Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Ruderal Plants Providing Bees Diversity on Rural Properties

PC Montagnana, MJO Campos

Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Rio Claro, São Paulo, Brazil

Article History

Edited by
Celso Martins, UFPB, Brazil
Received 24 October 2019
Initial acceptance 27 April 2020
Final acceptance 21 July 2020
Publication date 30 September 2020

Keywords
Plant diversity, crops, smallholder rural properties, Atlantic Forest.

Corresponding author
PC Montagnana
Departamento de Ecologia
Instituto de Biociências
Universidade Estadual Paulista Júlio de Mesquita Filho
Avenida 24 A, 1515, Bela Vista
CEP: 13.506-900, Rio Claro-SP, Brasil.
E-Mail: paula-eco@hotmail.com

Abstract

Many are the anthropogenic drivers of pollinator decline, but the loss of suitable habitats, among other effects caused by agricultural intensification, deserves special attention. Reduction in the availability of floral resources negatively affects bee communities, compromising bee species composition, foraging behavior, corporal size, and fitness. Our study aims to understand whether the presence of herbaceous plants, acting as foraging sites, next to crops contributes to bee species richness in smallholder rural properties. Bee sampling was performed on smallholder rural properties in the municipality of Guapiara, southern São Paulo state. Individuals who visited the flowers of ruderal plants and crops were collected, using an entomological net, for ten months. A total of 61 bee species were identified, with the highest species richness being sampled in ruderal plant flowers in the three properties studied. Only in one property, ruderal plants hosted a more diverse bee assemblage (Shannon-Wiener and taxonomic diversity indices), but species composition differed from that sampled in crop plants (Jaccard index) in all properties. Thirty-two species were sampled exclusively in ruderal plants, versus 9 only in crops and 20 species in both types of plants. Pollen analysis showed that of the 22 species of bees that were sampled only on flowers of ruderal plants, 9 species carried pollen of tomato and one species of bee carried pollen of kabocha squash. Ruderal plants can provide an alternative food resource for pollinators, enabling these insects to remain in or be attracted to crop areas, where, in addition to visiting such plants, they also visit the cultivated plant flowers. Allowing coexistence between crops and ruderal plants, provided that the issues of plant health are observed, is a simple and low-cost measure for farmers and will provide both economic and environmental benefits.

Introduction

In recent years, a growing number of studies have shown that the yields of many agricultural crops increase when pollinated by bees. The role of wild bees has garnered further attention due to the realization that human food security is directly dependent on this ecosystem service (Potts et al., 2016; Klein et al., 2018). According to Garibaldi et al. (2013), pollination by wild insects is more efficient than that carried out by honey bees alone; Mallinger et al. (2019) quantified the economic value of pollination by wild bees in sunflower crops in the United States at 56.7 million US dollars; two solitary bee species were identified as the most efficient pollinators of this crop. Crops such as strawberries (Herrmann et al., 2019; MacInnis & Forrest, 2019), rapeseed oil (Halinski et al., 2018; Perrot et al., 2018) and blueberry (Nicholson & Ricketts, 2019), among others, are also better pollinated by wild bees.

The anthropogenic drivers of pollinator decline are many (see Potts et al., 2010), but the loss of suitable habitats, among other effects of agricultural intensification, deserves special attention. Food and nesting resources are drastically reduced in intensively farmed landscapes because such areas have a high rate of land-use change and severe disturbances such as tillage, mowing, and grazing (Potts et al., 2010). Specialist species, whether habitat or dietary, experience the

ISSN: 0361-6525

greatest population decline (Biesmeijer et al., 2006). As the proportion of a landscape's agricultural cover increases, bee abundance and richness decrease, and much phylogenetic diversity is lost, leaving bee communities in such landscapes composed of more closely related species (Grab et al., 2019).

Reduction in the availability of floral resources, regardless of the responsible causes, affects bee communities negatively in terms of species composition, foraging behavior, corporal size, and fitness (Jha & Kremen, 2013; Campbell et al., 2018; Rollin et al., 2019). Larval development and offspring size suffer when females provide brood cells with little pollen or pollen of poor nutritional quality (Peterson & Roitberg, 2006; Vanderplanck et al., 2014; Moerman et al., 2015; Renauld et al., 2016). Since the amount and type of nutrients present in pollen and nectar tend to vary from one plant species to another (London-Shafir et al., 2003; Mao et al., 2013; Abbas et al., 2014) bees' access to a diverse flora ensures better-nourished insects.

Sites within rural properties that harbor plant diversity can help maintain pollinator populations and thus provide pollination services in agricultural landscapes (Albrecht et al., 2007), for both crops and native plants. Ghazoul (2006) found that there were more pollinator visits to *Raphanus raphanistrum* L. flowers when individuals of this species occurred close to individuals of three more herbaceous species. According to the author, his study confirmed the hypothesis that pollination facilitation exists among co-flowering plants whose floral displays differ, attracting greater pollinator diversity. Thus, in addition to being an alternative source of food, ruderal plants also contribute to greater diversity in environments at the farm scale, which favors a greater abundance of pollinators (Chateil & Porcher, 2015).

Our study aims to understand whether the presence of herbaceous plants, acting as foraging sites, next to crops contributes to bee species richness in smallholder rural properties. We believe that the bee species richness and diversity sampled on ruderal plant flowers are greater than those sampled on crop plants; bee species composition also differs between these two types of plants.

Material and Methods

Study area

The study was carried out at three smallholding rural properties in Guapiara municipality, southern São Paulo state, Brazil (Fig 1). According to Köppen typology, the climate in Guapiara is Cwa (humid temperate with dry winter and wet summer). The mean temperature is 15.8ºC in the coldest month (July) and 23.2ºC in the hottest month (February); the average rainfall is 53.3 mm in the driest month (August) and 217.3 mm in the wettest month (January) (CEPAGRI, 2014). Guapiara contains part of the Intervales State Park and is in the heart of the region that comprises the Mosaic of Conservation Units of Serra de Paranapiacaba, which preserves the largest remaining area of Atlantic Forest in Brazil, with 120,000 hectares of protected areas (Fundação Florestal, 2019).

