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Diet of *Xylocopa grisescens* Lepeletier (Hymenoptera, Apidae) in a Semiarid Area Cultivated with Passion Fruit (*Passiflora edulis* Sims)

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Abstract

The loss and fragmentation of native vegetation harm pollinators and reduce crop productivity. The passion fruit crop is an excellent model for investigating this issue, as its pollinators are especially sensitive to deforestation. This study aimed to investigate food sources used by *Xylocopa grisescens* Lepeletier in the surroundings of passion fruit orchards. Samples of larval provisions within trap nests and pollen in scopae were collected from August 2019 to March 2020 and were subjected to acetolysis. Additionally, adults were collected while foraging in vegetation surrounding orchards. Twenty-nine pollen types were found in the larval provisions. *Mimosa arenosa* (TRF = 54.96%) and *Anadenanthera colubrina* had the highest relative frequencies (TRF). *Passiflora edulis* pollen type was found in all samples but had low frequency. The trophic niche width (H') based on larval provisions was 1.34, and H' based on pollen of the scopae was 1.79. The carpenter bee *X. grisescens* exploited a diverse set of plants around the orchards but concentrated foraging on a limited number of species, which were replaced over time, indicating generalist foraging behavior and flexibility in selecting sources of floral resources. Our study reinforces the need to conserve native vegetation remnants to maintain carpenter bee populations, which can contribute to increased crop productivity and improved fruit quality.

Introduction

Yellow passion fruit (*Passiflora edulis* Sims) essentially depends on pollinators as it is a self-incompatible plant species. Therefore, cross-pollination becomes necessary for producing fruits and seeds (Giannini et al., 2015; Lucia et al., 2017; Martarello et al., 2021). The natural pollination of these flowers is mainly performed by large carpenter bees belonging to the genus *Xylocopa* (Yamamoto et al., 2012; Oliveira et al., 2014; Junqueira & Augusto, 2016; Lucia et al., 2017; Barros et al., 2024). Recent studies have indicated that this plant-pollinator system is vulnerable to decline due to climate change (Giannini et al., 2013; Bezerra et al., 2019). Orchards where the natural pollination rate was found to be less than 13% have a pollination deficit (Junqueira & Augusto, 2016). The low density of effective pollinators leads to low fruit set and/or increases the need for manual pollination (Junqueira et al., 2013; Barrera et al., 2020), which increases production costs and can result in high food prices (Wurz et al., 2021).

Brazil boasts extensive passion fruit cultivation, ranking as the world's largest producer (Junqueira & Augusto, 2016), with approximately 45 thousand hectares (IBGE, 2022). These areas require pollination services provided by carpenter bees. National production of this crop reached 698 kt in 2022 (IBGE, 2022). Data on the diet of these bee species are scarce and insufficient to support management plans.

Experiments to increase the abundance of carpenter bees in passion fruit orchards have shown positive results in increasing floral visit rates, which could reduce pollination deficits and increase fruit set (Oliveira-Filho & Freitas, 2003; Junqueira et al., 2013; Junqueira & Augusto, 2016; Silva & Freitas, 2018). Carpenter bee management for crop pollination

has been implemented in some countries, such as the Philippines, USA, Malaysia, and Brazil (Raju & Rao, 2006; Osterman et al., 2021), mainly by providing substrates for nesting in crop areas (Oliveira-Filho & Freitas, 2003; Pereira & Garófalo, 2010; Junqueira et al., 2013; Oliveira et al., 2014; Silva & Freitas, 2018).

Passion flowers only provide nectar for carpenter bees. Therefore, these populations depend on the vegetation around the orchards to obtain pollen to feed their larvae and, consequently, for their persistence in these agrosystems (Bernardino & Gaglianone, 2008; Silva et al., 2010; Yamamoto et al., 2012; Oliveira et al., 2014; Junqueira & Augusto, 2016; Lucia et al., 2017; Silva & Freitas, 2018). Since the scarcity of pollen sources in the habitat affected the number of generations per year of the carpenter bees (Raju & Rao, 2006; Moure & Melo, 2022), it is essential to identify plant sources of pollen necessary for the population maintenance of bee species with the potential to be managed as pollinators, such as *Xylocopa* species, to develop effective management plans.

