REVISTA DELOS DESARROLLO LOCAL SOSTENIBLE

Weeds as an essential component of sustainable meliponiculture practices in organic agroecosystem

As ervas daninhas como um componente essencial das práticas de meliponicultura sustentável no agroecossistema orgânico

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ABSTRACT
Knowledge of bee flora is key information for sustainable meliponiculture practices. Aiming to stablish the plant species used by the stingless bee *Tetragonisca angustula* used as food sources during the year in an organic agroecosystem, this study identified the pollen grains in the pollen loads of worker bees. The pollen load of bees returning to hives was collected bi-monthly for one year, summing 240 pollen loads analyzed by optical microscopy. We found 76 pollen types in the pollen loads, of which 60% were of weeds. The plants most frequently identified in the pollen loads were: *Alchornea, Arracacia xanthorrhiza, Baccharis, Bidens pilosa, Brassica juncea, Brassica oleracea, Byrsonima* sp., *Cecropia, Chamaecrista, Citrus* sp., *Crotalaria* sp., *Croton, Datura, Eucalyptus, Hyptis, Lippia alba, Mangifera indica, Melastomataceae* sp., *Momordica charantia, Myrsine, Pereskia aculeata, Persea americana, Piptadenia, Pisum sativum, Plinia peruviana, Pluchea,* Poaceae, *Prunus persica, Psidium guajava, Raphanus sativus, Rubus urticifolius, Schefflera,* and *Schinus*. Bees foraged mostly on weeds, especially during winter and autumn, and so the presence of weeds can be very beneficial to meliponiculture practices. The by-products of meliponiculture in agroecosystems are derived from diversified botanical sources, that is, wild honey of good quality because it is from an organic system.

Keywords: pollen sources, melissopalynology, stingless bee, *tetragonisca angustula*.

REVISTA DELOS DESARROLLO LOCAL SOSTENIBLE

RESUMO

O conhecimento da flora das abelhas é uma informação fundamental para práticas sustentáveis de meliponicultura. Com o objetivo de estabelecer as espécies vegetais utilizadas pela abelha sem ferrão Tetragonisca angustula usada como fonte de alimento durante o ano em um agroecossistema orgânico, esse estudo identificou os grãos de pólen nas cargas de pólen de abelhas operárias. A carga de pólen de abelhas que retornam às colmeias foi coletada bimensalmente durante um ano, somando 240 cargas de pólen analisadas por microscopia ótica. Encontramos 76 tipos de pólen nas cargas de pólen, dos quais 60% eram de ervas daninhas. As plantas mais frequentemente identificadas nas cargas de pólen foram: Alchornea, Arracacia xanthorrhiza, Baccharis, Bidens pilosa, Brassica juncea, Brassica oleracea, Byrsonima sp., Cecropia, Chamaecrista, Citrus sp., Croton, Datura, Eucalyptus, Hyptis, Lippia alba, Mangifera indica, Melastomataceae sp., Momordica charantia, Myrsine, Pereskia eata, Persea americana, Piptadenia americana Plinia peruviana, Plaqueia, Poaceae, Prunus persica, Psidium guajava, Raphanus sativus, Rubus urticifolius, Schefflera e Schinus. As abelhas se alimentavam principalmente de ervas daninhas, especialmente durante o inverno e o outono, e, portanto, a presença de ervas daninhas pode ser muito benéfica para as práticas de meliponicultura. Os subprodutos da meliponicultura em agroecossistemas derivam de fontes botânicas diversificadas, isto é, mel selvagem de boa qualidade porque é de um sistema orgânico.

Palavras-chave: fontes de pólen, melissopalinologia, abelha sem ferrão, tetragonisca angustula.

1 INTRODUCTION

Knowledge of the bee flora of ecosystems in which beekeeping, either meliponiculture or apiculture, is developed is of great importance. Variability of flora allows sustainable and profitable beekeeping (Marques & Muniz 2011). The botanical diversity of organic agroecosystems can be favorable for insects, especially as food sources for pollinators such as bees. Unlike in monocultures, the removal of weeds is discouraged in organic agroecosystems since it can harm the survival of native pollinator populations (Nicholls & Altieri 2013). There are several agronomic strategies to encourage weeds that are beneficial to pollinators. The cultivation of weeds at crop edges, as well as within fields, can conserve and enhance insect pollinators by offering a succession of blooming species throughout the year (Nicholls & Altieri 2013).