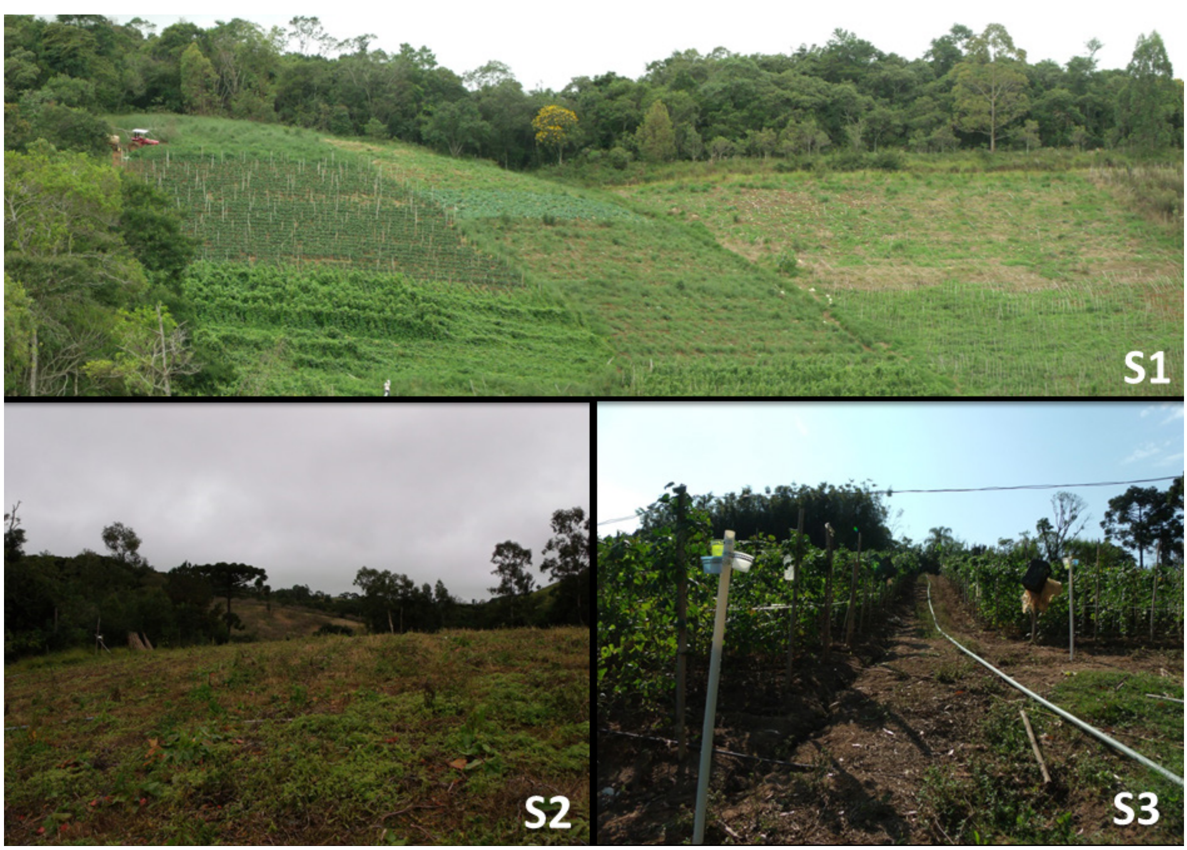

Fig 1. Smallholder rural properties overview. S1: property S1 showing cultivated and post-harvest areas. S2: property S2 showing a post-harvest area. S3: property S3 showing flat bean plantation.

Property S1 is the largest in the area (8.67 ha), followed by property S2 (4.20 ha) and S3 (3.24 ha). The longest Euclidean distance between the properties is 8.63 km (S2-S3), with properties S1 and S2 being closer (4.61 km); S1 and S3 are 6.91 km apart (Fig 2). In all, six crops were grown on the three rural properties over a year (zucchini, kabocha squash, tomato, flat bean, peach, and cucumber). However, on S1 property five crops were grown (S1 = kabocha squash, tomato, flat bean, peach, and cucumber), blooming from July/2012 to March/2013 (Table 1). Properties S2 and S3 had three crops each (S2 = flat bean, cucumber, and zucchini; S3 = flat bean, tomato, and kabocha squash), but with only one crop in common and different blooming periods (Fig 3). Blooming period refers to the time when there were crop flowers on the property, as there were plantations of the same crop with different ages within the properties. A common practice in the properties studied is to allow the free growth of ruderal plants in post-harvest areas and crop borders so that throughout the year there is a turnover of areas that concentrate a greater abundance of these plants.

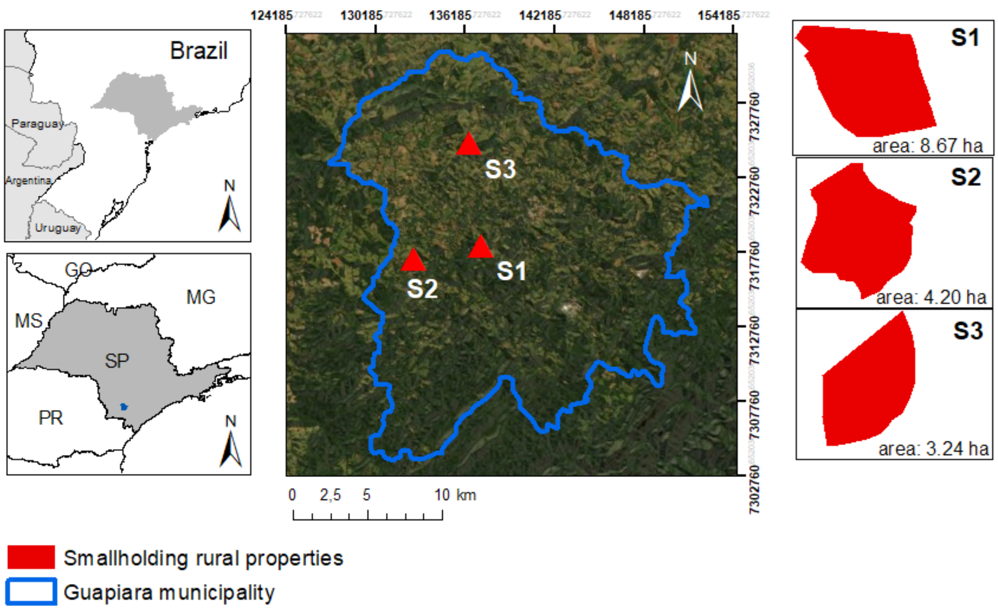

Fig 2. Study area location. South America, Brazil. Blue square indicating the study area in the São Paulo state.

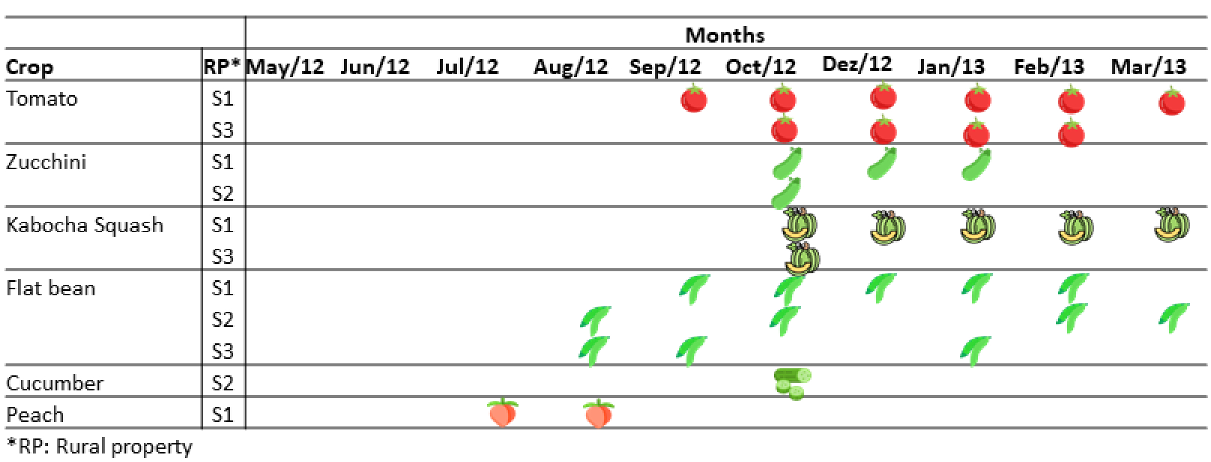

Fig 3. Crop plants blooming period at three smallholder rural properties in Guapiara, São Paulo state, Brazil, between May 2012 and March 2013.

