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Zoopollination of Pulque agave crop (*Agave salmiana*) in the central valleys of Mexico

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Abstract

Background: The cultivation of pulque agave (*Agave salmiana*) is ecologically and economically important in Mexico. However, traditional practices that remove plants before flowering for beverage production have limited knowledge of its pollinators.

Questions: This study aimed to identify potential pollinators of *A. salmiana* under cultivation conditions.

Studied species / data description: The focal species was *Agave salmiana*. Data included vertebrate floral visitors, pollen loads, and nectar production. Diversity was assessed using Shannon-Wiener, Simpson, Pielou indices, and Hill numbers.

Study site and dates: The study was conducted in cultivated landscapes of the Llanos de Apan, central Mexico, during 2023 and 2024.

Methods: Vertebrate visitors were recorded using camera traps. Pollination potential was evaluated through pollen load analysis, and nectar volume and timing were measured.

Results: Vertebrate visitors were dominated by hummingbirds (Trochilidae), with *Eugenes fulgens* showing the highest visitation rate. Nectar-feeding bats (Glossophaginae) were frequent nocturnal visitors and effectively contacted flowers. Insects, particularly *Apis mellifera*, were abundant but rarely contacted reproductive structures. All visitors carried *A. salmiana* pollen, with the highest loads recorded in *E. fulgens* and *Basilinna leucotis*. Nectar volume peaked in the afternoon and evening. Diversity values were comparable to those of wild agave species, although evenness was lower due to dominance by a few species.

Conclusions: *Agave salmiana* is pollinated by both diurnal and nocturnal vertebrates and is not specialized on either group. Flowering agaves in cultivation provide key floral resources that support local pollinator diversity in agricultural landscapes.

Keywords: Camera trap, floral visitor, Glossophaginae, nectar, pollen load, Trochilidae.

Resumen

Antecedentes: El cultivo del maguey pulquero (*Agave salmiana*) es ecológica y económicamente importante en México. Sin embargo, las prácticas tradicionales de cortar las plantas antes de la floración para la producción de pulque han limitado el conocimiento sobre sus polinizadores.

Preguntas: El objetivo fue identificar los polinizadores de *A. salmiana* en cultivo.

Especies estudiadas / descripción de los datos: La especie focal fue *Agave salmiana*. Se obtuvieron datos de visitantes florales, cargas de polen y producción de néctar. La diversidad se evaluó mediante los índices de Shannon–Wiener, Simpson, Pielou y números de Hill.

Sitio de estudio y fechas: El estudio se realizó en paisajes cultivados de los Llanos de Apan, centro de México, durante 2023 y 2024.

Métodos: Los visitantes se registraron mediante fototrampeo. El potencial de polinización se evaluó con análisis de cargas de polen y mediciones del volumen y temporalidad del néctar.

Resultados: Los colibríes (Trochilidae) dominaron los registros, con *Eugenes fulgens* como la especie más frecuente. Los murciélagos nectarívoros (Glossophaginae) fueron visitantes nocturnos comunes y contactaron eficazmente las flores. Los insectos, especialmente *Apis mellifera*, fueron abundantes pero con escaso contacto reproductivo. Todos los visitantes transportaron polen de *A. salmiana*. El néctar alcanzó su máximo por la tarde y noche. La diversidad de visitantes fue comparable con la de agaves silvestres, aunque con poca equitatividad.

Conclusiones: *Agave salmiana* es polinizado por vertebrados diurnos y nocturnos sin especialización marcada. Los agaves cultivados en floración aportan recursos clave para la diversidad local de polinizadores en paisajes agrícolas.

Palabras clave: Cámara-trampa, visitante floral, Glossophaginae, néctar, carga polínica, Trochilidae.

Zoopollination is pollination mediated by animals; this type of pollination is so successful that it is estimated that around 87.5 % of angiosperm species use it for sexual reproduction (Ollerton *et al*. 2011). Pollinators provide essential contributions in all terrestrial ecosystems; these include bees and other insects, hummingbirds, and bats, among others (Mayer *et al*. 2011). The plant - pollinator relationship is considered one of the most ecologically relevant interactions since many plants would not be able to produce seeds and reproduce without this process, and without pollen and nectar for the animals that feed on them, pollinator populations would decline (Ollerton *et al*. 2011). In general, plants and their different groups of pollinators are being threatened by human activities, including habitat destruction, introduction of pathogens and parasites, and climate change (Watanabe 2014).

In addition to its ecological importance, pollination has a vital relationship with human well-being as it is linked to agricultural production and therefore food security, since it is estimated that 87 out of 115 of the main crops worldwide depend on zoopollination (Porto *et al.* 2020). Although not all plants depend entirely on this process for their reproduction, when a pollinator mediates it, crop yield and quality are higher (Tremlett *et al*. 2020). Thus, the global estimated value of crop pollination ranges between US $267 - 657 billion annually (Porto *et al*. 2020). However, it is important to emphasize that there is a great lack of data on the zoopollination of commercially important crops, particularly in developing countries such as Mexico (Porto *et al*. 2020, Gobierno de México 2021).

One of the crops of notable importance for Mexico is agave. The relationship between agaves and humans has a long history that goes back at least 9,000 to 10,000 years (MacNeish 1992). This relationship has been so close that it has been considered symbiotic (Gentry 1982). The use of this plant has been a pillar for subsistence, complementing the cultivation of grasses (Evans 1990, Álvarez-Ríos *et al*. 2020). Agricultural systems have traditionally been made up of strips of agaves alongside annual crops such as maize, beans, and squash (Evans 1990, Álvarez-Duarte 2018, Álvarez-Ríos *et al*. 2020). It has been historically relevant in Mesoamerican and Aridoamerican cultures due to its multiple uses, some of which have persisted in rural and peri-urban communities (Altieri & Toledo 2011, Torres-García *et al*. 2019). Agaves have different uses, such as traditional medicine, food, fencing, fodder, construction, the making of garments and utensils, the extraction of juices and syrups, ritual uses, ornamental uses, as well as the production of alcoholic beverages such as tequila, mezcal, bacanora, and pulque (Gobierno de México 2021, Trejo *et al*. 2022).

