Research article

Floral visitors in the crop *Phaseolus coccineus* (Fabaceae) on the Altiplano of Puebla, Mexico: importance of agricultural management and flower color

Visitantes florales en el cultivo de *Phaseolus coccineus* (Fabaceae) en el Altiplano de Puebla, México: importancia del manejo agrícola y color de flor

Karina Alejandra Cué-Hernández[1], Abel Gil-Muñoz[1,3], Armando Aguirre-Jaimes[2], Pedro Antonio López[1], Oswaldo Rey Taboada-Gaytán[1]

Abstract:

Background and Aims: *Phaseolus coccineus* is an allogamous plant, with variation in flower color, and reproduction that depends on pollinators. The species is cultivated under two approaches: one in which agrochemicals are not used to control weeds and pests (traditional management) and another in which they are (technified management). No studies have evaluated the influence of agricultural management system and flower color on the floral visitors of this species; our objective was therefore to analyze how these factors affect the diversity and frequency of the floral visitors of *P. coccineus*.
Methods: A study was conducted with two landraces of *P. coccineus* (white-flowered and red-flowered), cultivated under two different management systems. Simultaneous observations were conducted for a period of eight days during the peak of flowering, quantifying the number of floral visitors and visits. Species richness and the effective number of species were calculated, the latter was used as a measure of diversity. Chi-square tests were applied, a correspondence analysis performed, and rank abundance curves constructed.
Key results: Forty-two morphospecies were recorded, grouped into six orders. *Apis mellifera* and *Hylocharis leucotis* were the predominant species. The numbers of visits recorded under traditional management were 1613 and 1105 in white and red flowers, respectively. Under the technified management, these values were 1427 and 815, respectively. There were consistent statistical differences between management systems, but not for flower color. In the white flowers, the traditional management was twice more diverse than the technified management. In the red flowers, this difference was 1.26 times.
Conclusions: The diversity and frequency of floral visitors of *P. coccineus* are favored by the traditional agricultural management, demonstrating the desirability of promoting this type of cultivation. Under the conditions studied, flower color did not influence the species richness or demonstrate any defined tendencies on the frequency of visits.
Key words: agricultural system, bees, hummingbirds, pollination, runner bean.

Resumen:

Antecedentes y Objetivos: *Phaseolus coccineus* es una planta alógama, con variación en color de la flor, que depende de polinizadores para su reproducción. La especie se cultiva bajo dos modalidades, una en la cual no se emplean agroquímicos para controlar arvenses y plagas (manejo tradicional) y otra en la que sí se usan (manejo tecnificado). No existen estudios que hayan evaluado la importancia del manejo agrícola y color de flor sobre los visitantes florales de esta especie, por lo que nuestro objetivo fue analizar cómo influyen estos factores en la diversidad y frecuencia de visitantes florales de *P. coccineus*.
Métodos: Se estudiaron dos variedades de *P. coccineus* (de flor blanca y roja), cultivadas bajo dos sistemas de manejo diferentes. En el pico de floración, durante ocho días, se realizaron observaciones simultáneas, contabilizando el número de visitantes florales y de visitas. Se calculó la riqueza específica y los números de especies efectivas como medida de diversidad, se aplicaron pruebas de chi-cuadrada, se practicó un análisis de correspondencias y se construyeron gráficos de rango-abundancia.
Resultados clave: Se registraron 42 morfoespecies, agrupadas en seis órdenes; predominaron *Apis mellifera* e *Hylocharis leucotis*. El número de visitas registradas fue de 1613 y 1105 en manejo tradicional de flores blancas y rojas, respectivamente, y de 1427 y 815 en el manejo tecnificado, existiendo diferencias estadísticas consistentes entre manejos, no así para color de flor. En flores blancas, el manejo tradicional fue dos veces más diverso que el tecnificado; en flores rojas, la diferencia fue de 1.26 veces.
Conclusiones: La diversidad y frecuencia de visitantes florales de *P. coccineus* se ven favorecidas por el manejo agrícola tradicional, ello fundamenta la conveniencia de fomentar esta modalidad de cultivo. Bajo las condiciones estudiadas, el color de flor no influyó en la riqueza de especies, ni mostró tendencias definidas en la frecuencia de visitas.
Palabras clave: abejas, colibríes, frijol ayocote, polinización, sistema agrícola.

[1]Colegio de Postgraduados Campus Puebla, Boulevard Forjadores de Puebla 205, Santiago Momoxpan, 72760 San Pedro Cholula, Puebla, México.
[2]Instituto de Ecología, A.C., Red de Interacciones Multitróficas, Carretera antigua a Coatepec 351, El Haya, 91070 Xalapa, Veracruz, México.
[3]Autor para la correspondencia: gila@colpos.mx

Received: April 8, 2022.
Reviewed: May 9, 2022.
Accepted by Marie-Stéphanie Samain: June 10, 2022.
Published Online first: July 7, 2022.
Published: Acta Botanica Mexicana 129 (2022).

To cite as: Cué-Hernández, K. A., A. Gil-Muñoz, A. Aguirre-Jaimes, P. A. López and O. R. Taboada-Gaytán. 2022. Floral visitors in the crop *Phaseolus coccineus* (Fabaceae) on the Altiplano of Puebla, Mexico: importance of agricultural management and flower color. Acta Botanica Mexicana 129: e2054. DOI: https://doi.org/10.21829/abm129.2022.2054

e-ISSN: 2448-7589

Introduction

The importance of animal-mediated pollination in agriculture is clear, since biotic pollination increases production in 87 of the 124 crops that provide 99% of the global volume of foods, and in 91 of the 107 crops commercialized in the world (excluding those that self-pollinate, are wind-pollinated or are parthenocarpic) and consumed by humans (Klein et al., 2007). Despite the importance of biotic pollination, different studies over the last decade have raised concerns over the diminution of pollinators worldwide (Biesmeijer et al., 2006; Potts et al., 2010; Burkle et al., 2013) and the grave consequences this could have for global biodiversity (Lundgren et al., 2016), agricultural production (Aizen and Harder, 2009) and food supply for the human population (Eilers et al., 2011).

The main factors that lead to the reduction or loss of pollinators include: a) land use change, which implies the modification and loss of the natural and semi-natural habitats (Potts et al., 2010), and b) intensification of agricultural activity (Altieri and Nicholls, 2013; Garibaldi et al., 2013). Land use change has been found to affect not only the pollinators, but also floral visitors in general. This was demonstrated by Deguines et al. (2012), who detected a negative affinity of the visitors (except hymenopterans) for urban areas and a positive affinity for agricultural and natural areas.

In terms of agricultural intensification, it has been documented in crops of *Coffea canephora* Pierre ex A. Froehner, that the employment of less intensive practices, such as the use of a greater diversity of shade trees, pruning of these trees (to increase the levels of light and presence of flowering herbaceous plants), increasing nesting sites, a minimal control of weeds and a reduction in the application of herbicides, could increase the diversity of bees (Klein et al., 2003). In *Theobroma cacao* L., most of the pollinators were found in traditional production systems, followed by conventional intensive and organic management systems (Salazar-Díaz and Torres-Coto, 2017). In vineyards, it was observed that organic management produced communities with a higher richness of herbaceous plant and lepidopteran species, compared to those under conventional management (Puig-Montserrat et al., 2017). Finally, in *Cajanus cajan* (L.) Millsp., the use of pesticides negatively affected the abundance of pollinators, while the application of fertilizers increased the presence of pests, for which reason it was recommended to utilize less aggressive management practices, such as the reduced use of pesticides and fertilizers (Otieno et al., 2011).

The use of pesticides has been shown to affect survival and reproductive success in the pollinators (Potts et al., 2010; Roulston and Goodell, 2011; Gill et al., 2012; Rundlöf et al., 2015), and the application of herbicides leads to the loss of nesting sites and important resources for the pollinators (for example, nectar, pollen, oils and aromatic compounds) (Holzschuh et al., 2008). Moreover, it has been documented that pollination services increase in line with the diversity and abundance of floral patches within the plots, leading to the presence of wild pollinators that are more efficient than the honeybee (*Apis mellifera* Linnaeus, 1758) (Shuler et al., 2005; Norfolk et al., 2016; Lucas et al., 2017; Villamil et al., 2018; Eeraerts et al., 2019).

To enable the process of pollination, plants present floral traits that influence the attraction of one or more specific groups of pollinators (Olesen and Jordano, 2002). One such trait is flower color, which acts as a signal for the pollinators searching for food, influencing their constancy, visitation rate and eventually the reproductive success of the plants (Chittka et al., 1994). The pollinators with visual capacities differentiated for the identification of colors include the bees and hummingbirds (de Camargo et al., 2019). The former have a trichromatic visual system that is more sensitive to ultra-violet, blue and green wavelengths (Menzel and Backhaus, 1991), while the latter have a tetrachromatic system that is sensitive to the violet and red wavelength range (Ödeen and Hastad, 2010). This suggests that flowers that reflect the color red are preferentially visited by hummingbirds and less attractive to bees (Cronk and Ojeda, 2008).

A good model with which to evaluate the importance of flower color and the type of agricultural management system for the floral visitors and potential pollinators is that of *Phaseolus coccineus* L. (Fabaceae), locally known as “frijol ayocote” (Fig. 1). This species is native to Mexico, and presents wild, feral, and cultivated populations (Burquez and Sarukhán, 1980). The latter are of particular relevance to the farmers of central-eastern Puebla, the principal region of ayocote production in Mexico (López-Báez et al., 2018), and also are important elements in the diet of different regions of the country. Unlike the common bean (*P. vulgaris* L.), *P. coccineus* requires cross-pollination, which is performed mainly by bees, bumblebees and hummingbirds, the majority of which are wild (Burquez and Sarukhán, 1980; Labuda, 2010). Studies of pollination in this crop have focused on the characterization of floral visitors (Burquez and Sarukhán, 1980) and the mechanisms of pollination (self-pollination and cross-pollination) in the first days of flowering (Williams and Free, 1975), as well as the efficiency of different pollinator species in terms of reproductive success (Free, 1966; Kendall and Smith, 1976; Pando et al., 2011; Tchuenguem et al., 2014). However, no studies have been directed towards determining whether the community of floral visitors of *P. coccineus* and its activity vary as a function of the type of agricultural management system and flower color.

Our objectives were therefore 1) to characterize the diversity of floral visitors present in the *P. coccineus* crop, and 2) to quantify the number of visits that occurred under two agricultural management systems (traditional and technified) and in two flower colors (white and red).

It was hypothesized that: 1) the richness and diversity of the floral visitors will be greater in the traditional management system because, by having a lesser environmental impact, conditions will be presented that favor the presence of visitors to the crops, and 2) the flower color will influence the richness, diversity and activity of the floral visitors since, given that insects and birds -such as the hummingbirds- perceive color in different wavelength ranges, the insects (particularly bees) will prefer to visit white flowers, while the hummingbirds will prefer red flowers.

Materials and Methods

Study area

The study was conducted in the locality of San Andrés Calpan in the Mexican state of Puebla, located at 19°06'28''N, 98°27'33''W and at 2430 m a.s.l. (INEGI, 2010) (Fig. 2). The predominant climate is temperate subhumid with summer rains (C(w_2)), which range from 900 to 1100 mm per year, while the mean annual temperature ranges from 12 to 18 °C (INEGI, 2010; 2019).

