



RESEARCH ARTICLE - ANTS

Characterizing the Use of Botanical Resources from Pollen Loads from *Apis mellifera*, and Stingless Bees' Pot-pollen Palynology: An Approach Using Network Analysis and Generalized Linear Models

LEANDRA SCHUASTZ BREDA^{1,4}, JOSÉ ELTON DE MELO NASCIMENTO², VAGNER DE ALENCAR ARNAUT DE TOLEDO²
VANDERLEI APARECIDO DE LIMA³, MARIA LURDES FELSNER^{3,4}

1 - Universidade Tecnológica Federal do Paraná – UTFPR/DV, Dois Vizinhos-PR, Brazil

2 - Universidade Estadual de Maringá – UEM, Maringá-PR, Brazil

3 - Universidade Tecnológica Federal do Paraná – UTFPR/PB, Pato Branco-PR, Brazil

4 - Universidade Estadual do Centro Oeste – UNICENTRO/CEDETEG, Guarapuava-PR, Brazil

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Corresponding author

Leandra Schuastz Breda 

Universidade Tecnológica Federal do Paraná – UTFPR/DV

Estrada p/ Boa Esperança, Km 04 -

Zona Rural, CEP: 85660-000. Dois

Vizinhos, Paraná, Brasil.

E-Mail: leandrasbreda@gmail.com

Abstract

Pollen loads gathered by honeybees and pot-pollen stored by stingless bees are functional foods with commercial potential. The botanical origin influences the physicochemical composition of the corbicular pollen, *Apis mellifera*'s bee bread, and the stingless bees' pot-pollen processed in their nests. Thus, it is vital to determine the factors affecting the botanical composition of these apicultural products. As no prior research on the subject has been conducted, this study examines seasonality and bee species' impact on the phytogeographic source of honeybee pollen loads and stingless bees' pot-pollen through the use of palynological analysis, Network Analysis, and Generalized Linear Models (GLM). Due to seasonal variations and abiotic factors, *Melipona quadrifasciata quadrifasciata*, *Melipona marginata*, and *Scaptotrigona bipunctata* stingless bees produced monofloral pot-pollen from plant species within the Myrtaceae, Euphorbiaceae, and Fabaceae families. In contrast, *A. mellifera* and *Tetragona clavipes* bees exhibited a more generalist pollen foraging behavior, resulting in heterofloral pollen loads or pot-pollen in most seasons studied. The GLM results indicate that factors including 'bee species' and 'season' impacted the volume and varieties of pollen gathered by honeybees and stingless bees. It is vital to acknowledge the production area's characteristics, such as climatic conditions, bee-friendly plants, flower types and quantity, and the behavioral patterns of the bee species regarding pollen foraging. These results highlight the significance of establishing best practices for producing monofloral and heterofloral pollen loads, or pot-pollen. This information may be utilized for commercial applications of *A. mellifera* pollen loads and stingless bee pot-pollen.

Introduction

Bees gather pollen from diverse plant species, which they then agglutinate with salivary secretions and/or nectar to form pellets. These pellets are stored inside the nests. Pollen

collection is crucial in developing and maintaining bee colonies, as it is their primary protein source (Giampieri et al., 2022).

Bee pollen contains essential nutrients, including proteins, amino acids, vitamins, fatty acids, carbohydrates, fibers, minerals, sterols, terpenes, pigments, and polyphenols.



Due to its diverse composition, bee pollen has been acknowledged as a functional food. It consists of biologically active substances with antibacterial, antifungal, and antioxidant properties that can be utilized for therapeutic and prophylactic objectives. These properties have the potential to effectively prevent well-known diseases, including cancer, cardiovascular diseases, and diabetes (Carpes et al., 2008; Martins et al., 2011; Gupta et al., 2011; Morgano et al., 2012; Arruda et al., 2013; Chamorro et al., 2017; Bárbara et al., 2018; Caldas et al., 2019; Castiglioni et al., 2019; Thakur & Nanda, 2020; Giampieri et al., 2022).

Recently, researchers globally have shown interest in investigating the beneficial health properties displayed by bee pollen and their potential use as constituents in the pharmaceutical and food sectors. However, using this technology for commercial purposes remains challenging due to the substantial variability in its composition, influenced by factors such as the plants visited by bees, environmental conditions, plant nutrition, harvesting location, time of year, preservation techniques, and storage conditions (Martins et al., 2011; Gupta et al., 2011; Nascimento et al., 2019).

Several reports in the literature have focused on characterizing the botanical and geographic origins of pollen loads or pot-pollen to understand the variability of its chemical composition. This understanding has facilitated the development of practices for obtaining bee pollen from specific plant sources, called monofloral bee pollen. Clear and concise descriptions of these practices have been formulated for improved apiculture and meliponiculture. Successful beekeeping activities require beekeepers to be acquainted with the native plants in the area of production, the time of year during which the pollen is gathered, the specific type of bee involved, and the procedures followed after collection (Ramírez-Arriaga, 2016; Chamorro et al., 2017; Mailula, and Nofemela, 2017; Castiglioni et al., 2019; Brodschneider et al., 2019; Thakur, and Nanda, 2020). Based on this information, generating monofloral pollen loads or pot-pollen that possess more consistent nutritional and compositional traits is feasible, resulting in a more appealing product for industrial purposes.

Information about plant species grown in production regions and utilized by bees is crucial in characterizing this bee product (Novais et al., 2009). Brazil's climates and floral resources favor apiculture and meliponiculture development (Martins et al., 2011). Nevertheless, due to the region's extensive climatic and floral diversity, the results of carried-out studies remain insufficient for the rapid and accurate characterization of bee products produced in each region of Brazil. Studies conducted in Brazil's southern region have mainly focused on the characteristics of pollen loads produced in Rio Grande do Sul and Santa Catarina states (Barth et al., 2010). Studies conducted in Paraná State regarding the botanical and chemical properties of pollen loads or pot-pollen are insufficiently developed (Carpes et al., 2008). However, these studies have sparked researchers' curiosity to delve further into characterization studies to enhance the advancement of apiculture and meliponiculture in the region.

The literature indicates a growing focus on examining the impact of varying seasons or climatic conditions on the pollen composition of different bee species, including honeybees and stingless bees, in specific regions of Brazil and globally. This research has been conducted by various authors, including Dimou and Trasyvoulou (2007), Bilisik et al. (2008), Negrão et al. (2014), Aleixo et al. (2016), Mattos et al. (2018), Negrão and Orsi (2018), Hrcir et al. (2019), Nascimento et al. (2019), and Melo Nascimento (2020). Different bee species exhibit specific flower preferences during pollen collection. These preferences are influenced by various factors, including colony size, larval development in hive nests, inflorescence type of plant species, flower shape, color, and odor, as well as the distance from the floral source of the hive (Hilário & Imperatriz-Fonseca, 2009; Aleixo et al., 2016). The climate of the producing region can impact the pollen foraging of bees, affecting both these apicultural products' chemical and botanical composition.

Mathematical and statistical modeling techniques provide insights into the relationships and their evolution over time in a particular producing region. For this purpose, Network Analysis has become a prevalent mathematical modeling technique. It has been utilized in various fields of study to uncover research patterns in bibliographic data, characterize trends in medical procedures and pharmacological or public health studies, and investigate environmental concerns (Hevey, 2018; Martín-Sanz et al., 2022; Wang et al., 2023). Additionally, it has been applied in ecological research to comprehend the interconnections among diverse species in a given ecosystem (Landi et al., 2018; Lander, 2019; Guimarães Jr., 2020; Borchardt et al., 2021). This type of mathematical modeling enables the creation of diverse graphical models that provide more precise depictions of the interrelations among the study variables and facilitate the recognition of behavior patterns and dynamics of the variables (Landi et al., 2018; Guimarães Jr., 2020).

No research has been conducted on the botanical origin of *A. mellifera* pollen loads or stingless bee' pot-pollen across various seasons in a clearly defined region (Maringá City) in Paraná State, Brazil. Furthermore, no study has been undertaken to explore how bee species and seasons impact the botanical origin of bee pollen. Palynology was utilized to identify the contribution of plant species in the botanical composition of pollen loads of *A. mellifera* and pot-pollen produced by four species of stingless bees (*M. marginata*, *M. quadrifasciata quadrifasciata*, *S. bipunctata*, and *T. clavipes*). We employed Network Analysis and GLM to investigate the influence of the factors 'bee species' and 'season' on the botanical variability of pollen loads and pot-pollen.

Materials and Methods

Entomological identification of the bees

The entomological identification of the bees examined in this study (*Apis mellifera* Linnaeus, 1758; *Melipona*

(*Eomelipona marginata* Lepeletier, 1836; *Melipona (Melipona) quadrifasciata quadrifasciata* Lepeletier, 1836; *Scaptotrigona bipunctata* (Lepeletier, 1836), and *Tetragona clavipes* (Fabricius, 1804)) was conducted by researcher Favízia Freitas de Oliveira in 2019. These bee species were deposited at the Dynamic Interdisciplinary Museum of the State University of Maringá (UEM) in the Yoko Terada collection in Maringá City, Brazil.

Pollen loads and pot-pollen sampling

Five colonies were utilized to produce samples of *A. mellifera* pollen loads in Langstroth hives. A pollen collector was installed at the hive entrance two days every two weeks to collect pollen loads. At the end of the collection period, the pollen loads were gathered and homogenized to create a monthly sample by combining the two samples obtained during the month after cleaning.

Stingless bee pot-pollen samples were produced in Inpa hives and collected monthly from five colonies of four stingless bee species. The pollen pots of the nest were opened with a spatula, and approximately 10 g of pot-pollen (when available) were harvested from each colony monthly.

