ECOLOGY, BEHAVIOR AND BIONOMICS

Floral Preferences and Climate Influence in Nectar and Pollen Foraging by *Melipona rufiventris* Lepeletier (Hymenoptera: Meliponini) in Ubatuba, São Paulo State, Brazil

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ABSTRACT - We describe the environment effects on the amount and quality of resources collected by *Melipona rufiventris* Lepeletier in the Atlantic Forest at Ubatuba city, São Paulo state, Brazil (44º 48'W, 23º 22'S). Bees carrying pollen and/or nectar were captured at nest entrances during 5 min every hour, from sunrise to sunset, once a month. Pollen loads were counted and saved for acetolysis. Nectar was collected, the volume was determined and the total dissolved solids were determined by refractometer. Air temperature, relative humidity and light intensity were also registered. The number of pollen loads reached its maximum value between 70% and 90% of relative humidity and 18 ºC and 23ºC; for nectar loads this range was broader, 50-90% and 20-30ºC. The number of pollen loads increased as relative humidity rose ($r_s = 0.401$; $P < 0.01$) and high temperatures had a strong negative influence on the number of pollen loads collected ($r_s = -0.228$; $P < 0.01$). The number of nectar loads positively correlated with temperature ($r_s = 0.244$; $P < 0.01$) and light intensity ($r_s = 0.414$; $P < 0.01$). The percentage of total dissolved solids (TDS) on nectar loads positively correlated with temperature and light intensity ($r_s = 0.361$; $P < 0.01$ and $r_s = 0.245$; $P < 0.01$), negatively correlated with relative humidity ($r_s = -0.629$; $P < 0.01$), and it increased along the day. Most nectar loads had TDS between 11% and 30%, with an average of 24.7%. The volume measures did not show any pattern. Important pollen sources were Sapindaceae, Anacardiaceae, Rubiaceae, Arecaceae, Solanaceae and Myrtaceae; nectar sources were Sapindaceae, Fabaceae, Rubiaceae, Arecaceae and Solanaceae.

KEY WORDS: Apidae, Atlantic Forest, environment influence, food resource, floral origin

Foraging behavior of bee species is related to resources abundance and to their local distribution, to species susceptibility to abiotic factors, their communication ability and to colony size (Sommeijer *et al* 1983, Ramalho *et al* 1985, 1989, Roubik 1989).

Stingless bees are generalists using resources of varied floral origin, which is essential for survival of their perennial colonies (Ramalho *et al* 1989, White *et al* 2001). Their foragers, however, exhibit floral constancy, and individuals usually restrict their visits to a type of flower during a certain foraging flight (Ramalho *et al* 1994, White *et al* 2001). This behavior makes these bees important potential pollinators in natural habitats and in agro-ecosystems (Roubik 1989).

Following this pattern, foragers of the genus *Melipona* usually carry pollen loads of a single floral origin and the species most visited by them belong to the plant families Myrtaceae, Melastomataceae and Solanaceae (Ramalho *et al* 1989, 1990, 1994, 2007, Wilms & Wiechers 1997). Myrtaceae is the most important plant family in tropical forests, both in number of individuals and in number of species (Mori *et al* 1983, Cesar & Monteiro 1995, Assis 1999). In the plain coast at State Park of the Serra do Mar, in Ubatuba, the most common plant families are Myrtaceae, Fabaceae, Rubiaceae, Euphorbiaceae, Lauraceae and Melastomataceae (Assis 1999).

Species of *Melipona* collect nectar loads with an average of 40% to 50% of sugar content, with lower concentrations being around 20% (Roubik & Buchmann 1984, Roubik *et al* 1995, Biesmeijer 1997). However, preferences in relation to concentration can vary, allowing the maintenance of more than one species in a same environment. To obtain larger metabolic earnings, bees should collect nectar with sugar concentrations of approximately 60% (Roubik & Buchmann 1984).

Stingless bees are among the most common visitors in the Atlantic Forest, but few studies have dealt with the resources they use (Ramalho *et al* 1989, Wilms *et al* 1996, Wilms & Wiechers 1997). According to Ramalho *et al* (1990), these bees are adapted to the seasonal rhythms of blooming and changes in the floral composition of the Neotropical habitats where they are found.

