

**Pollinating potential of bee floral visitors of *Spondias mombin* (Anacardiaceae)
cultivated in northeastern Brazil**

**Potencial polinizador das abelhas visitantes florais de *Spondias mombin* (Anacardiaceae)
cultivadas no nordeste brasileiro**

**Potencial polinizador de las abejas visitantes florales de *Spondias mombin*
(Anacardiaceae) cultivados en el noreste del Brasil**

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Abstract

To better understand the relationship between *Spondias mombin* (Anacardiaceae) with its floral visitors, we investigated stigma receptivity and flowering pattern, as well as the abundance, frequency and behavior of bees visiting its flowers, aiming to discuss their potential as pollinator of this plant species. We studied *S. mombin* in an orchard in Northeastern Brazil. Stigma receptivity coincided with the time bees were foraging, mainly for pollen. According to the bee behavior on flowers, their visiting hour, abundance, and frequency in the studied area, we concluded that *Apis mellifera* (Linnaeus), *Trigona spinipes* (Fabricius), *Xylocopa grisescens* (Lepeletier) and *Augochloropsis* sp. are potential pollinators of *S. mombin*. Also, *A. mellifera* and *T. spinipes* were the most abundant species in the area and collected floral resources with greater frequency, contributing more significantly to pollination. Due to the overwhelmingly presence of these two bee species in NE Brazil, we conclude they are the main potential pollinators of *S. mombin* in the region.

Keywords: Crop pollination; Yellow mombin; Pollinator behavior; *Apis mellifera*; Native bees; *Trigona spinipes*.

Resumo

Para compreender melhor a relação entre *Spondias mombin* (Anacardiaceae) e seus visitantes florais, investigamos a receptividade do estigma e o padrão de florescimento, bem como a abundância, frequência e o comportamento das abelhas que visitam suas flores, com o objetivo de discutir seu potencial como polinizadores desta espécie de planta. Estudamos essa relação em um pomar no Nordeste do Brasil. A receptividade do estigma coincidiu com o momento em que as abelhas visitavam as flores para coleta de recursos, principalmente pólen. De acordo com o comportamento das abelhas nas flores, horário de visitas, abundância e frequência na área estudada, concluímos que *Apis mellifera* (Linnaeus), *Trigona spinipes* (Fabricius), *Xylocopa grisescens* (Lepeletier) e *Augochloropsis* sp. são potenciais polinizadores de *S. mombin*. Além disso, *A. mellifera* e *T. spinipes* foram as espécies mais abundantes na área e coletaram recursos florais com maior frequência, contribuindo de forma mais significativa para a polinização. Devido à abundante presença dessas duas espécies de abelhas no Nordeste do Brasil, concluímos que elas são os principais potenciais polinizadores de *S. mombin* na região.

Palavras-chave: Polinização de culturas; Cajá; Comportamento do polinizador; *Apis mellifera*; Abelhas nativas; *Trigona spinipes*.

Resumen

Para comprender mejor la relación entre *Spondias mombin* (Anacardiaceae) y sus visitantes florales, investigamos la receptividad del estigma y el patrón de floración, así como la abundancia, frecuencia y comportamiento de las abejas que visitan sus flores, con el fin de discutir su potencial como polinizadores de esta frutífera. Estudiamos esta relación en un cultivo en el noreste de Brasil. La receptividad del estigma ocurrió en el momento en que las abejas visitaban las flores para recolectar recursos, principalmente polen. De acuerdo al comportamiento de las abejas en flores, horarios de visita, abundancia y frecuencia en el área estudiada, se concluye que *Apis mellifera* (Linnaeus), *Trigona spinipes* (Fabricius), *Xylocopa grisescens* (Lepeletier) y *Augochloropsis* sp. son polinizadores potenciales de *S. mombin*. Además, *A. mellifera* y *T. spinipes* fueron las especies más abundantes en el área y recolectaron recursos florales con mayor frecuencia, contribuyendo más significativamente a la polinización y la fructificación inicial que otras especies de abejas. Debido a la abundante presencia de estas dos especies de abejas en el noreste de Brasil, concluimos que son los principales potenciales polinizadores de *S. mombin* en la región.

Palabras clave: Polinización de cultivos; Jobo amarillo; Comportamiento de polinizadores; *Apis mellifera*; Abejas nativas; *Trigona spinipes*.

1. Introduction

Yellow mombin (*Spondias mombin* L., Anacardiaceae) is a typical tropical fruit tree occurring spontaneously in wild of tropical America (from Mexico to Brazil) (Mitchell & Daly, 2015), having also been naturalized in Africa (Schnell, 1976). In Brazil, it occurs in north (in the Amazon rainforest) and northeast (from semi-arid areas of Caatinga to areas of wet forest fragments) (Carneiro & Martins, 2012). *S. mombin* fruits have great socioeconomic importance for these regions, contributing to improve the livelihood of small-scale farmers or home-based producers (Mitchell & Daly, 2015). Its exploitation is based mainly on extractivism and fruits are harvested from trees that grow in the wild or in backyard orchards. Across the country, it is mostly consumed in the form of processed products (e.g., pulps, juices, jellies, ice cream, liqueurs and popsicles), supplied by local markets and major wholesale chains (Clarke, Schreckenberg, & Haq, 2011; Chang, Brattlof, & Ghukasyan, 2014).