The fresh pollen samples were then cleaned to remove contamination from harvesting (dead bees, bee larvae, and propolis). Each pot-pollen or pollen load sample was collected in Falcon tubes, identified by bee species, colony, and collection month, and stored at -20 °C until palynological analysis.

A. mellifera pollen load samples (n = 55) and stingless bee pot-pollen samples (n = 42 for *M. marginata*; n = 44 for *M. quadrifasciata quadrifasciata*; n = 58 for *S. bipunctata*; n = 46 for *T. clavipes*) were collected during the period between June 2018, and May 2019, in the Beekeeping and Meliponiculture Sector, at the Experimental Farm of the State University of Maringá, Paraná, Brazil (coordinates 23°15'15", and 23°33'27" S, and 51°50'05", and 52°05'59" W). The climate of Maringá city, located in the Southern region of Brazil, is classified as *Cfa* (h), mesothermal humid subtropical, with a rainfall rate higher than 1,500 mm over the year.

Palynological Analysis

Palynological analysis was conducted to identify the botanical origin of pollen loads and pot-pollen samples. Before analysis, the samples were thawed and placed in a forced-air oven at 55 °C for 72 hours. Subsequently, the samples were ground using a mortar and passed through a 1 mm sieve.

For palynological analysis, each sample was suspended in 70% alcohol for 24 hours. The suspension was centrifuged, and the alcohol was then discarded. After that, 4.0 mL of glacial acetic acid was added to the pollen material, and the suspension was left undisturbed for 24 hours, as suggested by Silva et al. (2014). The pollen material was then acetolysed with the method proposed by Erdtman (1960) and preserved in 50% glycerol. Slides were prepared with Kisser gelatin and sealed with nail polish for each pollen sample. Pollen types were identified in qualitative analyses by comparing them

with the reference slides of the study area deposited at the Bee Laboratory of the State University of Maringá, Maringá, Brazil, and with specialized literature on bee-collected pollen identification (Miranda & Andrade, 1990; Silva et al., 2010, 2014). The quantitative analysis involved counting the initial 400 pollen grains from each bee pollen sample, as Montero and Tormo (1990) described. The pollen grains were then classified according to Barth's (1970), Louveaux et al. (1970), and Louveaux et al. (1978) system, which includes four categories: dominant pollen (accounting for > 45% of the total pollen grains on the slide), accessory pollen (accounting for 15.0-45.0%), important isolated pollen (accounting for 3.0-15.0%), and occasional isolated pollen (accounting for < 3.0%).

Statistical analysis

To investigate whether "bee species" or "season" had a significant influence on the botanical composition of *A. mellifera* pollen loads and stingless bee pot-pollen samples, pollen analysis data (including pollen types from plant species, their count frequencies, and the number of pollen grains) were analyzed using generalized linear models (GLM) at the 95% confidence level using the R software environment. For the statistical analysis, the repeated pollen types in different months were counted every time they were observed in the evaluated samples. The 'number of pollen grains' was analyzed using the Poisson probability distribution with a log link function, whereas the 'count frequency' of pollen grains was analyzed using the Gamma probability distribution with a log link function.

Network analysis is a powerful mathematical modeling tool that facilitates the interpretation of the dynamics of ecological networks, allowing the evaluation of interactions between plants, between insects or between insects and plants, as well as environmental disturbances and their influence on the stability of ecosystems (Guimarães Jr., 2020; Borchardt et al., 2021). In our study, bipartite Network graphs with $k = 4$, weighted and multilayered, were constructed according to the methodology described by Guimarães Jr. (2020). In Network graphs, vertices or nodes show the interacting units, while edges or links (lines) show the interactions between pairs of units. In our work, in the Network graphs for each bee species, the nodes were represented by the seasons and the edges or links (lines) by the interactions between the pollen types collected and the seasons. Bipartite graphs represent a particular type of k -partite graph, in which k is the Number of partitions of nodes, and so interactions occur only between nodes of different partitions. In our work, k was equal to 4, representing the interactions of the pollen types collected by the bee species in the four seasons (spring, summer, autumn, and winter). Considering that the frequencies of pollen types collected over a year vary between seasons for each bee species, weighted multilayer graphs were constructed, which can express the foraging pattern of bee species in each season through the differences observed in the thickness of the lines.

Network analysis was conducted using Gephi 0.9.2 software to visually evaluate the impact of bee species and season (represented by the four seasons of the year) on the botanical composition of pollen loads and pot-pollen samples. In this investigation, the Network graphs were partitioned into four classes based on seasonality, i.e., winter, spring, summer, and autumn. The strength of the association between each pollen type and the season for each bee species is related to the count frequencies of the studied pollen types in the pollen loads and pot-pollen. In other words, it defines the intensity

of bee visitation to a given plant in a given season of the year, taking into account all the bee pollen samples collected within the study period (from June 2018 to May 2019).

Results

Palynological Analysis

The palynological analysis of *A. mellifera* pollen loads indicated the contribution of 19 botanical families and 41 pollen types, as shown in Table 1 and Figure 1.

Table 1. Pollen types found in pollen loads harvested by *A. mellifera* in different seasons of the year at the Maringá City, Paraná State, Brazil.

Pollen Type		Winter	Spring	Summer	Autumn
Family	Genus	(Jun/Jul/Aug)	(Sep/Oct/Nov)	(Dec/Jan/Feb)	(Mar/Apr/May)
Amaranthaceae	<i>Alternanthera</i> sp.				OIP
Amaranthaceae	<i>Amaranthus</i> sp.			OIP	
Anacardiaceae	<i>Schinus</i> sp.		OIP		OIP
Apocynaceae	<i>Aspidosperma</i> sp.		OIP		
Apocynaceae	<i>Tabernaemontana</i> sp.		OIP		
Asteraceae	<i>Ageratum</i> sp.		AP		OIP
Asteraceae	<i>Baccharis</i> sp.	IIP	AP	AP (Dec)/IIP (Feb)	OIP(Mar)/IIP (Abr)/ AP (May)
Asteraceae	<i>Bidens</i> sp.	OIP	AP (Nov)/OIP (Oct)	IIP	OIP
Asteraceae	<i>Cosmos</i> sp.			OIP	
Asteraceae	<i>Emilia</i> sp.	IIP	OIP		
Asteraceae	<i>Galinsoga</i> sp.		AP		
Asteraceae	<i>Mikania glomerata</i> sp.	OIP	OIP		
Asteraceae	<i>Sonchus</i> sp.			OIP	
Asteraceae	<i>Tridax</i> sp.			OIP	IIP
Asteraceae	<i>Vernonanthura</i> sp.			OIP	
Brassicaceae	<i>Raphanus</i> sp.	OIP (Jun)/ DP (Jul/Aug)	AP (Sep)/OIP (Oct)		AP (Mar/May)/OIP (Apr)
Cannabaceae	<i>Celtis</i> sp.				OIP
Commelinaceae	<i>Commelina</i> sp.		OIP		OIP (Mar)/ AP (May)
Euphorbiaceae	<i>Alchornea</i> sp.		IIP		
Euphorbiaceae	<i>Croton</i> sp.		OIP		
Fabaceae	<i>Albizia</i> sp.		OIP	IIP (Dec)/OIP (Jan)	
Fabaceae	<i>Mimosa</i> sp.	DP	OIP	DP (Dec/Jan)/ AP (Feb)	AP/DP (Apr)/IIP (May)/OIP
Fabaceae	<i>Senegalia</i> sp.			OIP	
Fabaceae	<i>Senna</i> sp.				OIP
Fabaceae	<i>Leucaena</i> sp.		OIP (Sep/Oct)/IIP (Nov)	OIP	OIP
Fabaceae	<i>Schizolobium</i> sp.	IIP	IIP(Sep)/OIP (Oct)		
Fabaceae	<i>Stylosanthes</i> sp.	OIP			OIP
Lamiaceae	<i>Mesosphaerum</i> sp.				OIP
Malvaceae	<i>Ceiba</i> sp.			OIP	OIP
Malvaceae	<i>Heliocarpus</i> sp.	IIP			
Moringaceae	<i>Moringa</i> sp.		OIP		
Myrtaceae	<i>Callistemon</i> sp.			OIP	OIP
Myrtaceae	<i>Eucalyptus</i> sp.	OIP (Jun)/ AP (Jul)	IIP (Sep/Nov)/OIP (Oct)	DP	AP (Mar/May)/IIP (Apr)
Myrtaceae	<i>Eugenia</i> sp.	AP		AP	
Myrtaceae	<i>Psidium</i> sp.	OIP	OIP (Sep)/ AP (Oct)/ IIP (Nov)		
Poaceae	<i>Zea</i> sp.	OIP	IIP	OIP (Dec)/IIP (Jan/Feb)	IIP (Mar)/OIP (Apr/May)
Petiveriaceae	<i>Gallesia</i> sp.			OIP	
Rhamnaceae	<i>Hovenia</i> sp.			OIP	
Rubiaceae	<i>Richardia</i> sp.		OIP		
Sapindaceae	<i>Serjania</i> sp.				OIP
Urticaceae	<i>Cecropia</i> sp.			OIP	

DP = dominant pollen (frequencies > 45%); AP = accessory pollen (frequencies between 15 to 45%); IIP = important isolated pollen (frequencies between 3 to 15%); OIP = occasional isolated pollen (frequencies < 3%); Jan = January; Feb = February; Mar = March; Apr = April; May = May; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.

