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EFECTIVIDAD DE POLINIZADORES EN ESPECIES DEL GÉNERO *SALVIA* (LAMIACEAE) CON SÍNDROMES FLORALES CONTRASTANTES

TESIS

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CONTENIDO

RESUMEN

Premisa del estudio: La adaptación floral al polinizador más efectivo no necesariamente impide las visitas de diferentes gremios de visitantes florales que pueden contribuir a la reproducción de las plantas. *Salvia* comprende alrededor de 1000 especies, en su mayoría exclusivamente asociadas con la polinización por abejas y colibríes, sin embargo, el desempeño de estos gremios rara vez ha sido analizado. En este trabajo, evaluamos el desempeño de distintos visitantes florales como polinizadores para determinar la validez de los síndromes florales en *Salvia*.

Métodos: Utilizando cinco especies representativas de la polinización por abejas, colibríes y polinización mixta, realizamos una serie de experimentos manipulativos para determinar si las especies de plantas dependen de los polinizadores, y para cuantificar la contribución de sus visitantes florales más frecuentes a la producción de frutos y semillas. Evaluamos a cada polinizador de acuerdo a su eficiencia y efectividad, y utilizamos análisis de escalamiento multidimensional no métrico para explorar la relación entre la morfología floral y el desempeño de los polinizadores.

Resultados clave: Encontramos que todas las especies de plantas dependen de los polinizadores para su reproducción. A pesar de ser visitadas por múltiples grupos funcionales, abejas, colibríes, y ambos grupos, fueron los polinizadores más efectivos de las especies melitófilas, ornitófilas, y la especie con características mixtas, respectivamente. No obstante, polinizadores que no coincidían con el síndrome floral fueron encontrados en todas las especiesde plantas, siendo en ocasiones más eficientes que aquellos que sí coincidieron con el síndrome. Los NMDS agruparon a cada especie de *Salvia* de acuerdo al síndrome floral propuesto en la literatura.

Conclusiones: En *Salvia*, los síndromes florales predicen adecuadamente los polinizadores efectivos primarios, sin embargo, polinizadores efectivos secundarios pueden tener un papel importante en la reproducción de las plantas, especialmente en especies con sistemas de polinización mixta. Nuestros resultados resaltan la importancia de considerar la producción de frutos y semillas como estimadores de la adecuación de las plantas para evaluar el desempeño de los visitantes florales e identificar a los polinizadores efectivos.

Palabras clave: Polinización, síndromes de polinización, eficiencia de polinizadores, efectividad de polinizadores, polinizadores primarios, polinizadores secundarios.

ABSTRACT

Premise of the study: Floral adaptation to the most effective pollinator may not necessarily preclude visitation by different visitor guilds that could contribute to plant reproduction. *Salvia* comprises ca. 1000 species almost exclusively associated with bee and hummingbird pollination, nevertheless, the performance of these visitors has rarely been analyzed. Here, we evaluated pollination performance of several floral visitors to assess the reliability of floral syndromes in *Salvia*.

Methods: Using five representative species corresponding to bee, hummingbird and mixed pollination syndrome, we conducted a series of manipulative experiments to determine whether plant species are pollinator dependent, and to quantify the contribution of frequent floral visitors to fruit and seed production. We scored pollinators according to their efficiency and effectiveness, and used non-metric multidimensional scaling (NMDS) to explore the relationship between floral morphology and pollinator performance.

Key results: We found that all plant species depend on pollinators for reproduction. Despite being visited by multiple guilds, bees, hummingbirds, and both functional groups were the most effective pollinators of melittophilous, ornitophilous and mixed species, respectively. However, pollinators not matching the floral syndrome were found in all plant species, and sometimes were more efficient than those matching. NMDS grouped each *Salvia* species according to floral syndromes proposed in the literature.

Conclusions: Floral syndromes can accurately predict the primary pollinators in *Salvia*, however, secondary pollinators may play a crucial role in plant reproduction, especially for mixed pollinated species. Our study highlights the importance of considering plant fitness surrogates such as fruit and seed production to assess floral visitor's performance and identify effective pollinators.

Key words: Pollination, pollinaiton syndromes, pollinator efficiency, pollinator effectiveness, primary pollinators, secondary pollinators.

Floral syndromes and pollinator performance in five Neotropical *Salvia* species: comparing melittophilous, ornitophilous and mixed phenotypes

Arturo Tavera et al.

