An Acad Bras Cienc (2025) 97(1): e20240521 DOI 10.1590/0001-3765202520240521
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ECOSYSTEMS

Efficiency of pantraps for monitoring bees diversity in Brazilian acerola orchards: the role of color diversity

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Abstract: Bee monitoring characterizes the local fauna and determines conservation measures. The study evaluated the influence of pantrap color on bee attraction and the difference in seasonal patterns of these insects. Collections were carried out in commercial plantations of *Malpighia emarginata* in the Northeast region of Brazil, using yellow, blue and white pantraps. A total of 1,449 bee specimens belonging to 59 species, four subfamilies of Apidae, 18 tribes and 31 genera were captured, with emphasis on the subfamily Apinae (89.6%). Blue pantraps captured 66.5% of the total sampled specimens, followed by white (19.6%) and yellow (13.9%). *Melitomella grisescens* (29.3%) was the most abundant species, followed by *Apis mellifera* (10.3%), *Melitoma segmentaria* (10.1%), *Ptilothrix plumata* (9.6%) and *Melitoma ipomoearum* (6.8%). With the exception of A. mellifera, all the most abundant species belonged to the Emphorini tribe (56%). Regarding the Centridini tribe, pantraps were efficient in collecting species, but not individuals. The months of September and November/2019 were the months when the lowest numbers of insects were collected and the lowest rainfall rates were recorded. In this sense, understanding new methodologies becomes essential to identify the diversity of pollinators for the construction of management and conservation plans.

Key words: agriculture, Apidae, biodiversity, bee sampling, tropical dry forest, West Indian cherry.

INTRODUCTION

Pollination by biotic agents is responsible for 70% of global food production, with an estimated annual economic value of these services provided by insects of around € 153 billion (Gallai et al. 2009, Klein et al. 2007). It is estimated that the yearly economic harm due to the loss of pollinators is on the order of US$ 10.5 billion, reaching US$ 334.1 billion in globally (Bauer & Wing 2010). Pollinator-dependent crops account for approximately 5–8% of current global agricultural production, with an annual market value of US$235 billion to US$577 billion (2015 US dollars1) worldwide, and is directly attributable to animal pollination (IPBES 2016). Thus, increasing agricultural production and reducing food insecurity depend on the conservation and management of the services provided by pollinators.

In agroecosystems, the occurrence of pollination deficit has been reported in different crops and is associated with failures in the flow of pollen, either in quantity or in the insufficient number of pollinator visits received per flower (Vaissière et al. 2009). *Malpighia emarginata* DC (Malpighiaceae) is an example of this situation, because although it is self-compatible, the species depends on bees from the Centridini tribe for good fruiting (Oliveira et al. 2013). Known in English variously as the Antilles cherry, Barbados cherry, West Indian cherry or Guarani cherry, and acerola in Brazilian Portuguese, this species is an important tropical species that produces fruits with high nutritional content and concentration of ascorbic acid (vitamin C) (Bezerra et al. 2017, Vilhena & Augusto 2007).

Brazil is considered the largest producer, consumer and exporter of acerola in the world, with about 46,000 tons/year, and the Northeast region of the country, where this study was carried out, is responsible for 82% of this production (Cavichioli et al. 2014, IBGE 2017). Most of this region is occupied by the Caatinga Biome, which presents marked seasonality, with two well-defined seasons throughout the year (dry and rainy), contributing to the phenology of *Malpighia emarginata* and the attractiveness of the areas. In cultivations of *M. emarginata*, Siqueira et al. (2011), in a semi-arid area, observed that even in similar management conditions and areas, differences in the fruiting rate throughout the year were observed. In this sense, Aguiar et al. (2013) stated that fluctuations in the abundance of floral resources in the caatinga affect the structure of the guild of bees that use these floral resources.

In the context of the global decline of pollination services to pollination, actions aimed at the management and conservation of these agents have been prioritized, so there is demand for consistent information that can be compared in different environments (Ekroos et al. 2014, Garibaldi et al. 2013). Collection using colored dish traps (pantraps) is one of the methods used for these studies because it is reliable, efficient, inexpensive, suitable for long-term studies and useful for comparisons by eliminating bias of the collector (Droege et al. 2010, Prado et al. 2017).

These traps can sample insects from a variety of habitats and different heights (at ground level, above ground level and at plant height), and can be used both in agricultural areas and in natural environments, in addition to complementing other active research methods (Nuttman et al. 2011). According to Gollan et al. (2011), the effectiveness of pantraps is attributed to the combination of different colors to sample the global biodiversity of insects, with the use of white, yellow and/or blue being ideal for sampling bees. Additionally, they are effective in assessing species abundance. and richness of diverse floral visitor communities (Abrahamczyk et al. 2010, Wilson et al. 2008).

The combination of trap colors has its functionality explained because they imitate the color of flowers and, therefore, are effective in collecting bees (Wilson et al. 2016). Campbell & Hanula (2007) mentioned that the color blue has the shortest wavelength used, which may explain why hymenoptera generally prefer pantraps of this color in most locations where this methodology was used. Gumbert (2000) found that both inexperienced and experienced bees show a particular preference for wavelengths between 400 and 420 nm (violet and dark blue). Dyer et al. (2016) observed a significant preference for the “white” and “blue” wavelength, with a higher preference for the wavelength for the blue spectrum in studies with *Tetragonula carbonaria*. These innate preferences promote the ability of pollinators to find flowers. It is worth remembering that there was an evolution in blue flowers with greater rewards bringing benefits to attract native pollinators.

Interestingly, Acharya et al. (2021) stated that the visual spectrum of bees and the measurement of light reflectance can explain the differences in bee capture rates. However, for Dyer & Chittka (2004) related that bees almost immediately learn to visit only target flowers that offer rewards, because they have large concentrations to detect flowers. These results demonstrate that even though the reflectance of the traps is not measured, as in this work, this visual recognition of bees can be responsible for successful capture in pantraps. Insects answer colors differently in environmental contexts and also within taxonomic group criteria, and the success of specific colors may vary between regions and habitats (Saunders & Luck 2013).

These taxonomic group criteria and the capture of specific genera by pantrap were also selected by Lorandi et al. (2023) in organic and conventional agroecosystems, in which the four most abundant subfamilies were Apinae, Halictinae, Andreninae and Megachilinae. Boyer et al. (2020) in studies with alfalfa (*Medicago sativa*), stated that pantraps were better at capturing bees of the genera Halictus and Augochlorella.