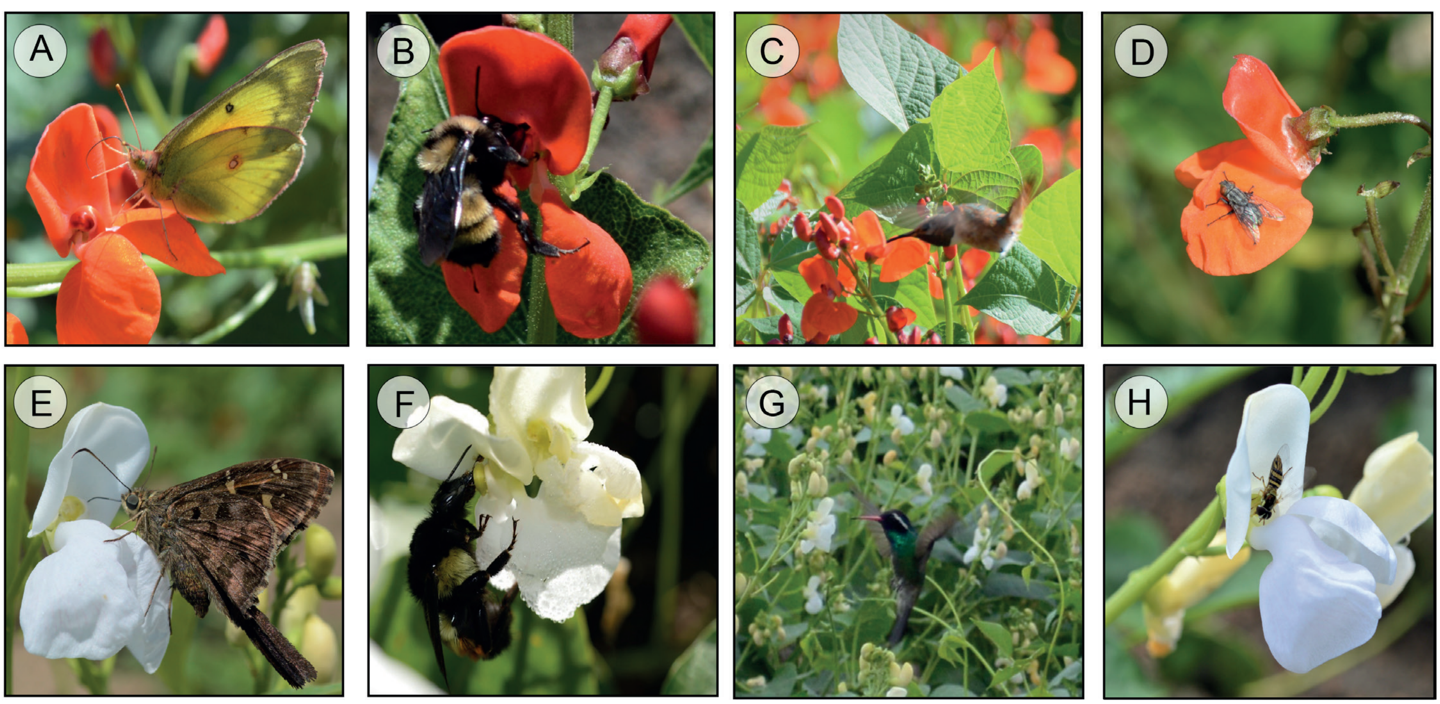

Figure 1: Examples of floral visitors of *Phaseolus coccineus* L.: A. *Colias eurytheme* Boisduval, 1852; B. *Bombus* sp. 1; C. *Selasphorus rufus* Gmelin, 1788; D. *Musca domestica* Linnaeus, 1758; E. *Urbanus dorantes* Stoll, 1790; F. *Bombus ephippiatus* Say, 1837; G. *Hylocharis leucotis* Bourcier & Mulsant, 1846; H. *Allograpta obliqua* Say, 1823. (Photos: K. A. Cué-Hernández).

Study fields

Two fields were selected, each of 1000 m^2, located at 100 m distance from each other and separated by strips of oat (*Avena sativa* L.) and maize (*Zea mays* L.). Apple (*Malus domestica* (Suckow) Borkh.) trees were present on the edges of each field. In each field, two landraces of *P. coccineus* were sown, identified as Landrace 89 (black seeds and red flowers) and Landrace 46 (white seeds and flowers). These landraces are part of the germplasm kept by the Plant Breeding Program at the Colegio de Postgraduados Campus Puebla. They were directly collected with peasants and assigned the codes P89NF and P46BC, respectively (López-Báez et al., 2018). Both accessions are routinely used in several research projects as well as by peasants. The growth habit of both landraces corresponded to an indeterminate bush type. Each field was divided into eight plots, allowing alternation of the two landraces of *P. coccineus*. In this way, patches of the landrace with red flowers were interspersed with patches of the landrace with white flowers, producing a checkered pattern. Each plot was formed by seven rows of 22 m in length and 0.75 m in width; two seeds were sown every 50 cm in each row. The four plots of each landrace in each field were considered as replications. Sowing was carried out on the 30th of May 2019.

Agronomic management

One field underwent the agronomic management known as "Technified" (TE), which consisted of the application of agrochemicals to control weeds and pests. Weeds were controlled at 25 days after sowing (DAS) with an herbicide (bentazone), at a dose rate of 2 l·ha^{-1}, while the pests were treated at 61 DAS with an insecticide (lambda-cyhalothrin) applied at a dose rate of 0.1 l·ha^{-1}. The second field underwent the agronomic management known as "Traditional" (TR), in which weeds were controlled manually and no pest control was conducted. Both fields were fertilized using the

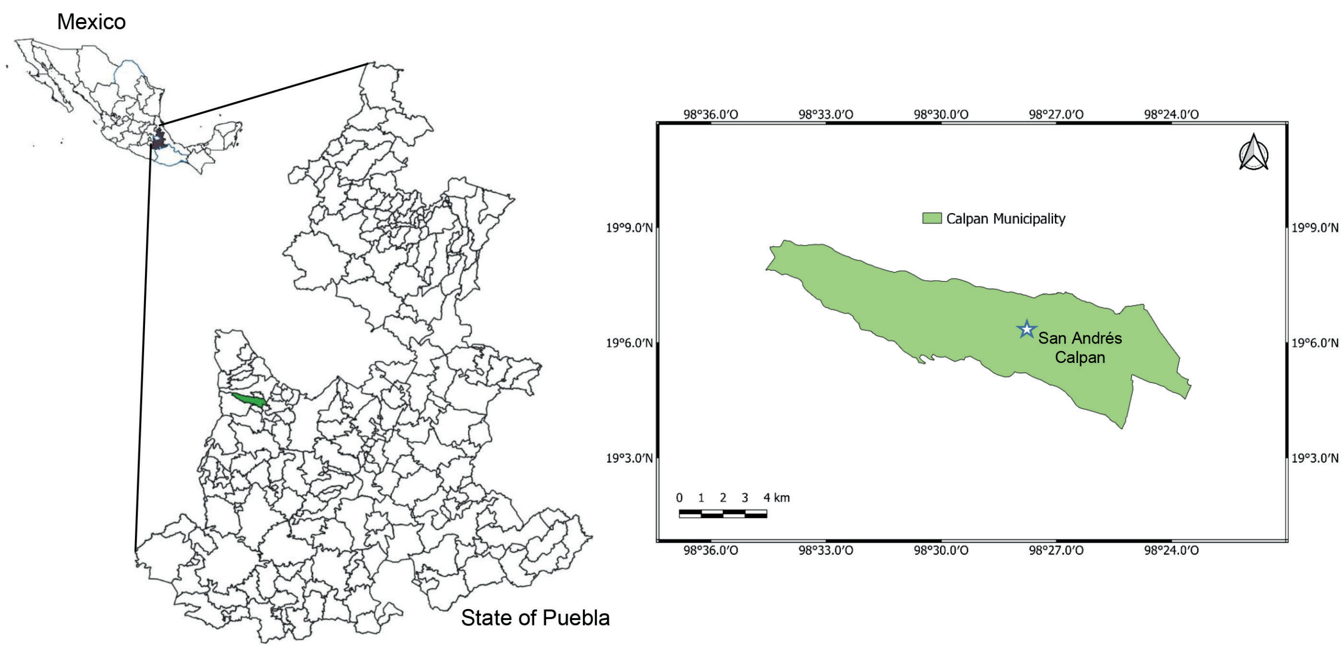

Figure 2: Geographic location of San Andrés Calpan, Puebla, Mexico.

formula 60N-60P-00K ($kg \cdot ha^{-1}$ of nitrogen, phosphorus and potassium) applied on a single occasion, at 34 DAS, using diammonium phosphate and urea as sources.

Observation and sampling of floral visitors

The observation and sampling of floral visitors in both management systems and landraces were conducted during the peak of flowering (from 69 to 76 DAS, between the months of August and September 2019). In this period, observations were made in each field, alternating the days of observation among them. In this way, four days were used for each management system. On each day, three observation points were randomly selected on the intersections of the plots of the checkered pattern, in order to simultaneously observe the two different landraces. At each selected point, for each landrace, a vertical plant observation area of 250 cm^2 (50 cm in length × 50 cm in width) was delimited (Pinilla-Gallego and Nates-Parra, 2015) using a wooden quadrat. The number of floral buds with corolla, flowers in anthesis and senescent flowers within each quadrat were counted. Observations of the floral visitors within the delimited area were then made during the time period from 08:00 to 17:00 h. For these observations, only flowers in anthesis were considered.

Observations of insects were conducted at 8, 10, 12, 14 and 16 h, while those of the birds were at 9, 11, 13 and 15 h. At each time and each point of observation, the frequency and identity of the insects or birds that visited the flowers were recorded for a period of 10 min. At the end of this period, 10 min were taken for moving to and location at the next point of observation, where the process was repeated.

To identify insects of Hymenoptera and Diptera, one or two individuals per species were collected using an entomological net while traversing the field during the time spent moving between observation points. Once captured, the insects were placed in a lethal chamber with ethyl acetate and then in jars with 70% alcohol, labeled with the name of the locality, date and time, for subsequent identification in the laboratory. There, they were mounted and identified to the lowest possible taxonomic level with the help of taxonomic keys: Fernández and Sharkey (2006) and Michener (2007) for Hymenoptera, and Brown et al. (2010) and González et al. (2016) for Diptera. For Lepidoptera, Coleoptera and Hemiptera, while moving between observation points, direct observations were made, and photographs were taken to later identify them to the lowest possible taxonomic level using the illustrated guides of Glassberg (2007) for Lepidoptera, Cervantes Mayagoita and Huacuja Zamudio (2020), Nájera Rincón and Souza (2010) for Coleoptera, and Corrales Castillo et al. (2017) for Hemiptera. Collected specimens were deposited in the entomological collection of the Instituto de Ecología, A.C. (IEXA). Hummingbirds were not collected, but rather photographed and identified *in situ* with the help of the specialized guide of Peterson and Chalif (2000).

Data analyses

The number of floral buds, flowers in anthesis, senescent flowers, and flowering structures (the sum of all the previous values) for white and red flowered landraces within each management system were compared by means of a Wilcoxon test (because the variables were not normally distributed). The program SAS University® v. 9.4 (SAS Institute Inc., 2020) was used.