Data collection

For a period of ten months, from May 2012 to March 2013 (except November 2012), we collected bees at three rural properties. Bees were collected from 8 a.m. to 1 p.m., always by two collectors, with a change of collectors every sampling month. Using an entomological net one collector sampled the bees walking the crop lines, and the other one collected the bees on ruderal flowers present on the crop edges and in transects open in post-harvest areas. When there were no crops in bloom on any of the three rural properties, the two collectors sampled the bees on ruderal flowers in the post-harvest and surrounding areas. Each day of collection involved four to five 1-hour sampling cycles at 20-minute intervals, totaling 50 hours/property. At the time of insect collection were noted the plant type where the capture occurred (whether cultivated

Table 1. Bee species sampled on crop flowers at three smallholder rural properties in Guapiara, São Paulo state, Brazil, between May 2012 and March 2013.

Crop	Bee species	Rural properties		
		S1	S2	S3
Tomato (*Lycopersicon esculentum* Mill.)	*Anthrenoides meridionalis* (Schrottky, 1906)	X		
	Augochloropsis cupreola (Cockerell, 1900)			X
	Augochloropsis electra (Smith, 1853)			X
	Augochloropsis sp. 2	X		
	Augochloropsis sp. 3	X		X
	Bombus (Fervidobombus) morio (Swederus, 1787)	X		X
	Bombus (Fervidobombus) pauloensis Friese, 1913	X		X
	Exomalopsis (Exomalopsis) analis (Spinola, 1853)	X		X
	M. (Melipona) quadrifasciata quadrifasciata Lepeletier, 1836			X
	Oxaea flavescens (Klug, 1807)			X
	Oxaea sp.			X
	Paratrigona subnuda Moure, 1947	X		X
	Psaenythia bergii (Holmberg, 1884)	X		
	Pseudaugochlora indistincta (Almeida, 2008)			X
	Tetragonisca angustula (Latreille, 1811)			X
	Trigona spinipes (Fabricius, 1793)	X		
	Xylocopa (Neoxylocopa) suspecta (Moure & Camargo, 1988)			X
	Xylocopa (Stenoxylocopa) artifex (Smith, 1874)	X		
Zucchini (*Cucurbita pepo* L.)	*Augochlora (Augochlora) amphitrite* (Schrottky, 1909)	X		
	Augochlora (Augochlora) foxiana (Cockerell, 1900)	X		
	Bombus (Fervidobombus) morio (Swederus, 1787)	X		
	Bombus (Fervidobombus) pauloensis Friese, 1913			
	Peponapis fervens (Smith, 1875)	X		
	Trigona hyalinata (Lepeletier, 1836)	X		
	Trigona spinipes (Fabricius, 1793)	X	X	
Kabocha Squash (*Cucurbita maxima* Duchesne)	*Apis mellifera* Linnaeus, 1758	X		
	Bombus (Fervidobombus) pauloensis Friese, 1913	X		
	Melissoptila thoracica (Smith, 1854)	X		
	Paratetrapedia fervida (Smith, 1879)	X		
	Peponapis fervens (Smith, 1875)	X	X	
	Trigona hyalinata (Lepeletier, 1836)	X		X
	Trigona spinipes (Fabricius, 1793)	X	X	
Flat bean (*Phaseolus vulgaris* L.)	*Apis mellifera* Linnaeus, 1758	X		X
	Augochlora (Augochlora) amphitrite (Schrottky, 1909)			X
	Bombus (Fervidobombus) morio (Swederus, 1787)	X	X	X
	Bombus (Fervidobombus) pauloensis Friese, 1913	X	X	X
	Paratrigona subnuda Moure, 1947		X	X
	Peponapis fervens (Smith, 1875)	X		
	Schwarziana quadripunctata (Lepeletier, 1836)	X		
	Trigona spinipes (Fabricius, 1793)	X	X	X
	Xylocopa (Neoxylocopa) brasilianorum (Linnaeus, 1767)		X	
	Xylocopa (Neoxylocopa) frontalis (Olivier, 1789)	X	X	
	Xylocopa (Stenoxylocopa) artifex (Smith, 1874)	X		X
Cucumber (*Cucumis sativus* L.)	*Bombus (Fervidobombus) morio* (Swederus, 1787)		X	
	Paratrigona subnuda Moure, 1947		X	
Peach (*Prunus persica* (L.) Batsch)	*Trigona hyalinata* (Lepeletier, 1836)	X		

or ruderal), the identity of the rural property (if number 1, 2 or 3), the collector (identified by the first letter of the name) and the number of the insect (based on the amount that each collector sampled). Bee specimens collected were euthanized in ethyl acetate and stored in 70% alcohol. In the laboratory, the specimens were pressed, appropriately tagged, and stored in entomological drawers. Species identification was carried out by Dr. Silvia Pedro from Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Brazil. The specimens are currently stored in the collection of the Grupo de Estudos em Ecologia e Conservação de Abelhas Silvestres laboratory, São Paulo State University (UNESP), Rio Claro, São Paulo, Brazil. The sampling of ruderal plants present in rural properties was also carried out monthly. Each new ruderal plant that was identified in bloom was photographed, flower buds were collected for pollen analysis and a sample of the plant for the confection of exsiccates. The same procedure was adopted for crop plants in bloom.

Pollen analysis

To complement the data of which plants were used as a food source for the bees sampled in the three rural properties, we carried out the analysis of pollen grains adhered to the specimens' bodies, scopes, and corbicles. The pollen samples were obtained from the body of 115 individuals collected on crop flowers and 267 individuals collected on ruderal flowers and transferred to test tubes containing 50 mL of ethanol solution 70%. After 24 hours, the ethanol was discarded and the samples were placed in 4 mL of glacial acetic acid for 24 hours (Silva et al., 2010). After that, the pollen material was submitted to the acetolysis process following the method described by Erdtman (1960), and when the acetolysis process was finished the samples were then put in an aqueous glycerine solution of 50%. After another 24 hours, small amounts of acetolized pollen were removed with cubes of glycerine jelly for the preparation of three microscopic slides per individual. Photomicrographs were taken and pollen types were identified by comparisons with the reference slides created with pollen samples collected from floral buds of the ruderal and crop plant species sampled in the three rural properties studied. Pollen grains removed from anthers contained in flower buds were submitted to the same process described above, with three microscopic slides per plant species. The ruderal plants sampled were identified using the book "Plantas daninhas do Brasil" (Lorenzi, 2008).