In the Northeast region of Brazil, studies using pantraps have been carried out in crops of apple (Moreira et al. 2016), cashew (Andrade 2014) and melon (Silva et al. 2013), but there are no studies of this type for acerola. The relationship with color association was also verified in other crops and in different regions. According to Saunders & Luck (2013), there was a greater preference for white pantraps in almond orchards (*Prunus dulcis*) that have white flowers. In the canola (*Brassica Napus* L.) area, Jaques et al. (2023) yellow pantraps were predominant, considering that the perception of insects' colors can be compromised by the confusion between the flower and the pantraps. In studies with alfalfa (*Medicago sativa*) Boyer et al. (2020) stated that there was a small difference between blue and white coloration in bee capture.Thus, the present survey using pantraps to sample pollinators of *Malpigia emarginata* is unprecedented. We hypothesize that blue traps will capture more bees due to their similarity to preferred floral cues, while white and yellow traps will capture fewer. Our main objective was to document the abundance and richness of bee species and their relationship with the colors of traps in acerola orchards, aiming to answer the following questions: (1) How does the use of different colored pantraps (blue, white, and yellow) affect the diversity and abundance of bees captured in acerola orchards? (2) Is there a difference in the composition of the sampled insect groups (subfamilies, tribes and species) among the three colors of pantraps? (3) Is there a difference in the seasonal patterns of insects sampled among the three pantrap colors?

MATERIAL AND METHODS

Study area and sites

The study was carried out in the most arid zone of the Northeast region of Brazil, in four agricultural areas with commercial plantations of *Malpighia emarginata* (Figure 1). Two were located in the municipality of Petrolina, state of Pernambuco, in the Senador Nilo Coelho Irrigation Project, with sprinkler irrigation. The first (Area PE-1, coord. 40°60'57 W; 9°34'19 S, size of 12 hectares) had 8.0 ha cultivated with *M. emarginata*, cultivars BRS Sertaneja, Junko, Costa Rica and Nikki. The second (Area PE-2, coord. 40°62'67 W; 9°33'18 S, size of 6 hectares) had 2.5 ha cultivated with cultivars BRS Sertaneja, Junko and Costa Rica. The second two areas were located in the municipality of Juazeiro, state of Bahia, in the Mandacaru Irrigation Project, with acerola orchards of the BRS Sertaneja and Junko cultivars throughout the area and microsprinkler irrigation. The first area (Area BA-1, coord. 40°40'03 W; 9°39'33 S) had 6 hectares and the second (Area BA-2, coord. 40°41'90 W; 9°39'86 S) had 5 hectares.

According to the Köppen classification, the region's climate is BSwh', that is, dry, xerophytic vegetation (Caatinga or Tropical Dry Forest), with dry winters and average temperature in the coldest month above 18 °C. In the last 20 years, the average annual rainfall in the region

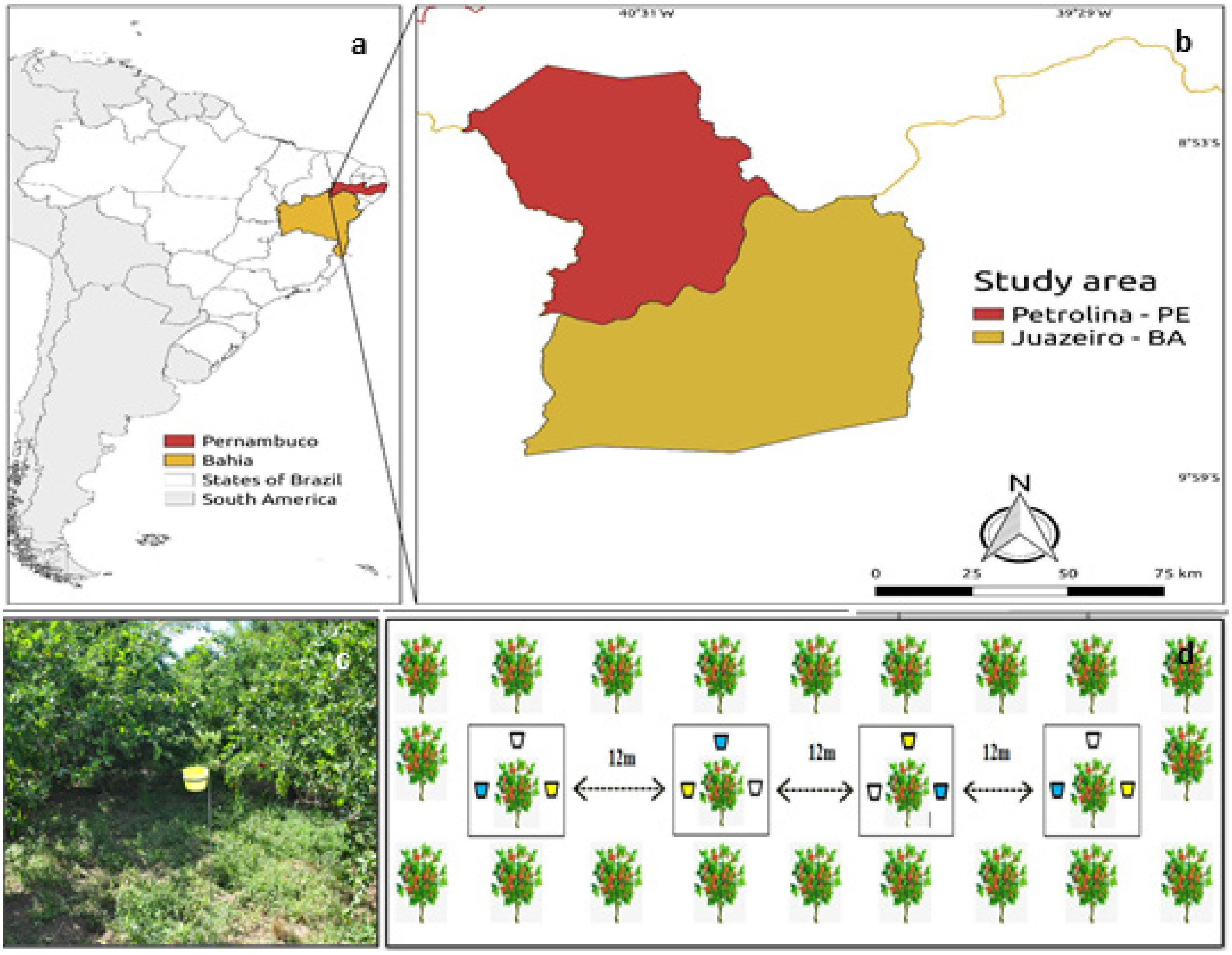

Figure 1. Location and methodology of the present study. a- map of Brazil with emphasis on the Northeast region (gray) and study site (red); b- detail of the municipalities of Petrolina, state of Pernambuco, and Juazeiro, state of Bahia, c- positioning of the pantraps in the field, fixed on a support 1m above the ground; d- layout of blocks with pantraps used for sampling insects.

was 529 mm, with the period from November to April being considered the rainy season (Teixeira 2010).