The flowers of *S. mombin* have strong scent during anthesis, high pollen production and presence of nectar, suggesting a bee pollination syndrome (Barreto, Leal, Anjos, &

Castro, 2006), but it attracts a great number of generalist insects (Ramos, 2009; Carneiro & Martins, 2012; Oliveira, Souza, & Freitas, 2012). The inflorescences have thousands of flowers and these flowers can be either androgynous or staminate and both types occur in the same inflorescence (Ramos, 2009; Carneiro & Martins, 2012; Oliveira et al, 2012), with the anthesis beginning around 05:30 am (Ramos, 2009; Oliveira et al, 2012).

Spondias mombin is self-incompatible and dependent on pollinators to set fruits (Carneiro & Martins, 2012; Oliveira et al, 2012). Its small flowers produce great amounts of floral resources, and their morphology (i.e., radial symmetry, open corolla, and exposed fertile structures) allows the collection of pollen and/or nectar by a great diversity of eusocial and solitary bees (Ramos, 2009; Carneiro & Martins, 2012).

Native stingless bees (Apidae: Meliponini) with small/medium sizes such as *Trigona spinipes* (Fabricius) in semiarid areas of Northeast of Brazil (Oliveira et al, 2012), *Tetragona goettei* (Friese) and *Trigona hyalinata* (Lepeletier) in the Amazon region (Ramos, 2009) and *Scaptotrigona tubiba* (Smith) in the tropical Atlantic rainforest in northeastern Brazil (Carneiro & Martins, 2012), have been considered potential pollinators of *S. mombin*. However, because its abundance, behavior, and ability to visit a large number of flowers, the honey bee, *Apis mellifera* (Linnaeus), has been considered the main effective pollinator of *S. mombin* (Carneiro & Martins, 2012; Oliveira et al, 2012). Nevertheless, more studies are necessary to better understand the floral biology of *S. mombin* and the role of its bee floral visitors on the pollination process of this plant. Thus, we investigated its stigma receptivity and flowering pattern, as well as the abundance, frequency and behavior of bees visiting flowers during collection of floral resources, aiming to discuss their potential as pollinators of this plant species in a xeric region of Brazil.

2. Methodology

This study refers to an experimental research developed under field conditions, in which the quantitative method was adopted, following the basic recommendations for this category of research, as cited by Pereira, Shitsuka, Parreira, & Shitsuka (2018). According to these same authors, the mathematical approach often becomes important because it makes possible to predict events. In this method, numerical data was collected using measurements of quantities that originate a set of data, which could be analyzed by means of statistical analysis, which is one of the mathematical techniques.

2.1 Study area

The study was carried out in the Northeastern semi-arid region of Brazil, in an orchard of *S. mombin* (5°12'9.8" S and 37°59'29.2" W), established in the Chapada do Apodi (Apodi plateau), situated in Limoeiro do Norte county, Ceará State. The trees in the orchard were ten-year-old clones grafted onto *S. tuberosa*, which were grown in a rainfed system. In total, 32 plants of the Lagoa Redonda clone, with a maximum of three meters in height, were distributed into eight plots, eight meters apart, each one containing four plants. There was another area, with the same size and display eight meters apart beside the study area, cultivated with a different clone (Gereau), which served as pollen donor, which will be described in the next section. This study was carried out during the entire flowering period of the trees between November 2009 and January 2010.

2.2 Flowering pattern and stigma receptivity

Flowers were studied from buds to senescence or initial fruit set, aiming to investigate the flowering pattern, anthesis, sexual expression, basic floral features, floral resources, and the stigma receptivity. We consider the senescence, the moment that the flower begins to wilt, presenting a change in the color of the petals, which coincided with the absence of floral resources available.

- Flowering pattern: the 32 plants were monitored before they come into blooming to the end of the flowering period, and records on the first flower opening were taken. For the panicle records, a total of 37 inflorescences were monitored since the opening of their first flower until there were no more flowers in the inflorescence, recording the time of floral resources presentation and the number of flowers per panicle. Panicles were randomly chosen among those that were situated in distance to the ground that allowed full visualization by the observer.
- Anthesis, sexual expression, basic floral features, and floral resources: 48 panicles in 32 trees (1-2 panicles/tree) that presented a great quantity of flower buds in the pre-anthesis phase were marked and observed until senescence. Observations for anthesis duration (i.e., opening and closing time of flower petals), basic floral morphology (i.e., flower types, presence of smell, flower color, number of flowers per panicle, flower type proportion in the panicle and arrangement of the floral types in the panicle), and available floral resources

were carried out in these flowers in 10 nonconsecutive days. Pollen presentation was determined using a magnifying glass that allowed to assign percentages from 0 to 100, according to the amount of pollen present on anthers, where: 0% indicated that anthers had no visible pollen and 100% indicated that anthers were completely covered with pollen. The presence of nectar was verified by observing the gloss of a liquid solution directly at the bottom of the floral nectary (Pacini & Nepi, 2007).