Some species of stingless bees, including the jataí (*Tetragonisca angustula*), have known potential as pollinators of plant species cultivated both in green houses and in open fields (Meléndez Ramírez et al. 2018, Slaa et al. 2006). Therefore, meliponiculture has become attractive to agricultural producers due to the fact that it is inexpensive compared to other activities, is not demanding in terms of space and work, provides by-products valued by the market, and is profitable in the short and medium term, in addition to being an important alternative for the conservation of agroecosystems as well as bee populations (Cortopassi-Laurino et al. 2006).

However, the methods of introducing and managing stingless bees in plantations has been little studied. That said, pollen identification is of fundamental importance to knowing the pollen types and forage plant species preferred by bees in their search for food. Analysis of the pollen transported in the corbiculae of bees or stored in pollen-pots can lead to an understanding of the network between bees and plants as food sources. Furthermore, this information can be used to plan actions that are beneficial for meliponiculture and beekeeping, as well as for managing agroecosystems.

Our goal with this study was to identify the pollen grains in the pollen loads of worker bees of *Tetragonisca angustula* (jataí) to determine the plants it uses as food sources. The results will highlight plants with potential for meliponiculture in organic agroecosystems in the mountainous region of the state of Espírito Santo, Brazil.

2 MATERIAL AND METHODS

The study was conducted on an organic family farm in the central mountainous region of the state of Espírito Santo, in the municipality of Santa Maria de Jetibá, Rio Possmoser District (20°07'48 "S, 40°50'13 "W), with an average altitude of 944 meters. The climate of the region is classified as subhumid temperate, with dry winters and hot summers, Cwa, according to the Köppen classification (Alvares et al. 2013). The farm was chosen because of its horticulture with varied cultivation of vegetables, various types of fruit trees and medicinal, spice and aromatic plants, as well as possessing five-hectares of preserved forest.

The stingless bee species selected for study was the jataí bee, *Tetragonisca angustula*, as it occurs naturally in different ecosystems in the state of Espírito Santo and its management practices are well known.

Five “INPA” (Instituto Nacional de Pesquisas da Amazônia) standard rational boxes were placed on a rural plot indicated for management for the rearing of *T. angustula*. The boxes were installed in a covered shed in the center of the cultivation area, where they were placed on a wooden shelf 1.5 meters high, with 0.5 meters between boxes.

Pollen was collected from the corbiculae of bees bi-monthly for one year (March 2019 to March 2020), to identify cultivated and non-cultivated plant species in their flowering period. Collecting occurred on the following dates to cover the four seasons of the year: March 03, 2019 and May 16, 2019 (autumn), July 11, 2019 and August 22, 2019 (winter), October 10, 2019 and December 10, 2019 (spring), January 30, 2020 and March 18, 2020 (summer). A total of eight collections were made during the experimental period, two per season and on average every 45 days, always in the morning from 8am to 12pm, the period when bees forage the most. An entomological net was used to catch bees at the entrance of the hive on their return from foraging in the field, without harming the insects. Tweezers and a stylus were used to remove the pollen load of six bees from each colony in the five hives, for a total of 30 samples per collection (A1 to A6; B1 to B6; C1 to C6; D1 to D6; E1 to E6), 60 samples per season and 240 samples for the one year of the study. Pollen loads were collected directly from both corbiculae of each bee and placed together in an identified and dated 15-ml centrifuge tube. The pollen was subsequently homogenized and stored in a refrigerator for later preparation of microscopy slides and analysis.

Collected pollen was prepared by the classical European method, without acetolysis (Louveaux et al. 1978), which does not remove the cytoplasmic content of the pollen.

Flowering plants were collected on the same dates of pollen load collection to prepare a reference pollen database for the study site. Pollen slides were made for the plant species listed in Ferreira et al. (2020) and in Ferreira et al. (2021) from pre-anthesis flower buds removed from specimens. Anthers were dissected under a stereomicroscope (Olympus SZX9) and pollen grains were acetolyzed according to Erdtman (1952). Three microscopy slides were prepared for each plant species.

The slides were deposited in the Pollen Database of the Recursos Vegetais e Opoterápicos (SRVO) laboratory of the Ezequiel Dias Foundation (FUNED). Photomicrographs of pollen grains were captured with a 5Mp Moticam camera coupled to an Olympus BX 50 microscope, using Image Pro10 Windows software.

Pollen grains from the pollen loads of bees were identified using an Olympus BX 50 optical microscope at SRVO of FUNED. Identification was done by comparison with the Reference Pollen Database as explained above, with the SRVO Reference Pollen Database, with specialized literature and with the help of a specialist palinotaxonomist (Cynthia F. P. Luz).