Data analysis

We compared the bee assemblages from flowers of ruderal and crop plants for each of the three properties. To estimate species richness, we used the Chao 1 index, which is based on abundance and is a function of the ratio of singletons to doubletons (Magurran, 2013). Average taxonomic breadth, ($\Delta+$; Clarke & Warwick, 1998; Andersson et al., 2013) and Shannon's (H') diversity index were calculated to estimate the sample diversity, while the dominance was calculated through the Simpson dominance index (D). Average taxonomic breadth is a diversity index that uses information from a Linnaean classification tree of the taxonomic relations between all sampled bee species. High $\Delta+$ values indicate a taxonomically broad, therefore taxonomically diverse, bee community; low $\Delta+$ values indicate a community composed of closely related species. Simpson dominance index ranges from 0, where all taxa are equally present, to 1, where one taxa dominates the community (Magurran, 2013). Finally, to verify whether bee species composition differed between the assemblage sampled from ruderal plants and that sampled from crop plants, we used the Jaccard's dissimilarity index (Magurran, 2013). The analyses were performed using RStudio software version 3.6.3 (R Core Team, 2020) and the package vegan (Oksanen et al., 2019).

Results

A total of 61 bee species were identified (families Apidae, Halictidae, Megachilidae, Colletidae, and Andrenidae) among the 666 specimens collected across the three properties (Table 2). Property S2 harbored the greatest bee species richness (n = 41 species), followed by properties S3 (n = 37) and S1 (n = 31). *Bombus pauloensis* was the most abundant species in the three rural properties (S1 = 66 specimens; S2 = 65; S3 = 30), with *Trigona spinipes* the second most abundant in two properties (S2 = 23 specimens; S3 = 28) and *Bombus morio* the second most abundant in the S1 property (S1 = 40 specimens).

Twenty-nine bee species were collected on crop flowers, where the tomato was the crop with the greatest bee species richness visiting its flowers (n = 18 species) (Table 1) and the bee species *Bombus morio* that visited more varieties of crops. Twenty-eight species of ruderal plants were identified overall, with 25 species occurring on property S1, 20 species on property S2, and 15 species on property S3 (Table 3). Pollen analysis showed that *Leonurus sibiricus* and *Sonchus oleraceus* were the ruderal species visited by the largest number of bee species, 22 species each. Meanwhile, the rural property with the greatest ruderal plants species richness (S1 = 25 ruderal species) was not the place where there was the greatest bee species richness (S2 = 41 bee species), nor the place with the greatest bee species richness visiting ruderal plants (S2 = 39 bee species). There were nine bee species collected only on crop flowers and 32 bee species collected only on ruderal flowers (Table 2); however, 20 bee species were collected on both types of plants. In the Discussion section, we will pay special attention to species that have visited both ruderal and crop plants.

Pollen analysis also showed that of the 22 species of bees that were sampled only on flowers of ruderal plants, 9 species carried pollen of tomato and one species of bee carried pollen of kabocha squash, being *Augochloropsis* sp 1, *Dialictus* sp., *Exomalopsis* (Exomalopsis) *planiceps*, *Exomalopsis* sp 2, *Megachile (Chrysosarus) pseudanthidioides, Melissodes (Ecplectica) nigroaenea, Neocorynura oiospermi, Psaenythia* sp, *Thygater (Thygater) analis*, and *Exomalopsis* sp 1, respectively. The same happened with the bee species that were collected only on crop flowers, of 9 species 5 carried pollen grains from ruderal plants: *Augochlora (Augochlora) foxiana*, *Augochloropsis electra*, *Exomalopsis (Exomalopsis) analis*, *Oxaea* sp., and *Paratetrapedia fervida*.

Table 2. Bee species, abundance and type of plant visited at three smallholder rural properties in Guapiara, São Paulo state, Brazil, between May 2012 and March 2013.

Species	Plants		Abundance/ Rural Property		
	Crop	Ruderal	S1	S2	S3
ANDRENIDAE					
OXAEINI					
Oxaea flavescens Klug, 1807	X				1
Oxaea sp	X				6
PANURGINAE					
PROTANDRENINI					
Anthrenoides meridionalis (Schrottky, 1906)	X	X	9	1	
Anthrenoides jordanensis Urban, 2007	X			1	
Cephalurgus anomalus Moure & Lucas de Oliveira, 1962		X		3	
Psaenythia bergii Holmberg, 1884	X	X	1	1	2
Psaenythia sp		X		3	
Rophitulus sp		X		1	
APIDAE					
APINAE					
APINI					
Apis mellifera Linnaeus, 1758	X	X	42	18	10
BOMBINI					
Bombus (Fervidobombus) brasiliensis Lepeletier, 1836		X		1	1
Bombus (Fervidobombus) morio (Swederus, 1787)	X	X	40	10	5
Bombus (Fervidobombus) pauloensis Friese, 1913	X	X	66	65	30
EMPHORINI					
Melitoma segmentaria (Fabricius, 1804)		X		1	
EUCERINI					
Melissodes (Ecplectica) nigroaenea (Smith, 1854)		X	3	4	2
Melissoptila richardiae Bertoni & Schrottky, 1910		X	1		1
Melissoptila thoracica (Smith, 1854)	X	X	4		3
Peponapis fervens (Smith, 1875)	X	X	7	3	5
Thygater (Thygater) analis (Lepeletier, 1841)		X		2	
Trichocerapis mirabilis (Smith, 1865)		X	1		
EUGLOSSINI					
Eulaema (Apeulaema) nigrita Lepeletier, 1841		X		1	
EXOMALOPSINI					
Exomalopsis (Exomalopsis) analis Spinola, 1853	X		9		11
Exomalopsis (Exomalopsis) planiceps Smith, 1879		X	1	1	
Exomalopsis (Exomalopsis) sp 1		X	3		
Exomalopsis (Exomalopsis) sp 2		X		1	
MELIPONINI					
Geotrigona subterranea (Friese, 1901)		X	4		3
M. (Melipona) quadrifasciata quadrifasciata Lepeletier, 1836	X	X		2	3
Paratrigona subnuda Moure, 1947	X	X	28	22	10
Schwarziana quadripunctata (Lepeletier, 1836)	X	X	10	6	1
Tetragonisca angustula (Latreille, 1811)	X	X		1	1
Trigona hyalinata (Lepeletier, 1836)	X	X	12		3

Table 2. Bee species, abundance and type of plant visited at three smallholder rural properties in Guapiara, São Paulo state, Brazil, between May 2012 and March 2013. (Continuation)