Bee sampling methods

To monitor the diversity of floral visitors for pollination of acerola plants using pantraps, we used a method adapted from FAO (2016), Halinski et al. (2018) and Shrestha et al. (2019). Collections were carried out monthly, from May 2019 to May 2020, in the four study areas, totaling 156 hours of sampling effort. The pantraps consisted of white plastic containers measuring 13.5 cm in diameter x 9 cm in height, painted internally with UV spray paint in yellow (Colorgin® Luminous 756), blue (Colorgin® Luminous 757) and white (original color).

The pans were filled with 650 ml of water and four drops of detergent to break the surface tension of the waterand were fixed to a metal support, placed about 1 m above the ground (Figure 1c), following the methodology of Nuttman et al. (2011) and Tuell & Isaacs (2009). According to the authors, the traps are most effective when placed at the level of the flowers, in the middle of the canopy, or at a height of one-third of the height of the plant. In the orchards

d

evaluated, the acerola trees were presented at three meters high, with the traps positioned at one-third of this height.

A set of three traps (one of each color) was placed at a distance of 2.5 m from a plant forming an equilateral triangle of 3.60 m on each side, to ensure independence between the samples from the containers (Droege et al. 2010). Eight sets were positioned linearly at a distance of 12 m from each other (Figure 1d), making a total of 24 pantraps per area. In each set, the position of the colors of the pantraps at the vertices of the triangles were alternated, to sample the range of microhabitats and the environmental heterogeneity of each agricultural system. The traps were always placed in the morning and removed the next morning, remaining in the field for 24 hours. The collected specimens were transferred to plastic bags containing 70% alcohol, packed in plastic organizer boxes for transport to the laboratory (Droege et al. 2010, Krug & Alves-Dos-Santos 2008).

The insects captured in the pantraps were taken to the Ecology Laboratory of Brazilian Agricultural Research Corporation (Embrapa Tropical Semi-Arid) and underwent a screening process to separate the bees from other types of insects. The bees were pinned, assembled and dried in an oven, later identified and labeled by origin, with individual and sequential numbering for spreadsheet purposes. Identifications were made down to the lowest possible taxonomic level, with the aid of taxonomic keys. The specimens were then separated into morphospecies and compared with reference material from the Entomological Collection of the Departmento de Sistemática e Ecologia da Universidade Federal da Paraíba (Gonçalves & Brandão 2008, Krug & Alves-Dos-Santos 2008). The collected specimens were deposited in the collection of the Laboratório de Ecologia da Embrapa Semiárido and in the entomological collection of the Departmento de Sistemática e Ecologia da Universidade Federal da Paraíba. The other insects are stored in 70% alcohol in the collection of Laboratório de Ecologia da Embrapa Semiárido.

Statistical analysis

To assess efficiency, capture per unit of effort (CPUE = number of bees/pantrap-day) was calculated by dividing the total number of bees captured by the number of traps X the number of days (Prado et al. 2017). All other statistical analyses were performed using the statistical package R version 4.2.1 (R Development Core Team 2022). To test data normality and assess whether the analyses would be parametric or nonparametric, the Shapiro-Wilk test was used. The analysis of abundance, average species richness and group of bees obtained by each pantrap color, seasonality and proportion of individuals collected by color throughout the year, were compared using the Friedman test (Friedman chi-squared), followed by a post hoc (Chi-square test) contained in one of the R base packages, with significance level $\alpha = 0.05$), along with the Wilcoxon to compare paired samples to identify the source of treatment difference. Average species accumulation curves (1000 randomizations) and nonparametric richness estimator (Jackknife 2) were performed in the statistical package R version 3.4.0 (R Development Core Team 2022). The analysis and graphical representation (plotted) were performed using the vegan package of the R program (Oksanen et al. 2017). Finally, the boxplots were built using the ggplot2 package of R (Wickham & Chang 2016).

RESULTS

A total of 1,449 bee specimens were captured (CPUE=1.16 ± 0.79), belonging to 59 species, distributed in four subfamilies of Apidae, 17 tribes and 31 genera. Among the captured individuals, 89.7% belonged to the Apinae subfamily, with a greater contribution from the Emphorini tribe, responsible for 62.5% of the total specimens collected. The other subfamilies were distributed as 5.1% for Halictinae, 3.9% for Megachilinae and 1.3% for Andreninae. The most diverse genera were *Augochlora* (8 species) and *Centris* (6 species), followed by *Melissodes* and *Melissoptila*, with 4 species each. *Melitomella grisescens* (n = 426, 29.3%) was the most abundant species, followed by *Apis mellifera* (n = 149, 10.3%), *Melitoma segmentaria* (n = 147, 10.1%), *Ptilothrix plumata* (n = 139, 9.6%) and *Melitoma ipomoearum* (n = 99, 6.8%) (Table I).

Of the total sampled specimens, 66.5% were captured in blue pantraps, followed by white and yellow pantraps with 19.6% and 13.9%, respectively. It is noteworthy that twice the number of individuals were captured in blue pantraps (n=963; CPUE=0.77 ± 0.61) than yellow and white pantraps together (Table I). As for richness, 84.7% of the total species were captured in blue pantraps, followed by white pantraps with 64.4% (Table I). The lowest richness was registered in the yellow pantraps, with 44.1% (Table I).

Analysis of the values obtained for the three colors together showed significant differences both for mean abundance (Chi-squared = 8.7692, df = 2, P-value = 0.01247) and richness (Chi-squared = 9.5704, df = 2, P-value = 0.008352) (Figure 2). In the pairwise analysis, significant differences were found for abundance and same was observed for richness (Table II).

In general, specimens of the four subfamilies were captured in the pantraps of all three colors. However, for Apinae, Halictinae and Megachilinae, the largest number of individuals was captured in the blue traps, with values of 66.3%, 59.5% and 94.6%, respectively, differing significantly from the other traps. In the subfamily Andreninae, the yellow pantraps captured the highest number of individuals (42.1%), although this did not differ significantly from the white pantrap (Table I).

At the level of tribes, 52.6% were sampled in traps with all three colors, and for Emphorini, Lithurgini, Eucerini, Augochlorini and Centridini there was a preference of the bees for the blue pantraps. Epicharitini and Ericrocidini were captured exclusively in blue traps, while Protepeolini and Megachilini were found exclusively in white traps, with no tribes found exclusively in yellow traps (Table I). In pairwise analyses (chi-square test) for the most abundant tribes (n≥ 40 individuals), significant differences were found when comparing the three colors (Table III).