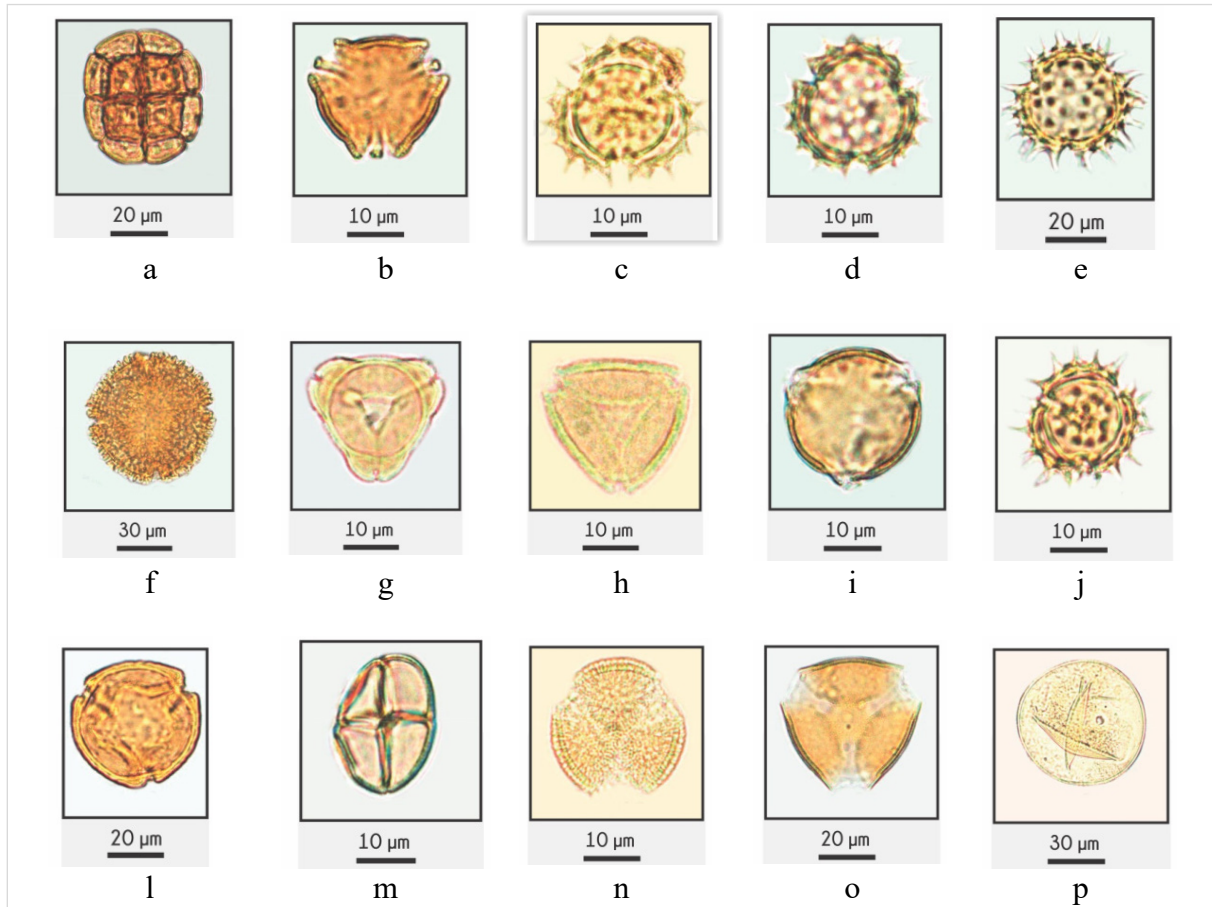


Fig 1. (a) *Albizia* sp. (Fabaceae); (b) *Alchornea* sp. (Euphorbiaceae); (c) *Ageratum* sp. (Asteraceae); (d) *Baccharis* sp. (Asteraceae); (e) *Bidens* sp. (Asteraceae); (f) *Ceiba* sp. (Malvaceae); (g) *Eucalyptus* sp. (Myrtaceae); (h) *Eugenia* sp. (Myrtaceae); (i) *Eriobotrya* sp. (Rosaceae); (j) *Galinsoga* sp. (Asteraceae); (l) *Melia* sp. (Meliaceae); (m) *Mimosa* sp. (Fabaceae); (n) *Raphanus* sp. (Brassicaceae); (o) *Senna* sp. (Fabaceae); (p) *Zea* sp. (Poaceae).

The largest contribution of pollen grains was observed for *Mimosa* sp. from the Fabaceae family. Thus, samples collected in winter, summer, and autumn were classified as monofloral pollen of *Mimosa* sp. Other families also contributed to the classification as dominant pollen, with *Raphanus* sp. (Brassicaceae) in winter and *Eucalyptus* sp. (Myrtaceae) in summer (Table 1). These families were also in pollen loads samples collected throughout the year and classified as accessory pollen. Other vital families were Asteraceae with pollen types, including *Ageratum* sp., *Baccharis* sp., *Bidens* sp., *Galinsoga* sp., and Commelinaceae with *Commelina* sp. These pollen types also contributed as accessory pollen to the botanical composition of the pollen loads of this bee species. Less frequently, pollen grains from the Asteraceae, Euphorbiaceae, Fabaceae, Malvaceae, Myrtaceae, and Poaceae families were detected and classified as important isolated pollen.

Seventeen botanical families and 32 pollen types were identified in the *M. marginata* pot-pollen samples using palynological data (see Table 2; Figure 1). Pollen belonging to the Euphorbiaceae, Fabaceae, and Myrtaceae families, which were identified as the dominant pollen types, were found

to contribute significantly to pollen loads. During the study period, monofloral pollen samples were obtained from various plant species: *Alchornea* sp. (Euphorbiaceae) during the winter, *Albizia* sp. (Fabaceae) during the summer, *Senna* sp. (Fabaceae) during the winter, and *Eucalyptus* sp. (Myrtaceae) during the spring, summer, and autumn seasons.

At intermediate frequencies (15-45%), and according to the classification of accessory pollen, pollen types of *Baccharis* sp. (Asteraceae), *Alchornea* sp. (Euphorbiaceae), *Albizia* sp. (Fabaceae), *Senna* sp. (Fabaceae), *Mimosa* sp. (Fabaceae), *Melia* sp. (Meliaceae), *Eucalyptus* sp. (Myrtaceae), *Psidium* sp. (Myrtaceae), *Pleroma* sp. (Melastomaceae), and *Serjania* sp. (Sapindaceae) were identified. Important isolated pollen types were classified from Arecaceae, Euphorbiaceae, Fabaceae, Magnoliaceae, Myrtaceae, Rosaceae, and Solanaceae.

The palynological data of *M. quadrifasciata* pot-pollen identified 17 pollen types and only eight botanical families (refer to Table 3 and Fig 1), revealing the lowest botanical richness of pollen types in all seasons.

The *M. quadrifasciata* pot-pollen was mainly composed of pollen grains from the Fabaceae and Myrtaceae families, classified as dominant pollen.

Table 2. Pollen types found in pot-pollen harvested by *M. marginata* in different seasons of the year at the Maringá City, Paraná State, Brazil.

Pollen Type		Winter	Spring	Summer	Autumn
Family	Genus	(Jun/Jul/Aug)	(Sep/Oct/Nov)	(Dec/Jan/Feb)	(Mar/Apr/May)
Anacardiaceae	<i>Schinus</i> sp.		OIP		
Arecaceae	<i>Archontophoenix</i> sp.		IIP (Sep)/OIP (Oct/Nov)		
Asteraceae	<i>Baccharis</i> sp.	AP			
Asteraceae	<i>Bidens</i> sp.	OIP	OIP		
Asteraceae	<i>Cosmos</i> sp.	OIP		OIP	
Brassicaceae	<i>Raphanus</i> sp.				OIP
Commelinaceae	<i>Commelina</i> sp.	OIP			
Euphorbiaceae	<i>Alchornea</i> sp.	DP /OIP (Aug)	IIP (Sep/Oct)/ AP (Nov)	IIP (Dec)/OIP (Fev)	OIP
Fabaceae	<i>Albizia</i> sp.		AP (Oct)/IIP (Nov)	DP (Dec/Jan)/ IIP (Feb)	
Fabaceae	<i>Leucaena</i> sp.	OIP	OIP	OIP	OIP
Fabaceae	<i>Machaerium</i> sp.				IIP
Fabaceae	<i>Mimosa</i> sp.	AP /IIP	OIP	OIP	
Fabaceae	<i>Schizolobium</i> sp.		OIP	OIP	
Fabaceae	<i>Senegalia</i> sp.		OIP		
Fabaceae	<i>Senna</i> sp.	DP /OIP (Jun)	AP		
Fabaceae	<i>Stylosanthes</i> sp.	IIP (Jun)/OIP (Aug)			OIP
Fabaceae	<i>Tipuana</i> sp.		IIP		
Magnoliaceae	<i>Magnolia</i> sp.			IIP	
Malvaceae	<i>Triumfetta</i> sp.				OIP
Malvaceae	<i>Sida</i> sp.	OIP			
Melastomataceae	<i>Pleroma</i> sp.			AP	
Meliaceae	<i>Melia</i> sp.	AP			
Myrtaceae	<i>Eucalyptus</i> sp.	AP (Jun)/IIP (Aug)	DP (Sep)/IIP (Oct/Nov)	IIP (Dec)/OIP (Jan)/ DP (Feb)	DP (Mar/Apr/May)
Myrtaceae	<i>Psidium</i> sp.		AP (Sep/Oct)/ IIP (Nov)		
Myrtaceae	<i>Callistemon</i> sp.	IIP (Jun)/OIP (Aug)			
Myrtaceae	<i>Eugenia</i> sp.	OIP			
Myrtaceae	<i>Syzygium</i> sp.		OIP		
Poaceae	<i>Zea</i> sp.	OIP			
Rosaceae	<i>Eriobotrya</i> sp.				OIP (Mar)/IIP (Apr)
Salicaceae	<i>Casearia</i> sp.	OIP			
Sapindaceae	<i>Serjania</i> sp.	OIP	AP		OIP (Mar)/ AP (Apr/May)
Solanaceae	<i>Solanum</i> sp.		IIP		

DP = dominant pollen (frequencies > 45.0%; AP = accessory pollen (frequencies between 15.0 to 45.0%); IIP = important isolated pollen (frequencies between 3.0 to 15.0%); OIP = occasional isolated pollen (frequencies < 3.0%); Jan = January; Feb = February; Mar = March; Apr = April; May = May; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.