Species	Plants		Abundance/ Rural Property		
	Crop	Ruderal	S1	S2	S3
Trigona spinipes (Fabricius, 1793)	X	X	23	23	28
TAPINOTASPIDINI					
Paratetrapedia fervida (Smith, 1879)	X		1		
TETRAPEDIINI					
Tetrapedia aff. *diversipes* Klug, 1810		X	1		
XYLOCOPINAE					
CERATINI					
Ceratina (Ceratinula) cf. *oxalidis* Schrottky, 1907		X		1	
XYLOCOPINI					
Xylocopa (Neoxylocopa) brasilianorum (Linnaeus, 1767)	X	X		2	1
Xylocopa (Neoxylocopa) frontalis (Olivier, 1789)	X	X	5	4	1
Xylocopa (Neoxylocopa) suspecta Moure & Camargo, 1988	X	X			2
Xylocopa (Stenoxylocopa) artifex Smith, 1874	X	X	3	3	2
COLLETIDAE					
COLLETINAE					
Colletes rugicollis Friese, 1900		X		1	
HALICTIDAE					
HALICTINAE					
AUGOCHLORINI					
Augochlora (Augochlora) amphitrite (Schrottky, 1909)	X	X	2	1	7
Augochlora (Augochlora) foxiana Cockerell, 1900	X		1		
Augochlora (Oxystoglossella) morrae Strand, 1910		X	1	1	1
Augochlora (Oxystoglossella) sp		X		1	
Augochloropsis cupreola (Cockerell, 1900)	X	X	3	2	2
Augochloropsis electra (Smith, 1853)	X				2
Augochloropsis nasuta Moure, 1944		X			1
Augochloropsis notophos (Vachal, 1903)		X		1	
Augochloropsis sp 1		X			2
Augochloropsis sp 2	X	X	7	3	2
Augochloropsis sp 3	X		1		1
Neocorynura oiospermi (Schrottky, 1909)		X	3	4	1
Pseudaugochlora indistincta Almeida, 2008	X				1
HALICTINI					
Dialictus creusa (Schrottky, 1909)		X		1	
Dialictus sp 1		X			1
Dialictus sp		X	1	2	3
Pseudagapostemon (Neagapostemon) cf. *cyanomelas* Cure, 1989		X			1
MEGACHILIDAE					
MEGACHILINAE					
ANTHIDIINI					
Anthidium manicatum (Linnaeus, 1758)		X	1	1	
MEGACHILINI					
Megachile (Chrysosarus) pseudanthidioides Moure, 1943		X		1	1
Megachile (Melanosarus) sp		X		2	
Megachile sp 1		X		2	
Megachile sp 2		X		1	

A comparison between the richness estimator Chao 1 and the sampled species' richness values indicates that there may be even more bee species in the study area (Table 4) and there were greater bee species on ruderal flowers than on crops. The assemblages of bees collected on ruderal plants are not more diverse than those of crop plants, nor are they taxonomically more diverse (Table 4). Property S2 differs from these results, due to a smaller number of bee species collected on crop plants; this difference may be a consequence of not having planted tomatoes that year, a mass-flowering crop and prevalent in the Guapiara municipality. Property S3 was the only one that showed greater dominance in the bee assemblage collected on ruderal plants. However, the composition of bee species differs between crop and ruderal for the three properties (Table 4).

Table 3. Ruderal plant species, resource available for pollinators according to literature data (P: pollen; N; nectar), and number of floral visiting bee species (according to pollen analysis) at three smallholder rural properties in Guapiara, São Paulo state, Brazil, between May 2012 and March 2013. In bold are the ruderal plant species visited by the greatest number of bee species.

Family	Species	Resource	Floral visiting bee species (N)	Rural properties S1	S2	S3
Apocynaceae	*Asclepias curassavica*	N/P	-	X	X	X
Asteraceae	*Ageratum conyzoides*	N/P	7	X	X	X
	Bidens alba	N/P	6	X	X	
	Bidens pilosa	N/P	11	X	X	X
	Emilia fosbergii	N/P	4	X		X
	Galinsoga paviflora	N/P	1	X	X	
	Senecio brasiliensis	N/P	4	X	X	
	Sonchus oleraceus	**N/P**	**22**	X	X	
	Synedrella nodiflora	N/P	2	X		X
	Tilesia baccata	N/P	2	X	X	
	Tithonia diversifolia	N/P	-		X	
	Vernonia westiniana	N/P	-	X		X
Bignoniaceae	*Pyrostegia venusta*	N/P	-			X
Boraginaceae	*Cordia curassavica*	N	-		X	
Brassicaceae	*Brassica rapa*	N/P	6	X		
Convulvulaceae	*Ipomea nill*	N/P	-	X	X	X
	Ipomea triloba	N/P	-	X		
Euphorbiaceae	*Ricinus communis*	P	1	X		X
Lamiaceae	***Leonurus sibiricus***	**N**	**22**	X	X	X
Malvaceae	*Sida rhombifolia*	P	4	X	X	
Phytolaccaceae	*Phytolacca americana*	N/P	-	X		X
Primulaceae	*Anagallis arvensis*	P	-	X	X	
Rosaceae	*Rubus rosifolius*	N/P	1	X	X	X
Solanceae	*Solanum americanum*	P	-	X	X	
	Solanum erianthum	P	1	X	X	
	Solanum paniculatum	P	2	X	X	X
Verbenaceae	*Lantana camara*	N	-	X	X	X
	Stachytarpheta cayennensis	N	4	X	X	X
Total species				**25**	**20**	**15**

Discussion

As we expected, species richness of bees visiting ruderal flowers was greater than the richness of bees visiting crop flowers, as well as differences in species composition. But we did not expect that there will be no differences concerning diversity (Shannon index) and average taxonomic breadth (Δ+). However, our results reinforce the importance of ruderal flora for increasing plant diversity on rural properties and thus contributing to bee diversity in agricultural landscapes. Studies conducted mostly in Europe and the United States have already evaluated the importance of plant diversity in agricultural areas, mainly in set-aside systems, and its positive effects on pollinator diversity (Carreck & Williams, 2002; Potts et al., 2003; Hopwood, 2008; Kuussaari et al., 2011; Tscharntke et al., 2011; Kovács-Hostyánszki et al., 2011; Torné-Noguera et al., 2014; Venturini et al., 2017).

Table 4. Richness estimators, diversity and dominance indexes for bees sampled from crop and ruderal plants at three smallholder rural properties in Guapiara, São Paulo state, Brazil, between May 2012 and March 2013.

	Property S1		Property S2		Property S3	
	Crop	Ruderal	Crop	Ruderal	Crop	Ruderal
Species richness	19	23	6	39	18	28
Chao 1	26	44.25	7	73.33	29.66	48.6
Shannon-Wiener (H')	2.35	2.4	1.76	2.69	2.3	2.95
Simpson (D)	0.85	0.87	0.8	0.86	0.84	0.92
Average taxonomic breadth (Δ+)	80.46	80.15	58.83	87.66	80.63	80.86
Jaccard (J)	0.7142		0.9052		0.7709	

Ruderal plants can provide an alternative food resource for pollinators, enabling these insects to remain in or be attracted to, crop areas where they visit the cultivated plant flowers as well as the ruderal plant flowers (Nicholls & Altieri, 2013). Our pollen analysis shows that both bee species that were sampled only on crop or ruderal flowers carried pollen grains from both types of plants, together with the result that 20 bee species were sampled in both types of plants, it strengthens the argument that ruderal plants are an alternative food source in agricultural landscapes (Luz et al., 2011; Luz et al., 2018; Santos et al., 2020). However, there is a lot of concern about ruderal plants competing with crop plants for pollinator visitation. For some crops, though, studies are showing that such competition does not occur (Lundin et al., 2017; Alomar et al., 2018; Knapp et al., 2019); instead, the presence of a richer ruderal plant community at the field scale favored a more diverse bee community (Norris et al., 2018; Knapp et al., 2019). In sunflower crops, for example, the presence of a diverse ruderal plant community within the crop field was responsible for increasing the diversity of flower visitors while also mitigating the negative effects of isolation on crops that were far from native vegetation remnants (Carvalheiro et al., 2011).