At the species level, the blue traps captured 15 unique species (25.4% of the total), while the yellow and white pantraps captured two (3.4%) and eight unique species (13.6%), respectively (Figure 3a). These unique species can be considered indicators, since they were associated with a certain trap color. Among the indicator species of blue pantraps, 11 were from the Apinae subfamily, indicating that this trap color is fundamental for sampling representatives of this group. Thus, the results obtained indicate that 42.4% of the collected bee species were recorded in only one of the trap colors. The data also showed that 30.5% of the total species (n=18) were collected by pantraps of all three colors. There was no observation of specimens collected jointly by the yellow and white pantraps (Figure 3a).

Among the species considered most abundant (n ≥ 99 individuals), *Melitoma segmentaria* (Fabricius, 1804) (χ^2 = 5.19 , P = 0.5191), *Melitoma ipomoearum* Ducke 1912 (χ^2 = 8.610 , P = 0.1967), *Melitomella grisescens* (Ducke, 1907) (χ^2 = 5.29 , P = 0.5069) and *Ptilothrix plumata* Smith, 1853 (χ^2 = 3.74, P = 0.7115) presented preference

Table I. Subfamilies, tribes and species of Apidae, with their respective abundance and richness, sampled by different sets of pantraps in commercial orchards of *Malpighia emarginata* in the Northeast region of Brazil. The colored markers (🟡 = yellow, 🔵 = blue and ● = white) highlight the tribes and species solely collected in each trap color. Significant differences are represented by lowercase letters according to the chi-square test.

Subfamilies/ Tribes	Scientific name	Pantrap Color				
		Yellow	Blue	White	Total	%
ANDRENINAE		8b	5a	6b	19	1.3
Calliopsini	*Acamptopoeum prinii* (Holmberg, 1884)	6	3	3	12	0.8
	Callonychium (Callonychium) brasiliense (Ducke, 1907) 🔵	0	1	0	1	0.1
Protomeliturgini	*Protomeliturga turnerae* (Ducke, 1907)	2	1	3	6	0.4
APINAE		186b	861a	252b	1299	89.7
Apini	*Apis mellifera* Linnaeus, 1758	52b	47a	50ab	149	10.3
Centridini	*Centris (Hemisiella) trigonoides* Lepeletier, 1841🔵	0	2	0	2	0.1
	Centris (Centris) aenea Lepeletier, 1841🔵	0	4	0	4	0.3
	Centris (Centris) caxiensis Ducke, 1907🔵	0	1	0	1	0.1
	Centris (Hemisiella) tarsata, Smith 1874	1	2	0	3	0.2
	Centris (Heterocentris) analis, Fabricius, 1804	1	8	0	9	0.6
	Centris (Trachina) fuscata Lepeletier, 1841🔵	0	2	0	2	0.1
Emphorini	*Ancyloscelis apiformis* (Fabricius, 1793)	39	11	26	76	5.3
	Diadasina riparia (Duck, 1907)	9	7	2	18	1.2
	Melitoma ipomoearum, Ducke 1912	2b	76a	21c	99	6.8
	Melitoma segmentaria (Fabricius, 1804)	6b	140a	1b	147	10.1
	Melitomella grisescens (Ducke,1907)	16b	341a	69b	426	29.4
	Ptilothrix plumata Smith, 1853	22b	97a	20b	139	9.6
Epicharitini🔵	*Epicharis (Epicharis) bicolor* Smith, 1854 🔵	0	1	0	1	0.1
Ericrocidini🔵	*Mesoplia rufipes* (Perty, 1833) 🔵	0	1	0	1	0.1
Eucerini	*Florilegus* Robertson, 1900 sp.1🔵	0	1	0	1	0.1
	Melissodes Latreille, 1829 sp.1 🔵	0	11	0	11	0.8
	Melissodes Latreille, 1829 sp.2	0	17	1	18	1.2
	Melissodes Latreille, 1829 sp.3	2	4	3	9	0.6
	Melissodes Latreille, 1829 sp.4 🔵	0	1	0	1	0.1
	Melissoptila Holmberg, 1884 sp.1	0	3	1	4	0.3
	Melissoptila Holmberg, 1884 sp.2 ●	0	0	1	1	0.1
	Melissoptila Holmberg, 1884 sp.3	1	5	1	7	0.5
	Melissoptila Holmberg, 1884 sp.4 ●	0	0	1	1	0.1
Euglossini	*Euglossa (Euglossa) cordata* (Linnaeus, 1758)	0	4	1	5	0.3
	Eulaema (Apeulaema) nigrita, Lepeletier, 1841 🔵	0	1	0	1	0.1
Exomalopsini	*Exomalopsis (Exomalopsis) analis* Spinola, 1853	9	17	10	36	2.5
	Exomalopsis (Exomalopsis) auropilosa Spinola, 1853	19	24	22	65	4.5

Table I. Continuation.

	Exomalopsis Spinola, 1853 sp.1	1	5	0	6	0.4
Meliponini	*Partamona* Schwarz, 1939 sp.	1	2	4	7	0.5
	Trigona fuscipennis Friese, 1900 🟡	1	0	0	1	0.1
	Trigona spinipes (Fabricius, 1793)	1	2	1	4	0.3
Protepeolini●	*Leiopodus* Smith, 1854 sp. ●	0	0	1	1	0.1
Xylocopini	*Ceratina* Latreille, 1802 sp.1●	2	3	5	10	0.7
	Ceratina (Crewella) maculifrons Smith, 1854	1	20	10	31	2.1
	Ceratina Latreille, 1802 sp.2 ●	0	0	1	1	0.1
	Xylocopa (Neoxylocopa) grisescens Lepeletier, 1841 🔵	0	1	0	1	0.1
HALICTINAE		6b	44a	24c	74	5.1
Augochlorini	*Augochlora (Augochlora)* Smith, 1853 🟡	1	0	0	1	0.1
	Augochlora (Oxystoglossella) modica Lepeco e Gonçalves, 2020	3	11	6	20	1.4
	Augochlora (Oxystoglossella) morrae Strand, 1910 ●	0	0	1	1	0.1
	Augochlora (Oxystoglossella) tenax Lepeco e Gonçalves, 2020 ●	0	0	1	1	0.1
	Augochlora Smith, 1853 sp.2	0	2	1	3	0.2
	Augochlora Smith, 1853 sp.3	0	5	1	6	0.4
	Augochlora Smith, 1853 sp.4 🔵	0	1	0	1	0.1
	Augochlora Smith, 1853 sp.5	1	2	0	3	0.2
	Augochloropsis Cockerell, 1897, sp.1	0	3	2	5	0.3
	Augochloropsis Cockerell, 1897, sp.2🔵	0	1	0	1	0.1
	Pseudaugochlora pandora Smith, 1853	0	4	2	6	0.4
	Pseudaugochlora Michener, 1954 sp1	1	1	0	2	0.1
Halictini	*Dialictus opacus* (Moure, 1940)	0	5	4	9	0.6
	Dialictus Robertson, 1902 sp.1	0	2	2	4	0.3
	Ceblurgus longipalpis Urban e Moure, 1993	0	7	4	11	0.8
MEGACHILINAE		2b	53a	2b	57	3,9
Lithurgini	*Lithurgus huberi* Ducke, 1907	2	51	0	53	3.6
	Microthurge friesei (Ducke, 1907) 🔵	0	2	0	2	0.1
Megachilini●	*Megachile (Chrysosarus)* (Mitchell, 1943) sp. ●	0	0	1	1	0.1
Unidentified●	Unidentified●	0	0	1	1	0.1
	ABUNDANCE	202	963	284	1449	100
	%	13.9	66.5	19.6	100	
	CPUE	0.16±0.08	0.77±0.61	0.23±0.15	1.16±0.79	
	RICHNESS	26	50	38	59	
	%	44.1	84.7	64.4	100	