Among this range of beverages, pulque in particular has a profound history in Mexico, as its use began with ancient pre-Hispanic civilizations. Pulque is a fermented drink obtained from the sap of agaves, mainly from the species *Agave salmiana* var. *salmiana*, a domesticated species traditionally and intensively cultivated in central Mexico (Arita & Wilson 1987, Higuera-Orbe *et al*. 2025). To obtain the sap of the agave, which is the raw material for pulque, the plant's reproductive structure is cut prematurely before flowering, so the plant only multiplies through "hijuelos" (clones). Although this traditional practice is essential for the immediate use of the crop, it leaves a gap in our understanding of the organisms that could carry out pollination and produce seeds in *A. salmiana.*

Agave salmiana has a monocarpic reproductive system, meaning it dies after reproducing. Its inflorescence can reach up to 8 - 10 m in height and is paniculate, with each panicle bearing more than 100 flowers (Estrella Ruiz 2005, García-Mendoza 2007). The flowering of *A. salmiana* can begin in March, with some individuals blooming as late as July (unpublished data). However, there is pollination research for other agave species (see Arizaga *et al*. 2000, Molina-Freaner & Eguiarte 2003, Flores-Torres & Galindo-Escamilla 2017) In *A. salmiana* is an aspect that until now has only been partially known and under conditions unrelated to agriculture (*i.e*., Martínez del Río & Eguiarte 1987, Gómez-Aíza & Zuria 2010).

For this reason, the objective of this study was to determine, through three complementary techniques -camera trapping, pollen load analysis, and nectar production dynamics- the pollinators of *A. salmiana* under cultivation conditions. Additionally, to support the interpretation of visitation patterns, floral traits were characterized. Identifying the pollinators of *A. salmiana* in cultivation is key for the people who immediately depend on the production of this plant, as well as for the health of the ecosystems where it grows.

Materials and methods

Study Site. The study sites are located within the region historically known as the "Llanos de Apan" in the biogeographic area called the Trans-Mexican Volcanic Belt. The Llanos de Apan extend geographically from the northeast of the State of Mexico to the southeast of the State of Hidalgo and the northwest of Tlaxcala. Until the 1940s, this region was home to the most important maguey estates in the country due to pulque production (Ramírez 2021). We conducted fieldwork in March, April, May, and June during the years 2023 and 2024 in two locations within this region: Rancho La Gaspareña, Singuilucan, Hidalgo (19° 58' 9.31'' N and 98° 30' 19.91'' W), and Hacienda Xochuca in Tlaxco, Tlaxcala (19° 37' 40'' N and 98° 11' 43'' W; Figure 1). In both places, *Agave salmiana* is cultivated mainly for pulque production; the crop is established in "metepantles," which are rows of maguey plants that separate one crop from another, such as barley, fava beans, or fruit trees. No agrochemicals are used at the sites.

Singuilucan, Hgo. has a temperature range between 10 and 16 °C and 400 - 1,100 mm of precipitation, with a predominantly temperate sub-humid climate. 57.5 % of land use is agriculture and 39.3 % of the vegetation is forest (INEGI 2010a). In contrast, Tlaxco, Tlax. has a temperature range between 10 and 14 °C, 600 - 900 mm of precipitation, and also a predominantly temperate sub-humid climate. 65.4 % of land use is agriculture and 25.9 % of the vegetation is forest (INEGI 2010b).

Data Analysis. We carried out the analysis at the zone level (Llanos de Apan) because both study sites belong to the same biogeographic region (Morrone 2019), share similar agricultural management practices (Miguel 2023), and have a common natural distribution of pollinators (Howell & Webb 1995, Arita & Santos del Prado 1999). This decision was statistically supported using a Kruskal-Wallis test, which assessed the homogeneity in the floral visitation rate between the sites studied.

Figure 1. Location of the study sites in central Mexico. The Llanos de Apan region highlighted by a black rectangle. The inset provides a detailed view of the study sites: Rancho La Gaspareña in Singuilucan, Hidalgo, and Hacienda Xochuca in Tlaxco, Tlaxcala.

Characterization of Agave salmiana *inflorescences*. To better understand the plant-animal interaction in cultivated agaves, certain aspects of the flowering of *A. salmiana* were characterized. The number of umbels on each individual was counted, and floral measurements were taken from seven cultivated individuals (two individuals from Tlaxcala and five individuals from Hidalgo). A total of 24 randomly selected flowers per individual were measured, totaling 168 flowers. Fifteen morphological traits were estimated based on Trejo *et al.* (2024), these traits are in detail in Table 1.

Tepal length was used as a key morphological variable because nectar accumulates at its base, thus determining the accessibility of the resource for floral visitors. This allows the relationship between tepal length and morphological traits of pollinators to be assessed, such as bill length in nectarivorous birds or snout and tongue length in nectar-feeding bats (Fleming *et al*. 2009, Muchhala & Thomson 2009, Tobias *et al*. 2022).