Consequently, *Albizia* sp. (Fabaceae) was the source of monofloral pot-pollen in summer, *Senna* sp. (Fabaceae) in winter, and *Eucalyptus* sp. (Myrtaceae) in summer and autumn. At the same time, *Psidium* sp. (Myrtaceae) was used in spring and winter, and *Eugenia* sp. (Myrtaceae) in winter. Accessory pollen types were identified from

Baccharis sp. (Asteraceae), *Albizia* sp. (Fabaceae), *Mimosa* sp. (Fabaceae), *Senna* sp. (Fabaceae), *Eucalyptus* sp. (Myrtaceae), and *Psidium* sp. (Myrtaceae). In addition, important isolated pollen types were identified from families such as Euphorbiaceae, Malvaceae, Meliaceae, Rosaceae, and Sapindaceae.

Table 3. Pollen types found in pot-pollen harvested by *M. quadrifasciata quadrifasciata* in different seasons of the year at the Maringá City, Paraná State, Brazil.

Pollen Type		Winter (Jun/Jul/Aug)	Spring (Sep/Oct/Nov)	Summer (Dec/Jan/Feb)	Autumn (Mar/Apr/May)
Family	Genus				
Asteraceae	<i>Baccharis</i> sp.			AP	
Euphorbiaceae	<i>Alchornea</i> sp.	IIP	OIP	IIP	
Fabaceae	<i>Albizia</i> sp.		IIP (Oct)/AP (Nov)	DP (Dec)/AP (Jan)/ OIP (Feb)	
Fabaceae	<i>Leucaena</i> sp.			OIP	
Fabaceae	<i>Mimosa</i> sp.	OIP		IIP	AP (Mar/Apr)
Fabaceae	<i>Senegalia</i> sp.			IIP	
Fabaceae	<i>Senna</i> sp.	DP (Jun)	AP	OIP	
Fabaceae	<i>Stylosanthes</i> sp.	OIP			
Malvaceae	<i>Heliocarpus</i> sp.	IIP			
Meliaceae	<i>Melia</i> sp.	OIP			
Meliaceae	<i>Cedrela</i> sp.	OIP			
Myrtaceae	<i>Eucalyptus</i> sp.	AP (Jun/Jul)	AP (Oct)/IIP (Nov)	IIP (Jan)/DP (Feb)	DP (Mar/Apr/May)
Myrtaceae	<i>Callistemon</i> sp.	OIP			
Myrtaceae	<i>Eugenia</i> sp.	DP (Jul)/IIP (Aug)			
Myrtaceae	<i>Psidium</i> sp.	DP	DP (Sep/Oct)/ AP (Nov)	OIP (Dec)/AP (Jan)	
Rosaceae	<i>Eriobotrya</i> sp.				OIP
Sapindaceae	<i>Serjania</i> sp.	OIP			

DP = dominant pollen (frequencies > 45%; AP = accessory pollen (frequencies between 15 to 45%); IIP = important isolated pollen (frequencies between 3 to 15%); OIP = occasional isolated pollen (frequencies < 3%); Jan = January; Feb = February; Mar = March; Apr = April; May = May; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.

Palynological analysis of *S. bipunctata* pot-pollen revealed 32 pollen types from 19 botanical families (Table 4; Figure 1). The pot-pollen of this bee species exhibited a greater prevalence of pollen types belonging to the Euphorbiaceae, Fabaceae, and Myrtaceae families. The pot-pollen composed mainly from *Alchornea* sp. (Euphorbiaceae) during winter, *Albizia* sp. (Fabaceae) during spring and summer, and *Eucalyptus* sp. (Myrtaceae) during winter, summer, and autumn were identified as monofloral pot-pollen.

Intermediate frequencies ranging from 15 to 45% were identified for several pollen types including *Alchornea* sp. (Euphorbiaceae), *Schizolobium* sp. (Fabaceae), *Tipuana* sp. (Fabaceae), *Moringa* sp. (Moringaceae), *Eucalyptus* sp. (Myrtaceae), *Hovenia* sp. (Rhamnaceae), and *Serjania* sp. (Sapindaceae). These pollen types were classified as accessory pollen. Important isolated pollen was identified from various plant families, including Amaranthaceae, Anacardiaceae, Apocynaceae, Areaceae, Asteraceae, Brassicaceae, Euphorbiaceae, Fabaceae, Malvaceae, Meliaceae, Moringaceae, Myrtaceae, Petiveriaceae, Rhamnaceae, Rosaceae, Rutaceae, Salicaceae, Sapindaceae, and Violaceae. Compared to

M. marginata and *M. quadrifasciata quadrifasciata* pot-pollen, *S. bipunctata* pot-pollen showed the highest richness of pollen types classified as accessory and important isolated pollens.

Palynological analysis of *T. clavipes* pot-pollen revealed the identification of 33 botanical families and 68 pollen types (see Table 5 and Figure 1). Across all bee species examined in this study, *T. clavipes* pot-pollen displayed the highest diversity in botanical families and pollen types throughout every season.

For this stingless bee, monofloral pot-pollen was collected from *Eriobotrya* sp. (Rosaceae) in autumn, *Raphanus* sp. (Brassicaceae), and *Alchornea* sp. (Euphorbiaceae) in winter. Pollen types of Amaranthaceae, Asteraceae, Brassicaceae, Euphorbiaceae, Fabaceae, Malvaceae, Myrtaceae, Piperaceae, Rosaceae, and Rutaceae families were identified as accessory pollen. The 33 identified families in *T. clavipes* pot-pollen contributed as isolated pollen. In addition, the *T. clavipes* pot-pollen was classified as heterofloral in most seasons due to the more outstanding contribution of different pollen types classified as accessory and isolated pollens.

Table 4. Pollen types found in pot-pollen harvested by *S. bipunctata* in different seasons of the year at the Maringá City, Paraná State, Brazil.

Pollen Type		Winter	Spring	Summer	Autumn
Family	Genus	(Jun/Jul/Aug)	(Sep/Oct/Nov)	(Dec/Jan/Feb)	(Mar/Apr/May)
Amaranthaceae	<i>Alternanthera</i> sp.		IIP		
Anacardiaceae	<i>Schinus</i> sp.		IIP		
Apocynaceae	<i>Aspidosperma</i> sp.		OIP		
Arecaceae	<i>Archontophoenix</i> sp.		OIP	OIP	
Asteraceae	<i>Baccharis</i> sp.		OIP		OIP
Brassicaceae	<i>Raphanus</i> sp.	OIP			
Euphorbiaceae	<i>Alchornea</i> sp.	DP (Jun)/AP (Jul)	OIP		
Euphorbiaceae	<i>Croton</i> sp.		IIP (Oct)/OIP (Nov)		
Fabaceae	<i>Albizia</i> sp.		DP (Oct)/OIP (Nov)	DP (Dec)/IIP (Jan)/ OIP (Feb)	
Fabaceae	<i>Bauhinia</i> sp.	IIP			
Fabaceae	<i>Leucaena</i> sp.		OIP	OIP	OIP
Fabaceae	<i>Macherium</i> sp.				IIP
Fabaceae	<i>Macroptilium</i> sp.		OIP		
Fabaceae	<i>Mimosa</i> sp.		OIP	IIP (Dec)/OIP (Jan)	
Fabaceae	<i>Schizolobium</i> sp.		AP (Sep)/OIP (Oct)/ IIP (Nov)		
Fabaceae	<i>Senegalia</i> sp.		IIP	OIP	
Fabaceae	<i>Tipuana</i> sp.		AP	AP (Dec)/OIP (Jan)	
Malvaceae	<i>Heliocarpus</i> sp.	IIP			
Malvaceae	<i>Ceiba</i> sp.		OIP		
Meliaceae	<i>Melia</i> sp.	IIP			
Moringaceae	<i>Moringa</i> sp.			AP	
Myrtaceae	<i>Callistemon</i> sp.				OIP
Myrtaceae	<i>Eucalyptus</i> sp.	IIP(Jun)/AP (Jul)/ DP (Aug)	AP (Sep/Nov)/ PII (Oct)	PII (Dec)/PD (Jan/Feb)	PD (Mar/Apr/May)
Myrtaceae	<i>Eugenia</i> sp.	PIO			
Myrtaceae	<i>Syzygium</i> sp.		IIP (Sep/Oct)/OIP (Nov)		
Petiveriaceae	<i>Gallesia</i> sp.				OIP
Rhamnaceae	<i>Hovenia</i> sp.		AP (Sep)/IIP (Oct)		
Rosaceae	<i>Eriobotrya</i> sp.				IIP (Mar/Apr) /OIP (May)
Rutaceae	<i>Murraya</i> sp.	IIP			
Salicaceae	<i>Casearia</i> sp.	IIP			
Sapindaceae	<i>Serjania</i> sp.	IIP (Jun)/AP (Jul)			IIP
Violaceae	<i>Pombalia</i> sp.	OIP			

DP = dominant pollen (frequencies > 45 %; AP = accessory pollen (frequencies between 15 to 45 %); IIP = important isolated pollen (frequencies between 3 to 15 %); OIP = occasional isolated pollen (frequencies < 3 %); Jan = January; Feb = February; Mar = March; Apr = April; May = May; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.