Data on plant species utilized in the diet of *Xylocopa* species were gathered from a few agroecosystems in Brazil. These data were collected through bee sampling during flower visits and/or through analysis of pollen loads and larval provisions (Silva, 2009; Silva et al., 2010; Carvalho et al., 2013; Oliveira et al., 2014; Junqueira et al., 2017; Araújo et al., 2021). Araújo et al. (2021) tested hypotheses regarding trophic niche partitioning between *Xylocopa grisescens* Lepeletier and *Xylocopa frontalis* (Olivier). They investigated the occurrence of individual specialization in the foraging of these diet-generalist species.

Given this knowledge gap concerning the trophic ecology of carpenter bee species with significant potential for crop pollination, the current study aimed to identify plant species that provide food resources necessary to maintain *X. grisescens* populations in a passion fruit cultivation area located in a semiarid region of Brazil. Additionally, changes in the diet of this carpenter bee were recorded over time.

Material and Methods

Study site

The present study was carried out in an area cultivated with passion fruit (*P. edulis*, Passifloraceae) and mango (*Mangifera indica*, Anacardiaceae) in Livramento de Nossa de Senhora County, Bahia State, Brazil (13°38'35'' S; 41°50'26'' W). Samplings were carried out in three orchards (approximately 3 hectares each) located 1 km from each other. The surroundings of the orchards were covered by seasonally dry vegetation (called "Caatinga") composed mainly of shrubs and small trees. The climate in this region is Aw-type, dry and warm all year long (Alvares et al., 2013). The annual mean temperature is 24.2 ºC, and the annual mean rainfall is 687.7 mm (Bahia, 2013). The rainy period was from January to March 2020. Data were obtained by the National Meteorology Institute (INMET) at Brumado Climatological Station, located 53 km from the study site.

Sampling and pollen sample processing

A 2m high and 1m long shelter was built to make trap nests available to the bees in each orchard. Each shelter had 50 bamboo cane-type trap nests, following Camillo et al. (1995) and Pereira & Garófalo (2010). Bamboo cane's lengths ranged from 160mm to 290mm, and diameters ranged from 20mm to 40mm. The nesting activity of *X. grisescens* in the trap nests was recorded monthly from August 2019 to March 2020. To assess the trophic dimension of the realized niche of this population in this time window, samples of larval provisions were collected monthly from thirteen different open brood cells, and all open nests in provisioning were sampled. In August, September, and October 2019, one nest was sampled per month. In December 2019 and January 2020, two nests were sampled each month, and in February and March 2020, three nests were sampled each month.

The interactions between *X. grisescens* and flowering plants were recorded along a 2 km long by 20 m wide transect through the vegetation near the orchards. The transect started at one of the edges of the orchard and followed through the natural vegetation (Caatinga) towards another orchard. It was continuously walked from 06:00 am to 04:00 pm, totaling 120 hours of sampling effort. Nine females were collected during flower visits to remove pollen samples from the scopae. They were transported individually in plastic containers, such as 50ml falcon tubes for centrifuges. The hind legs were cut in the laboratory, and scopae were stored separately in 70% ethanol. Floral branches of visited plants were collected and herborized for taxonomic identification. Bees' scopae and the samples of larval provisions were vortexed separately in a tube stirrer for approximately 10 minutes. This material was subjected to acetolysis (Erdtman, 1960) and assembled on five slides (for each sample) following Dórea et al. (2010). Glycerin gelatin was used on the slides to improve visualization; slides were covered with coverslips and sealed with paraffin (Salgado-Labouriau, 1961).

Data analysis

Pollen type identification and quantification were carried out by counting at least 1,000 pollen grains per sample, according to Vergeron (1964). Each nest was considered a sample to analyze larval provisions. To analyze pollen from the scopae, the hind legs of each bee were cut off and considered as one sample.