Of the 20 bee species in common between crop and ruderal plants (Table 1), some are well-studied crop pollinators. As is the case of bee genera *Apis*, *Bombus*, *Melipona*, *Trigona,* and *Xylocopa*, which are among effective pollinators of various crops, including those planted in our three rural properties studied (Giannini et al., 2015). *Apis mellifera*, *Bombus morio*, *Bombus pauloensis*, *Melipona quadrifasciata*, *Trigona spinipes,* and *Xylocopa frontalis* are among the 14 most important bee species for the pollination of Brazilian crops, considering the economic value of the pollination and the number of pollinated crops by them (Giannini et al., 2020). For good agricultural production, a diverse pollinator fauna is necessary (Garratt et al., 2014), which in turn is maintained by a diverse flora at crop scale and adjacent off-crop habitats (Medeiros et al., 2017; Thompson et al., 2019).

Tomato is the predominant crop in the Guapiara municipality, the longest time flowering period (6 months) and also with the greatest bee species richness as floral visitors. Due to its long flowering period and wide distribution in the municipality, it can be considered a mass-flowering crop and an important pollen source, mainly for native bee species since they are the only ones capable of performing buzz pollination releasing pollen from the poricidal anthers of its flowers (Nunes-Silva et al., 2010). Although studies indicate positive and negative effects of mass-flowering crops on bee species richness and abundance (Holzschuh, et al., 2011; Holzschuh et al., 2013; Diekotter et al., 2014; Holzschuh et al., 2016), it is recognized that a diversified flora in the region provide food resources in periods when the crops are not blooming, favoring pollinator populations permanence.

Plant diversity at crop scale is crucial for providing bees with diverse food sources, especially in agricultural regions with few remnants of native vegetation. Simplified landscapes, such as those dominated by monocultures, tend to harbor lower plant diversity and, therefore, lower quantity and quality of available food resources, which can compromise the fitness of bee individuals in the region. Renauld et al. (2016) verified that the offspring from the solitary bee *Andrena nasonii* Robertson, 1895 in homogeneous landscapes had a smaller body size than those born in landscapes with lower percentages of agricultural occupation. The availability of food resources throughout the seasons is closely related to plant diversity, which positively influences the fitness and population growth of social bees as well (Kaluza et al., 2018).

At the rural properties sampled in the present study, it is common practice for farmers to allow the free growth of ruderal plants in the post-harvest areas (Fig 4 C, E) and at the borders of the crop fields (Fig 4 A). Even though the practice is not intended to provide foraging places for bees, such sites are widely used by apicultural fauna (Fig 4 D, F). Measures such as those adopted by the farmers of Guapiara municipality favor the permanence of wild bee populations in agricultural areas, and they can potentially be replicated in other localities. Allowing ruderal plants to coexistence with crops, provided that the issues of plant health are observed, is a simple and low-cost measure for farmers and provides both economic and environmental benefits, as already presented by Carvalheiro et al. (2011). Our study has led us to conclude that ruderal plants help to enhance plant diversity at crop scale, which is crucial for providing bees with diverse food sources contributing to the permanence of these pollinators in agricultural landscapes.

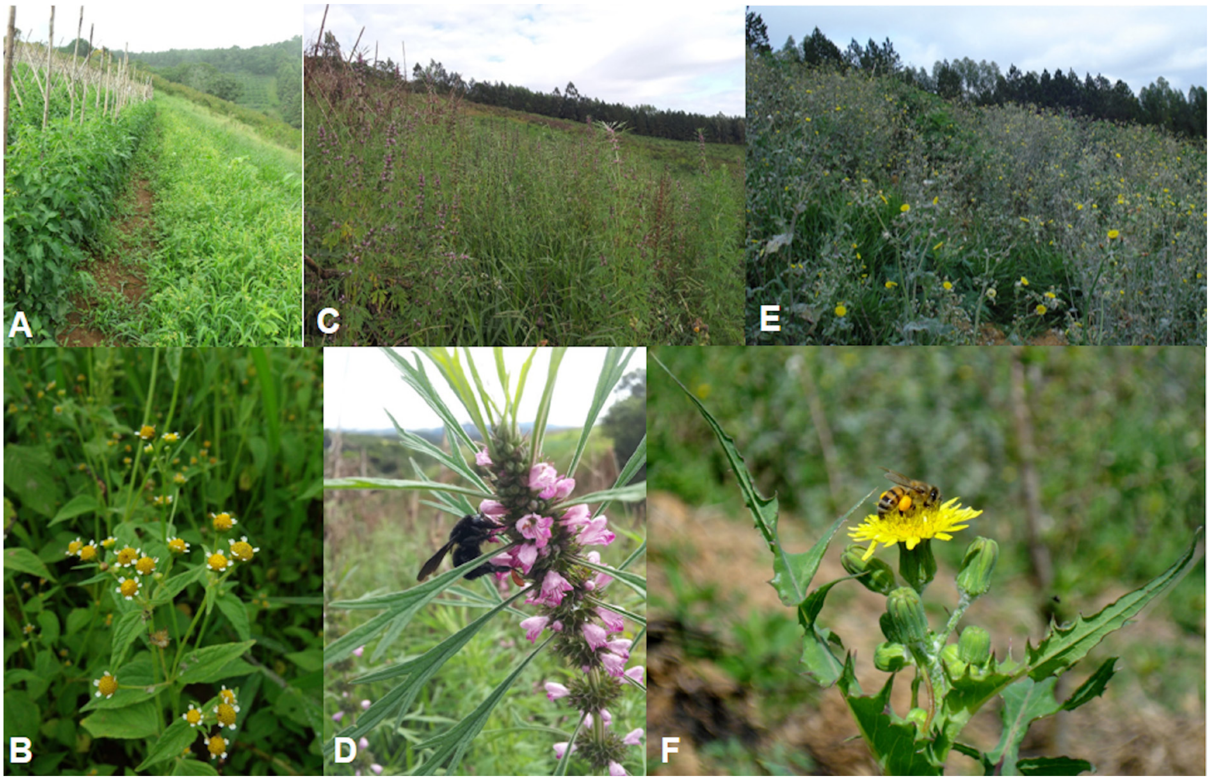

Fig 4. Ruderal plant growth sites at the studied smallholder rural properties. A: Ruderal plant strip, with a dominance of the species *Galinsoga parviflora*, next to tomato crop. B: *Galinsoga parviflora*. C: post-harvest site dominated by specimens of the ruderal plant *Leonurus sibiricus*. D: *Bombus* bee on *Leonurus sibiricus* inflorescence. E: post-harvest site dominated by specimens of the ruderal plant *Sonchus oleraceus*. F: *Apis mellifera* bee on *Sonchus oleraceus* inflorescence.

Acknowledgments

We thank the owners of the sites, Mr. Lauro, Paulo family, and Mr. José, who allowed us access to the plantations for the sampling of bee fauna. Funding provided by CNPq (project "Conservação e Manejo de Polinizadores para uma Agricultura Sustentável através da Abordagem Ecossistêmica"), Global Environmental Facility (GEF), the United Nations Environment Programme (UNEP), the Food and Agriculture Organization (FAO), the Brazilian Biodiversity Fund (FUNBIO), and the Brazilian Ministry of the Environment (MMA).