Once the floral visitors were identified, the specific richness and frequency of visits were calculated per species and order, for each agricultural management system and flower color. Chi square tests were performed to determine differences in the species richness and total number of visits (at the taxonomic level of order), for the different combinations of agricultural management system and flower color. The analyses were conducted with the program SAS University® v. 9.4 (SAS Institute Inc., 2020).

To analyze the diversity of floral visitors in each agricultural management system combined with flower color, the exponential of Shannon entropy index was used. This is expressed in units of effective species and allows direct comparisons to be made among communities (Jost, 2006; Moreno et al., 2011). These values were calculated with the software SPADE® (Chao and Shen, 2010). Rank-abundance graphs were produced to analyze the relative frequency and changes in the composition of species visiting each combination of management system and flower color. For this, the $\log_{10}$ of the number of visits of each species per agricultural management system and flower color was used, providing information relating to the distribution of the species without losing their identity and the visit frequencies of each species (Rocchini and Neteler, 2012). To determine whether the species had an affinity for any given management system and flower color, the number of visits per species was used to conduct a simple correspondence analysis, using the CORRESP procedure of SAS University® v. 9.4 (SAS Institute Inc., 2020).

The sampling efficiency of the species per management system and flower color was calculated from the non-parametric estimator Chao 2, since this does not fit a parametric mathematical model and considers the species observed in exactly one and two sampling units (Alfaro and Pizarro-Araya, 2017). To obtain the estimator, the program EstimateS® v. 9.1.0 (Colwell, 2013) was used. To eliminate the effect of the order in which the samples were added, 100 randomizations were conducted (Pineda-López and Verdú, 2013), considering the 12 observation points as sampling effort.

Results

No statistical differences were found for the number of floral buds ($-0.6642 \leq z \leq 0.6642$, $0.5066 \leq P \leq 1$), flowers in anthesis ($-0.9275 \leq z \leq 1.3093$, $0.1768 \leq P \leq 1$), senescent flowers ($-0.6742 \leq z \leq 0.4428$, $0.5002 \leq P \leq 1$) and total number of flowering structures ($-0.6642 \leq z \leq 0.4364$, $0.5066 \leq P \leq 1$) among landraces within each management system at all sampling dates. This implies that these variables did not influence the data recorded in the study. Mean values for flower buds, flowers in anthesis, and senescent flowers on each day were, respectively: 121.7, 16.3, 13.0 (first day); 125.7, 15.0, 14.8 (second day); 138.8, 17.3, 16.5 (third day); 74.0, 7.7, 11.0 (fourth day); 106.7, 14.5, 17.0 (fifth day); 13.8, 17.8, 75.3 (sixth day); 13.5, 12.7, 105.3 (seventh day); and 15.8, 12.3, 94.7 (eight day).

A total of 42 species of floral visitors were recorded, distributed in six orders: Hymenoptera (14 species), Lepidoptera (13 species), Diptera (seven species), Apodiformes (five species), Coleoptera (two species) and Hemiptera (one species). In the traditional management, a total of 1613 and 1105 visits were recorded in the white and red flowers, respectively. In the technified management, 1427 and 815 visits were recorded in the white and red flowers, respectively (Table 1).

The results of species richness per management system and flower color showed that, in the traditional management (TR) with white flowers, the following data were recorded per order: Hymenoptera (six species), Apodiformes (five species), Lepidoptera (five species), Diptera (four species) and Coleoptera (two species). In TR with red flowers, the data were: Lepidoptera (seven species), Hymenoptera (five species), Apodiformes and Diptera (four species each), Coleoptera and Hemiptera (one species each). In the technified management (TE) with white flowers, the data per order were: Hymenoptera (eight species), Lepidoptera (seven species), Diptera (five species), Coleoptera and Apodiformes (two species each). In TE with red flowers, the data were: Hymenoptera (11 species), Lepidoptera (six species), Diptera (three species), Coleoptera and Apodiformes (two species each). In general, the orders with the lowest number of species were Coleoptera and Hemiptera (Figs. 3A, B).

The patterns described above were maintained for the number of visits in the TR, in both white and red flowers, where there was a high frequency of visits of the orders Hymenoptera and Apodiformes. In TE, in both flower colors, the frequency of visits by Apodiformes was lower, while that of the orders Lepidoptera, Coleoptera and Diptera was higher than in TR.

The species richness was similar in the white flowers of the TR and TE plots (X^2=1.93, d.f.=4; P=0.760). This also occurred with the red flowers (X^2=4.39, d.f.=5; P=0.536), showing that the management system did not influence the species richness of the floral visitors found in each flower color. There were also no differences in the species richness recorded in white *vs.* red flowers in TR (X^2=1.87, d.f.=5; P=0.9678), and in TE (X^2=1.06, d.f.=4; P=0.955), indicating that, within each management system, flower color did not influence the species richness of the floral visitors.

On comparing the number of visits to white flowers between management systems (TR *vs.* TE), differences were found (X^2=480.24, d.f.=4; P<0.0001). The same was found when comparing the number of visits to red flowers between management systems (X^2=139.28, d.f.=5; P<0.0001). This indicates that the management system does affect the number of visits to flowers of either color. The more detailed analysis of the data showed that, in the case of white flowers in TE, greater numbers of visits by hymenopterans, lepidopterans, dipterans and coleopterans

Table 1: Records of species and their total frequency of visits to flowers of two landraces of *Phaseolus coccineus* L., under two agricultural management systems, in San Andrés Calpan, Puebla, Mexico.

Order	Species	Agricultural management			
		Traditional		Technified	
		Flower color		Flower color	
		White	Red	White	Red
Apodiformes	*Amazilia beryllina* Deppe, 1830	82	7	0	0
	Calothorax lucifer Swainson, 1827	34	0	0	0
	Colibri thalassinus Swainson, 1827	112	49	0	0
	Hylocharis leucotis Bourcier & Mulsant, 1846	272	209	108	52
	Selasphorus rufus Gmelin, 1788	241	100	35	35
Coleoptera	*Hippodamia* sp. 1	1	0	1	2
	Macrodactylus mexicanus Burmeister, 1855	3	2	16	2
Diptera	*Allograpta obliqua* Say, 1823	2	3	1	3
	Allograpta sp. 1	0	1	0	0
	Culex sp. 1	2	1	4	5
	Eristalis tenax Linnaeus, 1758	2	0	0	0
	Fannia canicularis Linnaeus, 1761	0	0	1	0
	Musca domestica Linnaeus, 1758	2	1	9	8
	Musca sp. 1	0	0	5	0
Hemiptera	*Euschistus* sp. 1	0	1	0	0
Hymenoptera	*Anthophora* sp. 1	0	1	3	4
	Anthophora sp. 2	0	0	0	1
	Apis mellifera Linnaeus, 1758	780	668	1144	631
	Bombus ephippiatus Say, 1837	4	0	0	0
	Bombus sp. 1	32	20	25	8
	Brachygastra mellifica Say, 1837	14	11	30	16
	Diadasia sp. 1	0	0	2	3
	Eucera sp. 1	0	0	0	1
	Peponapis sp. 1	9	15	5	12
	Peponapis sp. 2	0	0	0	4
	Sphex sp. 1	0	0	2	0
	Vespula squamosa Drury, 1770	0	0	1	0
	Xylocopa sp. 1	0	0	0	2
	Xylocopa sp. 2	3	0	0	1
Lepidoptera	*Autochton cellus* Boisduval & Le Conte, 1837	0	0	1	0
	Calephelis sp. 1	0	0	0	1
	Colias eurytheme Boisduval, 1852	0	0	0	5
	Eunica monima Stoll, 1782	0	2	0	0
	Hesperopsis alpheus Edwards, 1876	0	4	6	4
	Hylephila phyleus Drury, 1773	0	1	0	0
	Lerema accius Smith, 1797	1	0	0	0
	Lon melane Edwards, 1869	1	0	1	2
	Nathalis iole Boisduval, 1836	0	1	0	0
	Piruna sp. 1	0	1	2	0
	Polites vibex Geyer, 1832	3	5	12	10

Table 1: Continuation.

Order	Species	Agricultural management			
		Traditional		Technified	
		Flower color		Flower color	
		White	Red	White	Red
	Urbanus dorantes Stoll, 1790	8	0	4	0
	Vanessa cardui Linnaeus, 1758	5	2	9	3

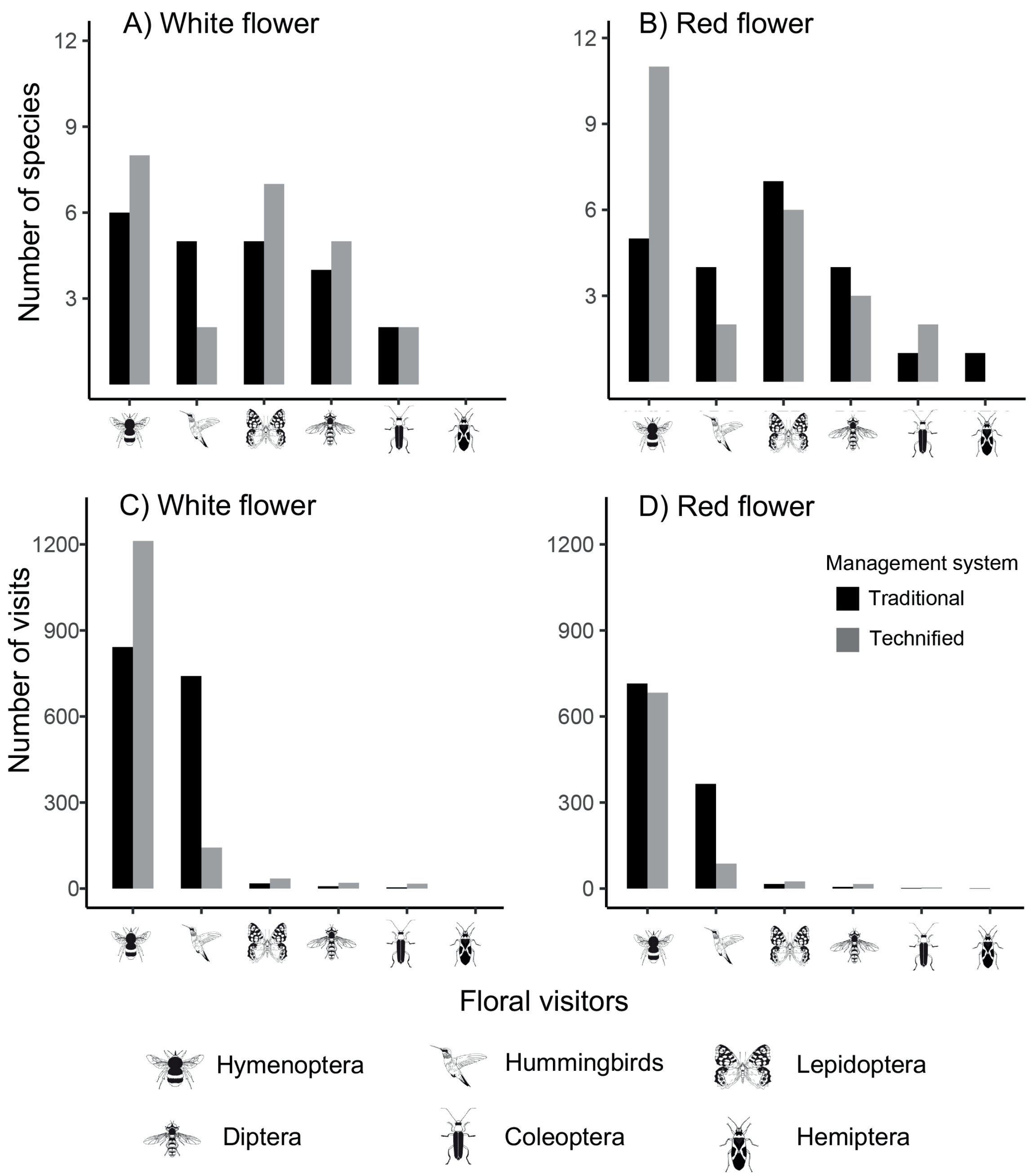

Figure 3: Records per taxonomic order and agronomic management system of the number of species visiting white (A) and red (B) flowers, and of the total number of visits in white (C) and red (D) flowers of *Phaseolus coccineus* L. (Icons of functional groups of floral visitors obtained from Divulgare, 2022).