for blue pantraps, with the difference being significant by the chi-square test. We stress that 95% of the individuals of the first species were collected in the blue traps, and only 4% in the yellow ones. With regard to the other three species, the blue traps captured between 70% and 80% of the specimens, Only *A. mellifera* did not have a preference, since it was equally found in traps of all three colors (Table I).

In relation to the Centridini tribe, whose species are considered the main pollinators of *M. emarginata*, the pantraps were not very efficient in the collection of individuals (n=21). This result was expected, since *M. emarginata* is pollinated by species that collect floral oils, such as Centridini. However, the traps captured individuals of six species, which can be considered efficient to sample the richness of the group.

With regard to the collection of bees by trap color, the group also preferred blue pantraps, with *Centris* (Hemisiella) *trigonoides* Lepeletier, 1841, *Centris* (Centris) *aenea* Lepeletier, 1841, *Centris* (Centris) *caxiensis* Ducke, 1907 and *Centris* (Trachina) *fuscata* Lepeletier, 1841 being captured exclusively in traps of this color (Table I).

The analysis of the sampling effort throughout the year showed that in February 2020 (n=287), shortly after the beginning of the rainy season, 19.8% of the total number of bees were captured in the pantraps, regardless of color, followed by May 2020 (n=198), when 13.7% of the total number of insects were captured. In general, the results showed fluctuation

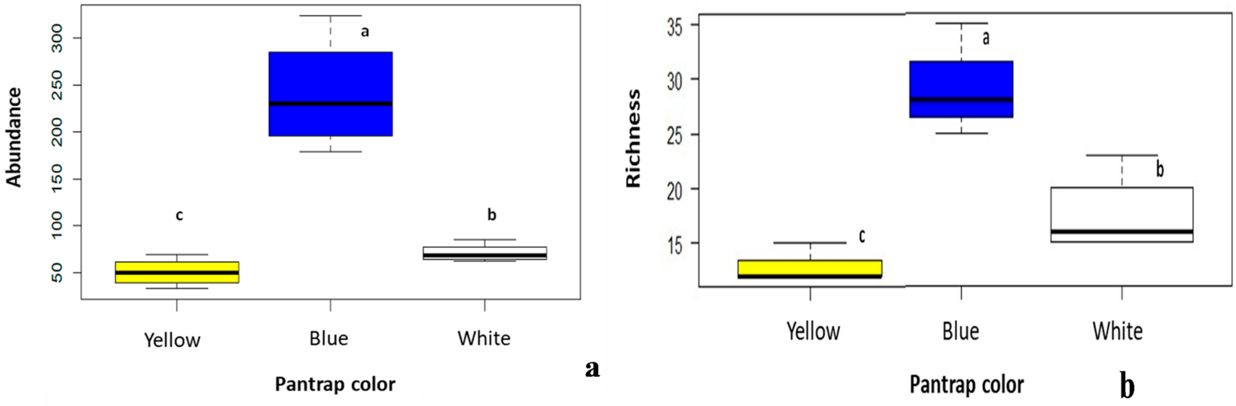

Figure 2. Comparison of average abundance (a) and richness (b) of bees in commercial orchards of *Malpighia emarginata* in Northeast Brazil. Significant differences are represented by lowercase letters, according to the Friedman test.

Table II. **Results of the chi-square test comparing pairwise the abundance and richness of species obtained by the pantraps nuclei, in acerola cultivation, in the Petrolina-PE/Juazeiro-BA Pole.**

Pairwise comparison	Abundance		Richness	
	χ^2	P-value	χ^2	P-value
Blue x Yellow	2.5787	0.4612	5.6709	0.01725
Blue x White	6.4590	0.0913	5.4634	0.01942
White x Yellow	5.0284	0.1697	4.5132	0.03364

throughout the year, with September and November 2019 being the months in which the lowest number of insects were collected and the lowest rainfall rates were recorded. As for the proportion of individuals collected by color throughout the year, the analyses (Friedman test) indicated variation, with the values found for February (χ_r^2= 7.5; df=2; P=0.003) differing significantly from the other months according to the chi-square test (Figure 3b).

Analyzing the monthly abundance by color indicated that the blue pantraps captured a greater number of individuals in all months except November 2019, when it was surpassed by the white pantrap, and in December 2019, when both captured similar numbers of insects (n=15 specimens, each). Regarding the white and yellow pantraps, there was alternation of prevalence during the months, and only in February 2020 was the quantity captured by the white pantraps (n=60 specimens) higher than that of the yellow pantraps (n=15 insects). In the other months, the amounts recorded in the pantraps of these two colors were similar (Figure 3b).

Considering the three colors together (Figure 4a), the method used was able to capture 65% of the expected species in the studied areas. Analysis of this parameter by pantrap color revealed that the curves also did not reach stability and showed only small difference between the sample designs represented, with the blue pantraps capturing 83% of the expected species (Figure 4c), while for yellow and white, these values were 60% and 54.5%, respectively (Figure 4b and 4d).

DISCUSSION

The decline in pollinator populations has made the monitoring of these agents, especially bees, increasingly important. Therefore, efforts have been made to standardize the methodology used, aiming at comparison in different environments. Among these methods, the use of pantraps has been highlighted for being considered efficient,

Table III- Results of the chi-square test comparing pairwise the abundance of the most abundant tribes obtained by the pantraps nuclei, in acerola cultivation, in the Petrolina-PE/Juazeiro-BA Pole. The numbers in bold indicate important differences.

