areas in the surroundings of passion fruit crops.

Material and Methods

This study was carried out in the area of the "Projeto de Irrigação Maniçoba" (Maniçoba Irrigation Project), in Juazeiro, state of Bahia, Brazil (09°24″S, 40°26″W), during 2006/2007. The project had a total area of 4,293 ha, occupied by 234 small farmers lots (1,889 ha) and 80 irrigated lots of agribusiness companies (2,379 ha) (CODEVASF 2005). In 2006, Juazeiro Municipality had 816-ha area of passion fruit crops (IBGE 2006). The climate is semiarid, with a mean annual rainfall of 520 mm, and rainfall concentrated from November to April (EMBRAPA 2007).

To survey the nests, three areas surrounding crops of yellow passion fruit (*Passiflora edulis*) were delimited. The choice of the areas took into account the distance to the São Francisco River and the conservation status of the vegetation. The areas were delineated by means of benchmark points done by GPS and then plotted on a map at the Laboratório de Geoprocessamento da Embrapa Semi-Árido (GIS Laboratory at Embrapa Semi-Arid). The first area (A) had 196 ha, with disturbed vegetation of open arboreous Caatinga, was close to the São Francisco River. The second area (B) had 14 ha, with less disturbed arboreous Caatinga vegetation, was 10.5 km far from the São Francisco River. The third area (C) had 49 ha, with vegetation physiognomy similar to area A, was 13.5 km distant from São Francisco River (Fig 1).

Nest location was conducted by covering the whole areas, inspecting all the dry trees and branches visually, and beating on the trunks of trees with an iron bar to check for the presence of bees. Three people sampled the areas A, B, and C during 40, 8, and 12 h, respectively. The sampling effort by area varied from 0.20 to 0.24 h/ha (areas A and C) to 0.57 h/ha (area B). The higher sampling effort in area B was necessary because this less disturbed area presented a higher density of trees. Thus, the sampling effort per tree can be considered equivalent or even lower than that in the other areas. A GPS was used to avoid concentration in one place and facilitate the orientation. The location of nests, plant species substrate, the number and diameter of nest entrances and height of entrances above the ground, and the *Xylocopa* species and the number of bees observed after beating the trunks were recorded. Voucher specimens were deposited at the Entomological Collection of Universidade Federal da Paraíba.

To evaluate the availability of nesting sites, a phytosociological analysis was carried out in areas A and B. The phytosociological plot survey method was used with eight plots randomly distributed in area A and four in area B. Each plot had dimensions of 10 m×20 m (200 m^2) and was separated by at least 50 m. Analysis of the vegetation structure (frequency, density, and dominance) included all individuals with a diameter at breast height (DBH) equal to or greater than 3 cm. The elements of horizontal and vertical structures were determined according to Rodal *et al* (1992). Phytosociological data analysis and Payandeh aggregation index (Pi) were obtained using "Mata Nativa" software (CIENTEC 2002). When $Pi<1$, the distribution is random or not aggregated; $1 <= Pi <= 1.5$ indicates a tendency for aggregation, and $Pi>1.5$ indicates an aggregated distribution. The value of importance (VI) is defined as the sum of the relative density, relative frequency, and relative dominance.

To study the relative frequency of the pollen types of plant species, pollen grains collected at the entrance of the nests or in the body of the bees were analyzed. These nests were in an urban area near crops of passion fruit and also in nests located inside the crop area and were not included in the samples of the three studied areas of Caatinga. Bees were collected using an insect net, and the pollen grains from various body parts were removed. The samples were placed on glass slides and prepared with glycerine jelly and stained with fuchsin. Under an optical microscope, 300 pollen grains were counted, and the relative frequency of passion fruit pollen and other pollen types was determined.

The Mann-Whitney *U* test was used to compare the diameter of branches used for nesting in different areas using BioEstat version 5.3.