The aim of this study was to investigate the nectar and pollen sources used by *Melipona rufiventris* Lepeletier (Hymenoptera: Meliponini), in an area of Atlantic Forest, and to describe the influence of climate conditions (temperature, relative humidity and light intensity) in the amount and quality of these resources.

Material and Methods

The study was carried out in a forest area on the coastal plain of Praia da Fazenda, located on the north of Ubatuba city, São Paulo state, Brazil, approximately at 44°48'W and 23°22'S. This plain is formed by a mosaic of vegetation types, which include beaches, dunes, swamps, marsh forests and forest formations on coastal belts and mountains. The climate is tropical with rainfall over the whole year (Af), annual mean rainfall is 2,650 mm, mean temperature is 21.9ºC and relative humidity is always higher than 85% (Köppen 1948, Herrera *et al* 1997).

By June 2000, two colonies of *M. rufiventris* were placed 5 m apart in an area called low restinga forest (Cesar & Monteiro 1995), close to a mangrove swamp (Assis 1999). One of these colonies was removed from a fig tree, inside the study area (colony 1); the other was transferred from São Simão, São Paulo state (colony 2). Colony strength was evaluated according to their population and brood combs size, and number of food pots (Kleinert 2005), as strong (colony 1) and intermediate (colony 2).

Samples were taken once a month from July 2000 to June 2001. Observations began with sunrise (ca. 5:00 am) and lasted until sunset (ca. 6:30 pm), for about 13h to 14h, depending on the length of day.

Every hour, colonies entrances were blocked for 5 min, and returning bees were collected with an aspirator. Abdomen contents of nectar collecting bees were extracted through their mandibles with capillary tubes, while the abdomen was pressed back-ventrally (Roubik & Buchmann 1984). Nectar volume and total sugar concentration were measured (the latter with a pocket refractometer). Samples with less than 5% concentration were recorded as water.

One of the corbiculae loads from pollen collecting bees was removed and conditioned for subsequent acetolysis and determination of floral origin (Erdtman 1960, Vergeron 1964). The same was done with the pollen present on the body of nectar collecting bees. Pollen loads from both colonies were randomly taken for analyses.

Pollen types were identified through the reference collection of the Laboratório de Abelhas, Universidade de São Paulo. Frequency of pollen grains was estimated by counts of 1,000 grains in two slides (Vergeron 1964). For each plant species, monthly mean pollen percentages were calculated.

When using pollen counts as a parameter, it is usually assumed that each pollen type is represented by the same volume. However, grain size is very variable, even inside a same plant family, and a simple counting would not represent its real contribution in the bee diet (Biesmeijer *et al* 1992).

Thus, the relative importance of each pollen type as food source was estimated through the coefficient of volumetric correction by Tasei (1973), as suggested by Silveira (1991):

$$Qi = \frac{(\text{Mean diameter of the species } i)^3}{(\text{Mean diameter of the species } s)^3}$$

where species *s* is that with smaller mean diameter and *i* any of the species observed in the sample.

Mean diameters were calculated by measuring the equatorial and polar distances of about 10 pollen grains from each species (Silveira 1991). The value of Q for each species was multiplied by the total number of grains of the species observed in a month. The resulting value (Qn) was transformed in percentage (% Qn).

Immediately before each sampling, temperature and relative humidity were measured with a digital termohigrometer, kept close to colony 1, and light intensity was measured close to the same colony with a hand light meter.

The correlation between abiotic factors and amount and quality of resources collected by bees was evaluated through Spearman non-parametric test (Zar 1999).

Results

Bees collected more pollen loads between 18ºC and 23ºC and between 70% and 90% of relative humidity. Nectar loads were collected in great amount in wide temperature and relative humidity ranges (20-30ºC and 50-90%, respectively). The number of collected pollen loads decreased as temperature rose ($rs = -0.228$; $P < 0.01$) and increased with relative humidity ($rs = 0.401$; $P < 0.01$). The number of nectar loads presented positive correlation with temperature and relative humidity only in colony 1 ($rs = 0.244$; $P < 0.01$ and $rs = 0.155$; $P < 0.05$, respectively); in colony 2, there was no correlation ($rs = 0.138$ and $rs = 0.067$). However, in both colonies, there were positive correlations between the number of nectar loads and light intensity ($rs_1 = 0.377$; $P < 0.01$ and $rs_2 = 0.456$; $P < 0.01$).