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INTRODUCTION

Biotic pollination is a key process for sexual reproduction in approximately 90% of angiosperm species (Ollerton et al., 2011; Stephens et al., 2023; Tong et al., 2023), and has been proposed as the main driver of floral diversification (Crepet, 1984; Bronstein et al., 2006; Kay & Sargent, 2009; Armbruster, 2014; Van der Niet et al., 2014). Thus, floral phenotypes should reflect adptation to its more effective pollinators (Stebbins, 1970). Pollination or floral syndromes represent specific combinations of floral attributes associated with pollination by particular animal groups (Faegri & Van der Pijl, 1979; Fenster et al., 2004). Floral syndromes have been widely used to infer the pollinator group in the absence of empirical evidence (Wilson et al., 2017; Dellinger, 2020). Furthermore, floral syndromes have been used to explain trait convergence among phylogenetically distant angiosperm taxa (Rosas-Guerrero et al., 2014; Ashworth et al., 2015; Dellinger, 2020).

Despite its ubiquity in pollination ecology, the floral syndrome concept has been questioned due to the apparent generalization of pollination systems (i. e., plant species are visited by multiple animal groups regardless of their floral phenotypes; Robertson, 1928; Herrera, 1996; Waser et al., 1996; Kingston & McQuillan, 2000; Ollerton et al., 2009; Wang et al., 2020). However, animals may differ in their ability to effectively transport pollen and contribute to plant reproductive success (Armbruster et al., 2000; Fenster et al., 2004; Rosas-Guerrero et al., 2014). Therefore, to differentiate between effective pollinators and floral visitors is a central task in plant-pollinator studies, which may help to determine whether plant species have generalized or specialized pollination systems (Rosas-Guerrero et al., 2014).

A common shortcoming of many pollination studies is to indiscriminately use the term 'pollinator', assuming that all floral visitors contribute to plant reproduction (de Santiago et al., 2019; Dellinger, 2020). Different studies have demonstrated that using surrogates of plant fitness considerably reduces the number of interacting partners for a plant species, leaving only those who actively contribute to pollen deposition, fruit, or seed production (e. g., Lopezaraiza et al., 2007; Benevides et al., 2013; King et al., 2013; Popic et al., 2013; Rosas-Guerrero et al., 2014; Ashworth et al., 2015; de Santiago et al., 2019). Regardless of the surrogate considered, some authors have proposed the term 'efficiency', as the per-visit contribution of a floral visitor; and 'effectiveness', as the combined effect of efficiency and visitation frequency to measure pollinator performance (Ne'eman et al, 2010; Willmer, 2011; Freitas, 2013; Armbruster, 2014). Assessing pollinator efficiency and effectiveness is crucial to determine which floral visitors are acting as potential selective agents and hence molding floral phenotypes (Rosas-Guerrero et al., 2014).

With a global distribution, *Salvia* (Lamiaceae) comprises ca. 1000 species (Kriebel et al., 2019; Moein et al., 2023), and due to its unique lever-like staminal pollen transfer mechanism and impressive phenotypic diversity, the genus has served as a model for evolutionary biologists and pollination ecologists (e. g., Claßen-Bockhoff et al., 2004; Walker & Sytsma, 2007; Fragoso-Martínez et al., 2018; Benítez Vieyra et al., 2014 and 2019; Drew et al., 2017; Kriebel et al., 2019 and 2020; Drew, 2020; Wester et al., 2020; Sazatornil et al., 2023). As in other plant clades (e. g., *Penstemon*: Wilson et al., 2004; Gesneriaceae: Martén-Rodríguez et al., 2009 and Serrano-Serrano et al., 2017; Ericaceae: Johnson, 2013; *Calceolaria*: Murúa & Espíndola, 2015; Balsaminaceae: Abrahamczyk et al., 2017), some authors have explored the relationship between different floral attributes and particular pollinator guilds in *Salvia* through different phylogenetic comparative approaches. The results of those studies have found strong support for pollinator-mediated floral evolution, where bees and hummingbirds (especially in the New World clades) act as the main pollinators for the genus (Fragoso-Martínez et al., 2018; Benítez Vieyra et al., 2014 and 2019; Kriebel et al., 2019 and 2020; Sazatornil et al., 2023).

Nearly half of *Salvia* species are placed within *Calosphace*, a subgenus restricted to America that comprises ca. 600 spp. (González-Gallegos et al., 2020). According to the most comprehensive floral syndrome classification for the clade (which is based on floral morphology and field observations for some plant species), 58% of the species are classified as melittophilous, 31% as ornitophilous and 11% have mixed or polymorphic phenotypes between two or more syndromes (Wester & Claßen-Bockhoff, 2011). Melittophilous species exhibit blue-purplish flowers with short corolla tubes, wide lower lips, and an active lever mechanism, while ornitophilous species usually exhibit reddish flowers with longer corolla tubes, narrower lower lips, and an inactive lever mechanism in some species (Wester & Claßen-Bockhoff, 2007 and 2011). Species with mixed or polymorphic phenotypes exhibit floral trait combinations not assignable to a single pollination syndrome (Wester & Claßen-Bockhoff, 2011).