Results

Nests of two carpenter bee species (*X. grisescens*, 190 nests, and *X. frontalis*, 128 nests) were recorded (Table 1). Only one

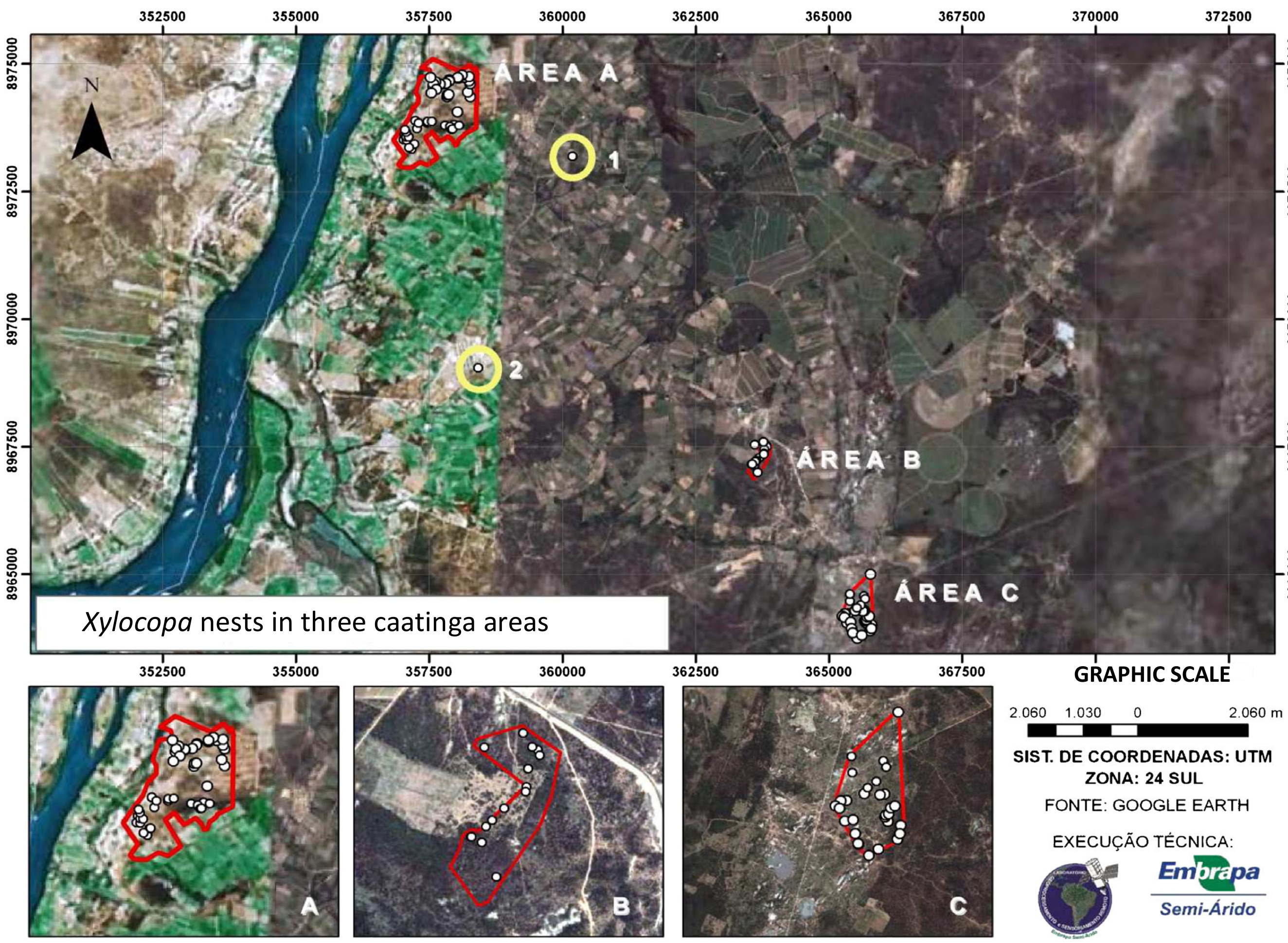

Fig 1 Area of Projeto Maniçoba, at Juazeiro, state of Bahia, Brazil, showing the location of areas (*A*, *B*, and *C*) sampled during the survey of nests of *Xylocopa* spp. (*1*) Nests within one cultivation of *Passiflora edulis*; (*2*) nests inside an urban area close to crops of *Passiflora edulis*. Some plants are superimposed on the map location of area B because they were too close to each other.