The results of the identification of pollen types in the pollen loads were subjected to exploratory analysis. A heatmap was created to show the frequency of occurence of the main pollen types identified in the samples (Galili et al. 2018). The greater the color intensity the greater the frequency of a plant species (in rows), by seasons of the year (in columns). Pollen spectra of seasons were grouped through hierarchical cluster analysis, using Ward's method and Euclidean distance to show the similarity among the pollen spectra. The number of pollen sources used by bees was compared among seasons of the year using the variable "number of pollen types", which is the diversity of the pollen loads of bees. The Kruskal-Wallis test was then performed, followed by Dunn's test for multiple comparisons between seasons. All analyses were performed in R software (R Core Team 2022).

3 RESULTS

In total, 76 pollen types distributed (Table I) in 31 plant families (Figure 1) were identified in the samples from the four seasons. The families with the greatest number of species identified in pollen loads were: Asteraceae, Myrtaceae, Euphorbiaceae, and Fabaceae (Figure 1). Fungi and bryophyte spores were also observed, mainly in pollen loads collected in winter and spring. The results indicate that the following plants have potential for meliponiculture in organic agroecosystems (Table I): *Alchornea, Arracacia xanthorrhiza, Baccharis, Bidens pilosa, Brassica juncea, Brassica oleracea, Byrsonima* sp., *Cecropia* (only pollen source), *Chamaecrista, Citrus* sp., *Crotalaria* sp., *Croton, Datura, Eucalyptus, Hyptis, Lippia alba, Mangifera indica*, *Melastomataceae* sp., *Momordica charantia, Myrsine, Pereskia aculeata, Persea americana, Piptadenia, Pisum sativum, Plinia peruviana, Pluchea,* Poaceae (only pollen source), *Prunus persica, Psidium guajava, Raphanus sativus, Rubus urticifolius, Schefflera,* and *Schinus*.

Only one pollen type was not identified to at least the family level. The total number of pollen types identified in autumn was 44, followed by winter with 37, summer with 26 and spring with 21 (Table I). Considering all pollen types in loads, weeds were more representative (60%) than cultivated species (Figure 2a). Weeds were visited by bees in all four seasons (Figure 2b) but were more representative than cultivated species only in winter (Figure 2b).

Analysis of the diversity of plant species/pollen types present in individual pollen loads revealed autumn to have the most diverse pollen loads with a mean of 12±5 pollen types, followed by winter with 8,5±4 pollen types, summer and spring with 3±1 pollen type for both season (Figure 3). The diversity of pollen loads differed significantly between winter and autumn (p-value <0.05) but not between spring and summer (Table II).

Autumn - A total of 44 pollen types were identified in pollen loads in autumn (Table I). Those with the highest frequencies (in parentheses) among samples were: *Eucalyptus* (30), *Schefflera* (26), *Bidens pilosa* (25), *Baccharis* (20), *Pereskia aculeata* (20), *Piptadenia* (18), Poaceae (18), *Alchornea* (17), *Arracacia xanthorrhiza* (16), *Pluchea* (14), *Datura* (13), *Lippia alba* (11), *Psidium guajava* (9), *Hyptis* (8), *Myrsine* (8), and *Prunus persica* (8). Autumn pollen loads had two to 19 pollen types (Figure 3). Weeds represented 65% of the pollen loads in this season (Figure 2B).

Winter - A total of 37 pollen types were identified in pollen loads in winter, with one pollen type not being identified (Table I). Those with the highest frequencies (in parentheses) among samples were: *Bidens pilosa* (17), *Raphanus sativus* (15), *Persea americana* (15), *Chamaecrista* (13), *Prunus persica* (13), *Brassica juncea* (11), *Citrus* (10), *Schefflera* (9), *Baccharis* (9), *Mangifera indica* (8), *Plinia peruviana* (8), *Schinus* (7), *Arracacia xanthorrhiza* (7), *Hyptis* (7), *Eucalyptus* (7), and Poaceae (7). Fungi spores were found in different pollen loads during this season. Winter pollen loads had two to 12 pollen types (Figure 3). Weeds represented 45% of the of the pollen in loads in this season (Figure 2B).

Spring - A total of 21 pollen types were identified in pollen loads in spring (Table I). Those with the highest frequencies (in parentheses) among samples were: *Alchornea* (28), *Rubus urticifolius* (8), *Brassica oleracea* (7), *Croton* (5), *Byrsonima* (4), *Persea americana* (3), and *Citrus* (3). Fungi and bryophyte spores were found in pollen loads during this season. Pollen loads had from one to six pollen types (Figure 3). Weeds represented 75% of the pollen in loads in this season (Figure 2B).