Concentration of total solutes in nectar presented positive correlation with temperature and light intensity ($rs = 0.361$; $P < 0.01$ and $rs = 0.245$; $P < 0.01$, respectively) and it increased slightly along the day (Fig 1). Relative humidity presented strong negative correlation with total solutes concentration ($rs = -0.629$; $P < 0.01$). *Melipona rufiventris* collected more frequently nectar loads with concentration between 11% and 30%, with average 24.7% (Fig 2). The amount of collected water was, proportionally, small.

The volume of the nectar load did not correlate with abiotic factors ($rs = -0.026$, to temperature; $rs = 0.001$, to relative humidity and; $rs = 0.014$, to ligth intensity) (Fig 3 - demonstrates how the volume varied with no specific pattern, mainly in colony 1).

The analysis of pollen loads showed that plant species increased or reduced their importance gradually along the months (*Online Supplementary Material 1*). From the 165 slides, 143 came from practically unifloral pollen loads, with the predominant grain species representing about 90% to

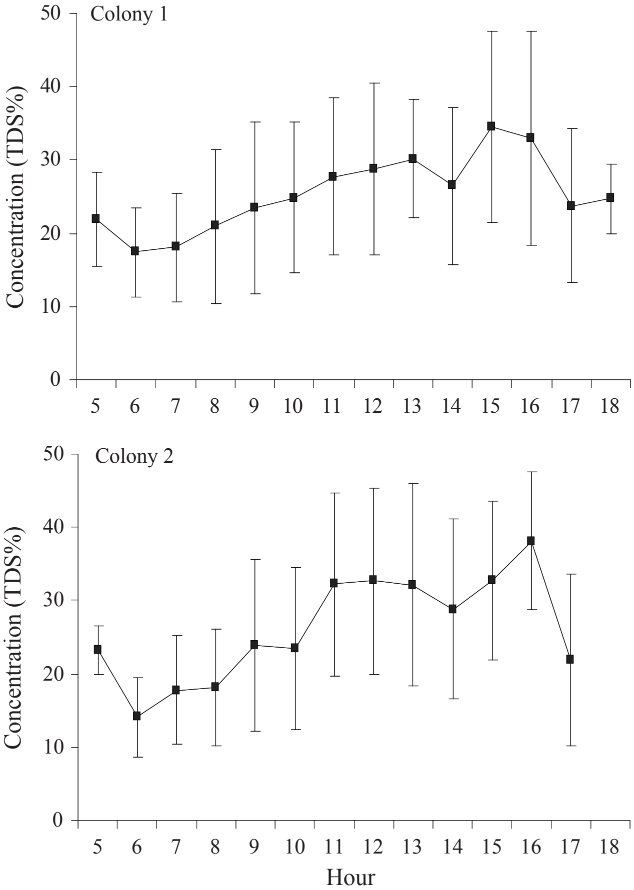

Fig 1 Variation along the day in sugar concentration (% STD) collected by *Melipona rufiventris* in Atlantic Forest, Ubatuba, SP, Brazil. Data represent mean ± SD.