Table 1. Measured characteristics of *Agave salmiana* flowers

Morphological trait	Mean (mm) ± e.d. (n = 168)
Total flower length	103.94 ± 15.56
Pedicel length	12.20 ± 3.47
Ovarian tube length	46.90 ± 3.37
Tepal length	44.51 ± 2.79
Flower tube diameter	10.86 ± 0.79
Oval length	39.76 ± 2.09
Distance from the ovary to the pistil	8.30 ± 1.06
Distance from the ovary to the filament	19.16 ± 1.99
Pistil length	55.72 ± 8.72
Pistil head length	4.21 ± 1.88
Pistil diameter	2.76 ± 0.39
Filament length	66.82 ± 3.65
Anther length	25.41 ± 4.10
Filter diameter	2.70 ± 0.51
Anther diameter	2.53 ± 0.41

Determination of Agave salmiana pollinators. All applicable national guidelines for the care and use of animals were followed under permission SEMARNAT SGPA/DGVS/ 07148 /21.

To identify the floral visitors of *A. salmiana* and comprehensively evaluate their potential as pollinators, three complementary techniques were used: camera trapping to directly observe floral visits, pollen collection from floral visitors to determine pollen transport effectiveness, and measurement of nectar production dynamics to understand the availability of the floral resource. Each of these procedures is detailed below.

Camera trapping.- Floral visitor filming was carried out using camera traps (Model HC-801A). These were programmed to take 10-second videos and remained active continuously for 3 to 5 days. Two sampling events were conducted between March and July of 2023 and two more between March and July of 2024. Four agave plants were filmed (n = 1 in Tlaxcala, n = 3 in Hidalgo), yielding a total of 272.36 hours of footage. Because the filming duration of each individual varied, visitation rates were standardized by the total number of recording hours. We identified visitors through video based on size, morphology, and coloration patterns. For quantifying visits and determining visitor roles, only organisms that made direct contact with the flowers and their reproductive parts were analyzed. The number of visits and the time of activity were recorded. Each visit was considered complete once the organism left the camera's field of view (Trejo-Salazar *et al*. 2015). Although insects, mainly *Apis mellifera*, were frequently recorded, most visits did not involve contact with reproductive structures. Therefore, insect visitors were documented but not included in subsequent diversity or pollination analyses, which focused on vertebrates.

Visitators diversity.- To characterize the structure of the floral visitor assemblage, we quantified diversity, dominance, and evenness using the Shannon-Wiener, Simpson, and Pielou indices, respectively. These indices were calculated using the *dplyr* package for data handling. To obtain diversity measures expressed in units of "effective number of species," we additionally calculated Hill numbers of orders q = 0, 1, and 2. These correspond to species richness (Q0), the effective number of common species derived from Shannon entropy (Q1), and the effective number of dominant species derived from Simpson diversity (Q2). For comparative purposes, we also computed Hill numbers for the floral visitor communities reported by Trejo-Salazar *et al*. (2015), using the Shannon and Simpson values provided in their publication, allowing direct comparison of diversity patterns between our system and previously documented agave pollination assemblages.

To assess sampling completeness, we constructed rarefaction and extrapolation curves using the iNEXT package (Chao *et al*. 2014, 2016). All diversity and rarefaction curve analyses were performed in R.

Pollen collection from floral visitors.- To capture bird visitors, mist nets were set up from 17:00 to 19:00 h and from 6:30 to 10:00 h; for bat capture, nets were active from 19:00 to 00:00 h. We deployed nets for three consecutive days during one period in 2023 and one in 2024 per site (Hidalgo and Tlaxcala), totaling 8,748 net hours. Upon capturing a visitor, we passed a fuchsin-stained gelatin cube over its head, neck, and bill or snout, to which the pollen adhered (Beattie 1971). We later melted this cube onto a microscope slide and transported it to the lab, where we quantified the quantity of *A. salmiana* pollen under a microscope. The samples were compared with a reference collection made at the study site to determine whether the animals carried the target pollen.

Nectar production dynamics.- Due to the height of agave stalks ($\bar{x}$ = 5.7 m ± 1.1, n = 10), scaffolding was used to collect nectar samples. To detect nectar secretion peaks, samples were taken every 2 hours over a continuous 24-hour period from ten randomly selected flowers from five different individuals, totaling 50 sampled flowers. Each flower was bagged with an organza bag before anthesis to prevent nectar removal by animals. We extracted nectar with insulin syringes and estimated its concentration using a handheld refractometer (Model RZ120, ATC brand).

Results

The statistical analysis using the Kruskal-Wallis test showed no significant differences in the floral visitation rate among the studied sites (H_1 = 3.00; P = 0.392), thus confirming the statistical homogeneity among locations. This result allowed for the consideration of a single study unit: the Llanos de Apan.

Characterization of Agave salmiana *inflorescences*. The morphological analysis of *A. salmiana* inflorescences showed that the scapes have an average of 23.7 umbels (n = 7). Regarding the flowers, they remain open all day and night (flowers begin to bloom at 22:00 h; Estrella Ruiz 2005), are yellow, and have a strong odor. The averages of the measurements taken, along with their standard deviation, are presented in Table 1. As shown in the table, the length of the tepal ranges between 4 and 6 cm (Figure 2). Based on literature (Gonzalez-Terrazas *et al.* 2012), we can assume that nectar-feeding bats distributed in these sites, with snouts not as long as those of the genus *Glossophaga*, would likely struggle to feed successfully on this agave species, since their snouts can access nectar in flowers around 3 cm deep. However, other species such as those of the genus *Leptonycteris*, with longer snouts, are capable of doing so. Regarding the birds recorded in the sampled agaves, we found that the total beak length does not exceed 3.5 cm, with *Eugenes fulgens* (Swainson 1827), *Lampornis clemenciae* (Lesson 1830), and *Melanerpes formicivorus* (Swainson 1827) being the three species with the longest beaks among the recorded visitors (3.34, 2.84, and 2.69 cm respectively; Tobias *et al*. 2022).