were recorded, while the number of visits by hummingbirds in TR was five times greater than in TE (Fig. 3C). In the red flowers in TE, more visits of lepidopterans, dipterans and coleopterans were recorded compared to those in TR, while the greatest quantity of visits by hymenopterans and hummingbirds was recorded in TR (Fig. 3D). The number of visits differed between white and red flowers in TR (X^2=49.95, d.f.=5; *P*<0.0001), indicating an effect of flower color in this management system: in white flowers, a greater number of visits of the recorded taxonomic orders was found, apart from Hemiptera (Figs. 3C, D). Comparison in this regard between the white and red flowers in TE was not significant (X^2=4.76, d.f.=4: *P*=0.312), for which reason flower color did not affect the number of visits in this management system.

The results of the correspondence analysis show that some species of floral visitors had greater affinity for a given combination of agricultural management system and flower color (X^2=1040.01, *P*<0.0001). Graphing the information of the two first dimensions (which explained 89.7% of the total variation) highlighted the separation between the management systems throughout Dimension 1, since combinations that involved TR were found at one extreme and those of TE were found at the other (Fig. 4). This indicated differences in the association of member species of the community of floral visitors.

On analysis of the relationships of the different species of floral visitors to each of the management system-flower color combinations, it was found that *Selasphorus rufus* Gmelin, 1788, *Amazilia beryllina* Deppe, 1830 (Apodiformes) and *Urbanus dorantes* Stoll, 1790 (Lepidoptera) were associated with TR with white flowers; while *Hylocharis leucotis* Bourcier & Mulsant, 1846 (Apodiformes) and *Bombus* sp. 1 (Hymenoptera) were associated with TR with red flowers. On the other hand, it was found in TE that *Allograpta obliqua* Say, 1823, *Allograpta* sp. 1, *Musca domestica* Linnaeus, 1758, *Culex* sp. 1 (Diptera), *Hylephila phyleus* Drury, 1773, *Vanessa cardui* Linnaeus, 1758 (Lepidoptera) and *Brachygastra mellifica* Say, 1837 (Hymenoptera) tended to be associated with red flowers, while *Peponapis* sp. 1 (Hymenoptera), *Macrodactylus mexicanus* Burmeister, 1855 (Coleoptera), *Hesperopsis alpheus* Edwards, 1876, *Piruna* sp. 1 (Lepidoptera) and *Fannia canicularis* Linnaeus, 1761 (Diptera) showed greater affinity with white flowers. The rest of the species had no clear affinity with any management system and flower color. It was also observed that the five species of hummingbirds were associated only with TR (Fig. 4).

The dominant species in both management systems and flower colors were *A. mellifera* and *H. leucotis* (Fig. 5). In TR, regardless of flower color, the third and fourth position corresponded to *S. rufus* and *Colibri thalassinus* Swainson, 1827 (Figs. 5A, C), respectively. Changes in the community structure and in the contribution to the total abundance between TR with white flowers and TR with red flowers were presented only in the fifth most dominant species, which was *A. beryllina* in the former and *Bombus* sp. 1 in the latter. In TE, the species *S. rufus* and *B. mellifica* occupied the third and fourth position (Figs. 5B, D). In both cases, the fifth most dominant species was different: *Bombus* sp. 1 in white flowers, and *Peponapis* sp. 1 in red flowers. These results indicate that, in all of the combinations of management system and flower color, the dominant species belonged to Hymenoptera, and that the presence of species of Apodiformes was greater in TR (Fig. 5).

The results of the effective number of species showed that TR with white flowers (^1D=5.2) was more diverse than TE with white flowers (^1D=2.6). In addition, TR with red flowers (^1D=3.9) was also more diverse than TE with red flowers (^1D=3.1). In white flowers, TR was twice as diverse as TE in terms of species of floral visitors; while in red flowers, TR was 1.26 times more diverse than TE in this regard. On comparison of the effective number of species in each management system, we found that TR with white flowers was 1.33 times more diverse than TR with red flowers and, in the case of TE, the plots with red flowers were 0.84 times more diverse than those with white flowers.

The sampling efficiencies obtained with the Chao 2 estimator were: 83% for TR with white flowers, 60% for TR with red flowers, 30% for TE with white flowers and 51% for TE with red flowers. These data highlight the need to conduct a greater sampling effort in order to capture the maximum number of potential species.

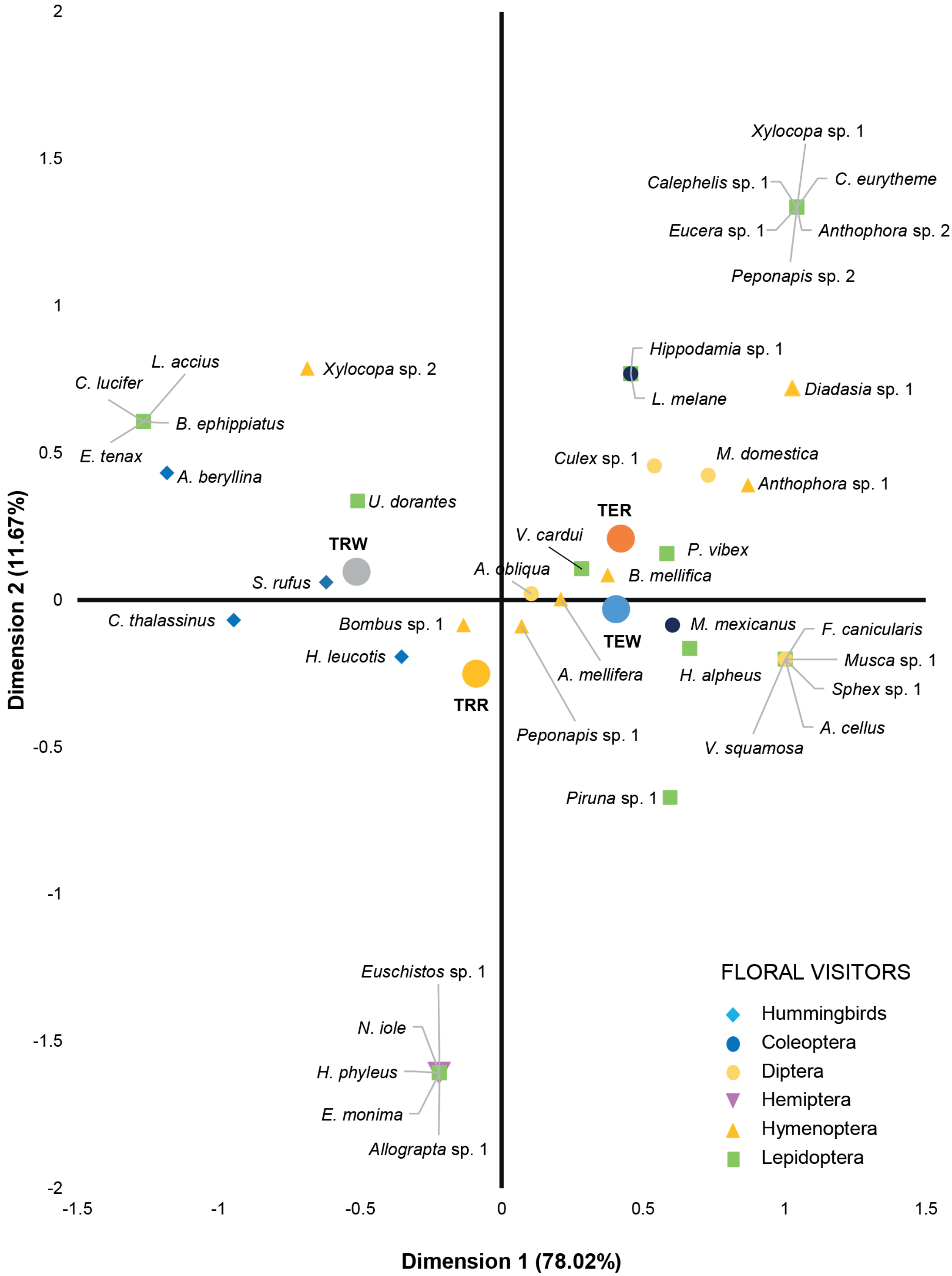

Figure 4: Correspondence analysis between the management system-flower color combinations (TRW=traditional management and white flowers, TRR=traditional management and red flowers, TEW=technified management and white flowers, TER=technified management and red flowers) and the species of floral visitors to the *Phaseolus coccineus* L. crop.

Discussion

The community of floral visitors associated with the *P. coccineus* crop was diverse

The species richness reported in this study (42 morphospecies) was greater than that found in studies conducted outside the distribution area of *P. coccineus,* particularly in cultivars in England, such as those of Free (1966), Blackwall (1971) and Kendall and Smith (1976), who found two, nine and five species, respectively. It was also greater than that observed in cultivars of Mexico, since it exceeded that reported by Burquez and Sarukhán (1980) in the state of Morelos (five species reported, including hummingbirds for the first time) and by Sousa-Peña (1992) in Chihuahua, where 16 species of visitors were found, including hummingbirds. The greater number of floral visitors found in our studies could be attributed to the combination of flowers present in the fields, as well as to the diversity of co-flowering species around the fields (Badillo-Montaño et al., 2018). It could also partly be due to the fact that the study was developed in a locality belonging to a region (the states of Puebla, Oaxaca and Chiapas) in which a high diversity of wild and domesticated forms of *P. coccineus* is concentrated (Delgado Salinas, 1988). Studies such as that of Genung et al. (2010) have documented that the genotypic diversity of a plant could have an indirect influence on the abundance and richness of the floral visitors.