Network Analysis

Network analysis was performed on all frequencies obtained from palynological analysis (Tables 1 to 5). This statistical analysis is an interesting way to graphically represent the richness of pollen types in the botanical composition and elucidate the foraging behavior and floral preferences of different bee species in a well-defined production region.

The thickness of the lines shown in the Network graphs (Figures 2 to 3) increases with the count frequencies of each pollen type in the botanical composition, indicating the importance of the diet. The interrelationships between the foraging patterns in all seasons were compared between *A. mellifera* and *T. clavipes* (Figure 2), and the following stingless bees *M. marginata*, *M. quadrifasciata quadrifasciata*, and *S. bipunctata* (Figure 3).

resources. This stingless bee species also preferred collecting *Eriobotrya* sp. pollen in autumn. When comparing the foraging patterns of these two bee species (*A. mellifera* and *T. clavipes*), a greater richness (number of different pollen types) was observed in the *T. clavipes* pot-pollen, including not identified taxa. This greater richness may result from good habitat quality in the study site. Diversity begets stability with increasing polleniferous species richness and allows more complex interactive networks in space and time (E.N. Silva, personal communication, 9 Jan 2024).

A different scenario was depicted for the specialized *M. marginata* (Figure 3-a), *M. quadrifasciata quadrifasciata* (Figure 3-b), and *S. bipunctata* (Figure 3-c) stingless bees. A similar foraging pattern was observed in the Network graphs, with preferences for pollen types of *Eucalyptus* sp., *Psidium* sp., *Senna* sp., *Albizia* sp., and *Alchornea* sp. in the different seasons. *M. marginata* and *M. quadrifasciata quadrifasciata* shared a preference for foraging pollen grains on *Eucalyptus* sp. in spring and *Albizia* sp. in winter, demonstrating that these plants support foraging by more than one stingless bee species.

Table 5. Pollen types found in pot-pollen harvested by *T. clavipes* in different seasons of the year at the Maringá City, Paraná State, Brazil.

Pollen Type		Winter	Spring	Summer	Autumn
Family	Genus	(Jun/Jul/Aug)	(Sep/Oct/Nov)	(Dec/Jan/Feb)	(Mar/Apr/May)
Acanthaceae	<i>Justicia</i> sp.	OIP			
Amaranthaceae	<i>Alternanthera</i> sp.		OIP	AP	
Amaranthaceae	<i>Amaranthus</i> sp.			IIP	OIP
Amaranthaceae	<i>Chamissoa</i> sp.			OIP	
Anacardiaceae	Unknown		OIP (Set)/IIP (Nov)		
Apocynaceae	<i>Tabernaemontana</i> sp.		IIP		
Arecaceae	<i>Archontophoenix</i> sp.		OIP	OIP	
Asteraceae	<i>Acmella</i> sp.				OIP
Asteraceae	<i>Baccharis</i> sp.	OIP		IIP	OIP (Apr)/AP (May)
Asteraceae	<i>Bidens</i> sp.	OIP		OIP	OIP
Asteraceae	<i>Cosmos</i> sp.			OIP	
Asteraceae	<i>Emilia</i> sp.	OIP	OIP		
Asteraceae	<i>Galinsoga</i> sp.			IIP	
Asteraceae	<i>Mikania glomerata</i> sp.	OIP			
Asteraceae	<i>Tridax</i> sp.	IIP (Jun)/OIP (Jul/Aug)	OIP		OIP
Asteraceae	<i>Vernonanthura</i> sp.	OIP			OIP
Bignoniaceae	<i>Dolichandra</i> sp.	OIP	IIP		
Bignoniaceae	<i>Mansoa</i> sp.			IIP	
Brassicaceae	<i>Raphanus</i> sp.	AP (Jul)/DP (Aug)	AP (Sep/Oct)/ IIP (Nov)	OIP	IIP (Mar/Apr)/ OIP (May)
Cannabaceae	<i>Celtis</i> sp.				OIP
Cannabaceae	<i>Trema</i> sp.			IIP	
Commelinaceae	<i>Commelina</i> sp.			OIP	
Cyperaceae	Unknown			IIP	
Cordiaceae.	<i>Cordia</i> sp.				OIP
Euphorbiaceae	<i>Alchornea</i> sp.	DP (Jun)/AP (Jul)/ OIP (Aug)	OIP (Sep)/AP (Oct)/ IIP (Nov)	OIP (Dec)/ AP (Jan)/IIP (Feb)	OIP (Mar)
Euphorbiaceae	<i>Croton</i> sp.		IIP (Oct)/AP (Nov)		
Fabaceae	<i>Bauhinia</i> sp.	OIP		OIP	
Fabaceae	<i>Machaerium</i> sp.	AP			AP (Apr)/IIP (May)
Fabaceae	<i>Senna</i> sp.	OPI (Jul)/IIP (Aug)		IIP	OIP
Fabaceae	<i>Cajanus</i> sp.	OIP			
Fabaceae	<i>Leucaena</i> sp.	OIP	IIP	OIP	OIP
Fabaceae	<i>Stylosanthes</i> sp.	OIP			OIP
Fabaceae	<i>Schizolobium</i> sp.		IIP	OIP	

DP = dominant pollen (frequencies > 45%); AP = accessory pollen (frequencies between 15 to 45%); IIP = important isolated pollen (frequencies between 3 to 15%); OIP = occasional isolated pollen (frequencies < 3%); Jan = January; Feb = February; Mar = March; Apr = April; May = May; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.

Similar foraging behavior was observed for *S. bipunctata* pot-pollen (Figure 3-c). These stingless bee species showed greater foraging for pollen types in spring. In contrast, they sought to collect pollen grains from a few specific pollen types (*Mimosa* sp. and *Senna* sp., both from the Fabaceae family) in autumn. None of these three stingless bee species used a

source plant over two seasons. The richness of polleniferous plants in Figure 2 decreased compared to Figure 3, and the line thickness shows less intense use of resources. *Eucalyptus* sp., again, is a crucial resource. These three species clearly occupy a narrow niche while competing with the other species, and they are specialists living on limited resources.

Table 5. Pollen types found in pot-pollen harvested by *T. clavipes* in different seasons of the year at the Maringá City, Paraná State, Brazil. (Continuation)

Pollen Type		Winter (Jun/Jul/Aug)	Spring (Sep/Oct/Nov)	Summer (Dec/Jan/Feb)	Autumn (Mar/Apr/May)
Family	Genus				
Fabaceae	<i>Delonix</i> sp.		IIP	OIP	
Fabaceae	<i>Poincianella</i> sp.	IIP	OIP (Dec/Feb)/ IIP (Jan)		
Fabaceae	<i>Albizia</i> sp.			OIP	
Fabaceae	<i>Tipuana</i> sp.			OIP	
Fabaceae	<i>Peltophorum</i> sp.			IIP	
Lamiaceae	<i>Leonotis</i> sp.	OIP			
Lamiaceae	<i>Mesosphaerum</i> sp.			OIP	
Magnoliaceae	<i>Magnolia</i> sp.	OIP			
Malpighiaceae	<i>Banisteriopsis</i> sp.	OIP			
Malvaceae	<i>Dombeya</i> sp.	OIP			
Malvaceae	<i>Ceiba</i> sp.			AP	OIP
Malvaceae	<i>Heliocarpus</i> sp.	IIP			
Malvaceae	<i>Pachira</i> sp.	OIP		OIP	
Malvaceae	<i>Sida</i> sp.	OIP			
Malvaceae	<i>Triumfetta</i> sp.				OIP
Meliaceae	<i>Melia</i> sp.	OIP		OIP	
Meliaceae	<i>Cedrela</i> sp.		OIP		
Moraceae	<i>Maclura</i> sp.	OIP			
Moringaceae	<i>Moringa</i> sp.	OIP		OIP	
Myrtaceae	<i>Eucalyptus</i> sp.	AP	AP (Sep)/OIP (Oct)/ IIP (Nov)	IIP	AP (Mar/May)/ OIP (Apr)
Myrtaceae	<i>Psidium</i> sp.		OIP		
Myrtaceae	<i>Eugenia</i> sp.				OIP
Passifloraceae	<i>Passiflora</i> sp.		OIP	OIP	
Petiveriaceae	<i>Gallesia</i> sp.			IIP	IIP (Mar)/OIP (Abr)
Piperaceae	<i>Piper</i> sp.	OIP	IIP	AP (Dec)/ IIP (Jan/Feb)	
Poaceae.	<i>Zea</i> sp.	IIP (Jun/Jul)/ OIP (Aug)	IIP	IIP (Dec/Feb)/ OIP (Jan)	OIP
Poaceae	<i>Urochloa</i> sp.	OIP		OIP	
Proteaceae	<i>Grevillea</i> sp.		OIP (Sep/Oct)/ IIP (Nov)		
Rhamnaceae	<i>Hovenia</i> sp.		OIP		
Rosaceae	<i>Eriobotrya</i> sp.				DP (Mar/May)/ AP (Apr)
Rutaceae	<i>Citrus</i> sp.		OIP		
Rutaceae	<i>Murraya</i> sp.	AP/IIP			
Salicaceae	<i>Casearia</i> sp.	OIP			
Sapindaceae	<i>Serjania</i> sp.	IIP (Jun)/OIP (Jul/Aug)			
Violaceae	<i>Pombalia</i> sp.	OIP			

DP = dominant pollen (frequencies > 45%; AP = accessory pollen (frequencies between 15 to 45%); IIP = important isolated pollen (frequencies between 3 to 15%); OIP = occasional isolated pollen (frequencies < 3%); Jan = January; Feb = February; Mar = March; Apr = April; May = May; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.