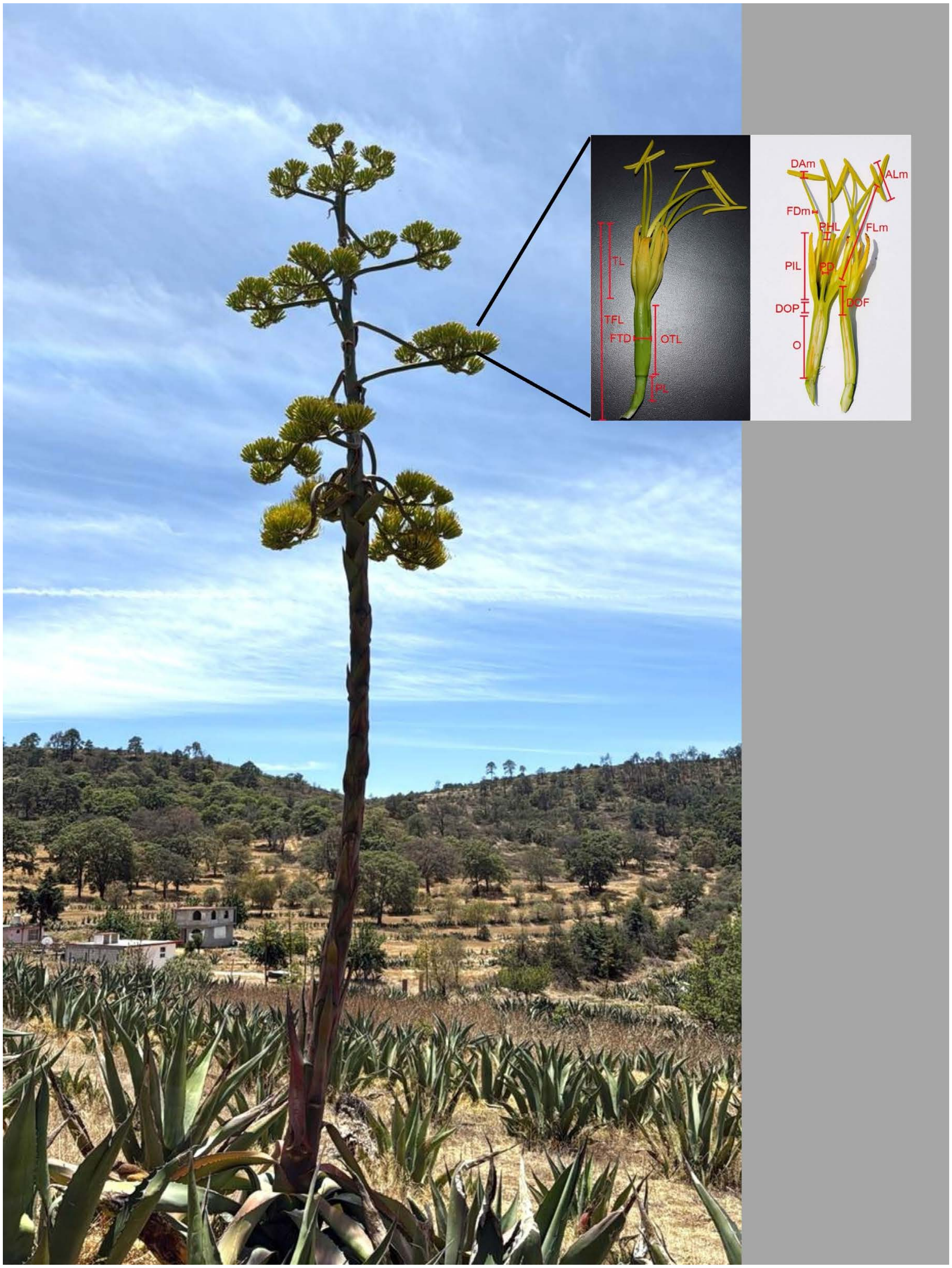

Figure 2. The scape or "quiote" of *Agave salmiana* is shown with the flower measures, taken and modify from Trejo *et al*. 2024: total flower length (TFL), Tepal length (TL), flower tube diameter (FTD), ovarian tube length (OTL), pedicel length (PL), anther diameter (DAm), anther length (ALm), filter diameter (FDm), filament length (FLm), pistil head length (PHL), pistil length (PIL), pistil diameter (PD), distance from the ovary to the pistil (DOP), distance from the ovary to the pistil (DOP), distance from the ovary to the filament (DOF), oval length (O).

Floral visitor diversity. The floral visitors identified in this study were the hummingbirds *Basilinna leucotis*, *Lampornis clemenciae*, *Eugenes fulgens*, *Selasphorus* sp.; Acorn woodpecker *Melanerpes formicivorus*; the orioles *Icterus wagleri*, *I. abeillei*, *I. parisorum*, *I. spurius*; and bats from the family Phyllostomidae, subfamily Glossophaginae (Figure 3). The standardized visitation rate for each recorded species is shown in Figure 3, where it can be seen that the hummingbirds *Eugenes fulgens* and *Lampornis clemenciae*, as well as bats from the subfamily Glossophaginae, had the highest visitation rates. Insects, mainly *Apis mellifera*, were also frequently recorded. This species accounted for ~99 % of insect visits and performed on average ~28.7 visits h^{-1}. However, video analyses showed that only ~2.5 visits h^{-1} involved contact with the reproductive structures, meaning that only 8.7 % of visits were potentially effective floral visits. In contrast, vertebrate visitors showed lower overall visitation rates but a consistently higher frequency of direct contact with anthers and stigmas. For these reasons, insects were considered ancillary floral visitors and were excluded from subsequent diversity and pollination analyses.