Floral visitors were understood as any animal that physically touched the flowers or the inflorescences (Hernández-Villa et al., 2020). These animals can play several roles in the flower, as is the case of pollinators, or have no relation at all with the reproductive process, even though some feed on nectar or pollen (Wäckers et al., 2007). Based on previous studies, some of the possible functions of various floral visitors recorded in this study are the following. Among the hymenopterans, *Apis mellifera* and several representatives of the genus *Bombus* Latreille, 1802, have been reported as pollinators of *P. coccineus* in England (Free and Racey, 1968; Blackwall, 1971; Kendall and Smith, 1976). In Mexico, *A. mellifera* was found as a constant visitor of the plant (Burquez and Sarukhán, 1980), and Sousa-Peña (1992) concluded that it is certainly a pollinator. Several *Bombus* species have also been mentioned as visitors of *P. coccineus*, some occasional and some frequent (Burquez and Sarukhán, 1980; Sousa-Peña, 1992). In addition, Burquez and Sarukhán (1980) reported two unidentified species of the genus *Anthophora* Latreille, 1803, as infrequent visitors, while Sousa-Peña (1992) observed another species that could be a potential pollinator. Finally, studies conducted in Cameroon showed that members of the genus *Xylocopa* Lepeletier, 1841, are efficient pollinators of *P. coccineus* (Pando et al., 2011; Tchuenguem et al., 2014).

As to the visitors of the orders Hemiptera and Coleoptera, *Euschistus* sp. 1 and *Macrodactylus mexicanus*, respectively, have been described as pests of several crops, including the genus *Phaseolus* L. (Panizzi and Slansky, 1985; Arce-Pérez and Morón, 2000), while members of the genus *Hippodamia* Dejean, 1837 (Coleoptera) are recognized as predators of aphids (Lara et al., 2022). With regard to the order Diptera, specifically the family Syrphidae, even though the adults tend to be close to flowers for mating and feeding (Ssymank et al., 2008) and some are considered as pollinators (Doyle et al., 2020), we found no reports of them as constant visitors or potential pollinators of *P. coccineus*. In the case of the order Lepidoptera, Sousa-Peña (1992) documented that some species, as *Colias eurytheme* Boisduval, 1852, and *Autochton cellus* Boisduval & Le Conte, 1837, (also observed in this study) are nectar thieves. Among the hummingbirds, *Hylocharis leucotis* is reported as an efficient pollinator of *P. coccineus* (Burquez and Sarukhán, 1980), *Selasphorus rufus* as a frequent visitor (Sousa-Peña, 1992) and *Calothorax lucifer* Swainson, 1827, as an occasional visitor (Burquez y Sarukhán, 1980; Sousa-Peña, 1992). In the literature, Blackwall (1971) mentioned to have observed thrips inside *P. coccineus* flowers and suggested they may have done some small contribution to pollination. In our study, we did not look for these small insects.

Among the floral visitors, dominant species belong to the orders Hymenoptera and Apodiformes. This result coincides with that reported by Burquez and Sarukhán (1980) and Sousa-Peña (1992). *Apis mellifera* (Hymenoptera) was the most dominant species in both agricultural management systems and flower colors, coinciding with that reported by Free (1966) and Koltowski (2004), but differing from that found by Pando et al. (2011) and Tchuenguem et al. (2014) in studies conducted in Poland and Africa, re-

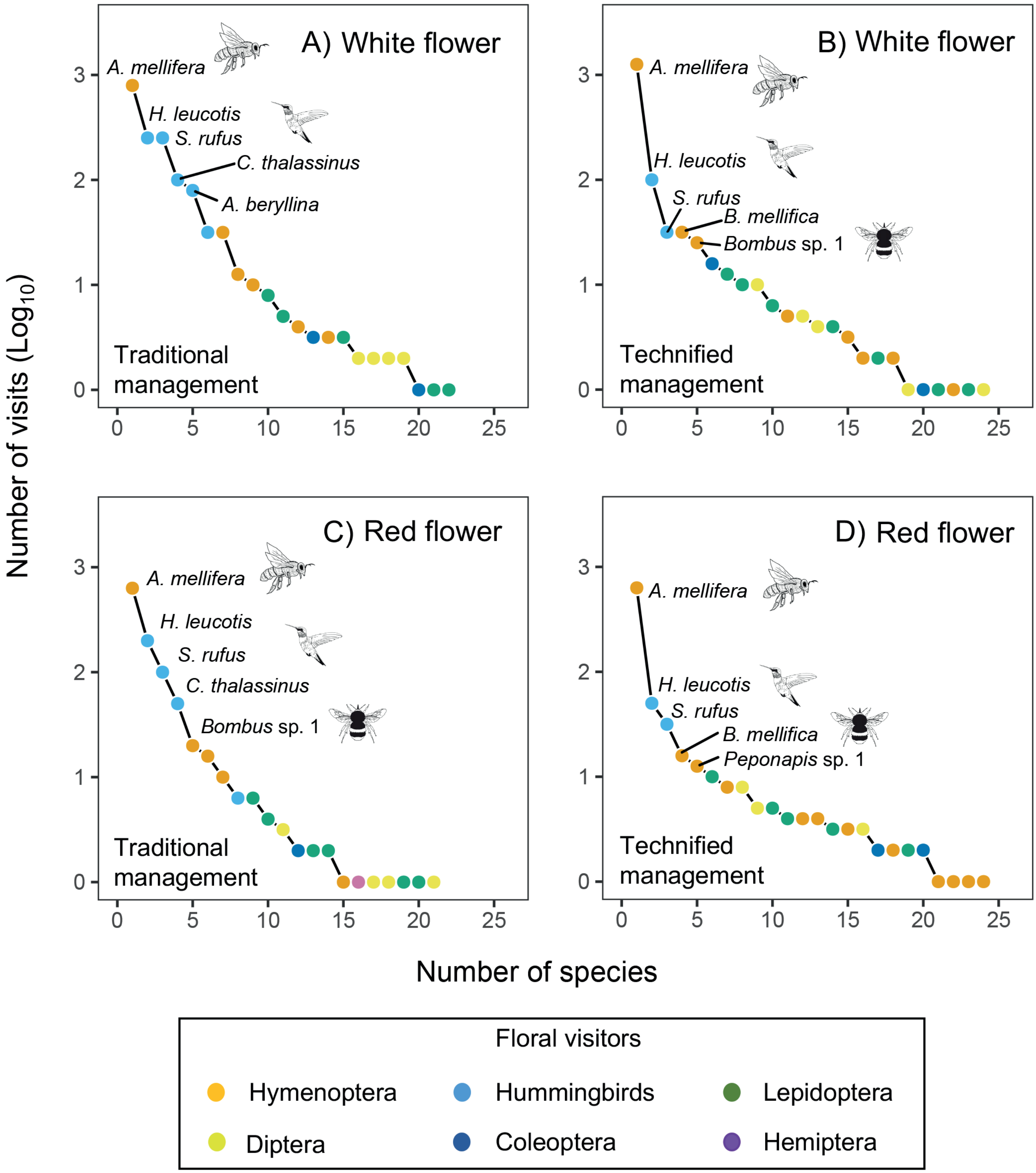

Figure 5: Rank abundance curves for floral visitors of *Phaseolus coccineus* L. under traditional (A) or technified (B) management with white flowers, and traditional (C) or technified (D) management with red flowers. (Icons of functional groups of floral visitors obtained from Divulgare, 2022).

spectively, that report carpenter bees of the genus *Xylocopa* as the most dominant species. *Apis mellifera* is a bee of generalist feeding habit and is the species most used for the production of honey and pollination of crops due to its ease of management (Nates-Parra, 2005), which probably accounts for its dominance. It is a species reported in many studies as associated with *P. coccineus*, serving as a pollinator, although on occasions it can also be a nectar thief (Free and Racey, 1968; Blackwall, 1971; Sousa-Peña, 1992; Koltowski, 2004).

Among the hymenopterans, another floral visitor for which a moderate number of visits were recorded was *Brachygastra mellifica*, a species for which no previous reports as a visitor of *P. coccineus* were found. According to Sugden and McAllen (1994), this wasp forages for nectar and honeydew on various plant species; nevertheless, the authors state that its potential as a pollinator does not seem great. They also report that *B. mellifica* shows a predatory behavior. Therefore, its presence may be due to the search for nectar or insect larvae to prey on. It is worth mentioning that *P. coccineus*, because of its sugar and pollen content in the flowers, figures among the list of good forage plants; therefore, it can attract various insects (Koltowski, 2004).

Hylocharis leucotis was the dominant hummingbird species. Burquez and Sarukhán (1980) report this species as an efficient pollinator in cultivars of *P. coccineus* in the Valles de Cuautla and Estado de México, at elevations greater than 2500 m a.s.l. *Hylocharis leucotis* has been considered a generalist species, due to the fact that its phenotypic characteristics, such as corporal weight (3.2-3.6 g) and (short) beak size, can confer the species with great mobility, and the potential to feed from a wide variety of flowers that are normally pollinated by insects (Maruyama et al., 2012; Rodriguez-Femat, 2014). In addition, these characteristics enable the species to participate in pollination of the crop since, on accessing the nectar chamber located in the basal part of the floral structures, grains of pollen adhere to it and can then be transferred from one flower to another (Sousa-Peña, 1992). The presence of another four hummingbird species not reported as floral visitors of *P. coccineus* in other studies outside Mexico is due to the fact, that the family to which these species belong (Trochillidae) is endemic to the American continent (Arizmendi and Berlanga, 2014).

Agronomic management system did not affect species richness, but did influence the number of visits per taxonomic order

The management system had no impact on the composition of the community of floral visitors but did influence the frequency of visits per taxonomic order. Independently of flower color, more visits were recorded in TR than in TE, particularly in species of the order Apodiformes (white flowers) and of Hymenoptera and Apodiformes (red flowers). This could be due to the fact, that the most intensive systems, in terms of technology application, exercise a greater negative impact on the habitat and wildlife (Viglizzo et al., 2002). It has been reported that the application of herbicides causes a loss of herbaceous plants that provide sites of shelter (e.g., nests) and food resources (nectar, pollen, oils and aromatic compounds) of importance to the pollinators (Holzschuh et al., 2008), and that insecticides, while they allow the control of crop pests, can contain chemical agents that reduce the abundance of other species, such as pollinators and natural enemies (Otieno et al., 2011).

The lower number of hummingbird visits under TE suggests that these animals present a greater sensitivity to this management system, either because of the application of agrochemicals, or through the presence of other taxonomic orders that exploit the same floral resources, increasing competition and reducing the quantity of nectar available. While it is known that their capacity for flight confers many hummingbird species with resistance to habitat transformation (MacGregor-Fors and Schondube, 2011; Bustamante-Castillo et al., 2018), the impact of agricultural practices remains unknown, particularly the effect of the use of pesticides on pollinator birds over time. It has been reported that, in hummingbirds (*Selasphorus rufus* -this study- and *Calypte anna* Lesson, 1829), associated with the cultivation of cranberries and exposed to neonicotinoid pesticides, residues of these chemicals can be found in the cloacal fluid and fecal granules (Bishop et al., 2018). The effect this could have on the birds is unknown, but studies conducted with sparrows that had consumed canola seeds treated with imidacloprid showed that they presented appetite and corporal weight loss, delayed migration, and problems of orientation during migration, increasing the risk of mortality and potentially compromising their opportunities for reproduction (Eng et al., 2017).