- **Stigma receptivity:** the stigma receptivity was studied during five consecutive days along a month by hydrogen peroxide test (Dafni, Pacini, & Nepi, 2005). In this test, at each 30 minutes time interval, 35 flowers had their stigmas immersed in a hydrogen peroxide (3%) solution and the receptivity of the stigma was verified by the presence of oxygen bubbles on the stigmatic surface. The tests were only carried out from 05:00 a.m. until 11:00 a.m., since, after that moment, the flowers no longer had pollen and already beginning senescence.

2.3 Abundance, frequency and behavior of bees on flowers

During 60 days, in the flowering peak, which occurred from mid December 2009 to the end of blooming, observations and notes on the abundance, frequency and pattern of foraging of the most abundant bee species visiting *S. mombin* in the area were taken.

Bees visiting *S. mombin* flowers were counted along 150 m transects using a hand tally counter during 10-minutes period, repeated twice per hour at intervals of 20 minutes (Vaissiere, Freitas, & Gemmill-Herren, 2011), we counting a total of 7.187 bees visiting the flowers during the period of observations. These counting were performed during twenty-five non-consecutive days, in four different flowering phases (100%, 75%, 50% and 25% of flowering), starting from the flowering peak (assigned here as 100%), between 05:00 and 08:00 a.m., period of the day bees visited *S. mombin* flowers in the orchard. Abundance comprised the quantity of bees from each species visiting flowers during all the studied period. Frequency referred to the number of bees present in the panicles according to the time of the day. At least one specimen of each bee species was collected and sent to taxonomic identification in Laboratory of Bionomy, Biogeography and Insect Systems (BIOSIS) of the Federal University of Bahia (UFBA).

Data on bee behavior on flowers were collected during fifteen non-consecutive days also from 05:00 to 08:00 a.m., at intervals of 30 minutes, beginning with the arrival of a bee in the inflorescence and ending when it left the flower or get out of the observer's sight (Vaissière et al., 2011). Specific observations of the foraging pattern of each bee species

visiting *S. mombin* flowers aiming to identify their potential pollinators were made: the kind of resources collected from flowers; time of visit; type of approach (if they touched the reproductive organs and, when touched, which part of their body contacted the anthers or stigma); time spent on a flower and panicle, and number of flowers and panicles visited per minute. In addition, the observer carried a lightweight aluminum ladder and, in some situations, used it to reach the canopy of the trees to register some of these behavioral variables.

2.4 Data analysis

We used descriptive statistics with means and standard error to analyze the data related to flowering pattern (i.e., number of flowers per inflorescence). The data on the abundance, frequency and behavior, of bees on the flowers of *S. mombin* were analyzed through analysis of variance (ANOVA) using the 'General Linear Models Procedure' (PROC GLM) and means were compared *a posteriori* by the Tukey test, at 5% probability, by the 'Statistical Analysis System' (SAS 9.3) software (SAS Institute Inc., 2001).

3. Results

3.1 Flowering pattern

The flowering of *S. mombin* began at the beginning of November (i.e., at the end of the dry season in the studied region) and lasted until the end of January (i.e., beginning of the rainy season in the studied region), comprising approximately three months of flowering. Inflorescences were arranged in terminal panicles and presented, on average, 900.7 flowers ($\pm 512.1 / n = 23$), reaching a maximum of 1500 flowers per panicle.

The three flower clusters closest to the base of the panicle were always composed exclusively of male (staminate) flowers and the remainders by hermaphrodite flowers, which characterizing an andromonoecious sexual system. Therefore, the proportion between male and hermaphrodite flowers in a panicle was 30% : 70%. Flowers are actinomorphic, present five white petals and released a strong sweet scent mainly at the beginning of anthesis, which occurred between 05:00 and 05:30 a.m., being the male flowers the first to open, followed by the hermaphrodite flowers. Around 06:00 a.m. all flowers were opened. The beginning of senescence was between 10:30 and 11:00 a.m. for both flower types. Hence, the anthesis

lasted, approximately, between 5.5 to 6.0 hours, with male flowers lasting some minutes longer than female flowers.

Spondias mombin flowers produced pollen and nectar as floral resources, with pollen being the most abundant resource. In the area of the present study, pollen release began early in the day, as soon as the anthesis began by 05:00 a.m. Pollen release peaked at 06:00 a.m. (Table 1), but from that moment the amount of pollen presented by the anthers reduced progressively until the fourth flowering stage of the anthesis (Table 1), when anthers were almost devoid of pollen grains, coinciding with the time of the beginning of the flower senescence.

Table 1 - Corolla stage, presence of nectar, stigma receptivity through H₂O₂ test and percentage of pollen release in flowers of *Spondias mombin* cultivated in an orchard in the semi-arid NE Brazil.