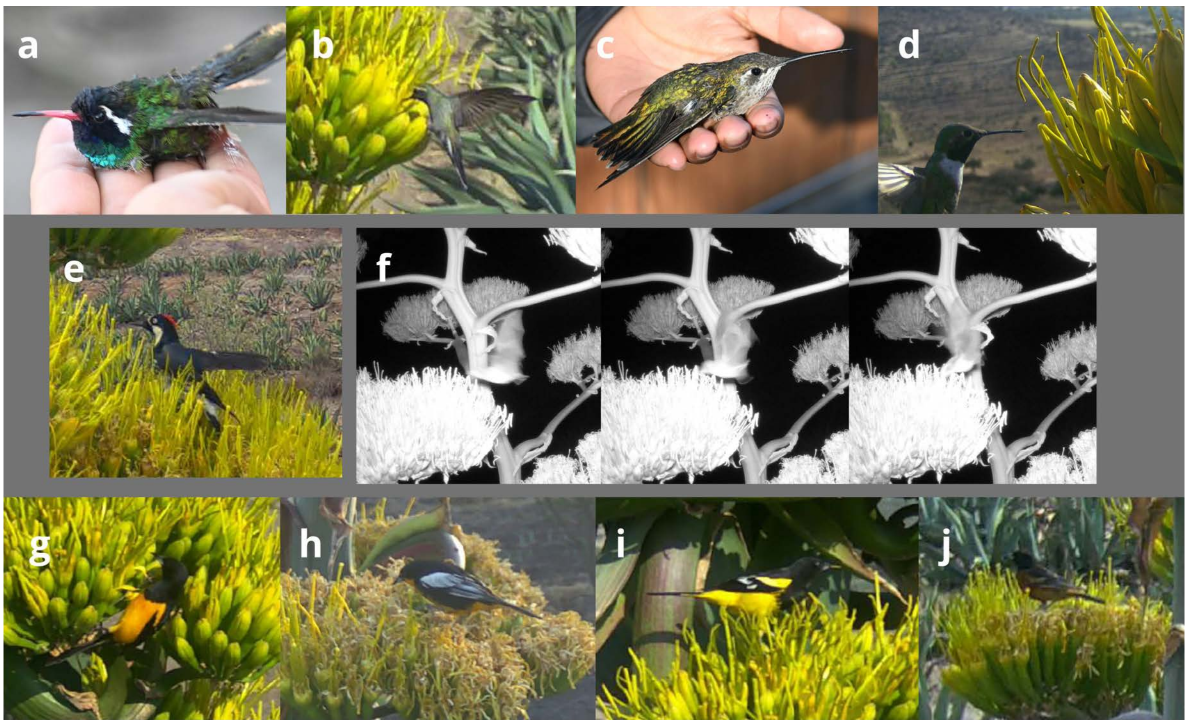

Figure 3. Floral visitors of *Agave salmiana,* in parenthesis the visitation rate: a) *Bassilina leucotis* (0.36), b) *Lampornis clemenciae* (1.09), c) *Eugenes fulgens* (3.23), d) *Selasphorus* sp. (0.02), e) *Melanerpes formicivorus* (0.05), f) bat from the subfamily Glossophaginae (2.43), g) *Icterus wagleri* (0.04), h) *I. abeillei* (0.02), i) *I. parisorum* (0.26), j) *I. spurius* (0.11).

Regarding the diversity indices, the Shannon-Wiener value of 1.50 reflects a community composed of several floral-visitor species with an uneven distribution of abundances. This is consistent with a marked dominance of a few taxa and the low contribution of the remaining species. The Simpson index of 0.72 similarly indicates that, although there is a moderate probability that two randomly selected individuals belong to different species, the community is still influenced by the disproportionate occurrence of a small subset of visitors. Finally, Pielou's evenness index of 0.65 corroborates this pattern by showing that species are not equally represented within the assemblage and that visit frequencies are concentrated in a limited number of taxa (see Figure 4).

Hill numbers provided a basis for comparing the effective diversity of floral visitors recorded in this study. In terms of richness (Q0), *A. salmiana* in the Llanos de Apan hosted 10 visitor species (Table 2).

The effective number of common species (Q1) revealed more nuanced patterns. *A. salmiana* showed a Q1 of 4.49. With respect to dominance (Q2), *A. salmiana* presented an effective number of dominant species of 3.62 (Table 2).