Summer – A total of 26 pollen types were identified in pollen loads in summer (Table I). Those with the highest frequencies (in parenthesis) among samples were: *Pluchea* (9), Poaceae (8), *Pisum sativum* (7), *Crotalaria* sp. (7), *Schefflera* (6), *Schinus* (5), Melastomataceae sp. (5), *Momordica charantia* (4), and *Cecropia* (4). Pollen loads had from one to four pollen types (Figure 3). Weeds represented 73% of the pollen loads in this season (Figure 2B).

4 DISCUSSION

The present study showed that sources for pollen, and possibly for nectar as well, for jataí bees in the studied agroecological system represent a large number of plant species throughout the year, most of which were weed species. Thus, the presence of weeds in organic agroecosystems is important for the maintenance and survival of bee populations.

Highly eusocial bee species, such as the *Tetragonisca angustula*, exploit a wide range of species as food sources, as they are generalists (Michener, 1974). There were at least 106 plant species available to bees throughout the year in the bee pasture of the studied organic agroecological system (Ferreira et al., 2020, 2021). The results show that *T. angustula* used approximately 72% of the existing plant species in the bee pasture, evidencing wide foraging among the available bee flora. Furthermore, more weeds were found in this environment (59) than cultivated species (47) (Ferreira et al. 2021, 2020). Among the 77 pollen types identified in the pollen loads of jataí bees throughout the year, the proportion pollen types of weeds in the pollen loads of *T. angustula* was higher (60%) than cultivated plants.

Organic agroecosystems characteristically have a huge diversity of non-cultivated species, or weeds. These species are known to be favorable as sources of floral resources for pollinating insects at all times of the year, whether in the harvest or off-season of cultivated species (Nicholls & Altieri, 2013). The present work evidenced this, showing the importance of weeds to worker bees during seasons with less floral resources, when, in many cases, it is necessary to offer artificial food to bees. Although weeds were more frequent in pollen loads than cultivated plants only during the winter, they were an important part of the diet of bees in all seasons. This highlights the role of this diversity of plants for the survival of these bees and, possibly, their dependence on foraging on these species during the winter and the off-season of cultivated species. Weeds are a rich source of nectar and pollen due to their great adaptability to edaphoclimatic conditions and are likely to be used by bee (Bretagnolle & Gaba 2015). When evaluating plant species with honey potential in the Paraguaçu river valley, municipality of Castro Alves, state of Bahia, (CARVALHO & MARCHINI 1999) found an important contribution of different weed species, such as *Commelina benghalensis*, *Croton campestris*, *Centratherum punctatum*, *Momordica charantia*, *Sida paniculata*, *Waltheria indica* and *Portulaca* spp., in the foraging of *Apis* bees and for honey production. Salis et al. (2015) evaluated the floral calendar of native honey plants in the Pantanal, state of Mato Grosso do Sul, and concluded that invasive plants have great potential as honey flora. Evaluating the foraging of bees, the authors concluded that 47% of the plant species visited are considered weeds, with emphasis on *Commelina erecta*, *Corchorus hirtus*, *Gomphrena celosioides*, *Hyptis suaveolens*, *Indigofera hirsuta*, *Malvastrum coromandelianum*, *Senna occidentalis*, *Sida rhombifolia* and *Wissadula hernandioides*. Surveying the flora of beekeeping interest in the municipality of Petrolina, state of Pernambuco, (Santos et al. 2006) observed that the weed species *Merremia aegyptia*, *Indigofera hirsuta*, *Macroptilium martii*, *Raphiodon echinus*, *Herissanthia crispa*, *Passiflora foetida*, *Richardia grandiflora*, *Waltheria rotundifolia* and *Tribulus cistoides* were widely visited by bees, making a great contribution to honey production. Kiill et al. (2000) found native bees visiting weedy flowers in areas cultivated with fruit trees in northeastern Brazil. These species, along with native plants, are part of the diet of these bees, especially in the dry season, when food sources are reduced, making weed species of great importance to bee foraging.

However, the present work did not assess the abundance of each plant species in the environment, and cultivated species are expected to be more abundant than non-cultivated species. This means that, due to the efficient recruitment system of eusocial bees to food sources, there is likely to be a concentration of foraging by bees on the most abundantly flowering plant species in the environment, especially during spring and summer. Several pollen loads had more than one pollen type present, indicating that individual worker bees visited different plants on the same collection trip.