Tribe	Pairwise comparison					
	Blue x Yellow		Blue x White		Yellow x White	
	χ^2	P-value	χ^2	P-value	χ^2	P-value
Emphorini	5.451	0.1416	3.956	0.2663	9.890	0.0195
Apini	6.405	0.0936	2.272	0.5180	5.428	0.1430
Exomalopsini	1.068	0.7848	4.301	0.2307	2.281	0.5161
Ancyloscelidini	8.282	0.0405	6.684	0.0827	3.882	0.2744
Lithurgini	1.708	0.6352				
Eucerini	1.554	0.6698	3.444	0.3281		
Augochlorini	5.400	0.1447	3.084	0.3788	2.313	0.51
Xylocopini	5.850	0.1191	6.644	0.08416		

reliable and easy to apply. Our results indicate that different trap colors influence the diversity and abundance of captured bees, with blue traps being the most effective. The color of traps can attract different species of bees and other pollinators, influencing both the diversity and the number of individuals captured.

In this study, the pantraps were efficient in monitoring bees captured in *Malpighia emarginata* orchards in the Northeast region of Brazil. The values obtained (1449 specimens of 59 species) are in the ranges of mean abundance (n= 1225 to 1829) and species richness (n= 42 to 66) of most studies using pantraps in agroecosystems (Almeida et al. 2019, Boyer et al. 2020, Castro et al. 2017, Moreira et al. 2016, Silva 2015, Silva et al. 2013) and in native areas (Abrahamczyk et al. 2010, Gollan et al. 2011). At the subfamily level, the results for abundance and richness showed significant presence of Apinae representatives, similar to what has been reported in other studies carried out in agroecosystems using the same method (Landaverde-González et al. 2017, Lorandi et al. 2023, Moreira et al. 2016). The other subfamilies contributed with values lower than 6%, similar to studies carried out with other crops (Thompson et al. 2020).

Among the captured individuals, 62.5% belonged to species of the Emphorini tribe, which can be explained by the habit of females to collect water to soften the earth during the construction of their nests on the ground. The abundance of *Apis mellifera* can also be explained by the habit of collecting water to cool their nests, particularly in the semiarid climate of the region (Michener 2007).

In general, blue traps were more efficient in capturing bees pollinating *M. emarginata*, with much higher values for both species abundance and richness. In Brazil's Northeast region, a similar results were reported in surveys carried out with pantraps in areas cultivated with *Cucumis melo* L. – Cucurbitaceae (Silva et al. 2013), *Malus pumila* Mill. – Rosaceae (Moreira et al. 2016), *Anacardium occidentale* L. (Andrade 2014), and *Gossypium hirsutum* L. – Malvaceae (Cruz & Freitas 2013). Abundance and richness studies carried out with the same methodology in agroecosystems in other countries have also produced similar results in surveys carried out in apple orchards (Joshi et al. 2015), almond orchards (Saunders & Luck 2013) and soybean fields (Clair et al. 2020).

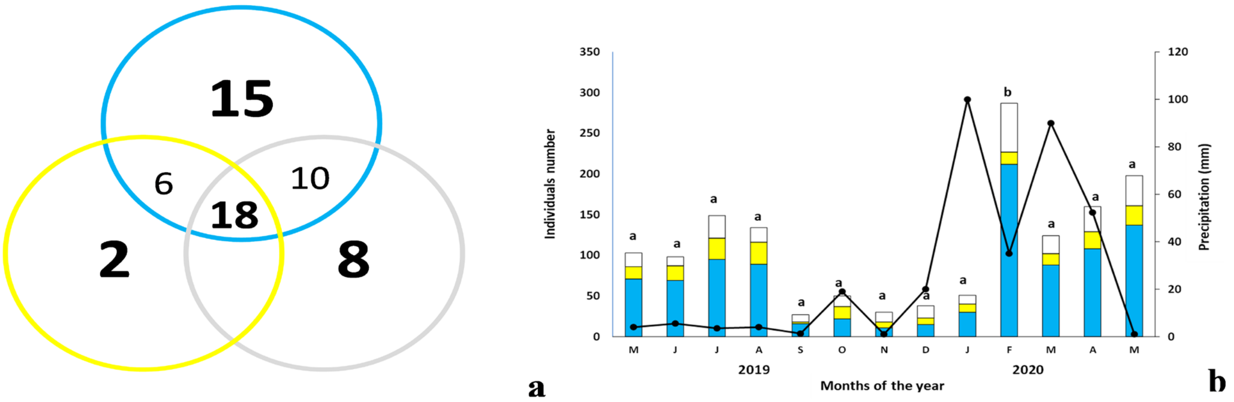

Figure 3. Bee diversity and seasonality in collections carried out with pantraps, from May 2019 to May 2020, in commercial orchards of *Malpighia emarginata*, in the Northeast region of Brazil. a- Venn diagram showing the number and proportion of morphospecies collected by blue, yellow and white traps; b- Monthly number of bee specimens collected in pantraps (bar) and graph of monthly accumulated precipitation (dashed line).

Several hypotheses have been raised to explain the preference for colors, from the relationship with the colors of the flowers visited, including their floral availability in the environment and type of habitat (Campbell & Hanula 2007, Dyer et al. 2016, Gumbert 2000, Gumbert & Kunze 2001) and ability to discern shorter wavelengths of light, up to the sociality level of the species (Sircom et al. 2018). Therefore, the preferred colors of blue and white in this study may be related to the bees' memory of the acerola flowers planted in these areas, which are of the Junko variety (pink/lilac color) and the sertaneja variety (white color), which which would explain the second highest capture observed in the white trap, contradicting the results of other studies with pantraps that claim that white traps are inferior to yellow and blue traps (Dec & Mouga 2014), explaining this correlation between flowers and trap color. However, as mentioned previously and although this is a reasonable speculation, the visual cues of flowers and how bees' preferences for flower color influenced trap choice were not directly tested in the current study.

According to Campbell & Hanula (2007), blue has the lowest wavelength among the three colors that are generally used in evaluations with pantraps (blue = 465 to 485 nm, white = 820 to 920 nm and yellow = 560 to 590 nm). This can explain the preference of bees for blue pantraps in most places where this technique has been used. Acharya et al. (2021) reported that blue traps have higher light reflectance, in the range of 300-500 nm, compared to the other colors, which probably plays an important role in attracting different species of bees to this color.