Authors Contribution

P. C. M. carried out the fieldwork and performed the statistical analysis; M. J. O. C. participated in the design of the study and, P. C. M. and M. J. O. C. drafted the manuscript. All authors gave final approval for publication.

References

Abbas, M., Klein, A.M., Ebeling, A. et al. (2014). Plant diversity effects on pollination and herbivorous insects can be linked to plant stoichiometry. Basic and Applied Ecology, 15: 169-178. doi: 10.1016/j.baae.2014.02.001

Albrecht, M., Duelli, P., Müller, C., Kleijn, D. & Schmid, B. (2007). The Swiss agri-environment scheme enhances pollinator diversity and plant reproductive success in nearby intensively managed farmland. Journal of Applied Ecology, 44: 813-822. doi: 10.1111/j.1365-2664.2007.01306.x

Alomar, D., González-Estévez, M.A., Traveset, A. et al. (2018). The intertwined effects of natural vegetation, local flower community, and pollinator diversity on the production of almond trees. Agriculture, Ecosystems and Environment, 264: 34–43. doi: 10.1016/j.agee.2018.05.004

Andersson, G.K.S., Birkhofer, K., Rundlöf, M., Smith, H.G. (2013). Landscape heterogeneity and farming practice alter the species composition and taxonomic breadth of pollinator communities. Basic and Applied Ecology, 14: 540-546. doi: 10.1016/j.baae.2013.08.003

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S. G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006). Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science, 313: 351-354. doi: 10.1126/science.1127863

Campbell, A.J., Carvalheiro, L.G., Maués, M.M., Jaffé, R., Giannini, T.C., Freitas, M.A.B., Coelho, B.W.T. & Menezes, C. (2018). Anthropogenic disturbance of tropical forests threatens pollination services to açaí palm in the Amazon river delta. Journal of Applied Ecology, 55: 1725-1736. doi: 10.1111/1365-2664.13086

Carreck, N.L. & Williams, I.H. (2002). Food for insects pollinators on farmland: insect visits to flowers of annual seed mixtures. Journal of Insect Conservation, 6: 13-23

Carvalheiro, L.G., Veldtman, R., Shenkute, A.G. et al. (2011). Natural and within-farmland biodiversity enhances crop productivity. Ecology Letters, 14: 251-259. doi: 10.1111/j. 1461-0248.2010.01579.x

CEPAGRI – Centro de Pesquisas Meteorológicas e Climáticas Aplicadas à Agricultura. (2014). http://cpa.unicamp.br/outras-informacoes/clima_muni_203.html (accessed date: 10 January, 2014).

Chateil, C. & Porcher, E. (2015). Landscape features are a better correlate of wild plant pollination than agricultural practices in an intensive cropping system. Agriculture, Ecosystems and Environment, 201: 51–57. doi: 10.1016/j. agee.2014.12.008

Clarke, K.R. & Warwick, R.M. (1998). A taxonomic distinctness index and its statistical properties. Journal of Applied Ecology, 35: 523-531.

Diekotter, T., Peter, F., Jauker, B., Wolters, V., Jauker, F. (2014). Mass-flowering crops increase richness of cavity-nesting bees and wasps in modern agro-ecosystems. Bioenergy, 6: 219-226. doi: 10.1111/gcbb.12080

Erdtman, G. (1960). The acetolysis method. A revised description. Svensk Botanisk Tidskrift, 39: 561-564.

Fundação Florestal (2019). https://guiadeareasprotegidas.sp.gov.br/ap/pe-intervales/ (accessed date: 15 March, 2019).

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R. et al. (2013). Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science, 339: 1608-1611. doi: 10.1126/science.1230200

Garratt, M. P.D., Coston, D. J., Truslove, C. L. et al. (2014). The identity of crop pollinators helps target conservation for improved ecosystem services. Biological Conservation, 169: 128-135. doi: 10.1016/j.biocon.2013.11.001

Ghazoul, J. (2006). Floral diversity and the facilitation of pollination. Journal of Ecology, 94: 295-304. doi: 10.1111/ j.1365-2745.2006.01098.x

Giannini, T.C., Boff, S., Cordeiro, G.D. et al. (2015). Crop pollinators in Brazil: a review of reported interactions. Apidologie, 46: 209-223. doi: 10.1007/s13592-014-0316-z

Giannini, T.C., Alves, D.A., Alves, R. et al. (2020). Unveiling the contribution of bee pollinators to Brazilian crops with implications for bee management. Apidologie, 51: 406-421. doi: 10.1007/s13592-019-00727-3

Grab, H., Branstetter, M.G., Amon, N. et al. (2019). Agriculturally dominated landscapes reduce bee phylogenetic diversity and pollination services. Science, 363: 282-284. doi: 10.1126/ science.aat6016

Halinski, R., dos Santos, C.F., Kaehler et al. (2018). Influence of Wild Bee Diversity on Canola Crop Yields. Sociobiology, 65: 751-759. doi: 10.13102/sociobiology.v65i4.3467

Herrmann, J.D., Beye, H., Broise1, C. et al. (2019). Positive effects of the pollinators *Osmia cornuta* (Megachilidae) and *Lucilia sericata* (Calliphoridae) on strawberry quality. Arthropod-Plant Interactions, 13: 71–77. doi: 10.1007/s11829-018-9636-7

Holzschuh, A., Dormann, C.F., Tscharntke, T. et al. (2011). Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. Proceedings of the Royal Society B. doi: 10.1098/rspb.2011.0268

Holzschuh, A. & Dormann, C.F. (2013). Mass-flowering crops enhance wild bee abundance. Oecologia, 172:477-484. doi: 10.1007/s00442-012-2515-5

Holzschuh, A., Dainese, M., Gonzáles-Varo, J.P. et al. (2016). Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. Ecology Letters, 19: 1228-1236. doi: 10.1111/ele.12657

Hopwood, J.L. (2008). The contribution of roadside grassland restorations to native bee conservation. Biological Conservation, 141: 2632-2640. doi: 10.1016/j.biocon.2008.07.026

Jha, S. & Kremen, C. (2013). Resource diversity and landscape-level homogeneity drive native bee foraging. Proceedings of the National Academy of Sciences of the United States of America, 110(2): 555-558. doi: 10.1073/pnas.1208682110

Kaluza, B.F., Wallace, H.M., Heard, T.A. et al. (2018). Social bees are fitter in more biodiverse environments. Scientific Reports, 8: 12353. doi: 10.1038/s41598-018-30126-0

Klein, A.M., Boreux, V., Fornoff, F. et al. (2018). Relevance of wild and managed bees for human well-being. Current Opinion in Insect Science, 26: 82–88. doi:10.1016/j.cois.2018.02.011

Knapp, J.L., Shaw, R.F. & Osborne, J.L. (2019). Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: Implications for pollination and bee conservation on farms. Basic and Applied Ecology, 34: 85-94. doi: 10.1016/j. baae.2018.09.003

Kovács-Hostyànszki, A., Körösi, A., Orci, K.M. et al. (2011). Set-aside promotes insect and plant diversity in a Central European country. Agriculture, Ecosystems and Environment, 141: 296-301. doi: 10.1016/j.agee.2011.03.004