Autumn was the season with the greatest species richness in pollen loads. It was also the season with the greatest number of plant species flowering in the study area, both cultivated (36%) and non-cultivated (35%) species (Ferreira et al., 2020, 2021). The greater richness of pollen types in pollen loads may have been due to a greater richness of floral resources in the bee pasture. On the other hand, the lowest number of pollen types in the pollen loads of bees was in summer, when about 31% of non-cultivated plant species were flowering in the bee pasture and only five cultivated species (10%), namely: bitter tomato (*Solanum aethiopicum*), carrot (Daucus carota), tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*) and eucalyptus (*Eucalyptus* sp.). Despite being fewer in number compared to non-cultivated species, the abundant flowering of individuals of these cultivated species may have led to a concentration of bee foraging in their flowers, so that more bees collected the pollen resource from the same floral source. In fact, when there is a massive supply of floral resources from the same source, eusocial bees tend to concentrate foraging on these more productive sources of food for them (Calaça et al. 2022).

Although the present study provides data about the identity of pollen grains in pollen loads of worker bees, it could be that these bees collected both nectar and pollen on their visits to flowers. The present approach, however, was not able to confirm which resource (pollen and/or nectar) was collected by the bees in flowers. Nonetheless, the plants listed here are of great importance for stingless bee beekeeping. The present study also did not quantify the contribution of each plant species to the diet of the bees, and it cannot state which plants these bees use in greater proportions for food. Finally, although the present study did not measure the efficiency of *T. angustula* as pollinator of the visited plant species, it is possible that these bees play an important hole in the reproduction of native plant species. Jataí bees are pollinators of several native (Calaça et al. 2022) and cultivated species (Antunes et al. 2007).

5 CONCLUSIONS

- Weeds are important food sources for jataí bees in the studied organic agroecosystem, although they also visited cultivated species.
- The by-products of meliponiculture in agroecosystems are diversified in their botanical sources, that is, wild honey of good quality because it is an organic system.
- The presence of weeds growing in and around crops can benefit bee health and conservation, promote sustainable economic activities such as meliponiculture, improve crop productivity and reduce pesticide exposure.

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ANNEXES

Figure 1. Number of pollen types identified in each pollen load of *Tetragonisca angustula* bees in an organic agroecosystem, Santa Maria de Jetibá, state of Espírito Santo, Brazil, by botanical family.

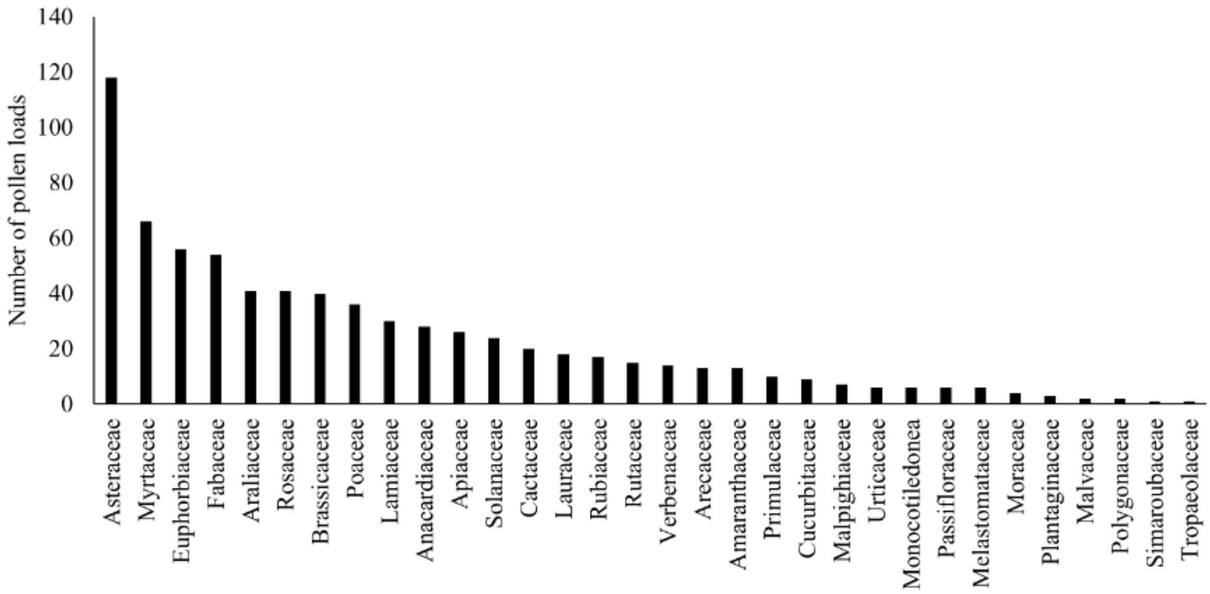

Figure 2. A. Relative frequency of cultivated species and weeds in analyzed pollen loads of *Tetragonisca angustula* (N=240 pollen loads). B. Relative frequency of cultivated species and weeds in analyzed pollen loads of *Tetragonisca angustula* for each season (autumn, winter, spring, summer; N=60 pollen loads each season).