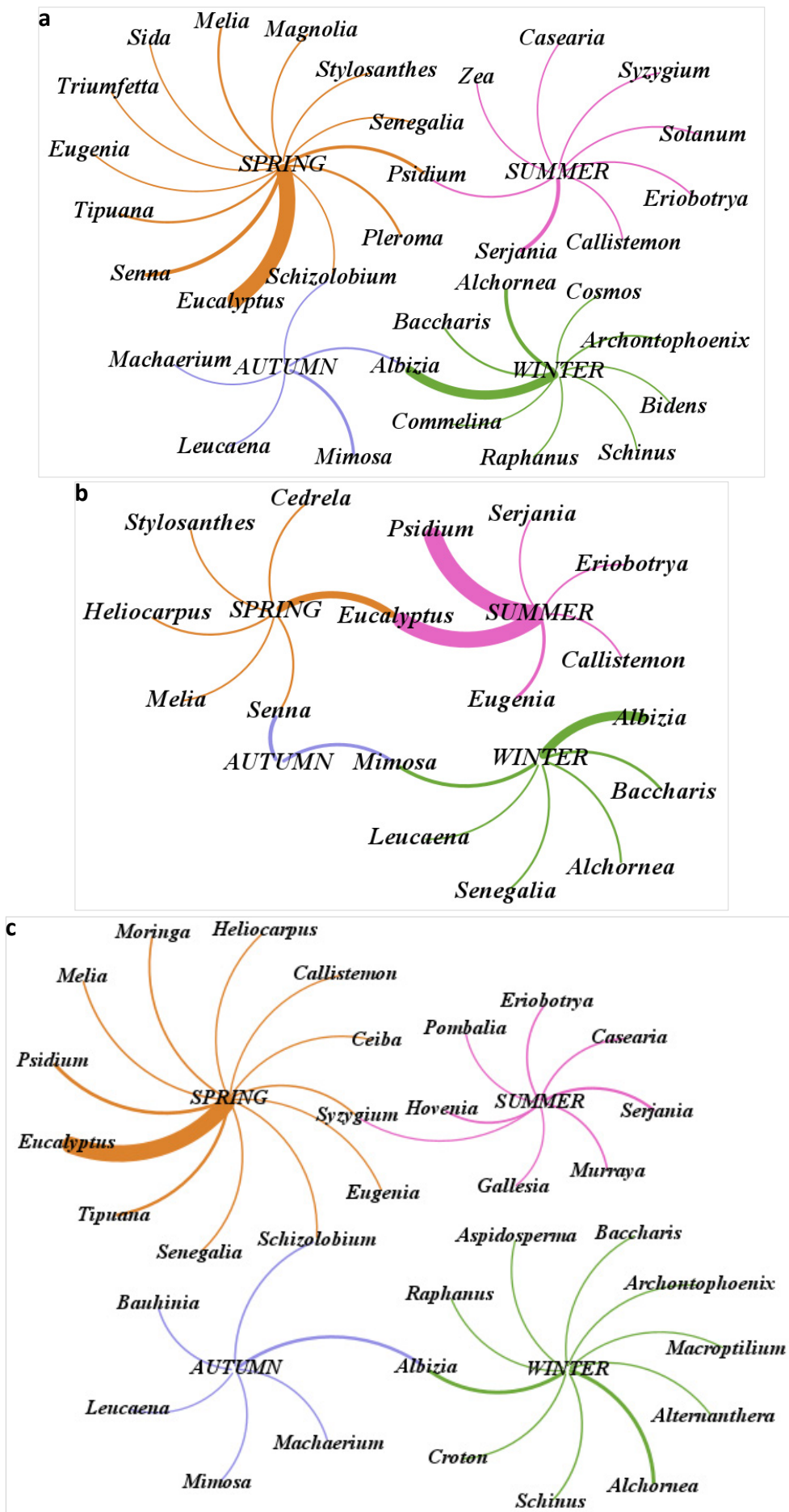


Fig 3. Network graphs of (a) *Melipona marginata*, (b) *Melipona quadrifasciata quadrifasciata*, and (c) *Scaptotrigona bipunctata* pot-pollen collected in different seasons. Thickness of the lines illustrates the contribution of frequencies of the pollen types found by the palynological analysis.

Pollen types from different sources were identified in pollen loads and pot-pollen in all seasons of the year (Tables 1-5; Figures 2 to 3). We conducted a generalized linear model (GLM) analysis at a 95.0 % confidence level to assess the importance of the ‘bee species’ and ‘season’ factors on the botanical composition of pollen loads and pot-pollen.

The GLM results (Table 6) indicate that the analyzed variables (bee species, seasons, and their interaction effect) significantly influenced the botanical composition of pollen loads and pot-pollen. The distribution percentage of each pollen type was calculated for different bee species and seasons (Table 7).

The factor ‘bee species’ significantly impacted the number of pollen types identified in the collected pollen loads and pot-pollen. Of the bee species examined, *T. clavipes* showed the higher diversity in pollen types (36.2%), followed by *A. mellifera* (22.6%), *M. marginata* (16.9%), *S. bipunctata* (14.8%), and *M. quadrifasciata quadrifasciata* (9.6%). These findings align with the results demonstrated in the palynological analysis and the Network graphs (Tables 1 to 5; Figures 2 to 3), indicating that *M. marginata*, *M. quadrifasciata quadrifasciata*, and *S. bipunctata* have less generalist foraging patterns across pollen types than *A. mellifera* and *T. clavipes*.

The variable ‘season’ (Table 7) demonstrates a well-proportioned dispersion of pollen types throughout all seasons.

Table 6. General Linear Model results. Wald’s chi-square test and significance for the factors ‘season’, ‘bee species’ and their interaction effect.

Variation sources	Type III		
	Wald’s chi-square	df	p-value
(Intercept)	2,626,900	1	0.000
Season	2,393	3	0.000
Bee species	23,658	4	0.000
Season * Bee species	9,296	12	0.000

Model: (Intercept), Season, Bee species, Season * Bee species

Spring showed a slightly higher percentage (28.7%), revealing the most diverse pollen types identified in pollen loads and pot-pollen from five bee species collected during the 12 months. Alternatively, autumn exhibited the lowest diversity of pollen types (20.7%) compared to the other seasons. The local climate may influence the foraging patterns of each bee species in the production area, as it impacts the availability of floral resources during different seasons. Therefore, the pollen-foraging patterns of bees are subject to change.

The region where production occurs is characterized by a warm, humid, and rainy climate throughout the year. According to information from INPE (Melo Nascimento, 2020), the highest rainfall rates were recorded during the spring (152, 279, and 144 mm) as compared to the rates during autumn (143, 49, and 86 mm). Furthermore, summer also recorded high rainfall rates (187, 118, and 143 mm), whereas winter had the lowest recorded rainfall rates (62, 5, and 224 mm). Mean relative humidity ranges for winter were recorded at 50.0-69.0%, for spring at 62.0-72.0%, for summer at 61.0-68.0%, and for autumn at 62.0-89.1%. The widest temperature range variation was observed in winter (6.6-33.2 °C) and spring (15.2-35.7 °C), with the least variability recorded in summer (17.2-38.1 °C) and autumn (12-31.8 °C).

A significant interaction effect between the ‘season’ and ‘bee species’ factors (Table 6) was observed at a statistically significant level ($p < 0.05$). This interaction effect indicates that the diversity and the number of pollen grains are influenced by the bee species that collected them and the season in which the pollen was obtained (Figure 4).

By evaluating the pattern shown in Figure 4, we can state that *A. mellifera* concentrated their foraging efforts during the winter while the stingless bee species in autumn and summer. Differences were also observed in the average number of pollen grains harvested among the stingless bee species. *M. quadrifasciata quadrifasciata* presented the highest average number of pollen grains compared to that determined for *T. clavipes*. Therefore, the factors ‘bee species’ and ‘season’ cannot be assessed separately in univariate inferences.

Table 7. Variability (%) of pollen types found in *A. mellifera* pollen loads, and stingless bees pot-pollen produced in different seasons.

