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

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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, fríjol ayocote, polinización, sistema agrícola.
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(w2)), which range from 900 to 1100 mm per year, while the mean annual temperature ranges from 12 to 18 °C (INEGI, 2010; 2019).

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**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 m², 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·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 two different landraces. At each selected point, for each landrace, a vertical plant observation area of 250 cm² (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 using the illustrated guides of Glassberg (2007) for Lepidoptera, Cervantes Mayagoitia 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 ≤ z ≤ 0.6642, 0.5066 ≤ P ≤ 1), considering the 12 observation points as sampling effort. Verdu, 2013), considering the 12 observation points as

100 randomizations were conducted (Pineda-López and the effect of the order in which the samples were added, Estim tense 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), Hy- menoptera (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), Cole- optera 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 Dip- tera was higher than in TR.

The species richness was similar in the white flowers of the TR and TE plots (X²=1.93, d.f.=4; P=0.760). This also occurred with the red flowers (X²=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 (X²=1.87, d.f.=5; P=0.9678), and in TE (X²=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 (X²=480.24, d.f.=4; P<0.0001). The same was found when comparing the number of visits to red flowers between management systems (X²=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.

<table>
<thead>
<tr>
<th>Order</th>
<th>Species</th>
<th>Agricultural management</th>
<th>Flower color</th>
<th>Flower color</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Traditional</td>
<td>Technified</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>White</td>
<td>Red</td>
</tr>
<tr>
<td>Apodiformes</td>
<td><em>Amazilia beryllina</em> Deppe, 1830</td>
<td>82</td>
<td>7</td>
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<td><em>Calathorax lucifer</em> Swainson, 1827</td>
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<td>272</td>
<td>209</td>
<td>108</td>
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<td><em>Selasphorus rufus</em> Gmelin, 1788</td>
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<td>1</td>
<td>9</td>
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<td><em>Musca</em> sp. 1</td>
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<td>668</td>
<td>1144</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Piruna</em> sp. 1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Polites vibex</em> Geyer, 1832</td>
<td>3</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 1: Continuation.

<table>
<thead>
<tr>
<th>Order</th>
<th>Species</th>
<th>Traditional</th>
<th>Technified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flower color</td>
<td>Flower color</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>Red</td>
<td>White</td>
</tr>
<tr>
<td>Urbanus dorantes Stoll, 1790</td>
<td>8</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Vanessa cardui Linnaeus, 1758</td>
<td>5</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

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 ($'D'=5.2$) was more diverse than TE with white flowers ($'D'=2.6$). In addition, TR with red flowers ($'D'=3.9$) was also more diverse than TE with red flowers ($'D'=3.1$). 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 conducted 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 *Brachygasta 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; Rodríguez-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 (Trochilidae) 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 ap-
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petite 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-cyhalothrin 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 wave-lengths. 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, dipter-
ans, 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.

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