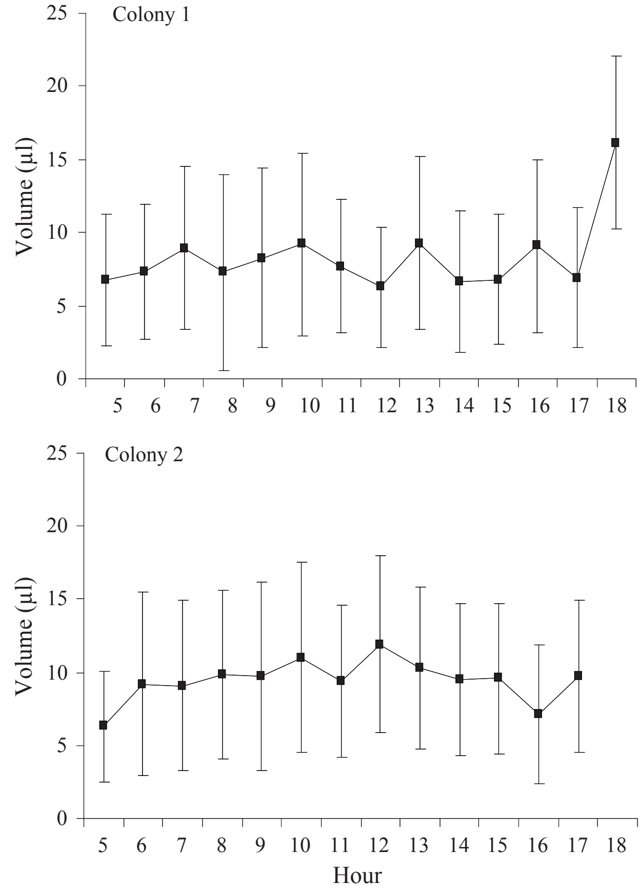

Fig 3 Variation along the day of nectar loads volume collected by *Melipona rufiventris,* in Atlantic Forest, Ubatuba (SP) Brazil. Data represent mean ± SD.

100%. There was no pollen collection in May/2001.

According to foraging frequency, the most important plant families were Sapindaceae, Myrtaceae, Anacardiaceae, Arecaceae, Solanaceae, Fabaceae and Melastomataceae. Myrtaceae was important from October to December, Arecaceae in September and October, Anacardiaceae and Melastomataceae in July and August. Sapindaceae was important from February to June, Solanaceae in January and March and Fabaceae in September and February (*Online Supplementary Material 1*). A great number of pollen types remained unidentified due to the small reference collection from areas of Atlantic Forest.

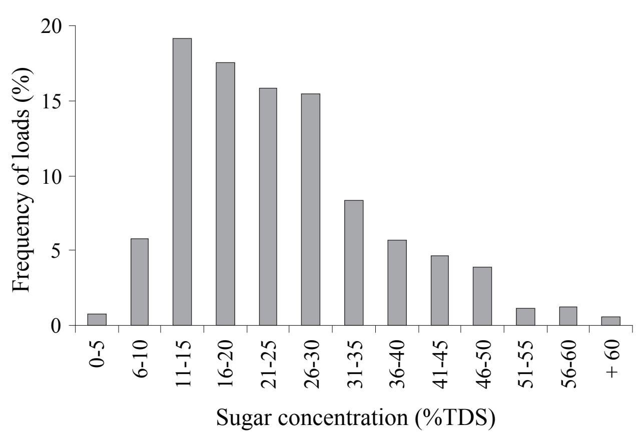

Fig 2 Sugar concentration (% STD) of nectar loads collected by returning workers of *Melipona rufiventris*, in Atlantic Forest, Ubatuba (SP) Brazil.

After volumetric correction, nearly the same plant families in changing order (Sapindaceae, Anacardiaceae, Rubiaceae, Arecaceae, Solanaceae and Myrtaceae) were the most important pollen sources for the bees. Their importance as food sources for bees varied along the study period (*Online Supplementary Material 2*). In spite of receiving frequent visits from bees, plant families with relatively small pollen diameters, like Melastomataceae and Fabaceae, were not important as food sources (p.ex. see *Mimosa bimucronata* at Feb/2001).

Few pollen grains were found in samples of only one colony, as *Lonchocarpus guileminianus, Schinus terebinthifolius*, Sapindaceae sp2, Rubiaceae sp1, not identified species nº 18 and nº 37 (Colony 1), and *Connarus* sp, *Alchornea* sp1 and not identified species nº 27 (colony 2).

Most pollen loads were collected between 5:00 am and 11:00 am. Exceptions were *Euterpe edulis*, *Trema micrantha,* and an unidentified species nº 3, collected between 4:00 pm and 5:00 pm, and Solanaceae sp1, collected along the whole day (6:00 am to 6:00 pm).