Studies into the innate and acquired recognition of bees in relation to colors have generated several articles with different species of bees. With *Bombus terrestrials*, Gumbert (2000) stated that both experienced

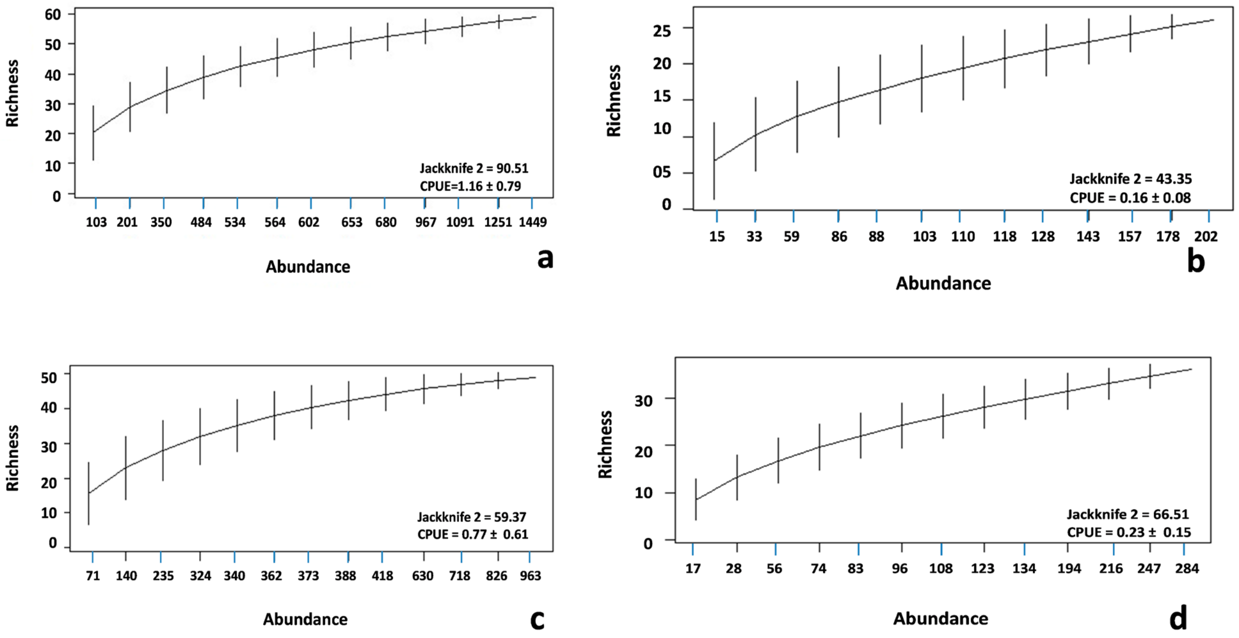

Figure 4. **Average accumulation curves of bee species collected with the three colors of pantraps (a) and in yellow (b), blue (c) and white (d) traps, in commercial orchards of acerola (*Malpighia emarginata*), in the Northeast region of Brazil (1000 randomizations). Vertical bars represent the standard deviation. Estimated species richness (Jackknife 2) is shown within each graph. CPUE = mean capture per unit effort (bees/pantrap-day) ± standard deviation.**

and inexperienced bees showed a particular preference for wavelengths between 400 and 420 nm (Violet and dark blue) and that the first foraging is maintained, and cannot be erased by other cumulative experiences with other colors. In studies with *Tetragonula carbonaria*, Dyer et al. (2016) observed a significant preference for the "blue" wavelength, followed by the "white" wavelength. In articles with *Chelostoma rapunculi* (Lepeletier 1841), Milet-Pinheiro et al. (2012) stated that both experienced and inexperienced foraging bees have an innate preference for the floral cues of their host plants. Furthermore, visual cues from plants were more attractive than olfactory cues to find their preferred flowers in experienced foraging bees. These innate preferences promote pollinators' ability to find flowers. suggesting that an evolution has occurred in blue flowers with greater rewards promoting benefits to attract native pollinators.

Bees' color vision can be tuned to relatively small spectral differences, showing that color vision is at least in part dependent on experience and behavioral plasticity, which plays an important role in how bees exploit color information (Reser et al. 2012).

In observations with *Apis mellifera*, Niggebrügge et al. (2009) stated that visual learning and discrimination are influenced by the quality of a stimulus, systematically varying the chromatic and achromatic properties of the stimuli, and that faster discrimination learning of color was correlated with reduced color similarity between stimuli. This "learning" and recognition of floral resources was studied by Bloch et al. (2001) who stated that in *Apis mellifera* it is possible that social and developmental factors influence expression in cells in different regions of the brain. Therefore, at birth, bees do not have a fully functional circadian system, limiting the foraging of these honey bees in the first days of life. This maturational factor is perhaps important in structuring the age-related division of labor, a fundamental characteristic in the organization of insect societies. However, although previous research suggests that bees have innate preferences for the blue spectrum, the present study only tested capture rates for different trap colors, and not the underlying cognitive mechanisms that influence bee behavior toward pantrap colors.

In general, greater capture has been observed in blue pantraps in open areas and in yellow pantraps in forested areas (Abrahamczyk et al. 2010, Gonçalves & Oliveira 2013). Abrahamczyk et al. (2010) verified an increase in the efficiency of blue traps with the reduction of canopy cover, while with the yellow traps the opposite occurred. They hypothesized that yellow traps may have stood out more due to the contrast to the environment with low lighting. On the other hand, Jaques et al. (2023) observed greater capture in yellow traps in canola and blueberry plantations, highlighting the influence of the type of crop on the composition of sampled species. In the present study, all areas sampled were open and located in irrigated acerola orchards in a semiarid region. Thus, the greater capture of bees in the blue traps must have been caused mainly by the environmental characteristics of the areas, including the bee communities and plant species present and characteristics of the sampled plant culture. Among the most abundant species, the results showed that the pattern held for most bees. With the exception of *Apis melifera*, which was equally sampled in the three colors, most species showed a marked preference for blue pantraps, with this preference being very pronounced in the four species of Emphorini (*Melitoma segmentaria, Melitomella grisescens, Melitoma ipomoearum* and *Ptilothrix plumata*). In Brazil, *M. segmentaria* was also the most abundant species captured pantraps in areas cultivated with *Solanum lycopersicum* L. - (Silva 2015) and *Anacardium occidentale* L. (Andrade 2014), with Solanaceae also registering its preference for blue traps. *Melitomella grisescens* was found in traps of all three colors in a survey carried out with *Gossypium hirsutum* L. – Malvaceae (Cruz & Freitas 2013). However, they observed a slight preference for blue pantraps, different from our observation. In a study carried out in an areas cultivated with *Malus pumila* Mill. – Rosaceae in the same region of the present study, Moreira et al. (2016) reported the almost exclusive presence of specimens of this genus in blue pantraps, agreeing with observations made for *M. emarginata*. However, the use of only blue pantraps would not be enough to sample the diversity of insects visiting *M. emarginata* plants The results showed that although the blue traps captured the highest number of unique species (25.4% of the total), some bee species were captured only in the white (13.6%) and yellow (3.4%) pantraps. Thus, the complementarity of the colors was fundamental to portray the composition of the apifauna of the acerola orchards. In surveys carried out in prairies, Geroff et al. (2014) reported that blue pantraps collected the highest species abundance and richness and that the yellow ones had the lowest quantities. The authors also mentioned the presence of indicator species that were associated with a certain trap color, with the greatest richness of these species being associated with blue pantraps, followed by white and yellow ones, similar to the results obtained here.