		N	Percentage (%)
Season	Winter	126	25.6
	Autumn	102	20.7
	Spring	141	28.7
	Summer	123	25.0
	Total	492	100.0
Factors	<i>A. mellifera</i>	111	22.6
	<i>M. marginata</i>	83	16.9
	<i>M. quadrifasciata quadrifasciata</i>	47	9.6
	<i>S. bipunctata</i>	73	14.8
	<i>T. clavipes</i>	178	36.2
	Total	492	100.0

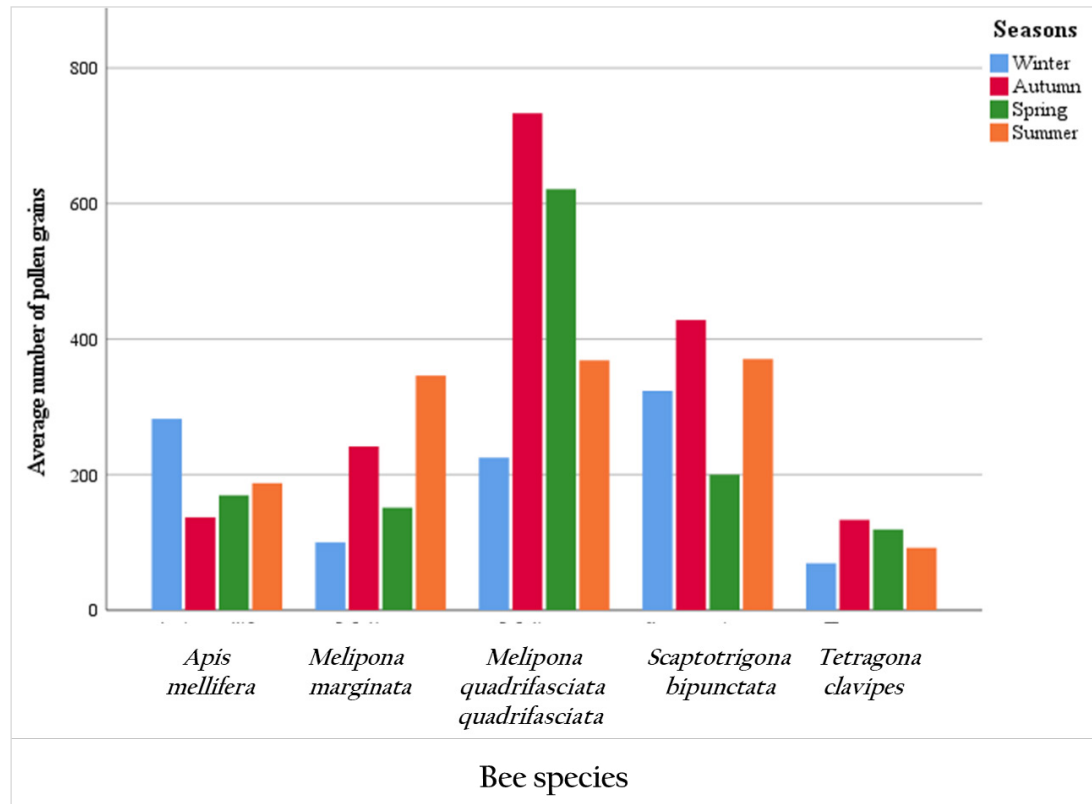


Fig 4. Contribution (%) of pollen types found in *Apis mellifera* pollen loads and stingless bee pot-pollen in different seasons. Illustration of the interaction effect between 'bee species' and 'season'.

Discussion

Palynological analysis of pollen loads and pot-pollen

A. mellifera is an exotic bee species widely spread in different biomes. It is well adapted to different climatic conditions and presents a generalist pollen and nectar harvesting behavior. This adaptation means that this bee species always seeks to exploit the most profitable floral sources in the producing region. For this reason, it collects pollens from different botanical origins following the food quality and quantity offered (Negrão & Orsi, 2018). This behavior can be observed in the continuous change of pollen types identified in honey and in pollen loads produced and reported in the literature for *A. mellifera* (Wilms & Wiechers, 1997; Kleinert & Giannini, 2012; Negrão & Orsi, 2018).

Despite exhibiting a generalist behavior concerning food collection, *A. mellifera* may intensify the foraging of certain plants during specific periods due to adequate climatic conditions and the availability of floral resources in the study area (Aleixo et al., 2016). Researchers have reported that these bees have concentrated their pollen harvesting efforts on bee plants of the Myrtaceae and Melastomaceae (Wilms & Wiechers, 1997), Myrtaceae, and Asteraceae (Negrão & Orsi, 2018), and Amaranthaceae, Araliaceae, Asteraceae, Myrtaceae, Poaceae, and Rutaceae families (Mattos et al., 2018). Similar pollen collection behavior was observed in our study as

reported in the literature regarding Myrtaceae pollen types, which were classified as dominant pollen in summer and as accessory pollen in spring and autumn. The most commonly found pollen types in *A. mellifera* pollen loads were *Eucalyptus* sp., *Eugenia* sp., and *Psidium* sp. from the Myrtaceae family. Our study also noted a prevalence of Brassicaceae pollen types in the winter and of Fabaceae pollen types in the winter, summer, and autumn, which were all classified as dominant. We hypothesize that *A. mellifera*'s year-round preference for Brassicaceae and Fabaceae plant species is linked to the abundance of floral resources resulting from the experimental farm's typical cultivation of these plants. Asteraceae was another important family identified in our work, and it was present in pollen loads at a lesser frequency in almost all seasons.

Stingless bees (Apidae, Meliponini) are eusocial bees that comprise more than 510 species living in predominantly tropical habitats around the globe. They are essential pollinators contributing to ecological forest conservation and the development of plants of agricultural interest (Hnrcir et al., 2019; Moura et al., 2022). In Brazil, there are 259 species of stingless bees, and 70 of them are in the Southern region, where Maringá is located in the Paraná State (Nogueira, 2023). They are characterized by a need to maintain a constant supply of food for the adult individuals and the support of the nest. They also present different morphological and biological characteristics concerning honey bees (Oliveira-Abreu et al., 2014).

Unlike the behavior exhibited by *A. mellifera*, the stingless bees do not migrate to other locals in search of more profitable sources when floral resources are scarce (Hrncir et al., 2019). So, when climatic conditions and food supply are favorable, stingless bees intensify the nectar and pollen harvesting and stock up on food in their nests to survive periods of floral resource scarcity. As a result of this behavior, several researchers have observed that stingless bees, despite being polylectic insects, show specific floral preferences during nectar and pollen harvesting, especially for massive flowering trees or plants that present flowers with poricidal anthers (Ramalho et al., 1990; Maya-Silva et al., 2014; Hrncir et al., 2019; Barth et al., 2020). Another strategy adopted by stingless bees to maximize the food harvest is performing the so-called “buzz pollination,” and, therefore, they can take advantage of exploring profitable pollen sources that are not available for other bees such as honey bees (Oliveira-Abreu et al., 2014).

Considering reports found in the literature, the main pollen sources for stingless bee species from the *Melipona* genus were pollen types from Myrtaceae, Euphorbiaceae, Fabaceae, Solanaceae, Leguminosae, Palmae, Rubiaceae, and Melastomataceae families (Wilms & Wiechers, 1997; Ramalho et al., 1990; Hrncir et al., 2019; Barth et al., 2020).

Few studies have reported *M. marginata*'s floral preferences in the nectar and pollen harvest (Kleinert-Giovannini & Imperatriz-Fonseca, 1987). This stingless bee species showed a preference for pollen types from Myrtaceae, Euphorbiaceae, and Solanaceae families, but other pollen types present in lesser frequency from Leguminosae, Melastomaceae, Moraceae, and Liliaceae families were also found.

Our study observed a similar behavior to that related to the literature: the choice of bee plants for pollen harvest by *M. marginata*. In their pot-pollen, dominant pollen types were Euphorbiaceae in the winter, Fabaceae in the winter and summer, and Myrtaceae in the spring, summer, and autumn. Other pollen types from Asteraceae, Euphorbiaceae, Fabaceae, Meliaceae, Myrtaceae, and Sapindaceae families were also found in intermediate counting frequencies, classified as accessory pollen.

Concerning bee plant species, *M. marginata* preferred *Eucalyptus* sp. (Myrtaceae), which was present in the pot-pollen in frequencies > 50.0% in most seasons. Kleinert-Giovannini and Imperatriz-Fonseca (1987) observed similar behavior. Although stingless bees present generalist pollen and nectar harvesting behavior, they can be temporarily selective and explore the most profitable and available floral sources in the region surrounding their hives (Aleixo et al., 2016; Wilson et al., 2021).

Comparing the species of bees of the genus *Melipona* studied in this work, we observed a greater interest in the literature in elucidating the foraging behavior of *M. quadrifasciata* than that shown for *M. marginata*. According to several researchers, some plant species from Asteraceae, Fabaceae, Melastomataceae, Myrtaceae, and Solanaceae families are preferred by *M. quadrifasciata* (Imperatriz-Fonseca et al., 1989; Maia-Silva et al., 2014; Oliveira-Abreu

et al., 2014; Hrncir et al., 2019; Barth et al., 2020). Considering these families, the massive flowering plant species were the most attractive to this stingless bee species. Therefore, they are important nectar and pollen sources accounting for up to 90% of colonies' annual food intake (Imperatriz-Fonseca et al., 1989; Ramalho et al., 1990; Maia-Silva et al., 2014; Hrncir et al., 2019; Barth et al., 2020).

Similar to what is reported in the literature, we identified the predominance of pollen types from Myrtaceae in all seasons. *M. quadrifasciata quadrifasciata* preferred to harvest pollen of *Eucalyptus* sp. during the summer and autumn, *Eugenia* sp. in the winter, and *Psidium* sp. in the winter and spring. Pollen types from Fabaceae were also found to be high in frequency in the winter and summer.

A comparison of pollen foraging behavior of *M. marginata* and *M. quadrifasciata quadrifasciata* showed similarities in most seasons. *Senna* sp. (Fabaceae) was found in high frequencies in the winter for both stingless bee species. The same pattern was observed for *Eucalyptus* sp. (Myrtaceae) in autumn and summer and for *Albizia* sp. (Fabaceae) in summer. These results indicated that these floral resources can support both stingless bee species in Maringá City, Paraná, Brazil.

Little information about bee plants used by *S. bipunctata* and *T. clavipes* for pot-pollen production can be found in the literature. According to Ramalho (2004) and Diniz and Buschini (2015), *S. bipunctata* preferred collecting pollen from massive flowering trees. This behavior can be associated with the need for great amounts of food resources to ensure its colonies' survival. Reports of pollen foraging behavior by *T. clavipes* are scarce. This stingless bee presents a more generalist food resource foraging behavior, usually visiting flowers of plant species of economic interest of the Myrtaceae family and native species in the producing region (Duarte et al., 2016).