tree species (*Commiphora leptophloeos*, Burseraceae) was used as a nest substrate by carpenter bees, despite the vegetation composition of areas A and B contains other tree species with different densities (Tables 2, 3). Nests were in dead and dry trunks and branches (Fig 2) with an average diameter of 5.3±2.0 cm (n=43) and an average height above the ground of 216.2±43.8 cm (n=43). Nest entrances had an average diameter of 1.5±0.4 cm (n=43). In all areas, the number of nests/tree varied from 1 to 20 (average 3.1±2.8, n=113, Fig 3a). Most of the nests (60.8%) were in trees with one to four nests, and trees with two nests were the most frequent (Fig 3a). Bees nested in branches from 1.6 to 11 cm in diameter (average 5.3±2.0), and most nested in branches with 3–5-cm diameter (Fig 3b). There was no significant difference between the areas regarding the diameter of branches used for nesting (Mann-Whitney U test, p>0.05).

In area A, 75 trees of *C. leptophloeos* with carpenter bees nests were recorded, and 180 nests were found, resulting in 2.4 nests/tree with nests and 0.9 nests/ha (Table 1, Fig 1). In area B, 22 trees of *C. leptophloeos* with nests were recorded, and 94 nests were found, resulting in 4.2 nests/tree with nests and 6.7 nests/ha. In area C, 44 nests in 16 trees were recorded (2.7 nests/tree, and 0.9 nests/ha, Table 1).

Regarding phytosociological parameters, in both studied areas, *C. leptophloeos* was the species that had the highest value of importance index among the plant species recorded in the arboreous stratum (Tables 2, 3). The relative density (% of trees/ha) for *C. leptophloeos* was distinct between both areas: (area A, disturbed, RD=8.24; and area B, less disturbed, RD=42.50). The same was observed for relative dominance (% of m^2/ha). In addition, the aggregation index of Payandeh indicated that this species had an aggregated spatial distribution (Pi=2.18).

During the observation of seven nests inside the crop area of *P. edulis* cultivation, we observed that females returning from foraging trips with a mass of pollen grains adhered to the dorsal region of the mesosoma performed cleaning behavior, shortly after throwing away the pollen out of the nest. The analysis of this pollen showed that over 80% of the grains were of *P. edulis*.

Table 1 Number of nests and bees of *Xylocopa* spp. in three areas of Caatinga, located around *Passiflora edulis* crops.

	Area A (disturbed)	Area B (less disturbed)	Area C (disturbed)
Size of the area (ha)	196	14	49
Number of trees with nests	75	22	16
Total number of nests	180	94	44
Number of nests of *X. grisescens*	108	56	26
Number of nests of *X. frontalis*	72	38	18
Total number of nests/ha	0.9	6.7	0.9
Total number of nests/tree	2.4	4.2	2.7
Total number of bees	186	95	39
Total number of bees/nest	1.0	1.0	0.9
Total number of bees/tree	2.4	4.3	2.4
Number of females of *X. grisescens*	117	56	23
Number of females of *X. grisescens*/ha	0.59	4.0	0.46
Number of females of *X. frontalis*	63	31	13
Number of females of *X. frontalis*/ha	0.32	2.21	0.26
Total number of females	180	87	36
Total number of males	6	8	3

In the urban area close to the crops, 40 nests were observed in a dead tree of *Ficus* sp. The analysis of the pollen grains taken from the body of the bees from these nests revealed that each species of carpenter bees visited five plant species, including *P. edulis*, whose flowers the carpenter bees were observed collecting nectar intensively (Table 4). Foraging for pollen occurred mainly in the morning, but no pollen from *P. edulis* was identified in their scopa. In the afternoon, bees foraged mainly for nectar, passively transporting high percentages of pollen of *P. edulis* mainly in the dorsal region of the mesosoma.