Given that the insecticide used (a pyrethroid, lambda-cyhalothrin) in TE is identified as dangerous to bees and other insects, a reduction in the number of visits of the different orders of insects was expected. This was the case only for two of the five orders in red flowered plants, and in none in white-flowered plants. Notably, *Apis mellifera* (the most numerous visiting insect) visited more the white flowers from TE than those of TR. This could perhaps be attributed to the fact that, by the time the observations were made, eight days had already elapsed since the insecticide application, and thus its effect had probably already diminished due to a lower concentration of the insecticide in the plant. In a study of passion fruit (*Passiflora edulis* Sims f. *flavicarpa* O. Deg.), it was found that the concentration of lambda-cyhalothrin in pulp and fruit decreased progressively after application, although traces could still be found up to 14 days later (Romero Ramírez and González Mejía, 2012). In a related study, Crenna et al. (2020) found that even though the initial concentrations of lambda-cyhalothrin in nectar and pollen after application are high (compared to boscalid), their dissipation rates are also high, resulting in a stronger attenuation during the flowering period. It has been reported that once the levels of lambda-cyalothrin decrease below the threshold for repellency, foraging honeybees can collect contaminated pollen (Dolezal et al., 2016); however, subtle physiological and behavioral effects may occur. Finally, it should be noted that pyrethroids are, on average, three times more toxic to bees by contact than by oral exposure, i.e., via residues present mainly in pollen, nectar or honey (Sanchez-Bayo and Goka, 2014) and that *A. mellifera* was the least sensitive species to cyhalothrin among nine species to which it was compared (Hardstone and Scott, 2010).

In the case of certain species of Lepidoptera, Coleoptera and Diptera found in the technified systems, it cannot be discounted that they could be associated with disturbance conditions, since with changing environmental conditions, the composition and diversity of species is modified, and those with life strategies that enable them to survive in disturbed environments therefore predominate (Flores and Sarandón, 2014).

Traditional management of the crop increased the diversity of floral visitors

This finding is consistent with that previously reported in coffee crops (Vergara and Badano, 2009), where more specialized management, in both shade and sun-grown crops, resulted in less diversity in terms of pollinators than traditional management. It is also similar to what was found in almond crops (Norfolk et al., 2016), where the abundance of wild pollinators was higher in crops where agrochemicals had not been used and honeybees had not been introduced. This was due to the abundance and richness of plant species that flower simultaneously with the almond trees, a situation that may also have occurred in the TR systems studied here. It is known that more diverse floral communities are associated with the provision of a greater diversity of resources (e.g., nectar, pollen) (Ghazoul, 2006) and that diversified agricultural systems support greater ecosystem services, such as biodiversity and pollination (Kremen and Miles, 2012), as well as maintain habitat sustainability (Altieri and Nicholls, 2013).

While the presence and visits of lepidopterans, dipterans and coleopterans were detected in the technified systems with both white and red flowers, it is important to highlight that these taxonomic groups seem to have little impact on the pollination of *P. coccineus*, due to their foraging behavior (contact with the flower and extraction of the nectar), which makes it difficult for the pollen to adhere to their bodies for transfer to other flowers (Burquez and Sarukhán, 1980). According to Sousa-Peña (1992), in the specific case of the lepidopterans, due to their low corporal weight, the position at which they land on the flower and the characteristics of their proboscises, they do not come in contact with the tip of the keel through which the stigma and pollen brush are exposed, for which reason they are considered nectar thieves. Considering these characteristics, the species of dipterans and other hymenopterans (that are not bees or bumblebees) could also be considered nectar thieves, since they do not make direct contact with the reproductive structures of the flowers.

Flower color did not influence species richness or present defined tendencies in terms of the number of visits or effective number of species

Flower color is one of the characteristics associated with pollination syndromes. The literature states that red flowers are frequently ornithophilous, and also present a copious production of dilute nectar along with large and wide tubular corollas (Cronk and Ojeda, 2008). It is also reported that yellow, blue or purple flowers with a wide lip and short flower tubes generally correspond to the bee pollination syndrome, or melittophyly (Fenster et al., 2004). It was therefore expected that flower color might be related in some way to the number of floral visitors or to the number of visits. However, our findings showed that species richness within each agronomic management system was statistically equal for both flower colors and that the effective number of species as a function of flower color varied according to the management system. Something similar occurred with the number of visits since, in particular the hummingbirds were expected to have a higher affinity for red flowers and the bees for white flowers, but all of the hummingbird species visited white flowers more than red flowers. In the case of bees, even though they tended to visit more frequently the white flowers, they did not show a marked affinity for any one color.

This could be due to the fact, that flower color is not the only characteristic involved in the attraction of pollinators (and floral visitors in this case). According to Rosas-Guerrero et al. (2014), floral syndromes also include the morphology, fragrance, size, rewards and phenology of the flower, among other attributes. On the other hand, it has been found that the characteristics of pollination syndromes are not all of equal importance. To this can be added the fact that, in several studies, flower color has been found to be an attribute that is not very informative or one that contradicts the expectations of traditional syndromes (Dellinger, 2020). Regarding the red color of the flowers, Cronk and Ojeda (2008) highlight two aspects: i) that it is not necessary to attract birds (there are examples showing them to be effective pollinators of species with orange, yellow and white flowers), and ii) that bees may perceive (and visit) some flowers that humans perceive as red, if these also present some reflectance in the shorter wavelengths. Other possible explanations may be a differential nectar supply between floral morphs, which could generate differential rates of visitation and thus pollen deposition (Valois-Cuesta et al., 2011), and the fact that, under natural conditions, flower choice may be influenced by the presence and abundance of other co-pollinators that could potentially compete for the floral resources (Lázaro et al., 2009).

Conclusions

Our study shows that there is a considerable diversity of floral visitors associated with the runner bean crop, and that the bees and hummingbirds are predominant among them. It also shows that the traditional management system that excludes the application of herbicides and insecticides favors a greater diversity of floral visitors and presents a higher frequency of visits, particularly of hummingbirds. This indicates that less technified management systems contribute to the maintenance of agroecosystem health, and very possibly to pollination services. The results also suggest that flower color did not modify species richness or have any consistent effect on the number of visits or on the structure of the floral visitor community. In order to assess the contribution of each floral visitor and to elucidate the potential pollinators of *P. coccineus* in the study region, future studies must assess pollen loads, nectar composition, as well as study the morphological coupling between the hummingbird species and *P. coccineus* flowers. This will provide an insight into the relationship between the patterns of visitation and their effects on pollination. Finally, we also believe that further studies of the impact of agricultural practices on the biotic pollination of other crops for which Mesoamerica is their center of origin are desirable, since this will contribute to the conservation of agricultural species relevant to food security as well as that of their respective biological vectors.

Author contributions

KCH, AGM, AAJ, PAL and OTG contributed to the study design. OTG provided the seeds. AGM, OTG and KCH supervised and sowed the experiments and participated in the crop management, KCH conducted the fieldwork and the taxonomic determination of the hymenopterans, dipterans, coleopterans, hemipterans and hummingbirds. AGM, PAL and KCH performed the statistical data analyses and AAJ produced the figures. AGM, AAJ and KCH redacted the discussion. All authors have revised and approved the final version of the manuscript.

Funding

This study was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT), (Graduate grant No. 441216 to KCH) and by the Colegio de Postgraduados, through Apoyo a Actividades Académicas y de Estudiantes.

Acknowledgements

We gratefully thank Eladio Espinoza Técuatl, producer of runner bean, for granting access to the fields in which we conducted the study, as well as the installations of the Farm “María Isabel” for development of the fieldwork. Thanks go to Sarahi Nocelo Rojas, José Hernández Cortés and Hugo García Perea for their collaboration in the sowing and crop management, to Natalia Pérez Ariza for identification of the lepidopteran species, to Betsabé Ruiz-Guerra for the review of previous drafts, and Keith MacMillan for helping with the English version. We also thank the reviewers and editors for their valuable comments.

Literature cited

Aizen, M. A. and L. D. Harder. 2009. The global stock of domesticated honeybees is growing slower than agricultural demand for pollination. Current Biology 19(11): 915-918. DOI: https://doi.org/10.1016/j.cub.2009.03.071

Alfaro, F. M. and J. Pizarro-Araya. 2017. Estimación de la riqueza de coleópteros epigeos de la Reserva Nacional Pingüino de Humboldt (Regiones de Atacama y Coquimbo, Chile). Gayana 81(2): 39-51. DOI: https://doi.org/10.4067/S0717-65382017000200039

Altieri, M. A. and C. I. Nicholls. 2013. Agroecología y resiliencia al cambio climático: principios y consideraciones metodológicas. Agroecología 8(1): 7-20.

Arce-Pérez, R. and M. A. Morón. 2000. Taxonomía y distribución de las especies de *Macrodactylus* Latreille (Coleoptera: Melolonthidae) en México y Estados Unidos de América. Acta Zoológica Mexicana (n.s.) 79: 123-239. DOI: https://doi.org/10.21829/azm.2000.79791913

Arizmendi, M. C. and H. Berlanga. 2014. Colibríes de México y Norteamérica. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). México, D.F., México. 160 pp.

Badillo-Montaño, R., A. Aguirre and M. A. Munguía-Rosas. 2018. Pollinator-mediated interactions between cultivated papaya and co-flowering plant species. Ecology and Evolution 9(1): 587-597. DOI: https://doi.org/10.1002/ece3.4781

Biesmeijer, J., S. P. M. Roberts, M. Reemer, R. Ohlemüller, T. M. J. Edwards, M. Peeters, A. P. Schaffers, S. G. Potts, R. J. M. C. Kleukers, C. Thomas, J. Settele and W. Kunin. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313(5785): 351-354. DOI: https://doi.org/10.1126/science.1127863

Bishop, C. A., A. J. Moran, M. C. Toshack, E. Elle, F. Maisonneuve and J. E. Elliott. 2018. Hummingbirds and bumblebees exposed to neonicotinoid and organophosphate insecticides in the Fraser Valley, British Columbia, Canada. Environmental Toxicology and Chemistry 37(8): 2143-2152. DOI: https://doi.org/10.1002/etc.4174

Blackwall, F. L. C. 1971. A study of plant/insect relationships and pod-setting in the runner bean (*Phaseolus multiflorus*). Journal of Horticultural Science 46(4): 365-379. DOI: https://doi.org/10.1080/00221589.1971.11514416

Brown, B. V., A. Borkent, J. M. Cumming, D. M. Wood, N. E. Woodley and M. A. Zumbado (eds.). 2010. Manual of Central American Diptera. Volume 2. NRC Research Press. Ottawa, Canada. Pp. 763-1282.

Burkle, L. A., J. C. Marlin and T. M. Knight. 2013. Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function. Science 339(6127): 1611-1615. DOI: https://doi.org/10.1126/science.1232728

Burquez, A. and J. Sarukhán. 1980. Biología de poblaciones silvestres y cultivadas de *Phaseolus coccineus* L.: I. Relaciones planta-polinizador. Boletín de la Sociedad Botánica de México 39: 5-24. DOI: https://doi.org/10.17129/botsci.1173

Bustamante-Castillo, M., B. E. Hernández-Baños and M. del C. Arizmendi. 2018. Hummingbird diversity and assemblage composition in a disturbed tropical dry forest of Guatemala. Tropical Conservation Science 11: 1-15. DOI: https://doi.org/10.1177%2F1940082918793303

Cervantes Mayagoitia, J. F. and A. H. Huacuja Zamudio. 2020. Guía de los Ácaros e Insectos Herbívoros de México. Volumen 4.