Anthesis stages (Time)	Corolla*	Presence of nectar	Stigma (H ₂ O ₂ test)	Pollen amount (%)
1° (05h00 - 06h30)	PO	Present	Receptive	100
2° (06h30 - 08h00)	TO	Present	Receptive	70
3° (08h00 - 09h30)	TO	Present	Receptive	30
4° (09h30 - 11h00)	BS	Absent	Receptive	10

*PO= partiality open; TO = totally open; BS = beginning of senescence.

Source: Authors.

3.2 Stigma receptivity

The hydrogen peroxide test showed that the stigma was receptive since the beginning of anthesis (05:00 a.m.) until the senescence of the flowers (11:00 a.m.), with the stigmatic surface reacting with hydrogen peroxide solution at the same intensity along all tested periods (Table 1).

3.3 Abundance, frequency and behavior of bee species on flowers

The bees were the main floral visitors and potential pollinators of *S. mombin* in the studied area, with foraging pattern at the flowers varied greatly among species, with significant differences ($p < 0.05$) among them, for the behavioral variables studied (Table 2).

Augochloropsis sp. is a non-social green-metallic native bee that was the less abundant species in the studied area (0.22%) (Table 3). Its visitation, to collect a small amount of floral

resources (pollen and nectar), began at 07:00 a.m., later than other bee species, and extended to around 08:00 a.m., reaching the foraging peak at 07:30 a.m. They visited, on average, only two flowers ($\pm 0.6 / n = 6$) in the same panicle per minute (Table 2) and was the one that spent more time in the same flower (15.0 ± 0.8 p < 0.05; df = 96; F value = 70.50), and in the same panicle (99.6 ± 15.7 ; p < 0.05; df = 86; F value = 18.20) during resources collection. During the visits to flowers, they transferred pollen from stamens to the hairs on their ventral abdominal region, and, when visiting other hermaphrodite flowers, they touched the stigmas with their abdomen performing pollination (Figure 1-A1 and A2).

The stingless bee *Plebeia flavocincta* (Cockerell) is a small bee with low abundance in the studied area (0.56%). It visited flowers of *S. mombin* to collect nectar and pollen, from 06:00 a.m. to 08:00 a.m., reaching the foraging peak between 06:30 and 07:00 a.m. This species was observed along the entire flowering period (Table 3). Foraging bees spent about 9.9 seconds ($\pm 1.0 / n = 12$) on the same flower, collecting pollen, storing it on their corbiculae and, during the same visit, consuming nectar. These bees usually stood on the top of the anthers while collecting the pollen grains. During collection they removed the grains from the anthers; flew around a while for better packing the pollen grains in their corbiculae and returned to flowers of the same panicle. This species was one of those that visited less flowers per minute (2.9 ± 0.6 ; p < 0.05; df = 90; F value = 116.74) (Table 2) and did not contribute to the transfer of the pollen grains to the stigmas of the hermaphrodite flowers.

Xylocopa grisescens (Lepeletier) is a non-social native large-sized bee that also visited the flowers of *S. mombin* on study area, but in much smaller proportion (2.01%). Females of *X. grisescens* initiated the resource collection on flowers at 05:00 a.m., reaching the foraging peak early in the morning, at 05:30 a.m., visiting the flowers of *S. mombin* exclusively to collect pollen until 07:00 a.m. They landed heavily on panicles, collecting in quite rough movements as many pollen grains as possible from several flowers at the same time (Figure 1-B1 and B2). Then, the bee moved to another panicle with large number of open flowers, and repeating the same behavior, visiting several panicles per minute ($4.0 \pm 0.8 / n = 16$). This species spent less time per flower (1.6 ± 0.5 ; p < 0.05; df = 96; F value = 70.50), less time per panicle (96.3 ± 2.0 ; p < 0.05; df = 86; F value = 18.20) and visited more panicles per minute (4.0 ± 0.8 ; p < 0.05; df = 80; F value = 22.50) than other studied species (Table 2). The bees were also very active, going from one side of the orchard to the other in fast flights, spending little time on each flower (1.6 ± 0.5 ; p < 0.05; df = 96; F value = 70.50), touching on reproductive structures of *S. mombin* flowers by the ventral abdominal region of individuals, performing pollination.

The stingless bee *Trigona spinipes* (Fabricius) was the second most abundant species on the area of study (30.1%) ($p < 0.05$; $df = 114$; F value = 1.88), starting the resource collection on flowers (Figure 1-C1). at 05:00 a.m., reaching the foraging peak between 05:30 and 06:00 a.m. *Trigona spinipes* was present during all flowering phases of *S. mombin*, collecting floral resources in the orchard and visited the flowers from until 08:00 a.m. Its abundance varied little along the flowering period. When compared to *A. mellifera*, the most abundant species in the area, the amount of *T. spinipes* bees foraging in the field only differed significantly when 100% and 75% of the trees were flowering ($p < 0.05$; $df = 114$; F value = 1.88) (Table 3). Foragers of *T. spinipes* spent 4.4 seconds on each flower (± 0.8 s / $n = 33$) focusing mainly on pollen collection, despite nectar collection had also been observed (Figure 1-C2). This species spent an average of 68.3 seconds on each panicle (± 18.3 s / $n = 30$), visiting several flowers per minute (8.1 ± 1.5 / $n = 10$) (Table 2) and transferred pollen grains between flowers during floral resource collection, performing pollination.