Our results indicated that pollen from Myrtaceae, Fabaceae, and Euphorbiaceae families were the primary and most attractive floral sources for *S. bipunctata*. Pollen types of *Eucalyptus* sp. (Myrtaceae) were predominant in winter, summer, and autumn; *Albizia* sp. (Fabaceae) in spring and summer, and *Alchornea* sp. (Euphorbiaceae) in winter. *S. bipunctata* pot-pollen was rich in pollens from the Sapindaceae, Moringaceae, and Rhamnaceae families.

The pollen foraging behavior of *S. bipunctata* was very similar to that observed for *M. marginata* and *M. quadrifasciata quadrifasciata*, especially in the winter, summer, and autumn. Our study shows that *S. bipunctata* preferred to collect pollen from the same floral sources as the two *Melipona* species studied here. These results suggest that plant species from the Myrtaceae, Euphorbiaceae, and Fabaceae families have sufficient pollen quantity and quality to support several stingless bee species without significant competition between them. Similar behavior was observed by Hilgert-Moreira et al. (2014) in pollen harvest from *Eucalyptus* sp. by stingless bees. These overlapping floral resources during pollen foraging by stingless bees is a common strategy adopted by bees from the *Melipona* genus (Ramalho, 1990).

For *T. clavipes*, as mentioned earlier, very little is known about its pollen-foraging behavior compared to reports for other stingless bee species. Duarte (2012) identified 17 botanical families and 38 pollen types in *T. clavipes* pot-pollen in the Southeast region of Brazil. Among the families reported, Fabaceae had more pollen types, followed by Asteraceae, Euphorbiaceae, Malvaceae, Piperaceae, and Poaceae. Our results suggested a similar pollen foraging behavior, showing the same preference for some of the families described by Duarte (2012). Frequencies > 45.0% in the pollen spectra show the predominance of *Raphanus* sp. (Brassicaceae), and *Alchornea* sp. (Euphorbiaceae) in winter, and of *Eriobotrya* sp. (Rosaceae) in autumn. Pollen types from Myrtaceae, Euphorbiaceae, Fabaceae, Brassicaceae, and Rosaceae were found in most seasons, with pollen frequencies between 15.0 and 45.0%. In comparison with the palynological analysis of *A. mellifera* pollen loads and pot-pollen of other stingless bee species previously described, *T. clavipes* presented a greater richness of families but lower frequencies of the pollen count in all seasons, indicating a more generalist pollen foraging pattern. Some similarities were observed in pollen foraging behavior between *A. mellifera* and *T. clavipes*, which shared the same floral preferences by pollen types of *Raphanus* sp. (Brassicaceae) in winter and spring, and *Eucalyptus* sp. (Myrtaceae) in winter, and autumn. The sharing of these floral resources between *A. mellifera* and *T. clavipes* was previously observed by Pedro and Camargo (1991) in the Southeast region of Brazil.

Influence of season and bee species on the botanical origin of pollen loads and pot-pollen

It is recognized in the literature that the botanical composition of corbicular pollen and pot-pollen is influenced by several factors, including climatic conditions, availability of bee flora, seasons of year, and bee species (Hilario & Imperatriz-Fonseca, 2009; Aleixo et al., 2016; Mattos & Soares, 2018; Negrão & Orsi, 2018; Hrcir et al., 2019). Our study investigated the influence of two factors ('season' and 'bee species') on pollen foraging behavior. It was demonstrated that both factors are important ($p < 0.05$), as well as having a significant interaction effect on them ($p < 0.05$), suggesting that they cannot be analyzed separately.

The influence of the 'bee species' factor is better explained when differences in pollen foraging patterns of *A. mellifera* and stingless bee species are compared. As previously described by our results, *A. mellifera* and *T. clavipes* present a more generalist pollen harvesting behavior, seeking the most profitable floral sources available in the region throughout the year. Then, they collected a greater number of pollen types. At the same time, *M. marginata*, *M. quadrifasciata*, and *S. bipunctata* showed a more specialized pollen foraging pattern, concentrating their efforts on their preferred floral resources, pollen types from Myrtaceae, Euphorbiaceae, and Fabaceae families.

Because stingless bees cannot move their nests from one place to another, they collect greater pollen and nectar quantities and stockpile food whenever there is a great availability of attractive plant sources in the environment to maintain their colonies in times of scarce floral resources (Oliveira-Abreu et al., 2014). Consequently, they concentrate their food collection process on plant species capable of providing greater pollen and nectar sources, such as massive flowering trees or flowering plants with poricidal anthers (Hrcir et al., 2019; Oliveira-Abreu et al., 2014; Wilson et al., 2021).

The factor 'season' can explain the changes observed in the pollen harvesting pattern of the preferred floral sources of *A. mellifera* and the four stingless bee species in each season. *A. mellifera* concentrates its efforts on pollen harvesting from plant species of the Fabaceae and Brassicaceae families in winter, Fabaceae and Myrtaceae in summer, and Fabaceae in autumn, and the greatest richness of pollen types of different families in spring. Similarly, the stingless bees studied in this work also changed their pollen harvesting pattern, seeking more profitable floral sources each season.

This behavior presented by different bee species can be associated with variations in climatic conditions, including temperature, humidity, and rainfall rates in the producing region in each season of the year. High rainfall levels and extreme temperatures affected bees' flight activity during pollen harvesting, whereas high temperatures require that different bee species adopt specific heat dissipation mechanisms during flight. Then, variations in rainfall rates and temperatures during the day can affect the pollen harvesting pattern of different bee species (Aleixo et al., 2016).

In addition, variations in climatic conditions in each season can affect the flowering phenology of plant species grown in the studied region (Oliveira-Abreu et al., 2014). Rainfall rates affect the abundance of individual plants, mainly in tropical environments where flowering plants and pollen availability for bees are strongly associated with seasonal variations in this parameter (Aleixo et al., 2016). As observed in Mattos et al. (2018), areas with minimal rainfall exhibit minimal temperature fluctuation due to an even spread of rainfall throughout the seasons and a low relative humidity and solar radiation. Consequently, these regions are most favorable for pollen loads and pot-pollen production.

Interaction effects between 'bee species', and 'season' can be explained by considering the biological characteristics of each bee species, its specific capacity of adaptation to climate, and available floral resource variations in each season in the producing region. It was verified that *A. mellifera* concentrated its pollen grains harvesting in the winter (the driest season and with the lowest minimum temperatures); this behavior was also observed by Negrão et al. (2014) and Negrão and Orsi (2018). On the other hand, *M. marginata*, *M. quadrifasciata*, *S. bipunctata*, and *T. clavipes* collected a greater quantity of pollen grains in autumn and summer

(seasons with the highest temperatures and rainfall rates). The literature has recognized that *A. mellifera* is the best bee species to adapt to extreme climatic conditions. In contrast, stingless bee species start the pollen harvesting process at different environmental minimal temperatures based on their body size, color, and individual thermoregulation mechanisms (Maia-Silva et al., 2014; Oliveira-Abreu et al., 2014). In addition, some bee species, such as stingless bees, tend to decrease larval production, i.e., to perform reproductive diapause in the coldest seasons of the year (winter and spring), thus decreasing the number of pollen grains collected in those seasons. Bee species undergoing reproductive diapause, such as stingless bees, often collect more pollen grains in summer and autumn. In contrast, those not undergoing this process tend to collect more pollen grains in winter and spring (Hilário & Imperatriz-Fonseca, 2009).

Conclusions

A. mellifera and *T. clavipes* exhibited the most generalized pollen-foraging behavior towards plants in the surrounding area of the Beekeeping and Meliponiculture Sector of the Experimental Farm of the State University of Maringá at Maringá City, Brazil. Additionally, the pollen loads and pot-pollen samples from these two bee species displayed a greater diversity of pollen types than those observed for *M. marginata* and *M. quadrifasciata quadrifasciata*, particularly during the spring and summer seasons. The stingless bees, *M. marginata*, and *M. quadrifasciata quadrifasciata*, selected pollen types derived from the Myrtaceae, and Fabaceae families. This behavior facilitated categorizing the majority of pot-pollen as monofloral. Monofloral pollen loads and pot-pollen production are desired in beekeeping and meliponiculture due to their potential application in the pharmaceutical and food sectors. The prevalence of a particular plant species within the botanical origin of commercial pollen loads, bee bread, and pot-pollen streamlines the standardization of its nutraceutical and functional properties while minimizing its compositional variability.

The botanical composition of the analyzed honeybee pollen loads and stingless bee pot-pollen is significantly affected by the combined influence of 'season' and 'bee species'. Therefore, these factors cannot be individually evaluated. Understanding the impact of environmental factors such as climatic conditions, plant variety, and seasonal changes on a range of bee species, including those that have received limited attention, like *S. bipunctata* and *T. clavipes*, is crucial to developing effective practices for pollen loads and pot-pollen production. Additionally, these biological and ecological implications can improve regional beekeeping and meliponiculture while providing valuable insights into the production of bee products with distinctive therapeutic characteristics and significant commercial potential.

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Authors' Contribution

LSB: investigation, methodology; writing-original draft.
JEMN: formal analysis; investigation; methodology.
VAAT: funding acquisition, resources, supervision.
VAL: conceptualization; investigation; methodology; writing-review & editing.
MLF: conceptualization; writing-original draft; writing-review & editing; supervision.

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