Discussion

The two species of *Xylocopa* studied excavated galleries for nesting exclusively in branches of *C. leptophloeos* in both areas. In addition, *C. leptophloeos* was also reported as the most used tree species for nesting by stingless bees (Meliponini) in Caatinga, although these bees use the living parts of the trees (Martins *et al* 2004). Five tree species (*Poincianella microphylla*, *Cnidoscolus quercifolius*, *Aspidosperma pyrifolium*, *Jatropha mollissima*, and *Jatropha ribifolia*) had a relative density higher than that of

Table 2 Plant species sampled and phytosociological parameters recorded in area A (disturbed) in Projeto Maniçoba, Juazeiro, Bahia, Brazil.

Plant species	*D*	RD	*F*	RF	Do	RDo	VI	VI%
Commiphora leptophloeos	43.75	8.24	62.5	10.87	4.48	49.96	69.06	23.02
Cnidoscolus quercifolius	106.25	20.00	100.0	17.39	1.22	13.55	50.94	16.98
Poincianella microphylla	118.75	22.35	62.5	10.87	0.30	3.32	36.54	12.18
Jatropha ribifolia	50.00	9.41	100.0	17.39	0.00	0.00	26.80	8.93
Jatropha mollissima	62.50	11.76	62.5	10.87	0.08	0.85	23.48	7.83
Spondias tuberose	18.75	3.53	37.5	6.52	1.18	13.12	23.17	7.72
Mimosa tenuiflora	43.75	8.24	50.0	8.70	0.22	2.49	19.42	6.47
Schinopsis brasiliensis.	12.50	2.35	25.0	4.35	1.04	11.61	18.31	6.10
Aspidosperma pyrifolium	50.00	9.41	25.0	4.35	0.17	1.93	15.69	5.23
Cordia oncocalyx	6.25	1.18	12.5	2.17	0.15	1.63	4.98	1.66
Amburana cearensis	6.25	1.18	12.5	2.17	0.14	1.50	4.86	1.62
Erythroxylum nummularia	6.25	1.18	12.5	2.17	0.00	0.04	3.39	1.13
Cnidoscolus urens	6.25	1.18	12.5	2.17	0.00	0.00	3.35	1.12
	531	100	575	100	9	100	300	100

D density (ind/ha), *RD* relative density (%), *F* frequency (%), *RF* relative frequency (%), *Do* dominance (m^2 /ha), *RDo* relative dominance (%), *VI* value of importance (%), *VI%* relative value of importance (%).

Table 3 Plant species sampled and phytosociological parameters recorded in area B (less disturbed) in Projeto Maniçoba, Juazeiro, Bahia, Brazil.

Plant species	*D*	RD	*F*	RF	Do	RDo	VI	VI%
Commiphora leptophloeos	212.5	42.50	100	22.22	13.56	93.34	158.06	52.69
Mimosa tenuiflora	100.0	20.00	75	16.67	0.27	1.88	38.55	12.85
Poincianella microphylla	62.5	12.50	50	11.11	0.08	0.56	24.17	8.06
Jatropha ribifolia	37.5	7.50	75	16.67	0.00	0.00	24.17	8.06
Pseudobombax simplicifolium	25.0	5.00	50	11.11	0.49	3.34	19.46	6.49
Jatropha mollissima	25.0	5.00	50	11.11	0.02	0.11	16.22	5.41
Sapium glandulosum	25.0	5.00	25	5.56	0.11	0.77	11.33	3.78
Cassia sp.	12.5	2.50	25	5.56	0.00	0.00	8.06	2.69
	500	100	450	100	14.530	100	300	100

D density (ind/ha), *RD* relative density (%), *F* frequency (%), *RF* relative frequency (%), *Do* dominance (m^2 /ha), *RDo* relative dominance (%), *VI* value of importance (%), *VI%* relative value of importance (%).