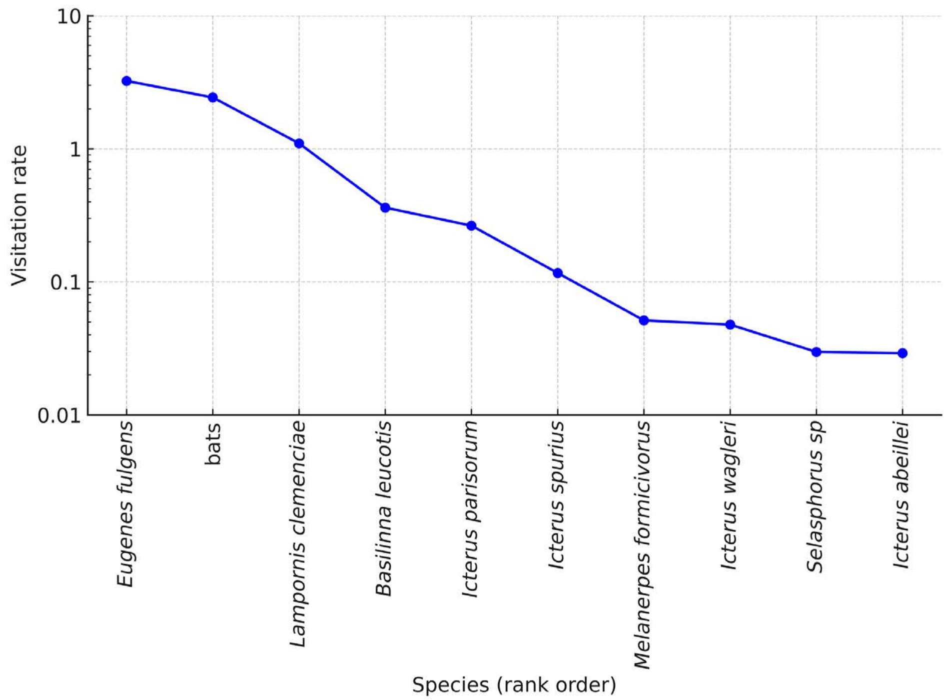

Figure 4. Rank-abundance curve of floral visitors recorded in cultivated agaves. The graph shows the abundance per species (ordered from highest to lowest abundance) on a logarithmic scale.

Table 2. Hill diversity values (Q0 - Q2) for wild agave species and cultivated *A. salmiana*.

	***A. difformis*, El Durazno (Trejo-Salazar et al. 2015)**	***A. difformis*, San Bernardo (Trejo-Salazar et al. 2015)**	***A. striata*, San Lucas (Trejo-Salazar et al. 2015)**	***A. striata*, San Bernardo (Trejo-Salazar et al. 2015)**	***A. garciae-mendozae*, San Bernardo (Trejo-Salazar et al. 2015)**	***A. salmiana*, Llanos de Apan, this study**
Q0	7	13	13	12	8	10
Q1	2.65	7.69	6.49	5.49	4.28	4.49
Q2	2.01	4.81	4.78	3.76	3.29	3.62

The rarefaction curve (Figure S1) reached an asymptote at three families, indicating that the sampling was sufficient to capture the diversity in this system. We conducted the rarefaction analysis at the family level because, although diurnal visitors were identified to species level, nocturnal ones could only be identified to the family level. When there is unequal taxonomic resolution among groups, it is recommended to standardize the analysis to the highest taxonomic level available -in this case, the family level- in order to ensure an objective comparison and avoid artificial bias in diversity estimates (Gotelli & Colwell 2001). In this regard, the taxonomic identification of nectar-feeding bats was based on detailed observations of video recordings, which confirmed their belonging to the family Phyllostomidae, specifically to the subfamily Glossophaginae, characterized by elongated snout, and presence of a nose leaf (Freeman 1995, Gonzalez-Terrazas *et al*. 2012).

Pollen load. All captured visitors carried pollen of *A. salmiana*, with the hummingbird *Eugenes fulgens* standing out, carrying between 1 and 151 pollen grains, followed by *Basilinna leucotis* with 45 grains, while *Icterus parisorum* and *Icterus wagleri* carried smaller loads (11 and 1 grains, respectively; Table 3).

Table 3. Species from which pollen load samples were taken, along with the corresponding quantity.

Bird species	Number of pollen grains
Icterus wagleri (n = 1)	1
Icterus parisorum (n = 1)	11
Basilinna leucotis (n = 1)	45
Eugenes fulgens (n = 10)	1-151

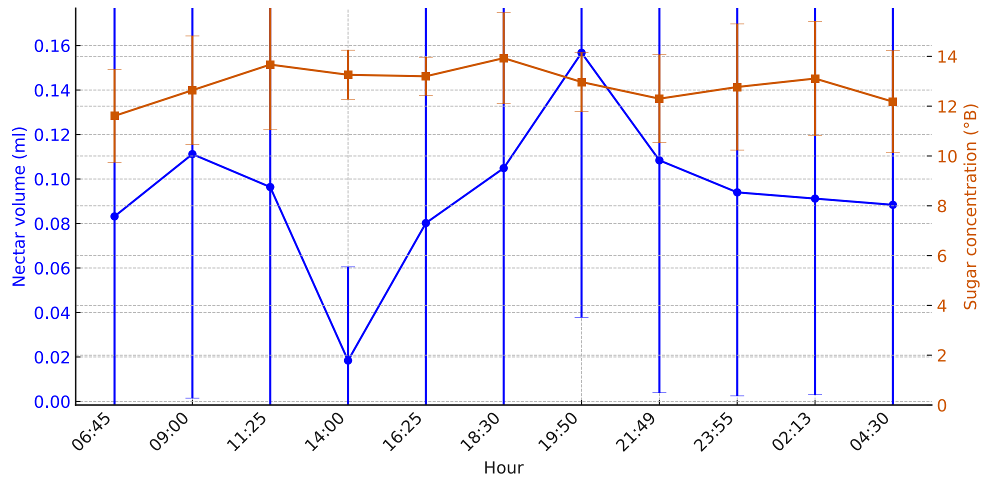

Figure 5. Variation in nectar quantity (blue, ml) and concentration (orange, °B) of *Agave salmiana* over 24 hours. Lines indicate the mean and bars represent the standard error.