The most abundant bee in the flowers of *S. mombin* was *Apis mellifera* (Linnaeus) (67.04%) ($p < 0.05$; $df = 114$; F value = 1.88). This species initiated the resource collection on flowers at 05:00 a.m., reaching the foraging peak between 05:30 and 06:00 a.m. The exotic honey bee (*A. mellifera*) foragers were observed in the canopy of the trees visiting flowers throughout the flowering period of the study, regardless the percentage of flowering (Table 3). Honey bee foragers visited *S. mombin* flowers, only for pollen collection, and never nectar (Figure 1-D1 and D2), until 07:30 a.m. Their abundance at flowers varied greatly according to the period of flowering, even though, this species was significantly more abundant ($p < 0.05$; $df = 114$; F value = 1.88) than the other species at all flowering phases (Table 3). Honey bee foragers spent less time on each flower than all species studied (1.9 s ± 0.7 ; $p < 0.05$; $df = 96$; F value = 70.50), visiting 2.3 panicles per minute (± 0.8 / $n = 28$) and collecting large pollen loads and packing them in their corbiculae (Figure 1-D3 and D4). During pollen collection, honey bee foragers visited several flowers, always touching the stigma region and transferring pollen grains between flowers, performing pollination, was the one that visited the greatest number of flowers per minute (18.3 ± 3.2 ; $p < 0.05$; $df = 90$; F value = 116.74) (Table 2).

Table 2 - Behavior of five bee species on flowers of *Spondias mombin* in an orchard established in the Chapada do Apodi (Apodi plateau), Ceará state, in the semi-arid NE Brazil.

Variables	Floral Visitors									
	<i>Augochloropsis</i> sp.		<i>Plebeia</i> <i>flavocincta</i>		<i>Xylocopa</i> <i>grisescens</i>		<i>Trigona</i> <i>spinipes</i>		<i>Apis</i> <i>mellifera</i>	
	n	X ± S.E.	n	X ± S.E.	n	X ± S.E.	n	X ± S.E.	n	X ± S.E.
TF	5	15.0 ± 0.8a	12	9.9 ± 1.0b	11	1.6 ± 0.5d	33	4.4 ± 0.8c	36	1.9 ± 0.7d
TP	6	99.6 ± 15.7a	12	72.5 ± 12.2b	10	6.3 ± 2.0c	30	68.6 ± 18.3b	28	54.6 ± 18.2b
FM	6	2.0 ± 0.6d	12	2.9 ± 0.6d	10	12.3 ± 2.1b	30	8.1 ± 1.5c	32	18.3 ± 3.2a
PM	4	1.0 ± 0.0c	9	1.1 ± 0.3cb	16	4.0 ± 0.8a	26	1.5 ± 0.6cb	28	2.3 ± 0.8b

TF: time bee spent on flowers; TP: time bee spent on panicles; FM: number of flowers visited per minute; PM: number of panicles visited per minute.

Means followed by different letters in each line differ significantly between them ($p < 0.05$).

Source: Authors.

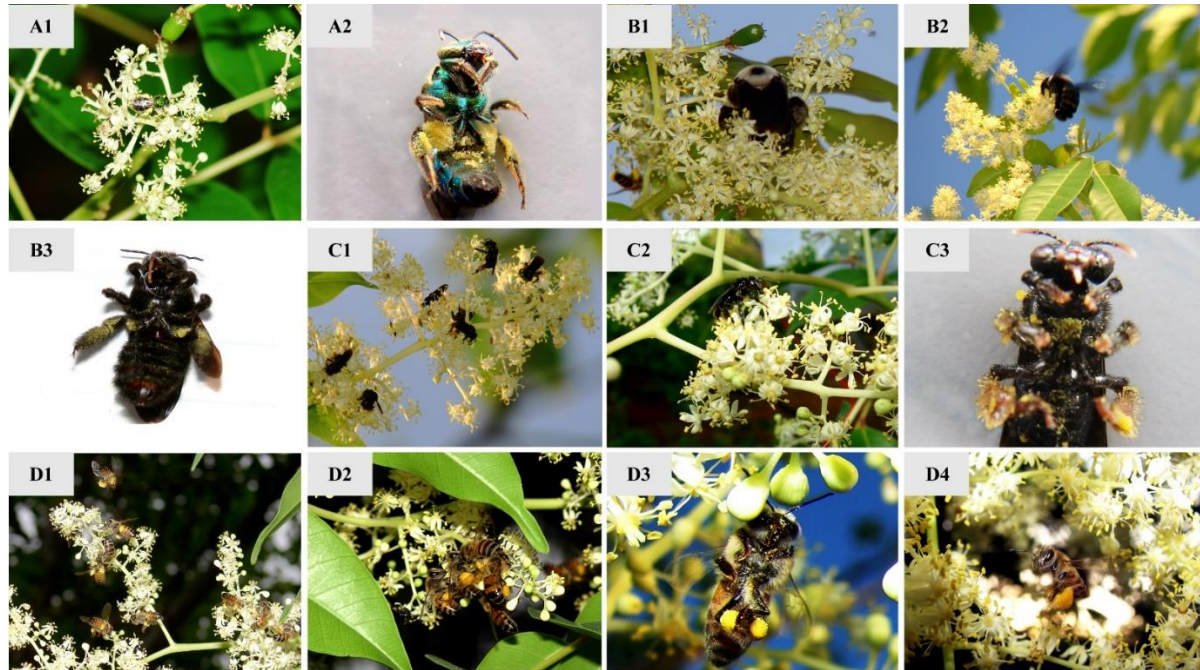
Based on all these described observations, *Augochloropsis* sp., *X. grisescens*, *T. spinipes* and *A. mellifera* were considered potential pollinators due to the relative abundance, frequency, and foraging behavior compatible (Table 2 and Table 3) with the reproductive biology of *S. mombin*. Only *P. flavocincta* was considered a floral visitor since they did not touch the stigma during visits, thus only collecting pollen from anthers.