C. leptophloeos in area A, indicating that selection for nesting substrate was independent of plant density. *Cnidoscolus quercifolius* and the *Jatropha* species are small trees or shrubs that contain much latex in the stems, used to treat external ulcers and snake bites, respectively, which may be avoided by the bees (Agra *et al* 2008). Trees of *C. leptophloeos* present dead wood, smooth and easy to be excavated, being more attractive to nesting females of *Xylocopa*, as reported for other substrates used for nesting by carpenter bees (Camillo & Garófalo 1982, Silva & Viana

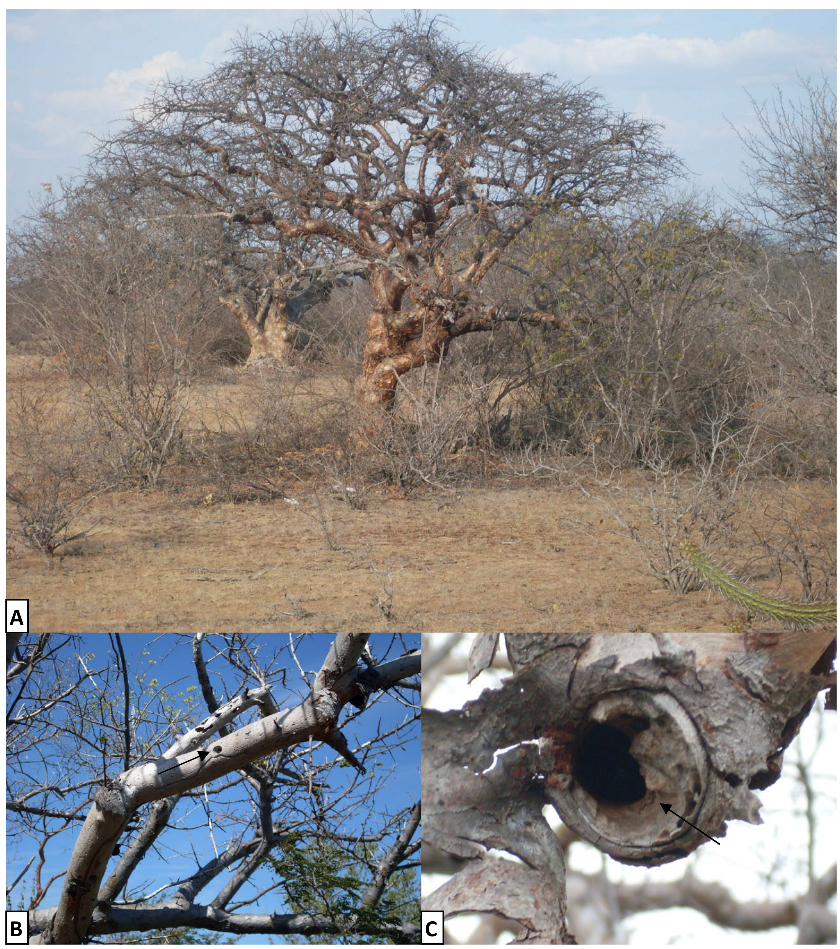

Fig 2 **a** General view of *Commiphora leptophloeos* (Burseraceae) tree. **b**, **c** Details of nest entrances (*arrow*) of *Xylocopa* spp. in dead and dry trunks and branches.