The reference collection of the Plant Micromorphology Laboratory (LAMIV) of the Universidade Estadual de Feira de Santana (UEFS) was used for pollen type identification, as well as pollen catalogs (Santos et al., 2006; Silva et al., 2010; Santos, 2011). The relative frequency (f) of each pollen type was calculated for each sample by applying the algorithm $f = ni/N \times 100$, where *ni* is the number of grains of the pollen type *i* in the sample, and N is the total number of grains counted in the sample. Relative frequencies were calculated monthly (MRF) and the whole sampling time (TRF – total relative frequency).

The Shannon-Wiener index (H′= − ∑ pk x lnpk) was used to calculate trophic niche width based on the pollen from scopae and from the nests, separately; pk is the number of grains of each pollen type divided by the total number of pollen grains counted in each month, and lnpk is the neperian logarithm of pk. The H' index was also used to calculate niche width based on flower visits data (Shannon, 1948).

Results

Nest provisioning was recorded from August 2019 to March 2020, except for November 2019. A total of 15,996 pollen grains were counted in the samples of larval provisions and allowed the identification of 29 pollen types (Table 1). The botanical family Fabaceae accounted for the highest

Table 1. Pollen spectrum found in larval provisions of *Xylocopa*. TRF: total relative frequency, MRF: monthly relative frequency of the pollen types.

Family	Pollen Type	TRF (%)	Monthly relative frequency of pollen types − MRF (%)						
			Aug	Set	Oct	Dec	Jan	Feb	Mar
Acanthaceae	*Ruellia*	0.12				0.73			
Anacardiaceae	*Spondias*	0.05				0.19			0.07
Cactaceae	*Opuntia*	0.20					0.45	0.69	
Convolvulaceae	*Evolvulus*	0.23			0.57	1.16			
	Jacquemontia	1.53	4.18	1.05	1.90	2.40	0.45		1.97
	Merremia	2.18		0.19	4.48	4.83	5.30	1.04	0.80
Euphorbiaceae	*Cnidoscolus urens*	0.06				0.23		0.12	
	Croton	0.04		0.19	0.29				
Fabaceae	*Aeschynomene*	0.93		0.06		0.04		4.32	0.02
	Anadenanthera colubrina	11.95		0.31	68.00	38.05		5.76	0.37
	Caesalpinia pulcherrima	0.25			0.67	1.12			0.10
	Chamaecrista	2.99	0.51		0.76	1.74	10.94	5.58	0.32
	Crotalaria	2.51	8.87	0.12	6.95	4.52	1.15	1.34	0.45
	Fabaceae type 1	0.53					4.20		
	Machaerium hirtum	2.00	10.03		0.57	2.13	2.40	1.94	0.17
	Mimosa arenosa	54.96	38.67	50.87	7.62	17.98	50.07	73.61	85.39
	Mimosa misera	5.89	6.13	1.24		13.11		3.31	9.67
	Phaseolus	7.38	17.82	44.38	5.71	5.80	0.30		
	Piptadenia stipulacea	2.27					18.14		
	Prosopsis juliflora	0.74			1.43	4.02			
	Senna	0.03					0.25		
	Senna multijuga	0.93					5.20	1.34	
Malpighiaceae	*Malpighia emarginata*	0.07		0.06		0.12	0.25	0.06	
Malvaceae	*Pseudobombax* type 1	0.01	0.14				0.05		
	Pseudobombax grandiflorum	1.01	11.54	0.06					
	Pseudobombax type 2	0.18	2.02						
Myrtaceae	*Eugenia*	0.16						0.78	
Passifloraceae	*Passiflora edulis*	0.79	0.07	1.48	1.05	1.82	0.85	0.12	0.57
Poaceae	Poaceae type1	0.02							0.07
H'		1.34	1.79	0.95	1.28	2.01	1.60	1.13	0.60
J'		0.51	0.75	0.38	0.50	0.70	0.61	0.43	0.23

pollen type richness in larval provisions, with 14 pollen types representing 90% of the quantified pollen grains. *Mimosa arenosa, Anadenanthera colubrina,* and the *Phaseolus* type had the highest total relative frequencies (TRF) at 54.96%, 11.95%, and 7.38%, respectively (Table 1). Eight pollen types reached a monthly relative frequency (MRF) higher than 10% in the larval provisions (Supplementary Material 1). Seven of these were related to plants in the Fabaceae family (*Mimosa arenosa*, *Anadenanthera colubrina, Phaseolus, Piptadenia stipulacea, Mimosa misera, Machaerium hirtum,* and the *Chamaecrista* type), and one was related to the Malvaceae family (*Pseudobombax grandiflorum*) (Table 1). On the other hand, the pollen type *Passiflora edulis* was found in all nests but was always represented by a low percentage of the pollen grains.