Ácaros e insectos antófagos y carpófagos de importancia agrícola y forestal. Universidad Autónoma Metropolitana. Cd. Mx., México. Pp. 108-114.

Chao, A. and T. J. Shen. 2010. SPADE®. Species prediction and diversity estimation. National Tsing Hua University. Hsin-Chu, Taiwan. http://140.114.36.3/wordpress/software_download/softwarespader_online/ (consulted May, 2020).

Chittka, L., A. Shmida, N. Troje and R. Menzel. 1994. Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. Vision Research 34(11): 1489-1508. DOI: https://doi.org/10.1016/0042-6989(94)90151-1

Colwell, R. K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Ver. 9. User's Guide and application. University of Connecticut. Storrs, USA. http://viceroy.eeb.uconn.edu/estimates (consulted May, 2020).

Corrales Castillo, J., A. González Herrera, A. Rodríguez Arrieta, A. Vargas Martínez and K. Villalobos Mora. 2017. Principales plagas de artrópodos en el cultivo de frijol en Costa Rica; Guía ilustrada de artrópodos adultos en campo y grano almacenado. Universidad Nacional. Heredia, Costa Rica. Pp. 45-48.

Crenna, E., O. Jolliet, E. Collina, S. Sala and P. Fantke. 2020. Characterizing honey bee exposure and effects from pesticides for chemical prioritization and life cycle assessment. Environment International 138: 105642. DOI: https://doi.org/10.1016/j.envint.2020.105642

Cronk, Q. and I. Ojeda. 2008. Bird-pollinated flowers in an evolutionary and molecular context. Journal of Experimental Botany 59(4): 715-727. DOI: https://doi.org/10.1093/jxb/ern009

de Camargo, M. G. G., K. Lunau, M. A. Batalha, S. Brings, V. L. G. de Brito and L. P. C. Morellato. 2019. How flower colour signals allure bees and hummingbirds: a community-level test of the bee avoidance hypothesis. New Phytologist 222(2):1112-1122. DOI: https://doi.org/10.1111/nph.15594

Deguines, N., R. Julliard, M. de Flores and C. Fontaine. 2012. The whereabouts of flower visitors: contrasting land-use preferences revealed by a country-wide survey based on citizen science. PLoS ONE 7(9): e45822. DOI: https://doi.org/10.1371/journal.pone.0045822

Delgado Salinas, A. 1988. Variation, taxonomy, domestication, and germplasm potentialities in *Phaseolus coccineus*. In: Gepts, P. (ed.). Genetic resources in *Phaseolus* beans. Their maintenance, domestication, evolution and utilization. Kluwer Academic Publishers. Dordrecht, Holland. Pp. 441-463.

Dellinger, A. S. 2020. Pollination syndromes in the 21st century: where do we stand and where may we go? New Phytologist 228(4): 1193-1213. DOI: https://doi.org/10.1111/nph.16793

Divulgare. 2022. Divulgare, Functional groups of pollinators. http://www.divulgare.net (consulted February, 2022).

Dolezal, A. G., J. Carrillo-Tripp, W. A. Miller, B. C. Bonning and A. L. Toth. 2016. Pollen contaminated with field-relevant levels of cyhalothrin affects honey bee survival, nutritional physiology, and pollen consumption behavior. Journal of Economic Entomology 109(1): 41-48. DOI: https://doi.org/10.1093/jee/tov301

Doyle, T., W. L. S. Hawkes, R. Massy, G. D. Powney, M. H. M. Menz and K. R. Wotton. 2020. Pollination by hoverflies in the Anthropocene. Proceedings of the Royal Society B: Biological Sciences 287(1927): 20200508. DOI: https://doi.org/10.1098/rspb.2020.0508

Eeraerts, M., G. Smagghe and I. Meeus. 2019. Pollinator diversity, floral resources and semi-natural habitat, instead of honeybees and intensive agriculture, enhance pollination service to sweet cherry. Agriculture, Ecosystems & Environment 284: 106586. DOI: https://doi.org/10.1016/j.agee.2019.106586

Eilers, E. J., C. Kremen, S. S. Greenleaf, A. K. Garbe and A. M. Klein. 2011. Contribution of pollinator mediated crops to nutrients in the human food supply. PloS ONE 6(6): e21363. DOI: https://doi.org/10.1371/journal.pone.0021363

Eng, M. L., B. J. M. Stutchbury and C. A. Morrissey. 2017. Imidacloprid and chlorpyrifos insecticides impair migratory ability in a seed-eating songbird. Scientific Reports 7: 15176. DOI: https://doi.org/10.1038/s41598-017-15446-x

Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution and Systematics 35(1): 375-403. DOI: https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Fernández, F. and M. J. Sharkey. 2006. Introducción a los Hymenoptera de la Región Neotropical. Sociedad Colombiana de Entomología y Universidad Nacional de Colombia. Bogotá, Colombia. Pp. 443-539.

Flores, C. C. and S. J. Sarandón. 2014. Desarrollo y evolución de los ecosistemas. In: Sarandón, S. J. and C. C. Flores (eds.). Agroecología: bases teóricas para el diseño y manejo de agroecosistemas sustentables. Universidad de La Plata. Buenos Aires, Argentina. Pp. 159-189.

Free, J. B. 1966. The pollination of the beans *Phaseolus multiflorus* and *Phaseolus vulgaris* by honeybees. Journal of Apicultural Research 5(2): 87-91. DOI: https://doi.org/10.1080/00218839.1966.11100139

Free, J. B. and P. A. Racey. 1968. The pollination of runner beans (*Phaseolus multiflorus*) in a glasshouse. Journal of Apicultural Research 7(2): 67-69. DOI: https://doi.org/10.1080/00218839.1968.11100191

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, L. G. Carvalheiro, L. D. Harder, O. Afik, I. Bartomeus, F. Benjamin, V. Boreux, D. Cariveau, N. P. Chacoff, J. H. Dudenhöffer, B. M. Freitas, J. Ghazoul, S. Greenleaf, J. Hipólito, A. Holzschuh, B. Howlett, R. Isaacs, S. K. Javorek, C. M. Kennedy, K. M. Krewenka, S. Krishnan, Y. Mandelik, M. M. Mayfield, I. Motzke, T. Munyuli, B. A. Nault, M. Otieno, J. Petersen, G. Pisanty, S. G. Potts, R. Rader, T. H. Ricketts, M. Rundlöf, C. L. Seymour, C. Schüepp, H. Szentgyörgyi, H. Taki, T. Tscharntke, C. H. Vergara, B. F. Viana, T. C. Wanger, C. Westphal, N. Williams and A. M. Klein. 2013. Wild pollinators enhance fruit set of crops regardless of honeybee abundance. Science 339(6127): 1608-1611. DOI: https://doi.org/10.1126/science.1230200

Genung, M. A., J. P. Lessard, C. B. Brown, W. A. Bunn, M. A. Cregger, Wm. N. Reynolds, E. Felker-Quinn, M. L. Stevenson, A. S. Hartley, G. M. Crutsinger, J. A. Schweitzer and J. K. Bailey. 2010. Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. PloS ONE 5(1): e8711. DOI: https://doi.org/10.1371/journal.pone.0008711

Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology 94(2): 295-304. DOI: https://doi.org/10.1111/j.1365-2745.2006.01098.x

Gill, R. J., O. Ramos-Rodriguez and N. E. Raine. 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature 491: 105-108. DOI: https://doi.org/10.1038/nature11585

Glassberg, J. A. 2007. A swift guide to the butterflies of Mexico and Central America. Sunstreak Books. Morristown, New Jersey, USA. 266 pp.

González, C. R., C. Reyes, M. I. Jercic, V. Rada, M. Saldarriaga, C. Pavletic and A. Parra. 2016. Manual de culícidos (Diptera: Culicidae) de la zona Norte y Centro de Chile, incluyendo Isla de Pascua. Instituto de Salud Pública de Chile, Ministerio de Salud de Chile. Santiago, Chile. Pp. 24-40.

Hardstone, M. C. and J. G. Scott. 2010. Is *Apis mellifera* more sensitive to insecticides than other insects? Pest Management Science 66(11): 1171-80. DOI: https://doi.org/10.1002/ps.2001

Hernández-Villa, V., H. Vibrans, E. Uscanga-Mortera and A. Aguirre-Jaimes. 2020. Floral visitors and pollinator dependence are related to floral display size and plant height in native weeds of central Mexico. Flora 262: 151505. DOI: https://doi.org/10.1016/j.flora.2019.151505

Holzschuh, A., I. Steffan-Dewenter and T. Tscharntke. 2008. Agricultural landscapes with organic crops support higher pollinator diversity. Oikos 117(3): 354-361. DOI: https://doi.org/10.1111/J.2007.0030-1299.16303.X

INEGI. 2010. Prontuario de información geográfica municipal de los Estados Unidos Mexicanos: Calpan, Puebla. Instituto Nacional de Estadística Geografía e Informática. Aguascalientes, México. 9 pp. https://www.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/21/21026.pdf (consulted January, 2021).

INEGI. 2019. Informe técnico de la Cuenca Hidrológica Río Alto Atoyac. Humedales. Instituto Nacional de Estadística Geografía e Informática. Aguascalientes, México. 78 pp. https://www.inegi.org.mx/contenido/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/702825189884.pdf (consulted July, 2021).

Jost, L. 2006. Entropy and diversity. Oikos 113(2): 363-375. DOI: https://doi.org/10.1111/j.2006.0030-1299.14714.x

Kendall, D. A. and B. D. Smith. 1976. The pollinating efficiency of honeybee and bumblebee visits to flowers of the runner bean *Phaseolus coccineus* L. Journal of Applied Ecology 13(3): 749-752. DOI: https://doi.org/10.2307/2402252

Klein, A., I. Steffan-Dewenter and T. Tscharntke. 2003. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. Journal of Applied Ecology 40(5): 837-845. DOI: https://doi.org/10.1046/j.1365-2664.2003.00847.x

Klein, A. M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen and T. Tscharntke. 2007.

Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B 274: 303-313. DOI: https://doi.org/10.1098/rspb.2006.3721

Koltowski, Z. 2004. Flowering biology, nectar secretion and insect foraging of the runner bean (*Phaseolus coccineus* L.). Journal of Apicultural Science 48(2): 53-60.

Kremen, C. and A. Miles. 2012. Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. Ecology and Society 17(4): 1-40. DOI: https://doi.org/10.5751/ES-05035-170440

Labuda, H. 2010. Runner bean (*Phaseolus coccineus* L.) biology and use. Acta Scientiarum Polonorum Hortorum Cultus 9(3): 117-132.

Lara, L. A., S. Godínez-Cortés, R. I. Torres, E. Padrón-Torres, J. Reyes, F. A. González-González, V. Hernández, E. D. González-Nieto and H. Hernández. 2022. Coccinélidos (Coleoptera: Coccinellidae) asociados a cítricos del centro-sur de Tamaulipas, México. Acta Zoológica Mexicana (nueva serie) 38(1): 1-10. DOI: https://doi.org/10.21829/azm.2022.3812454

Lázaro, A., R. Lundgren and Ø. Totland. 2009. Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. Oikos 118(5): 691-702. DOI: https://doi.org/10.1111/j.1600-0706.2008.17168.x

López-Báez, L. I., O. R. Taboada-Gaytán, A. Gil-Muñoz, P. A. López, E. Ortiz-Torres, M. L. P. Vargas-Vázquez and R. Díaz-Cervantes. 2018. Diversidad morfoagronómica del frijol ayocote en el altiplano centro-oriente de Puebla. Revista Fitotecnia Mexicana 41(4-A): 487-497.