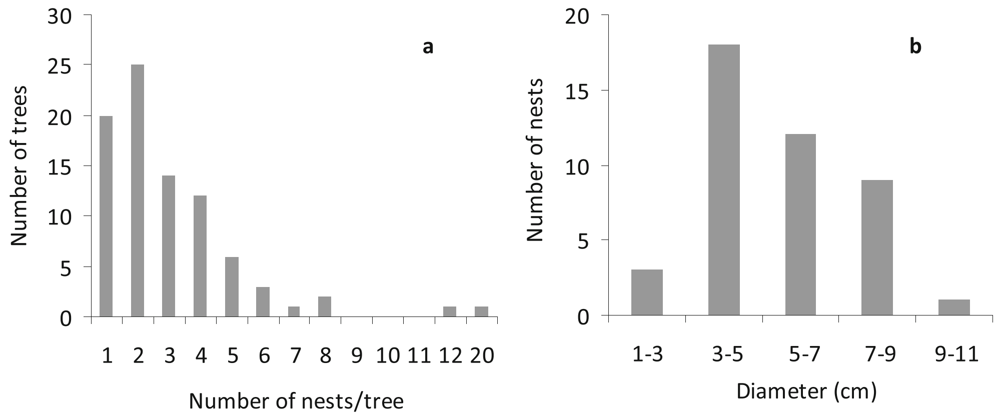

Fig 3 **a** Frequency distribution of the number of nests of *Xylocopa* spp. per tree of *Commiphora leptophloeos* (Burseraceae) in the Caatinga. **b** Frequency distribution of the diameter class of tree branches of *Commiphora leptophloeos* (Burseraceae) with nests of *Xylocopa* spp.

2002, Chaves-Alves *et al* 2011). According to Anzenberger (1977), substrate selection for nesting *Xylocopa* females seems to be more related to plant species characteristics than to bee species. Thus, the substrates are selected mainly for smoothness, texture, and lack of hard fibers that make nest excavation difficult (Camillo 2003).

The density of *C. leptophloeos* was high (212 plants/ha) in the area with less disturbed vegetation (area B), as well as its value of importance, indicating higher availability of substrates for nesting carpenter bees. Additionally, it is very reasonable to suppose that in this less disturbed vegetation, the trees of *C. leptophloeos* are older and larger, which also increase the availability of substrate for carpenter bees by the presence of many dead branches. One evidence of this is the higher absolute and relative dominance (m^2/ha) of this plant species in area B, showing that the tree trunks are larger in this area.

Table 4 Percentage of pollen grains types of *Passiflora edulis* and other plant species found in pollen removed from the body of females of *Xylocopa grisescens* and *Xylocopa frontalis* nesting in an urban area near crops of passion fruit.

	Bee species	Location of the pollen grains	*P. edulis* pollen (%)	Other species pollen (%)	Different pollen types (%)
Morning					
1	*X. grisescens*	Dorsal region of metasoma and mesosoma	0	100	A (2.0) B (98.0)
2	*X. grisescens*	Metasoma, scopa and ventral region of metasoma and mesosoma	0	100	A (100)
3	*X. grisescens*	Scopa, metasoma	0	100	B (2.6) D (97.4)
4	*X. grisescens*	Scopa and ventral region of metasoma	0	100	A (9.3) D (90.7)
5	*X. grisescens*	Scopa	0	100	A (8.0) C (92.0)
6	*X. frontalis*	Scopa	0	100	A (22.3) B (34.7) E (43.0)
Afternoon					
1	*X. grisescens*	Dorsal region of metasoma and scope	1.0	99.0	D
2	*X. grisescens*	Dorsal region of metasoma and head	3.3	96.0	E
3	*X. grisescens*	Dorsal region of metasoma	100	0	–
4	*X. grisescens*	Dorsal region of metasoma	42.4	57.6	A (12.0) D (45.6)
5	*X. grisescens*	Dorsal region of metasoma	33.4	66.6	D
6	*X. frontalis*	Dorsal region of metasoma	92.3	7.7	E
7	*X. frontalis*	Dorsal region of metasoma	100	0	0
8	*X. frontalis*	Scopa	0	100	D