In the samples obtained from the scopae of *X. grisescens* females collected visiting flowers were counted 8,289 pollen grains (Table 2), and 18 pollen types were found, among them: *Machaerium hirtum* (TRF = 19.46%), *Senna multijuga* (12.95%), *Chamaecrista* type (9.95%), *Senna* type 1 (9.77%), *Malpighia emarginata* (9.73%) and *Crotalaria* type 1 (8.62%). The pollen type *Passiflora edulis* was recorded in all samples (TRF = 4.68%). Pollen types related to Fabaceae represented 70% of the pollen grains counted in samples from the scopa of *X. grisescens* (Table 2).

The trophic niche width of *X. grisescens* based on larval provisions (H′) was 1.34, and monthly variation ranged from 0.60 to 2.01. Equitability (J') based on larval provisions samples was 0.51 (Table 1). The niche width based on pollen loads in the scopae was H' = 1.79 (range 1.02 to 2.12), and equitability (J') was 0.76 (Table 2). Additionally, 184 interactions between *X. grisescens* and nine plant species were recorded (Table 3). The niche width based on flower visits data was 1.94.

Table 2. Pollen types found in the scopae of *Xylocopa grisescens.* TRF: total relative frequency, MRF: Monthly relative frequency of the pollen types.

Plant Family	Pollen type	TRF (%)	Monthly relative frequency of the pollen types − MRF (%)			
			Oct	Nov	Dec	Jan
Anacardiaceae	*Spondias*	0.46	1.91			
Cactaceae	*Opuntia*	0.19		0.61		
Convolvulaceae	*Merremia*	2.67	9.19	1.42		
Euphorbiaceae	*Cnidoscolus urens*	0.30	0.77	0.36		
Fabaceae	*Anadenanthera colubrina*	6.95		22.73	0.00	
	Aeschynomene	0.79		2.59	0.00	
	Chamaecrista	9.95	24.11	3.62	9.55	5.44
	Crotalaria	8.62	26.30	3.23		3.81
	Machaerium hirtum	19.46	22.36	7.66	51.42	15.72
	Mimosa arenosa	6.20	7.72	5.24	1.00	8.05
	Mimosa misera	4.63		15.13	0.00	
	Senna	9.77				30.34
	Senna multijuga	12.95		23.02	37.18	3.50
Malpighiaceae	*Malpighia emarginata*	9.73	2.56	0.52		27.79
Malvaceae	*Pseudobombax* type 2	0.14		0.45		
Myrtaceae	*Eugenia*	2.24		2.39	0.00	4.67
Malvaceae	*Sida*	0.26	0.73	0.26		
Passifloraceae	*Passiflora edulis*	4.68	4.35	10.77	0.85	0.68
H'		1.79	1.83	2.12	1.02	1.79
J'		0.76	0.79	0.78	0.63	0.81

Table 3. Plant species visited by *Xylocopa grisescens* and number of visits recorded on flowers of each plant species.

Botanical family	Plant species visited	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Euphorbiaceae	*Jatropha urens* L.			4	2								
Fabaceae	*Senna multijuga* (Rich.) H.S.Irwin & Barneby						4	2	3	4	2	5	2
	Senna acuruensis (Benth.) H.S.Irwin & Barneby	6									3		
	Peltophorum dubium (Spreng.) Taub.	8											
	Prosopis juliflora (Sw.) DC.	9	12				7	10					
	Cenostigma pyramidale (Tul.) Gagnon GP. Lewis	13								5	8	10	15
	Coursetia sp.				5								
	Inga sp.							3	9	6	4		
	Crotalaria sp.					7	11	5					