Kuussaari, M., Hyvönen, T. & Härmä, O. (2011). Pollinator insects benefit from rotational fallows. Agriculture, Ecosystems and Environment 143: 28-36. doi: 10.1016/j.agee.2011.03.006

London-Shafir, I., Shafir, S. & Eisikowitch, D. (2003). Amygdalin in almond nectar and pollen – facts and possible roles. Plant Systematics Evolution, 238: 87–95. doi:10.1007/s00606-003-0272-y

Lorenzi, H. (2008). Plantas daninhas do Brasil. 4º ed., Instituto Plantarum de Estudos da Flora, 640p. ISBN: 8586714275

Lundin, O., Ward, K.L., Artz, D.R. et al. (2017). Wildflower plantings do not compete with neighboring almond orchards for pollinator visits. Environmental Entomology, 46: 559–564. doi: 10.1093/ee/nvx052

Luz, C. F. P., Fernandes-Salomão, T. M., Lage, L. G. A. et al. (2011). Pollen sources for *Melipona capixaba* Moure & Camargo: An endangered Brazilian stingless bee. Psyche. doi: 10.1155/2011/107303

Luz, C.F.P., Fidalgo, A.O., Silva, S.A.Y. et al. (2018). Floral resources and risk of exposure to pesticides for Melipona quadrifasciata anthidioides Lepeletier 1836 in a Cerrado of São Paulo (Brazil). Grana, 57: 377-400. doi: 10.1080/00173134.2018.1433716

MacInnis, G. & Forrest, J.R.K. (2019). Pollination by wild bees yields larger strawberries than pollination by honey bees. Journal of Applied Ecology, 56: 824-832. doi: 10.1111/1365-2664.13344

Magurran, A.E. (2013). Medindo a diversidade biológica. Editora UFPR:Curitiba, 261 p

Mallinger, R.E., Bradshaw, J., Varenhorst, A.J. & Prasifka, J.R. (2019). Native solitary bees provide economically significant pollination services to confection sunflowers (*Helianthus annuus* L.) (Asterales: Asteraceae) grown across the northern great plains. Journal of Economic Entomology, 112: 40–48. doi: 10.1093/jee/toy322

Mao, W., Schuler, M.A. & Berenbaum, M.R. (2013). Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*. Proceedings of the National Academy of Sciences of the United States of America, 110: 8842-8846. doi: 10.1073/pnas.1525259113

Medeiros, H.R., Hoshino, A.T., Ribeiro, M.C. et al. (2017). Non-crop habitats modulate alpha and beta diversity of flower flies (Diptera , Syrphidae) in Brazilian agricultural landscapes. Biodiversity and Conservation, 27: 1309-1326. doi: 10.1007/s10531-017-1495-5

Moerman, R., Vanderplanck, M., Roger, N., Declèves, S., Whathelet, B., Rasmont, P., Fournier, D. & Michez, D. (2015). Growth rate of bumblebee larvae is related to pollen amino acids. Journal of Economic Entomology, 109: 25-30. doi: 10.1093/jee/tov279

Nicholls, C. I. & Altieri, M. A. (2013). Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. Agronomy for Sustainable Development 33: 257 274. doi: 10.1007/s13593-012-0092-y

Nicholson, C.C. & Ricketts, T.H. (2019). Wild pollinators improve production, uniformity, and timing of blueberry crops. Agriculture, Ecosystems and Environment, 272: 29–37. doi: 10.1016/j.agee.2018.10.018

Norris, S.L., Blackshaw, R.P., Critchley, C.N. R. et al. (2018). Intercropping flowering plants in maize systems increases pollinator diversity. Agricultural and Forest Entomology, 20: 246–254. doi: 10.1111/afe.12251

Nunes-Silva P, Hrncir M, Imperatriz-Fonseca, VL (2010) A polinização por vibração. Oecologia Australis, 14: 140–151. doi:10.4257/ oeco.2010.1401.07

Oksanen, J., Blanchet, G.F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B, Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. and Wagner, H. (2019). vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Perrot, T., Gaba, S., Roncoroni, M. et al. (2018). Bees increase oilseed rape yield under real field conditions. Agriculture, Ecosystems and Environment, 266: 39–48. doi: 10.1016/j.agee.2018.07.020

Peterson, J.H. & Roitberg, B.D. (2006). Impact of resource levels on sex ratio and resource allocation in the solitary bee, *Megachile rotundata*. Environmental Entomology, 35: 1404-1410. doi: 10.1093/ee/35.5.1404

Potts, S.G., Vulliamy, B., Dafni, A. et al. (2003). Linking bees and flowers: How do floral communities structure pollinator communities? Ecology, 84: 2628-2642. doi: 10.1890/02-0136

Potts, S.G., Biesmeijer, J.C., Kremen, C. et al. (2010). Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution, 25: 345-353. doi:10.1016/j.tree.2010.01.007

Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T. et al. (2016). Safeguarding pollinators and their values to human well-being. Nature, 540: 220-229. doi: 10.1038/nature20588

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Renauld, M., Hutchinson, A., Loeb, G. et al. (2016). Landscape Simplification Constrains Adult Size in a Native Ground-Nesting Bee. PLoS ONE, 11: e0150946. doi:10.1371/journal.pone.0150946

Rollin, O., Pérez-Méndez, N., Bretagnolle, V. et al. (2019). Preserving habitat quality at local and landscape scales increases wild bee diversity in intensive farming systems. Agriculture, Ecosystems and Environment, 275: 73–80. doi: 10.1016/j.agee.2019.01.012

Santos, A.A., Parizotto, D., Schlindwein, C. et al. (2020). Nesting biology and flower preferences of Megachile (Sayapis) zaptlana. Journal of Apicultural Research. doi: 10.1080/00218839.2019.1703084

Silva, C.I, Arista, M., Ortiz, P.L., Bauermann, S.G, Evaldt, A.C.P. & Oliveira, P.E. (2010). Catálogo polínico: palinologia aplicada em estudos de conservação de abelhas do gênero Xylocopa no Triângulo Mineiro. EDUFU, Uberlândia.

Thompson, H., Schneider, C., Maus, C. et al. (2019). Prevalence and abundance of bees visiting major conventionally-managed agricultural crops in Brazil. Journal of Apicultural Research. doi: 10.1080/00218839.2019.1655132

Torné-Noguera, A., Rodrigo, A., Arnan, X. et al. (2014). Determinants of spatial distribution in a bee community: nesting resources, flower resources, and body size. PLoS ONE, 9(5): 1-10. doi: 10.1371/journal.pone.0097255

Tscharntke, T., Batáry, P. & Dormann, C.F. (2011). Set-aside management: How do succession, sowing patters and landscape context affect biodiversity? Agriculture, Ecosystems and Environment, 143: 37-44. doi: 10.1016/j.agee.2010.11.025

Vanderplanck, M., Moerman, R., Rasmont, P. et al. (2014). How does pollen chemistry impact development and feeding behavior of polylectic bees? PLoS ONE, 9: 1-9. doi: 10.1371/journal.pone.0086209

Venturini, E.M., Drummond, F.A., Hoshide, A.K. et al. (2017). Pollination reservoirs for wild bee habitat enhancement in cropping systems: a review. Agroecology and Sustainable Food Systems, 41: 101-142. doi: 10.1080/21683565.2016.1258377