The distribution of the nests of *Xylocopa* was concentrated in some trees and places in each area. This pattern of spatial distribution of nests is mainly related to two factors: (1) characteristics of the reproductive biology of carpenter bees, especially the reactivation of maternal nests and philopatry (Camillo & Garófalo 1989, Hogendoorn & Velthuis 1993, Junqueira *et al* 2012); and (2) the aggregate spatial distribution pattern of the used substrate (*C. leptophloeos*), similar to that reported for three species of carpenter bees (Silva & Viana 2002). Additionally, Gerling *et al* (1989) argued that the aggregate distribution of nests can be determined not only by the availability of substrate, but also by the presence of successful parental nests and chemical attractants.

The availability of substrates for nesting in the sampled areas, especially in the less disturbed area, is significant, indicating that the availability of substrate is not a limiting factor for nesting *Xylocopa* species in these areas. Zanella & Martins (2003) argued that it is likely that the fluctuations in the availability of floral resources, associated with the highly seasonal rainfall and a long dry season, represents the main limiting factor for population growth of solitary bee species in the semiarid region of northeastern Brazil.

The analysis of the pollen grains adhered to the body of the bees showed that females of *X. grisescens* and *X. frontalis* carried much pollen of passion fruit (*P. edulis*), which is passively deposited on the bee's body during nectar foraging. Most of these pollen grains are disposed outside the nest by foraging females, a behavior also recorded for *Xylocopa mordax* Smith in crops of *P. edulis* (Cobert & Willmer 1980). Our observations are consistent with the results of the analysis of pollen loads carried in the body of *X. grisescens* and *X. frontalis*, in a Brazilian tropical savanna, in which high proportions of pollen of *P. edulis* were found in the body of foraging females (52% and 40%, respectively), while the representation of this type pollen was quite modest in the brood cells of both species (4.8% and 0.8%, respectively) (Silva 2009).

Although the crops of *P. edulis* provide much nectar for carpenter bees for most part of the year (Kiill *et al* 2006) and there is availability of nesting substrate in the vicinity of the crops, these bees need a wide variety of floral resources to ensure their pollen needs, as pointed out by other authors (Silva 2009, Siqueira *et al* 2009, Giannini *et al* 2013). In this sense, urgent efforts are required to ensure the conservation of the fragments of Caatinga vegetation, so that the availability of the primary substrate for these nesting bees, *C. leptophloeos*, is not threatened, jeopardizing the populations of these important pollinators. This tree species is used by local craftsmen to manufacture gargoyles, other handicrafts, and medicinal use. The extraction endangers the availability of substrate, not only for nesting *Xylocopa* bees, but also for species of stingless bees (Meliponini) (Martins *et al* 2004). Although there is a government recommendation against cutting down the trees of *C. leptophloeos*, there is no any federal legislation ensuring the conservation of this important species. As suggested by Junqueira *et al* (2013), the benefits of nest management and introduction of *Xylocopa* nests in crop areas may depend on the ability of the surrounding area to sustain a population of introduced bees. Of course, these areas containing *C. leptophloeos* trees are also important as a repository of nests to be used in management programs.

Several studies have shown a relationship between the availability of native vegetation or seminatural habitats around crops and the abundance and diversity of floral visitors (Kremen *et al* 2002, Goulson 2003, Klein *et al* 2003, Vilhena *et al* 2012, Le Féon *et al* 2010, Yamamoto *et al* 2012). In the study area, native vegetation is distributed in small fragments in the vicinity of the crops and presumably contributes in the maintenance of populations of pollinators, including the carpenter bees, providing food resources and nesting sites for the bees. At present, these areas are not subject to management actions that enhance the preservation and multiplication of species that provide pollination services. Furthermore, the conventional farming practices used in the region, which includes the use of agrochemicals, without good control and monitoring, increases the possibility of poisoning of floral visitors.

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