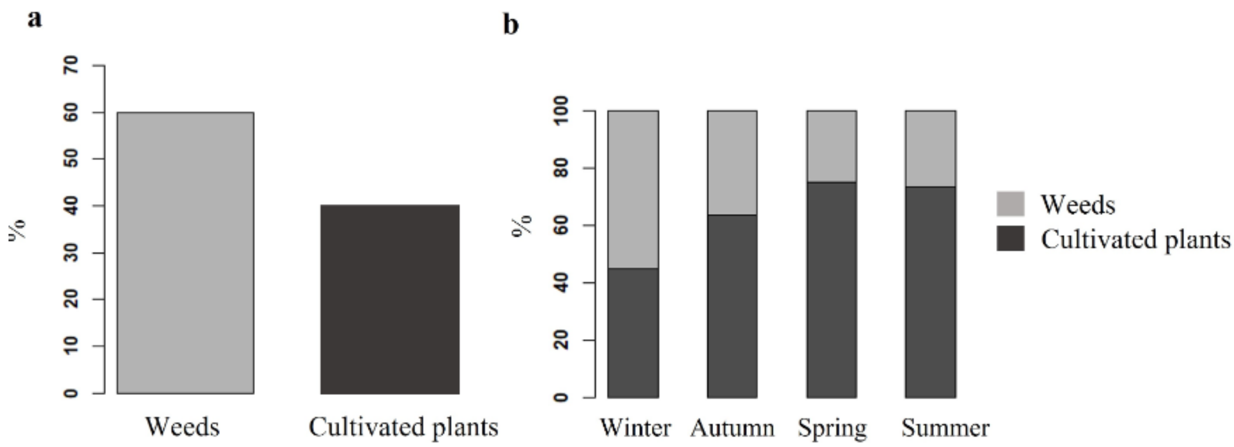

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Figure 3. Boxplot of the number of pollen types per pollen load collected during the four seasons of the year (autumn, winter, spring, summer; N=60 pollen loads each season).