Allophylus petiolatus (Sapindaceae), *Cupania* sp. (Sapindaceae), *Schizolobium parahyba* (Fabaceae), *Palicourea* sp.(Rubiaceae), *Euterpe edulis* (Arecaceae), Sapindaceae sp2, Solanaceae sp1, Rubiaceae sp1 and sp2, and not identified species nº 27 and nº 38 were dominant in samples collected to determine the floral origin of the nectar.

Discussion

Several authors have observed an increase of nectar concentration collected by bees along the day, but the relationship among nectar loads concentration, temperature and relative humidity was not always taken into account (Corbet *et al* 1979, Roubik & Buchmann 1984, de Bruijn *et al* 1991, Roubik *et al* 1995, Biesmeijer 1997). Biesmeijer *et al* (1999b) observed that temperature can explain part of the variation of sugar concentration from nectar collected by bees, noticing that in dry environments the nectar produced by the main sources of food for bees was more concentrated.

As noted previously (Roubik & Buchman 1984), all bees should prefer nectar with sugar concentration around 60% because of the larger caloric earnings, in spite of high viscosity. Even when bees collected nectar with sugar concentration as high as or higher than this, the average nectar concentration was between 40% and 50%, and the lowest concentrations were around 20% (Roubik & Buchmann 1984, Roubik *et al* 1995). According to Roubik *et al* (1995), stingless bee foragers tend to use all nectar concentrations, even as low as 5% or as high as 67%. Great part of the nectar loads collected by *M. rufiventris* foragers presented values between 11% and 30%, as observed for other species of the same genus in Central Panama (Roubik & Buchmann 1984) and Costa Rica (Biesmeijer *et al* 1999b).

Several factors can affect sugar concentration in nectar collected by bees, such as the distance between flowers and the nest, flower availability and morphology. Colony state, competition between foragers, its recruitment ability and nestmates disposition in accepting nectar are also important (Heinrich 1975, Waddington 1980, Nuñez 1982, Roubik & Buchmann 1984, Biesmeijer *et al* 1999a). Certain *Melipona* species may be regarded as specialists in nectar with high sugar concentration, while others collect nectar of lower and varied concentrations (Roubik & Buchmann 1984, Roubik *et al* 1995, Biesmeijer *et al* 1999a, b). These patterns affect species occurrence, turning some of them mutually excluding.

Sugar concentration in nectar changes from low to high along the day. This pattern is evident in the loads collected by foragers of *M. rufiventris* and in most species studied by Roubik & Buchmann (1984). However, this species concentrated its activity in the morning, when temperature was relatively low and humidity was high. The peak of nectar collection occurred between 9:25 am and 9:55 am, while that of pollen collection was around 7:30 am. At this time of day, nectar produced by flowers has a larger chance of not being exhausted, presenting therefore lower concentrations.

Wilms & Wiechers (1997) observed in Brazilian Atlantic Forest pollen foraging from Myrtaceae and Melastomataceae flowers before 10:00 am. These authors noticed a predominance of pollen from these families also in honey samples from *Melipona,* in Boracéia, SP.

Melipona species that do not concentrate their foraging activities in early morning tend to collect larger amounts of pollen and nectar in the afternoon, when nectar concentration is higher (Roubik & Buchmann 1984, Biesmeijer 1997).

As described for *Melipona rufiventris*, Ramalho *et al* (1994) also observed pollen collection from a single source by *Melipona quadrifasciata* Lepeletier and *M. scutellaris* Latreille. According to them, floral constancy is related to foraging efficiency and depends on the diversity and dispersion patterns of the available flowers and on the ability of bees to recognize pollen of a certain morphology.

Wilms *et al* (1996) studying several eusocial bee species in Boracéia observed that Asteraceae and Myrtaceae were their main food sources, and that *Melipona bicolor* Lepeletier*, M. quadrifasciata* and *M. rufiventris* shared the same trophic niche among them, but not with the other bees. Several species of *Melipona* are frequent visitors of Myrtaceae (Absy & Kerr 1977, Absy *et al* 1980, Sommeijer *et al* 1983, Falcão *et al* 1988, 1992, Guibu *et al* 1988, Imperatriz-Fonseca *et al* 1989). Myrtaceae and Arecaceae families are frequently visited by *Melipona* species*,* as well as by other bees, while Mimosaceae, Melastomataceae and Solanaceae are mostly visited by *Melipona* than by other species (Ramalho *et al* 1989, 1990, 2007).