Discussion

The analysis of pollen in larval provisions and scopae, added to flower visits data, indicated that *X. grisescens* used a range of plant species around the orchards to get floral resources and concentrated foraging efforts on a limited subset of plant species. Equitability values based on larval provisions were quite variable and very low in some months, indicating that only a few pollen types were overrepresented in some monthly samples. There was high substitution of pollen sources, which were essential for a short time. Silva (2009) also reported a few high-frequency pollen types in brood cells of *Xylocopa* species, which also points towards foraging concentration in a few plants. A similar trend was reported for *X. grisescens* and *X. frontalis* in Brazilian savanna areas (Araujo et al., 2021), for *X. frontalis* (Junqueira et al., 2017), and *Xylocopa augusti* Lepeletier de Saint Fargeau (Lucia et al., 2017).

The pollen type *Mimosa arenosa* is related to plant species that emerged as essential sources for *X. grisescens*. Some studies show the importance of this plant species and other bee species in the semiarid region of northeastern Brazil (Oliveira et al., 2010; Silva et al., 2018). *Mimosa* was considered one of the most essential plant genera for the maintenance of *X. grisescens, X. frontalis, Xylocopa hirsuttissima* Maidl, and *Xylocopa suspecta* Moure & Camargo populations in passion fruit crops surrounded by savanna vegetation in Brazil (Silva, 2009).

Several pollen types recorded at low relative frequency may be related to plants that have a secondary contribution to the bees' diet (Imperatriz-Fonseca et al., 1993) or to nectar sources (Silva, 2009; Lucia et al., 2017). The latter is the case with passion fruit pollen type, which was found at low frequency in all brood cells analyzed. Similar data were obtained for *X. augusti* by Lucia et al. (2017), who pointed out that *Passiflora caerulea* is just a source of nectar for this carpenter bee. Foraging behavior studies have also shown that *P. edulis* is a nectar-only source for several species of *Xylocopa* (Bernardino & Gaglianone, 2008; Silva, 2009; Silva & Freitas, 2018). *Prosopis juliflora,* an exotic plant species in Brazil (Andrade et al., 2009), received many visits of *X. grisescens*, although the frequency of the pollen type related to this plant species was also low in larval provisions. This plant species proved to be an essential source of floral resources for carpenter bees, probably because it is a locally abundant resource, as it has dense inflorescences with many flowers, which provide pollen and nectar for bees (Sousa et al., 2016).

The combination of pollen from scopae and flower visits data indicated using 36 plant species by *X. grisescens* in the areas around the passion fruit orchards studied in Caatinga vegetation. Literature data suggest that this species uses an even more extensive set of plants to gather floral resources. For example, Silva (2009) found a larger number (78) of pollen types when she analyzed larval provisions, feces, and body of *X. grisescens* females in a fragment of savanna vegetation close to a passion fruit orchard.

The carpenter bee *X. grisescens* showed polylectic foraging, gathering pollen from several phylogenetically unrelated plant species, flexibility in exploiting food sources, and substituting sources of food resources throughout the year. Despite the generalist foraging behavior, foraging was concentrated in a few plant species. The current study expands the knowledge base about plants important for maintaining carpenter bee populations in agricultural areas in a semiarid region, the Brazilian *Caatinga* ecosystem. As previously highlighted by other authors, the surrounding vegetation plays a fundamental role in maintaining carpenter bee populations in areas cultivated with passion fruit since the pollen is foraged from elsewhere on the broader landscape, and this resource is limiting the population growth of bee species. Our data

have applications in habitat management to make them more friendly for pollinators. Our recommendation for improving *X. grisescens* abundance, and thus the pollination rate of this crop in the Brazilian semiarid region, is that farmers ensure that the orchard surroundings have plant species that function as pollen sources for carpenter bees, as well as ensure the availability of nectar sources at times of low passion fruit flowering.