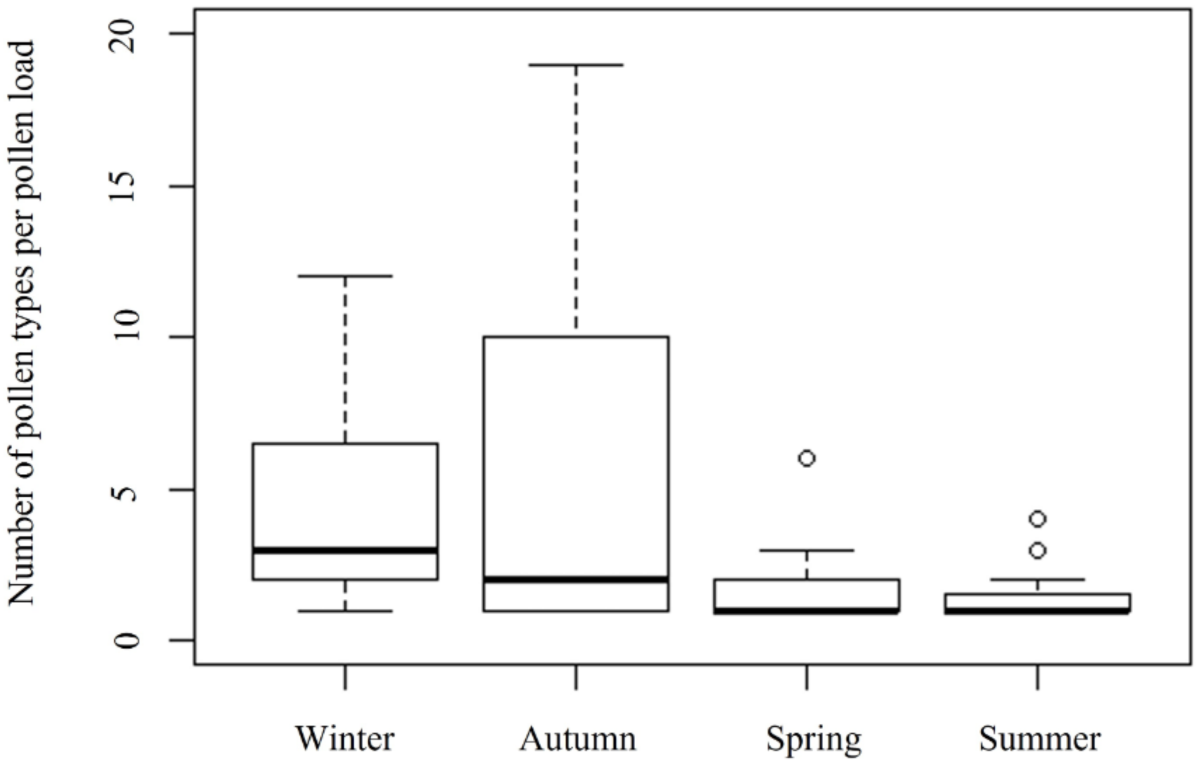

Figure 4. Heatmap showing the frequency of occurrence of the main pollen types identified in the pollen loads of *Tetragonisca angustula*. Hierarchical cluster analysis using Ward's method and Euclidean distance, to show the similarity among the pollen spectra of the four seasons of the year.