Table 3 - Abundance and relative abundance (%) of bee visitors on flowers of *Spondias mombin* according to the flowering phases (100%, 75%, 50% and 25%) in an orchard established in the semi-arid NE Brazil.

Flowering Phases	Floral Visitors				
	<i>Augochloropsis</i> sp.	<i>Plebeia flavocincta</i>	<i>Xylocopa grisescens</i>	<i>Trigona spinipes</i>	<i>Apis mellifera</i>
	X ± S.E.	X ± S.E.	X ± S.E.	X ± S.E.	X ± S.E.
100%	0.0 ± 0.0d (0.00%)	1.0 ± 0.2d (0.28%)	6.5 ± 3.5c (1.84%)	72.6 ± 3.5b (20.57%)	272.8 ± 73.5a (77.29%)
75%	0.4 ± 0.1d (0.10%)	1.5 ± 0.5d (0.40%)	6.0 ± 2.8c (1.58%)	102.3 ± 33.5b (26.95%)	269.3 ± 62.8a (70.96%)
50%	1.1 ± 0.7c (0.30%)	1.0 ± 0.6c (0.27%)	4.8 ± 1.4c (1.28%)	152.7 ± 22.5b (41.15%)	211.5 ± 63.0ab (56.99%)
25%	0.6 ± 0.2c (0.50%)	1.6 ± 0.8c (1.31%)	4.1 ± 1.7c (3.36%)	38.6 ± 16.8b (31.72%)	76.8 ± 13.8a (62.94%)
Relative abundance (General means)	0.22%	0.56%	2.01%	30.10%	67.04%

Means followed by different letters in each line differ significantly between them (p < 0.05).
 Source: Authors.

Figure 1 - (A1) *Augochloropsis* sp. visiting flowers of Yellow mombin (*Spondias mombin*); (A2) Pollen grains adhered to the ventral parts of *Augochloropsis* sp.; (B1) Female *Xylocopa grisescens* visiting and pollinating flowers; (B2) Several flowers being contacted at the same time by a female *X. grisescens*; (B3) Pollen grains adhered to the ventral parts of *X. grisescens*; (C1) Native stingless bee *Trigona spinipes* collecting floral resources in *S. mombin*; (C2) *T. spinipes* workers collecting nectar from flowers of *S. mombin*; (C3) Pollen grains in the body of a *T. spinipes* worker; (D1) Many *Apis mellifera* workers collecting pollen from flowers of *S. mombin*; (D2) Great number of *A. mellifera* workers visiting flowers for pollen at the same panicle; (D3) *A. mellifera* worker packing pollen during a foraging trip to *S. mombin* flowers; (D4) Large pollen load of a *A. mellifera* worker foraging in *S. mombin* flowers.



Source: Authors.

4. Discussion

4.1 Flowering pattern

All observed trees of *S. mombin* showed a continuous and simultaneous flowering, like other species of *Spondias* L. in Neotropical regions (Mitchell & Daly, 2015). This flowering pattern contributes to ensure pollination in the area and aims to attract a great diversity of pollinators. Based on our observations and in other studies about floral biology, *S. mombin* is pollinated primarily by Hymenoptera, mostly bees and some wasps (Nadia, Machado, & Lopes, 2007; Oliveira et al., 2012; Mitchell & Daly, 2015).

Spondias mombin flowers produced pollen and nectar as floral resources, with pollen being the most abundant resource, which attracts great numbers of bees (Ramos, 2009;

Carneiro & Martins, 2012; Oliveira et al., 2012; Mitchell & Daly, 2015). Carneiro and Martins (2012) described *S. mombin* as a synchronous flowering tree with nocturnally dehiscent flowers that produce a rich pollen resource and attract nocturnal and diurnal bee species, mainly honey bees and stingless bees. In the area of the present study however, pollen release began early in the day, but not during the night.