Regarding frequency, *M. rufiventris* seems to have floral preferences similar to other species of the same genus and to individuals of its own species observed in other areas. On the other hand, the importance of Melastomataceae family was lower than observed by Ramalho *et al* (1989, 1990). These results match the absence of observations of bees of this species in flowers of Melastomataceae at the study area, which indicates a low frequency of visits restricted to a short period of the year.

After volumetric correction, food resources offered by important plant families to *Melipona* species were smaller than expected. Myrtaceae, in spite of being frequently visited, had a relative contribution around 20% or less due to the small volume of their grains Melastomataceae and Fabaceae also contributed very little to the diet of *M. rufiventris*. On the other hand, Anacardiaceae (*Schinus terebinthifolius*), Rubiaceae and, in lower intensity, Sapindaceae and Solanaceae, presented an increased contribution after volumetric correction, highlightening the importance of using this correction method.

According to Roubik (1989), the flowering periodicity in tropical forest seems to prevent the extreme dependence of a bee in relation to a floral species. Data on *M. rufiventris* show that it visits dominant species in open and in regeneration areas as well as those that are characteristic from restinga areas and from those close to the hillsides. This flexible behavior not only enhances colonies survival in this changing environment, but also the reproduction and consequent preservation of some of the plant species capable to attract them.

Future studies approaching foraging patterns of native bees and their relation with the floral community and climate patterns should be useful to assess the conservation status of communities. These results can help in planning and monitoring actions of conservation and restoration at different scales.

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Online Supplementary Material 1

Floral resources from pollen loads of *Melipona rufiventris*, in Atlantic Forest at Ubatuba, SP, Brazil. Data refer to the mean percentage of each species observed in 9 to 24 slides.

Families/species	Months											
	Jul/00	A	S	O	N	D	Jan/01	F	M	A	M	J
Anacardiaceae												
Alchornea sp1						8.29						
Alchornea sp2							0.04	4.22	8.40			
Schinus terebinthifolius	46.15	41.62	0.04	0.18		0.007						
Arecaeae												
Euterpe edulis	14.00		32.98	59.24	0.81	1.83						
Asteraceae												
Vernonia sp.	0.008	6.54		0.01	0.007	0.007						
sp1	0.06	0.68	0.02	0.12	0.006				0.35			
sp2												0.008
Cecropiaceae												
Cecropia sp.	0.015	0.97										
Connaraceae												
Connarus sp.					15.84							
Fabaceae												
Mimosa bimucronata	0.07				0.16			50.01				
Lonchocarpus guileminianus			25.16									
Schizolobium parahyba					4.26	0.13						
Melastomataceae												
Tibouchina pulchra								0.34				8.32
sp1		49.88	0.007	0.02	0.07							
Myrtaceae												
Gomidesia schaueriana				16.46			5.29	3.86	0.02	3.53		1.07
sp1	4.62	0.21	16.61	0.12		33.73						
sp2					55.01	19.04						
Rubiaceae												
Palicourea sp.						16.50						
sp1						7.59						
sp2												13.94
Sapindaceae												
Allophylus petiolatus	0.11		0.01		0.007	0.02	0.03	18.94	48.38	86.70		0.64
Cupania oblongifolia												48.76
Serjania sp.								18.60	0.025	8.21		
sp1	0.025											
sp2					14.02							

Continue

Families/species	Months											
	Jul/00	A	S	O	N	D	Jan/01	F	M	A	M	J
Solanaceae												
sp1							90.27		42.83	0.45		
Sterculiaceae												
Dombeya sp.							4.37			0.01		
Ulmaceae												
Trema micrantha	0.008		8.34									
Not identified 1	0.02		0.008									
Not identified 3	34.72	0.02	0.18	0.17		0.08						
Not identified 4	0.08	0.04										
Not identified 5	0.04	0.02				0.04						
Not identified 6	0.01											
Not identified 8		0.008										
Not identified 9		0.008										
Not identified 11	0.01											
Not identified 12	0.008											
Not identified 14			0.025									
Not identified 15				23.62	0.006	0.08						
Not identified 16				0.04								
Not identified 17				0.03								
Not identified 18				0.008								
Not identified 19			8.24		0.008							
Not identified 20					0.008							
Not identified 22					9.77							
Not identified 27						12.66						
Not identified 29												0.34
Not identified 31												1.91
Not identified 32												0.008
Not identified 35									0.05			
Not identified 36									0.016			
Not identified 37								0.02		0.03		
Not identified 38										0.90		
Not identified 39										0.14		
Not identified 40										0.02		
Not identified 41								0.02				
Not identified 42								4.00				
Number of slides	12	12	12	12	12	12	23	24	12	24	0	9