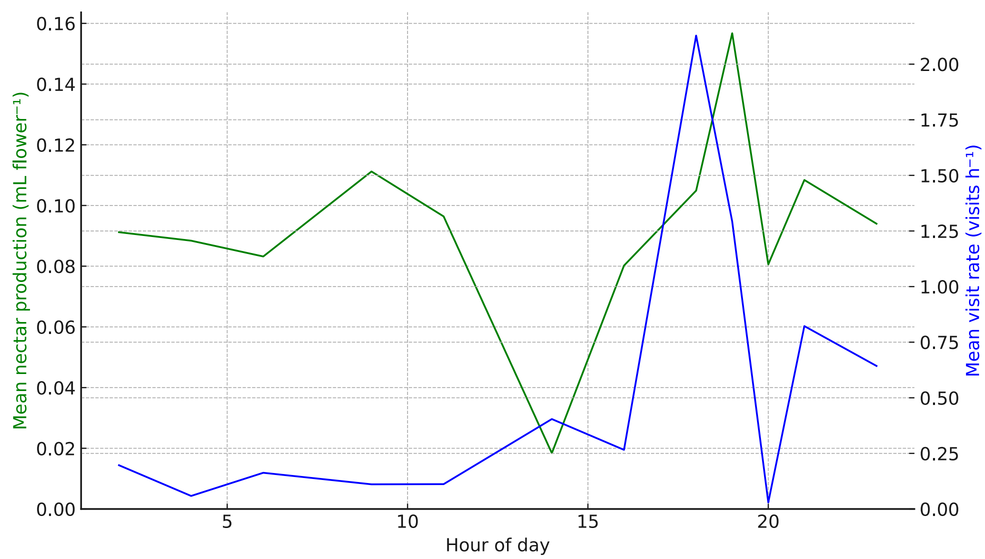

Figure 6. Relationship between visitation rate (blue line) and nectar quantity (green line) in *Agave salmiana.*

Nectar production dynamics. Nectar production in *A. salmiana* reached its minimum volume at 14:00 h and peaked at 19:50 h, with a total accumulated volume of 1.03 ml over 24 hours, showing a clear evening and nighttime increase in resource availability. On the other hand, the average sugar concentration in nectar was 12.8 °B ± 1.8 S.E., with slight variations observed in concentration -higher values at certain times- but not as pronounced as the changes in nectar quantity (Figure 5).

When relating nectar quantity to visitation rate (Figure 6), we found that nectar production in *A. salmiana* decreased toward midday (14:00 h; 0.018 mL flower^{-1}) and then continuously increase, reaching its peak at 19:00 h (0.157 mL flower^{-1}). Visitor activity was low during the early morning hours (2 - 6 h; ≤ 0.20 visits h^{-1}), increased sharply at dusk with a marked peak at 18:00 h (2.13 visits h^{-1}), and showed a second, more moderate peak at 21:00 h (0.82 visits h^{-1}). A Spearman correlation test showed that the relationship between nectar and visits was positive but not significant (P = 0.36; P = 0.25).

Discussion

Characterization of Agave salmiana inflorescences. Bats with long snouts can extract nectar from deeper parts than species with short snouts (Gonzalez-Terrazas *et al*. 2012). Regarding the birds recorded on agaves as floral visitors, although we found that their total bill length does not exceed 3.5 cm (Tobias *et al.* 2022) -a length shorter than the tepal length- birds such as hummingbirds and woodpeckers have tongues that can extend beyond the edge of the beak, which may allow them to reach targets (nectar or arthropods) that would be impossible to access with the beak alone (Erdoğan & Iwasaki 2013). *A. salmiana* has flowers where the tepals measure between 4 and 6 cm, which reinforces the findings of the other approaches used to identify the pollinators of *A. salmiana* in this study, showing that vertebrates are the main pollinators, rather than small animals such as insects.

Diversity of floral visitors. The diversity patterns observed in the floral-visitor assemblage of *Agave salmiana* reveal a community in which several species participate, but their contributions are uneven. The combined evidence from Shannon, Simpson, Pielou's evenness, and Hill numbers of points to a structurally simple assemblage dominated by a small group of highly active visitors.

The Hill number framework provides particularly meaningful insight into this structure. Although *A. salmiana* hosted 10 visitor species (Q0), an intermediate value relative to the inflorescences analyzed by Trejo-Salazar *et al*. (2015), which ranged from 7 to 13 species. Thus, the richness observed for *A. salmiana* exceeded that reported for *A. garciae-mendozae* (8 species) but remained below the levels documented for *A. difformis* and *A. striata* (12–13 species). The effective diversity metrics reveal that only a subset of these species contributes substantially to overall visitation. The Q1 value (4.49) suggests that the community behaves as though only four or five species are "common". While *A. difformis* (San Bernardo) and *A. striata* (San Lucas) exhibited relatively high values (7.69 and 6.49, respectively), indicative of more equitable and diverse communities, *A. salmiana* showed a Q1 of 4.49. This value falls within the midrange of those reported by Trejo-Salazar *et al*., (2015) comparable to *A. striata* from San Bernardo (5.49) and slightly higher than *A. garciae-mendozae* (4.28). While the Q2 value (3.62) indicates that an even smaller group functions as the dominant core of the assemblage, again an intermediate level relative to the wild agaves examined by Trejo-Salazar *et al*. (2015). This degree of dominance was slightly higher than that of *A. garciae-mendozae* (3.29) but lower than that recorded for *A. difformis* (4.81) and *A. striata* (4.78).

Comparisons with the wild agave species studied by Trejo-Salazar *et al*. (2015) further contextualize these trends. The effective number of common and dominant species in *A. salmiana* resembles the intermediate values documented for *A. garciae-mendozae*, *A. difformis*, and *A. striata*-agaves whose inflorescences also support communities structured around a limited number of frequent visitors. Thus, even in an agricultural landscape, *A. salmiana* does not exhibit a depauperate or homogenized visitor community. Instead, its assemblage mirrors patterns found in natural environments, where nectar-feeding bats and a few diurnal taxa consistently account for most floral interactions.