In the Brazilian Amazon region, Ramos (2009) found an average of 5940 flowers per panicle in *S. mombin*, but in an area of tropical Atlantic rainforest in NE Brazil, Carneiro and Martins (2012) reported 1708 flowers per panicle. Therefore, our finding in a semiarid region, with 900.7 flowers per panicle, was the smallest average of the three studies. Perhaps the humid climate of the Amazon or the tropical Atlantic rainforest favors blooming, increasing the number of flowers per panicles when compared to *S. mombin* cultivated in the dry climate of NE Brazil (Ramos, 2009).

Previous studies on floral biology of *S. mombin* reported functionally hermaphroditic, staminate, and pistillate flowers on the same plant, but strongly protandrous and self-incompatible (Stacy et al., 1996; Bawa, 1974; Lozano, 1986; Mitchell & Daly, 2015). However, our results differed from those studies, revealing the absence of pistillate flowers type in panicles and a low proportion of male : hermaphrodite flowers (30% : 70%). On the other hand, an opposite male : hermaphrodite flowers proportion (70% : 30%) was observed by Ramos (2009), while a more equitable proportion among these same floral types (55% : 45%) was found by Carneiro and Martins (2012). These differences regarding flower type and their proportions may be attributed to genetic characteristics specific to the studied clone genotype.

Regarding the anthesis of both type of flowers, our findings were similar to Ramos (2009) only for the beginning of the anthesis, which was recorded by this author as starting around 5:30 a.m. in both floral types. On the other hand, our results differed from Ramos (2009) for the time of flower senescence, which recorded the male flowers remaining open throughout the day, senescing only in the next morning, and the hermaphrodites remaining open for two or three days after the flower opening. Probably, these differences could be attributed by the definition of senescence by Ramos (2009), which considered it as the moment that flowers drop from the panicle, while we considered it as the time when flowers begins to wilt, presenting a change in the color of the petals and absence of floral resources available.

4.2 *Stigma receptivity*

Contrasting with our findings, in which the stigma was receptive since the beginning of anthesis, Ramos (2009) suggested that the stigmas of *S. mombin* trees, in the Amazon region, are receptive from one hour after flower opening, based in the hydrogen peroxide test. In other study with *S. tuberosa*, Nadia et al. (2007) found that pollen tubes originating from hand pollination can grow in the stigmas of one and two days-old, suggesting they are still receptive. In our study the stigma receptivity, based in the hydrogen peroxide test, showed a great amount of oxygen bubbles in the stigmatic surface since the flower opening until 11:00 a.m., indicating enzyme activity and, consequently, stigmatic receptivity (Dafni & Maués, 1998), as observed in others studies (Fonseca, Santos, & Vieira, 2015; Dey, Mondal, & Mandal, 2016).

4.3 *Abundance, frequency and behavior of bee species on flowers*

The small size, the great pollen amount, the presence of a strong scent and the open and shallow corolla, which allow an easy access by the visitors of *S. mombin* flowers (Ramos, 2009; Oliveira et al., 2012; Carneiro & Martins, 2012; Mitchell & Daly, 2015) have placed *S. mombin* as an important plant for native bees and honey bees. Indeed, bees were the main floral visitors and potential pollinators of *S. mombin* in the studied area.

Studies have showed *S. mombin* as a pollen source for some stingless bees (Marques-Souza, Absy & Kerr, 2007; Oliveira et al, 2012; Carneiro & Martins, 2012) and honey bees (Oliveira et al., 2012; Carneiro & Martins, 2012). In our study, we corroborate these findings, but we also found non-social bees of the genera *Xylocopa* and *Augochloropsis* using *S. mombin* flowers to collect floral resources. Pollen collection was more frequent than nectar collection by bee fauna present in *S. mombin* flowers on the study area, but some species were also observed collecting nectar (*T. spinipes*, *P. flavocincta* and *Augochloropsis* sp.). However, no bees collected exclusively nectar. These results differ from Ramos (2009) and Carneiro and Martins (2012), which identified the bee fauna visiting *S. mombin* only for pollen gathering.

Augochloropsis sp. collected both pollen and nectar as floral resources, touching the reproductive parts of the flowers. Thus, *Augochloropsis* sp. must be considered as a pollinator of *S. mombin*. However, due to its low occurrence in the area and its small abundance and frequency at the flowers, we suggest that this species contributed little to pollination and

consequently to set fruits. Besides that, when this bee species spent more time in the same flower and in the same panicle (Table 3), it probably increased the transfer of pollen grains from the same plant (geitonogamy), which do not favor the best pollination requirements of *S. mombin* (Ramos, 2009; Carneiro & Martins, 2012; Oliveira et al., 2012).

The native bee specie *P. flavocincta* visited flowers of *S. mombin* to collect nectar and pollen. It was the species which began foraging later in the day than any of the other bee species but remained in the field after all other species had gone. According to Silva, Meneses and Freitas (2019), the small size of *P. flavocincta* force this species to wait higher temperatures to warm up and fly when comparing to larger bee species, but also allows it to stay longer in the field exploring minor amounts of resources which are no longer satisfactory to larger bees. Although these bees visited the flowers when their stigma was still receptive, their small sizes and foraging behavior did not allow them to touch the stigmas and pollinate the flowers. Thus, *P. flavocincta* should be considered only as a floral visitor and not as an effective pollinator of *S. mombin*.