Fidalgo A O, Kleinert A M P (2010) Floral Preferences and Climate Influence in Nectar and Pollen Foraging by *Melipona rufiventris* Lepeletier (Hymenoptera: Meliponini) in Ubatuba, São Paulo State, Brazil. Neotrop Entomol 39(6): 879-884.

Online Supplementary Material 2

Floral resources from pollen loads of *Melipona rufiventris*, in Atlantic Forest at Ubatuba, SP, Brazil. Data refer to the Qn percentage of each species observed in a total from 9 to 24 slides. Qn = volumetric corretion.

Families/species	Months											
	Jul/00	A	S	O	N	D	Jan/01	F	M	A	M	J
Anacardiaceae												
Alchornea sp1						2.40						
Alchornea sp2							0.02	5.99	5.00			
Schinus terebinthifolius	81.19	90.48	0.16	0.37		0.05						
Arecaceae												
Euterpe edulis	15.57		53.58	67.87	3.01	1.95						
Asteraceae												
Vernonia sp.	0.02	1.25		0.05	0.13	0.06						
sp1	0.02	0.06	0.04	0.11	0.02				0.01			
sp2												0.01
Cecropiaceae												
Cecropia sp.	0.0004	0.002										
Connaraceae												
Connarus sp.					21.38							
Fabaceae												
Mimosa bimucronata	0.0003				0.01			6.23				
Lonchocarpus guileminianus			12.06									
Schizolobium parahyba					8.16	0.01						
Melastomataceae												
Tibouchina pulchra								0.22				2.11
sp1		8.15	0.003	0.005	0.09							
Myrtaceae												
Gomidesia schaueriana				5.18			1.74	0.16	0.01	2.52		0.49
sp1	0.51	0.02	4.49	0.02		13.41						
sp2					28.01	3.42						
Rubiaceae												
Palicourea sp.						55.29						
sp1						21.10						
sp2												89.26
Sapindaceae												
Allophylus petiolatus	0.05		0.01		0.02	0.02	0.01	59.84	39.58	81.26		0.06
Cupania oblongifolia												6.72
Serjania sp.								23.74	0.04	15.37		
sp1	?											
sp2					32.94							

Continue

Families/species	Months											
	Jul/00	A	S	O	N	D	Jan/01	F	M	A	M	J
Solanaceae												
sp1							98.06		55.08	0.20		
Sterculiaceae												
Dombeya sp.							0.16			0.21		
Ulmaceae												
Trema micrantha	0.01		18.46									
Not identified 1	0.001		0.001									
Not identified 3	2.61	0.001	0.03	0.01		0.01						
Not identified 4	0.01	0.004										
Not identified 5	0.001	0.001				0.004						
Not identified 6	0.003											
Not identified 8		0.002										
Not identified 9		0.02										
Not identified 11	0.004											
Not identified 12	0.004											
Not identified 14			0.07									
Not identified 15				26.31	0.05	0.19						
Not identified 16				0.04								
Not identified 17				0.03								
Not identified 18				0.004								
Not identified 19			11.10		0.04							
Not identified 20					?							
Not identified 22					6.16							
Not identified 27						2.03						
Not identified 29												0.06
Not identified 31												1.27
Not identified 32												0.02
Not identified 35									0.20			
Not identified 36									0.10			
Not identified 37								0.005		0.02		
Not identified 38										0.33		
Not identified 39										0.04		
Not identified 40										0.05		
Not identified 41								0.002				
Not identified 42								3.82				
Number of slides	12	12	12	12	12	12	23	24	12	24	0	9