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Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Availability of data and material/ Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by JSC, CMLA, MCD, FARS, PMV and WMA. The first draft of the manuscript was written by JSC and CMLA, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Suppplementary Material

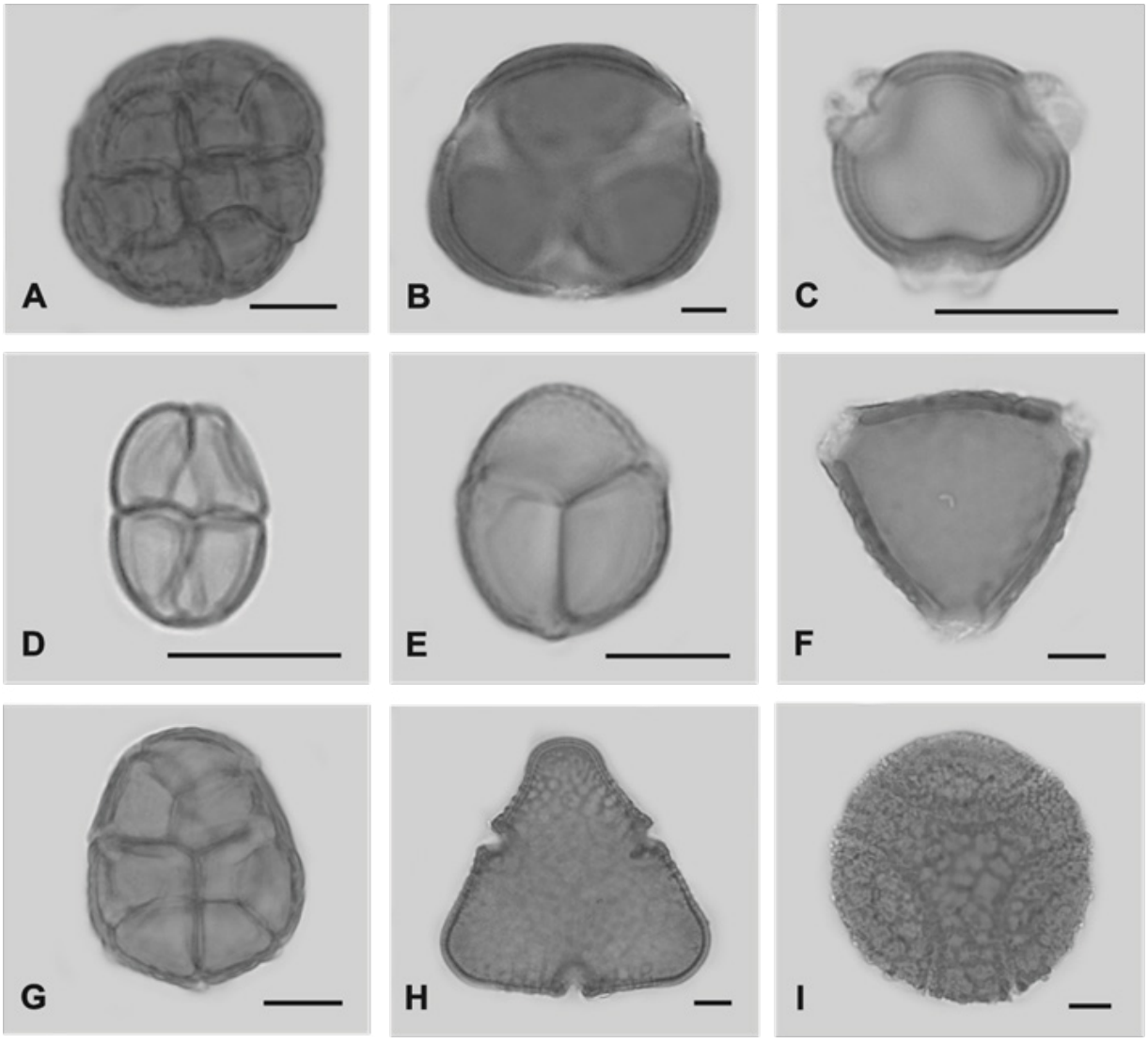

Fig S1. Pollen types reached a total relative frequency of 10%. Fabaceae: A- *Anadenanthera colubrina;* B-*Chamaecrista;* C-*Machaerium hirtum;* D-*Mimosa arenosa;* E-*Mimosa misera;* F-*Phaseolus* type*;* G-*Piptadenia stipulacea;* Malvaceae: H-*Pseudobombax grandiflorum*; Passifloraceae: I- *Passiflora edulis* (scale = 10 m).