Lucas, A., J. C. Bull, N. De Vere, P. J. Neyland and D. W. Forman. 2017. Flower resource and land management drives hoverfly communities and bee abundance in seminatural and agricultural grasslands. Ecology and Evolution 7(19): 8073-8086. DOI: https://doi.org/10.1002/ece3.3303

Lundgren, R., Ø. Totland and A. Lázaro. 2016. Experimental simulation of pollinator decline causes community-wide reductions in seedling diversity and abundance. Ecology 97(6): 1420-1430. DOI: https://doi.org/10.1890/15-0787.1

MacGregor-Fors, I. and J. E. Schondube. 2011. Gray *vs.* green urbanization: Relative importance of urban features for urban bird communities. Basic and Applied Ecology 12(4): 372-381. DOI: https://doi.org/10.1016/j.baae.2011.04.003

Maruyama, P. K., L. N. Custódio and P. E. Oliveira. 2012. When hummingbirds are the thieves: visitation effect on the reproduction of Neotropical snowbell *Styrax ferrugineus* Nees & Mart (Styracaceae). Acta Botanica Brasilica 26(1): 58-64. DOI: https://doi.org/10.1590/s0102-33062012000100007

Menzel, R. and W. Backhaus. 1991. Colour vision in insects. In: Gouras, P. (ed.). Vision and visual dysfunction. Vol. VI The perception of colour. Macmillan Press. London, UK. Pp. 262-293.

Michener, C. D. 2007. The bees of the world. The Johns Hopkins University Press. Baltimore, Maryland, USA. Pp. 558-831.

Moreno, C. E., F. Barragán, E. Pineda and N. P. Pavón. 2011. Reanálisis de la diversidad alfa: alternativas para interpretar y comparar información sobre comunidades ecológicas. Revista Mexicana de Biodiversidad 82(4): 1249-1261. DOI: https://doi.org/10.22201/ib.20078706e.2011.4.745

Nájera Rincón, M. B. and B. Souza. 2010. Insectos benéficos: guía para su identificación. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias y Universidad Federal de Lavras. Uruapan, México. Pp. 13-14.

Nates-Parra, G. 2005. Abejas silvestres y polinización. Manejo Integrado de Plagas y Agroecología 75: 7-20.

Norfolk, O., M. P. Eichhorn and F. Gilbert. 2016. Flowering ground vegetation benefits wild pollinators and fruit set of almond within arid smallholder orchards. Insect Conservation and Diversity 9(3): 236-243. DOI: https://doi.org/10.1111/icad.12162

Ödeen, A. and O. Håstad. 2010. Pollinating birds differ in spectral sensitivity. Journal of Comparative Physiology 196(2): 91-96. DOI: https://doi.org/10.1007/s00359-009-0474-z

Olesen, J. M. and P. Jordano. 2002. Geographic patterns in plant-pollinator mutualistic networks. Ecology 83(9): 2416-2424. DOI: https://doi.org/10.1890/0012-9658(2002)083[2416:GPIPPM]2.0.CO;2

Otieno, M., B. A. Woodcock, A. Wilby, I. N. Vogiatzakis, A. L. Mauchline, M. W. Gikungu and S. G. Potts. 2011. Local management and landscape drivers of pollination and biological control services in a Kenyan agro-ecosystem. Biological Conservation 144(10): 2424-2431. DOI: https://doi.org/10.1016/j.biocon.2011.06.013

Pando, J. B., F. N. T. Fohouo and J. L. Tamesse. 2011. Foraging and pollination behaviour of *Xylocopa calens* Lepeletier (Hymenoptera: Apidae) on *Phaseolus coccineus* L. (Fabaceae) flowers at Yaounde (Cameroon). Entomological

Research 41(5): 185-193. DOI: https://doi.org/10.1111/j.1748-5967.2011.00334.x

Panizzi, A. R. and F. Slansky. 1985. Review of phytophagous pentatomids (Hemiptera: Pentatomidae) associated with soybean in the Americas. The Florida Entomologist 68(1): 184-214. DOI: https://doi.org/10.2307/3494344

Peterson, R. T. and E. L. Chalif. 2000. Aves de México. Guía de campo. Ed. Diana. Cd. Mx., México. Pp. 176-195.

Pineda-López, R. and J. R. Verdú. 2013. Cuaderno de prácticas. Medición de la biodiversidad: diversidad alfa, beta y gama. Universidad Autónoma de Querétaro y Universidad de Alicante. Ed. Universitaria. Querétaro, México. 120 pp.

Pinilla-Gallego, M. S. and G. Nates-Parra. 2015. Visitantes florales y polinizadores en poblaciones silvestres de agraz (*Vaccinium meridionale*) del bosque andino colombiano. Revista Colombiana de Entomología 41(1): 112-119.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution 25(6): 345-353. DOI: https://doi.org/10.1016/j.tree.2010.01.007

Puig-Montserrat, X., C. Stefanescu, I. Torre, J. Palet, E. Fabregas, J. Dantart, A. Arrizabalaga and C. Flaquer. 2017. Effects of organic and conventional crop management on vineyard biodiversity. Agriculture Ecosystems & Environment 243: 19-26. DOI: https://doi.org/10.1016/j.agee.2017.04.005

Rocchini, D. and M. Neteler. 2012. Spectral rank-abundance for measuring landscape diversity. International Journal of Remote Sensing 33(14): 4458-4470. DOI: https://doi.org/10.1080/01431161.2011.648286

Rodríguez-Femat, J. F. 2014. Coexistencia de cuatro especies de colibríes en la Reserva Ecológica Huitepec, San Cristóbal de Las Casas, Chiapas. Tesis de maestría. El Colegio de la Frontera Sur. San Cristóbal de Las Casas, Chiapas, México. 57 pp.

Romero Ramírez, A. C. and A. González Mejía. 2012. Ensayo piloto de investigación relacionado con la determinación de períodos de carencia de residuos de plaguicidas en el cultivo de maracuyá (*Passiflora edulis* f. *flavicarpa*). Documento de Trabajo CIAT no. 128. Ministerio de Agricultura y Desarrollo Rural de Colombia y Centro Internacional de Agricultura Tropical. Cali, Colombia. 102 pp.

Rosas-Guerrero, V., R. Aguilar, S. Martén-Rodríguez, L. Ashworth, M. Lopezaraiza-Mikel, J. M. Bastida and M. Quesada. 2014. A quantitative review of pollination syndromes: do floral traits predict effective pollinators? Ecology Letters 17: 388-400. DOI: https://doi.org/10.1111/ele.12224

Roulston, T. H. and K. Goodell. 2011. The role of resources and risks in regulating wild bee populations. Annual Review of Entomology 56(1): 293-312. DOI: https://doi.org/10.1146/annurev-ento-120709-144802

Rundlöf, M., G. K. S. Andersson, R. Bommarco, I. Fries, V. Hederström, L. Herbertsson, O. Jonsson, B. K. Klatt, T. R. Pedersen, J. Yourstone and H. G. Smith. 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature 521(7550): 77-80. DOI: https://doi.org/10.1038/nature14420

Salazar-Díaz, R. and V. Torres-Coto. 2017. Estudio de la dinámica de polinizadores del cultivo de cacao (*Theobroma cacao*) en tres sistemas de producción. Revista Tecnología en Marcha 30(1): 90-100. DOI: https://doi.org/10.18845/tm.v30i1.3088

Sanchez-Bayo, F. and K. Goka. 2014. Pesticide residues and bees - A risk assessment. PLoS ONE 9(4): e94482. DOI: https://doi.org/10.1371/journal.pone.0094482

SAS Institute Inc. 2020. SAS University Edition Ver. 9.4. Statistical Analysis System Institute. Cary, USA. https://www.sas.com/es_mx/software/university-edition.html (consulted May, 2020).

Shuler, R. E., T. A. H. Roulston and G. E. Farris. 2005. Farming practices influence wild pollinator populations on squash and pumpkin. Journal of Economic Entomology 98(3): 790-795. DOI: https://doi.org/10.1603/0022-0493-98.3.790

Sousa-Peña, M. 1992. Polinización en el complejo *Phaseolus coccineus L.* (Fabaceae). Tesis de licenciatura. Facultad de Ciencias, Universidad Nacional Autónoma de México. México, D.F., México. 68 pp.

Ssymank, A., C. A. Kearns, T. Pape and F. C. Thompson. 2008. Pollinating flies (Diptera): A major contribution to plant diversity and agricultural production. Biodiversity 9(1-2): 86-89. DOI: https://doi.org/10.1080/14888386.2008.9712892

Sugden, E. A. and R. L. McAllen. 1994. Observations on foraging, population and nest biology of the Mexican honey wasp, *Brachygastra mellifica* (Say) in Texas (Vespidae: Polybiinae). Journal of the Kansas Entomological Society 67(2): 141-155

Tchuenguem, F. F. N., T. S. Fameni and D. F. Brückner. 2014. Foraging and pollination behaviour of *Xylocopa olivacea*

(Hymenoptera: Apidae) on *Phaseolus coccineus* (Fabaceae) flowers at Ngaoundéré (Cameroon). International Journal of Tropical Insect Science 34(2): 127-137. DOI: https://doi.org/10.1017/S1742758414000241

Valois-Cuesta, H., P. J. Soriano and J. F. Ornelas. 2011. Asymmetrical legitimate pollination in distylous *Palicourea demissa* (Rubiaceae): the role of nectar production and pollinator visitation. Journal of Tropical Ecology 27(4): 393-404. DOI: https://doi.org/10.1017/S0266467411000150

Vergara, C. H. and E. I. Badano. 2009. Pollinator diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. Agriculture, Ecosystems and Environment 129(1-3): 117-123. DOI: https://doi.org/10.1016/j.agee.2008.08.001

Viglizzo, E., A. Pordomingo, M. Castro and F. Lértora. 2002. La sustentabilidad ambiental de la agricultura pampeana ¿oportunidad o pesadilla? Ciencia Hoy 12(68): 38-51.

Villamil, L., M. Astier, Y. Merlín, R. Ayala-Barajas, E. Ramírez-García, J. Martínez-Cruz and M. E. Gavito. 2018. Management practices and diversity of flower visitors and herbaceous plants in conventional and organic avocado orchards in Michoacán, Mexico. Agroecology and Sustainable Food Systems 42(5): 530-551. DOI: https://doi.org/10.1080/21683565.2017.1410874

Wäckers, F. L., J. Romeis and P. van Rijn. 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. Annual Review of Entomology 52(1): 301-323. DOI: https://doi.org/10.1146/annurev.ento.52.110405.091352

Williams, I. H. and J. B. Free. 1975. The pollination and set of the early flowers of runner bean (*Phaseolus coccineus* L.). Journal of Horticultural Science 50(4): 405-413. DOI: https://doi.org/10.1080/00221589.1975.11514649