Regarding the species of bees from the Centridini tribe, the main pollinator of acerola trees, the pantraps proved to be inefficient in collecting individuals (n=21 species), although it was efficient in recording richness. The literature reports that the use of pantraps alone may not accurately estimate the pollinator fauna in a given area, so it is advisable to combine it with other collection methods (Cane et al. 2000). In the same region as the present study, in a survey of the diversity of bees in areas cultivated with *M. emarginata*, using collection with the aid of an entomological net to capture visitors on the plants or in flight near the cultivated areas and species nesting in trap nests, Kiill et al. (2023) recorded 11 species of bees, of which 10 were of the genus Centris, confirming the importance of this group as pollinators of the trees. Comparison of those results with those obtained here revealed that the traps captured 60% of the species, with *Centris* (Trachina) *perforator* Smith 1874); *Centris* (Melacentris) *obsoleta* Lepeletier, 1841; *Centris* (Ptilotopus) *maranhensis* Ducke, 1910; *Centris* (Paracentris) *xanthomelaena* Moure & Castro, 2001; and *Centris* (Ptilotopus) *sponsa* Smith, 1854 not being captured using pantraps. In general, most of these species can be considered large, similar to *Xylocopa* (Neoxylocopa) *grisescens*, which in the present study was represented by a single specimen, indicating that these traps are possibly not efficient for bees of this size. The same was reported by Gonzalez et al. (2020), who stated that large bees were rare, with a single individual of *Xylocopa violacea* (L.) (Apidae, Xylocopini) being captured, not reflecting the abundance of this bee in the area, which was often found throughout the day foraging.

Of the total Centridini species captured, 71% were found exclusively in the blue traps, with none of them being recorded in the white traps. Since the flowers of *M. emarginata* of the BRS Sertaneja variety are white, we expected that pantraps of this color could be more attractive to these bees, which did not occur. Goes et al. (2021) reported a similar finding and suggested that this result could be associated with the competition of pantraps with the flowers of the crop. Thompson et al. (2020) reported that the highest proportion of solitary species was found in pantraps positioned outside the cultivated area, indicating possible competition with the flowers to attract these bees.

Although pantraps were efficient in sampling Centridini species richness, they may not be sufficient to fully capture representatives of this group, especially large-bodied floral oil-collecting bees. Thus, future surveys should consider combining this type of sampling with other active collection methods (e.g., nets, direct observation) to better portray the presence and contribution of these pollinators.

Regarding the monthly abundance by color, the blue pantraps captured the greatest number of specimens throughout the year, and there was a close relationship between climate factors and the quantity collected by the traps, indicating marked seasonality. Here it is worth mentioning that the areas studied are located in the Northeast region of Brazil, which is characterized by semiarid climate conditions, typical of dry forests (Caatinga), with two well-defined seasons (dry and rainy) throughout the year. These conditions directly influence the phenology of the plants and the reproductive cycle of the bees, which have adapted to these seasonal patterns. Although the *M. emarginata* orchards are irrigated, the plants are influenced by local climate conditions, with the intensified flowering during the rainy season. Siqueira et al. (2011), in observations made in acerola cultivations in this region, reported differences in bee activity, and consequently in the fruiting rate in observations made at the end of the dry season and beginning of the rainy season, in agreement with the results reported here. Aguiar et al. (2013) commented that the structure of the guild of bees using floral resources in the Caatinga biome can be affected by fluctuations in the abundance of floral resources used by bees, similar to our results. In monitoring with trap nests, Melo & Zanella (2012) reported that the highest abundance of occupied nests was recorded in the rainy season, attributed to the greater availability of resources necessary for the development and nesting of bees.

Regarding the species accumulation curves and the nonparametric richness estimator (Jackknife 2), the absence of asymptotes in the curves was recorded both in the analysis of the three colors of the pantraps together, and in the analysis of the colors separately, indicating that the sampling carried out was not sufficient to record the totality of species expected in *M. emarginata* orchards. Other studies carried out in Brazil (Krug et al. 2021, Moreira et al. 2016) also have reported that this pattern occurs with other collection methods, and must be related to the high diversity of these environments. On the other hand, the fact that the curves for each of the trap colors showed the same pattern provided greater security to the analysis of the efficiency presented by the three pantrap colors.

CONCLUSIONS

The results presented in this study reinforce the efficiency of using pantraps to sample Apidae bees. The color of the traps influenced the attraction of bees, with blue pantraps being more attractive (first question). However, the combination of the three colors was essential to increase the richness of sampled species by almost 20% (second question). There was variation in capture throughout the year, probably related to precipitation (third question). Finally, we found that the use of this method in acerola orchards allows the diagnosis of potential floral visitors present in the area and its surroundings. However, by sampling specific groups, such as bees from the Centridini tribe, it is necessary to use other collection methods, such as entomological nets, to learn about the fauna and pollinators (Cane et al. 2000), depending on the crop under study (Jaques et al. 2023). Thus, studies aimed at monitoring pollinator diversity in agroecosystems are essential to support the development of sustainability and productivity in agriculture, compatible with environmental services, seeking ecological intensification, strengthening and diversification of these systems (Ricketts et al. 2008, Potts et al. 2016).

Acknowledgments

A postgraduate scholarship was granted to the corresponding author by the Office to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank Ms. Jean Miguel Alves dos Santos (Universidade Federal da Paraiba - UFPB), Dr. Patrícia Luiza de Oliveira Rebouças (Universidade do Estado da Bahia - UNEB) and Dr. Vinina Silva Ferreira (Universidade Federal do Vale do São Francisco - UNIVASF), for their support in identifying the bees.

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How to cite

COELHO MS, KIILL LHP & MARTINS CF. 2025. Efficiency of pantraps for monitoring bees diversity in Brazilian acerola orchards: the role of color diversity. An Acad Bras Cienc 97: e20240521. DOI 10.1590/0001-3765202520240521.

Manuscript received on May 18, 2024;
accepted for publication on January 6, 2025

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MARCIA S. COELHO: was responsible for the study design, methodology development, data collection, practical experiments and data analysis, produced the article's writing and final review of the text. CELSO F. MARTINS: collaborated in the discussion of the results and critical review of the manuscript, mainly in the literature analysis and structuring of the article, and also helped with the grammatical review and preparation of graphs. LÚCIA GELENA P. KIILL: was responsible for the preparation of the project, study design and methodology, contributed to the statistical analysis of the data and helped in the interpretation of the results, especially in comparison with previous studies, participated in the writing of the results and conclusion sections.