Xylocopa grisescens is a potential efficient pollinator for *S. mombin* because body size is closely related to flight range and mobility and therefore access to the floral resources, large bees can also carry greater pollen loads and visit more flowers per unit time than small ones (Streinzer, Huber, & Spaethe, 2016; Oliveira, Freitas, Scheper, & Kleijn, 2016; Wright, Roberts, & Collins, 2015; Veiga, Menezes, Venturieri, & Contrera, 2013). In *S. mombin* orchards this behavior may result in a much greater and more effective contribution to cross-pollination, since this species visited several panicles from different trees during collection. Indeed, as showed by Carneiro and Martins (2012) and by the study of Oliveira et al. (2012), which was conducted simultaneously and in the same orchard of the present work, cross-pollination with pollen from male flowers of a different clone showed a higher rate of initial fruit set. Thus, *X. grisescens* should be considered as an effective pollinator of *S. mombin*, performing pollination by transferring the pollen grains that were adhered to the hairs of its ventral region (Figure 1-B3). Because of the many features cited above, the genus *Xylocopa* is recognized as one of the most effective pollinators of agricultural crops (Giannini et al., 2015).

Trigona spinipes foragers, during flower visits, touched the reproductive organs with the ventral region of their bodies (Figure 1-C3), transferring pollen grains when moving to the next flowers, and effectively pollinating them. Due to the great abundance (Figure 1-C1) and frequency, even with few trees blooming, and also due to its behavior and time of visit while the stigma was still receptive, *T. spinipes* should also be considered an effective pollinator of

S. mombin. In a research studying *Spondias tuberosa* by Barreto et al. (2006), a large number of *T. spinipes* foragers were found collecting mainly pollen in the flowers and their movements favored pollination. This bee species is widely distributed in South America and due to its ability to visit a vast number of flowers of different plant species, *T. spinipes* is considered as a super generalist bee, and an efficient crop pollinator (Giannini et al., 2015; Jaffé et al., 2015).

At the flowering peak we could observe a greater number of *A. mellifera* workers visiting *S. mombin* flowers, than other bees present in the study area, and a possible competition between species (Roubik, 2009; Roubik & Villanueva-Gutiérrez, 2009). This result was similar to Carneiro and Martins (2012), which found honey bees with the greatest relative frequency of visitation. On the other hand, Ramos (2009) recorded the stingless bees *Tetragona goettei* and *Trigona hyalinata* with a relative frequency greater than honey bees. *Apis mellifera* foragers walked over several hermaphrodite flowers during collection of floral resources and touched their reproductive structures with the ventral region of body, transferring the adhered pollen grains to the stigma and pollinating the flowers. Oliveira et al. (2012) and Carneiro and Martins (2012) had pointed out the importance of *A. mellifera* for the pollination of *S. mombin*.

Therefore, due to the abundance frequency, behavior, time of foraging, number of flowers visited per minute and approach to the flowers, the honey bee should be considered as the most effective pollinators of *S. mombin* in the area of this study, Northeast Brazil. Oliveira et al. (2012) showed that *A. mellifera* produced five times more fruit than *T. spinipes* and almost more than twice as much when compared to the natural pollination of the orchard. It is not known to what extent the qualitative aspects (e.g., fruit weight, amount of pulp, seed germination capacity, soluble solids, etc.) of *S. mombin* fruits depend on the type of pollination.

Our study has some limitations. Unlike the previous works with this plant species which were conducted in natural settings where plants are isolated or sparsely distributed in the landscape, ours is the first study to evaluate floral biology of *S. mombin* and its floral visitors with potential for pollination in an orchard where there is a higher number of plants and flowers than in populations growing wild. Also, the orchard where this study was conducted was formed by clone trees situation where genetic variability among trees is greatly minimized and floral visitors find much higher uniformity in flower numbers, density, and floral resources than in natural populations. Both situations hindered a fair comparison between our study and the others. However, it is important to highlight that this work brings

new and important information to the pollination of *S. mombin* under cultivation as this plant species is quickly turning from an extractivist resource to an agricultural crop.

5. Conclusion

Based on bee behavior in the flowers, visiting hours, abundance, and frequency in the studied orchard, we concluded that *A. mellifera*, *T. spinipes*, *X. grisescens* and *Augochloropsis* sp. are potential pollinators of *S. mombin*. Also, due to the high number of foragers of *A. mellifera* and *T. spinipes* we concluded that these two bee species are the main potential pollinators of *S. mombin* in the region.

Future studies must be carried out to fill knowledge gaps in this study. For example, seeking a better understanding of the phenology and reproductive biology of different cultivars in different regions and their interaction with their floral visitors, as well as investigating the ideal arrangement between different clones within a plantation to take advantage of the foraging behavior of pollinators in favor of improving the orchard natural levels of pollination.

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