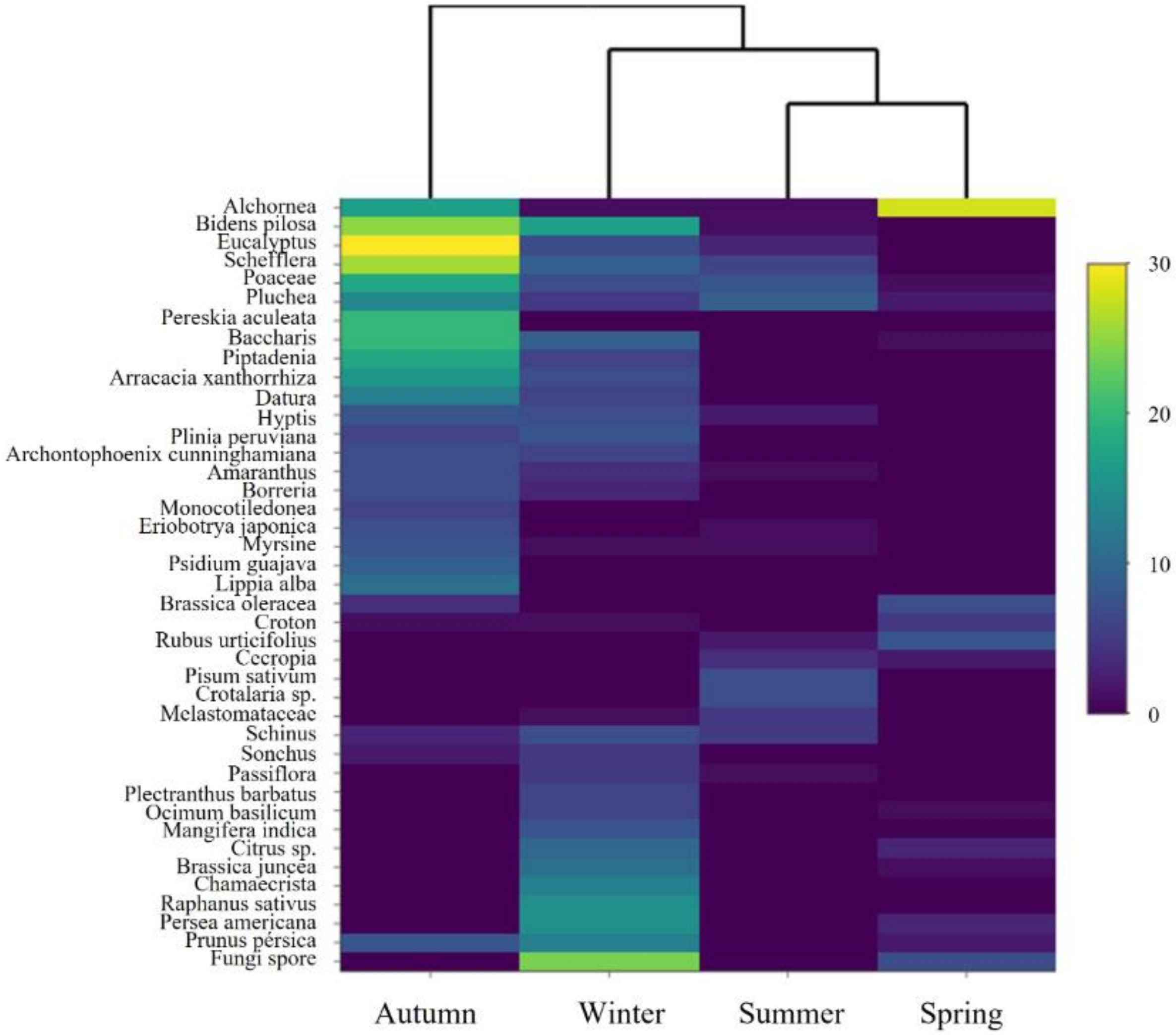

Table I. Pollen types identified in the sampled pollen loads of *Tetragonisca angustula* (jataí) collected over a period of one year in an organic agroecosystem, Santa Maria de Jetibá, Espírito Santo, Brazil (each season had N=60 pollen loads).

Family/Pollen Type	Autumn	Winter	Spring	Summer
Amaranthaceae				
Alternanthera		1		
Amaranthus	7	4		1
Anacardiaceae				
Tapirira	4		1	
Mangifera indica		8		
Schinus	3	7		5
Apiaceae				
Coriandrum sativum	1			
Daucuscarota				2
Arracacia xanthorrhiza	16	7		

Apocynaceae				
Prestonia/Mandevilla	4			
Araliaceae				
Schefflera	26	9		6
Arecaceae				
Archontophoenix cunninghamiana	7	6		
Asteraceae				
Lactuca sativa	1			
Cosmos caudatus	2			
Senecio brasiliensis		2		
Vernonia	1	1	1	
Sonchus	2	5		
Pluchea	14	5	2	9
Baccharis	2	9	1	
Bidens pilosa	25	17		1
Brassicaceae				
Eruca sativa				2
Brassica oleracea	4		7	
Brassica juncea		11	1	
Raphanus sativus		15		
Cactaceae				
Pereskia aculeata	2			
Cucurbitaceae				
Sechium edule	1		1	
Citrullus lanatus	2			
Momordica charantia			1	4
Euphorbiaceae				
Ricinus communis				1
Dalechampia		1		
Croton	1	1	5	
Alchornea	17	1	28	1
Fabaceae				
Mucuna	1			
Chamaecrista mucronata			2	
Pisum sativum				7
Crotalaria sp.				7
Chamaecrista		13		
Piptadenia	18	6		
Lamiaceae				
Plectranthus barbatus		6		
Ocimum basilicum		6	1	
Hyptis	8	7		2
Lauraceae				
Persea americana		15	3	
Malpighiaceae				
Banisteriopsis	1			
Malpighia emarginata	1			
Malpighiaceae sp.				1
Byrsonima sp.			4	
Malvaceae				
Sida rhombifolia	2			
Melastomataceae				
Melastomataceae sp.		1		5
Monocotiledonea				
Monocotiledonea	6			
Moraceae				

Moraceae sp.	4			
Myrtaceae				
Myrtaceae sp.	1			
Myrtaceae sp.1	2			
Psidium guajava	9			
Plinia peruviana	6	8		
Eucalyptus	3	7		3
Passifloraceae				
Passiflora		5		1
Plantaginaceae				
Plantago	3			
Poaceae				
Zea mays		2		
Poaceae	18	7	1	8
Polygonaceae				
Polygonum punctatum				2
Primulaceae				
Myrsine	8	1		1
Rosaceae				
Eriobotrya japonica	7			1
Rubus urticifolius			8	2
Prunus persica	8	13	2	
Rubiaceae				
Rubiaceae				1
Emmeorhiza umbellata		1		
Spermacoce			1	
Borreria	7	3		
Rutaceae				
Citrus sp1		2		
Citrus sp		1	3	
Simaroubaceae				
Simarouba amara	1			
Solanaceae				
Nicandra physalodes	5			
Datura	13	6		
Tropaeolaceae				
Tropaeolum majus				1
Urticaceae				
Cecropia			2	4
Verbenaceae				
Lantana camara			1	2
Lippia alba	11			
Total	44	37	21	26

Table II. P-values for Dunn's test of multiple comparisons after the Kruskal-Wallis test of the number of pollen types identified in each season of the study (autumn, winter, spring, summer). Significance level adopted was 0.05 (N=60 samples per season).

Seasons	Winter	Autumn	Spring
Autumn	0.05	-	< 0.001
Spring	< 0.001	< 0.001	-
Summer	< 0.001	< 0.001	0.344*

*not significantly different.