When comparing the bird species recorded in this work that frequently visit the Pulque agave with other studies conducted in natural and urban areas (Martínez Del Río & Eguiarte 1986, Gómez-Aíza & Zuria 2010), it was found that the same bird families (Icteridae and Trochilidae), and specifically the hummingbird *E. fulgens*, show a high visitation rate to this agave, highlighting their importance for the sexual reproduction of the species. This consistency occurs despite differences in geographic areas and management conditions. Regarding the role of bats, due to their high visitation rate, the greater amount of nocturnal nectar secretion, and the way these flying mammals feed -frequently contacting the reproductive parts of the flower (see Figure S2)- they should not be ruled out as potential pollinators of this agave. The limited information on this group in relation to the Pulque agave indicates that bats were just as efficient as birds in pollinating the plant (Estrella Ruiz 2005).

As for non-vertebrate visitors, our recordings and direct observations indicate that insects -primarily *Apis mellifera*- behave predominantly as nectar thieves, as previously suggested by Estrella Ruiz (2005). The floral morphology of *A. salmiana* facilitates this behavior: the elongated floral tube and the position of nectar at its base allow insects to enter, move along the corolla, and extract nectar with minimal or no contact with reproductive structures. This is consistent with the traits of flowers prone to nectar robbery (Polatto & Alves-Junior 2022). Quantitatively, we found that only a small fraction of *A. mellifera* visits (~8.7 %) resulted in contact with anthers or stigmas, indicating that insects may contribute marginally to pollen transfer. However, this contribution is likely limited relative to vertebrates, which consistently contacted reproductive structures and carried substantially higher pollen loads.

Nectar theft may also influence the pollination process indirectly by reducing nectar availability for effective vertebrate pollinators and modifying their foraging behavior (Zhang *et al*. 2014, Polatto & Alves-Junior 2022). These dynamics may be particularly relevant in cultivated systems where only a small proportion of plants are allowed to flower. Taken together, these observations suggest that insects play a secondary and context-dependent role in the pollination of *A. salmiana*: although they are frequent visitors and occasionally contact reproductive structures, their predominant behavior as nectar thieves and their comparatively low pollination efficiency indicate that vertebrates remain the principal pollinators in this system. Further work is needed to determine whether insect visitors might increase their functional importance under different management or environmental conditions.

Pollen load. Following Avalos *et al.* (2011), of the four species from which pollen samples were obtained, three -*Icterus parisorum*, *Basilinna leucotis*, and *Eugenes fulgens*- carried a high pollen load, suggesting a greater likelihood that they act as pollinators of the Pulque agave.

To our knowledge, there are no studies that have estimated pollen load in birds of the genus *Icterus*, although these birds have been considered pollinators of this and other agave species (Martínez Del Río & Eguiarte 1987, Ornelas *et al*. 2002). Due to the limited knowledge in this area regarding orioles, we recommend future studies to deepen this information by combining visitation rates and pollen loads to determine whether *Icterus wagleri* is a possible pollinator of *A. salmiana*.

Nectar production dynamics. There is little information on the nectar production dynamics of *A. salmiana*. In wild conditions, the average amount of nectar was greater than what was found in this study (2.8 ml Estrella Ruiz 2005 *vs*. 1 ml in this study), as well as the concentration (15.1 °B Estrella Ruiz 2005 *vs*. 12.8 °B in this study), suggesting that nectar production dynamics may differ under different management conditions such as agriculture. This is an underexplored topic, but Pamminger *et al.* (2019), when comparing nectar concentrations in cultivated and wildflowers, observed that concentrations did not differ between communities, though they found greater variability in nectar quality in uncultivated plants. Studying whether nectar production dynamics differ under various plant management conditions could lead to new proposals in the field of crop production and conservation, as well as better understanding the impacts of different types of plant management.

The highest nectar production in *A. salmiana* occurred at night, a pattern consistent with other agave species (Rocha *et al.* 2005). Nectar secretion occurs in specific rhythms -its temporal patterns of secretion and cessation define nectar production dynamics. This parameter is usually linked to the foraging behavior of floral visitors (Pacini & Nepi 2007). The approximate one-hour lag between the peak in visits (18:00 h) and the peak in nectar production (19:00 h) suggests that pollinators respond to resource availability, but their activity is also modulated by twilight and the emergence of nectar-feeding bats.

Pollinators of Agave salmiana. *Agave salmiana* exhibits a generalist pattern regarding its vertebrate pollinators; its prolonged anthesis and nectar production dynamics do not appear to be specialized exclusively for diurnal or nocturnal visitors. The high pollen load observed and the high visitation rate recorded indicate that the hummingbird *Eugenes fulgens* plays a significant role as a potential pollinator. Similarly, nectar-feeding bats, due to their high visitation rate and frequent contact with the reproductive parts of the flowers, also emerge as primary pollinators of this agave.

The diversity and abundance of floral visitors we recorded was relatively high, considering that less than 5 % of agave individuals in the crop are allowed to flower. These findings suggest that even limited flowering represents an important resource for local fauna. Nevertheless, the moderate values of the diversity indices indicate that the community is structured around a few dominant species, reinforcing the ecological relevance of maintaining flowering individuals within agricultural systems to sustain both dominant and less frequent visitors.

Finally, since some of our results suggest that cultivated agaves differ from wild agaves in traits such as nectar production, it would be important to conduct studies in intensive crops, as the crop where sampling was conducted did not reflect high-intensity management. Such studies could provide valuable information for developing more targeted agricultural management strategies that integrate both productivity and biodiversity conservation.

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Supplementary material

Supplemental data for this article can be accessed here: https://doi.org/10.17129/botsci.3807

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