Floral visitation has been well documented in *Salvia*, where bees and hummingbirds appear to be the main visitors for melittophilous and ornitophilous species, respectively (e. g., Visco & Capon, 1970; Dieringer et al., 1991; Arizmendi et al., 2007; Celep et al., 2014 and 2020; Wester & Claßen-Bockhoff, 2011). Nevertheless, there is growing evidence of frequent floral visitation by different functional groups, such as butterflies (Tavera et al., 2023), flies (Celep et al., 2014 and 2020; Saravia-Nava et al., 2023), hawkmoths (Xiao et al., 2023b), and hummingbirds (Cairampoma & Martel, 2012; Espino-Espino et al., 2014; Barrionuevo et al., 2021) in melittophilous species, and to a lesser extent, bees (Arizmendi et al., 2007; Espino-Espino et al., 2014; Saravia-Nava et al., 2023) and butterflies (Grases & Ramírez, 1998) in ornitophilous species.

There are still very few studies that have quantitatively assessed pollinator performance under natural conditions to test the reliability of floral syndromes in *Salvia*. For instance, Cairampoma et al. (2020) analyzed the pollination ability of *Caupolicana* cf. *piurensis* (Colletidae) and *Thaumastura cora* (Trochilidae), the two main floral visitors of *S. rhombifolia*, a melittophilous sage of Peru. Using single-visit experiments, the authors found that *C.* cf. *piurensis* and *T. cora* contributed similarly to plant's reproductive success, concluding that both pollinator species were equally efficient (Cairampoma et al., 2020). In another study on *S. daiguii*, a bee-pollinated species endemic to Hunan Province in China, Xiao et al. (2023b) quantified the pollen grains that *Apis cerana* (Apidae) and *Macroglossum bombylans* (Sphingidae) deposited on the stigma of *S. daiguii* flowers after a single visit, to determine whether both floral visitors were effective pollinators. The authors found that despite being less efficient than *A. cerana*, *M. bombylans* contributed to pollen deposition and was a secondary pollinator. Similarly, Saravia-Nava et al. (2023) determined the contribution of different pollinator guilds to pollen deposition and pollen grains germination in four Andean species of *Salvia*, finding evidence of bees acting as primary and secondary pollinators in ornitophilous species, and syrphid flies (Syrphidae) acting as primary pollinators in melittophilous species.

As previously mentioned, mounting evidence suggests that even plant clades strongly associated with particular pollinator-mediated diversification as the genus *Salvia* interact with diverse floral visitor assemblages, however, studies analyzing pollination success are still lacking. Detailed observations that consider ultimate pollination proxies such as fruit and seed production may render a more insightful view of the role of particular pollinator guilds in plant sexual reproduction (de Santiago-Hernández et al., 2019). Here, we combine a set of manipulative pollination experiments with multivariate analysis of floral morphological traits and pollinator performance on five phenotypically contrasting species of *Salvia* subgen. *Calosphace* with melittophilous (two species), ornitophilous (two species) and mixed floral syndromes (one species) to answer the following questions: i) Do plant species depend on pollinators for reproductive success? ii) Do effective pollinators match with those expected by the floral syndrome? iii) Do morphological floral traits allow grouping *Salvia* species according to the floral syndromes proposed in literature? Accordingly, we formulated the following predictions: i) we expect *Salvia* species to be highly dependent on pollinators for fruit and seed production; ii) we expect to find a strong association between floral syndromes and the most effective floral visitors, with bees as the main pollinators for melittophilous species, hummingbirds for ornitophilous species, and more than one functional group for the mixed/polymorphic species; iii) we expect to observe a clear separation between melittophilous and ornitophilous species in the multivariate ordination space, with the mixed pollinated species placed between the melittophilous and ornitophilous clusters.

MATERIALS AND METHODS

Study site

Fieldwork was conducted in three sites located south of the city of Morelia, Michoacán, Mexico (Fig.1) during the flowering seasons (July – March) of 2022-2023 and 2023-2024.

The three sites were located within the Trans-Mexican Volcanic Belt, between 2130 and 2700 m.a.s.l., with mixed pine-oak forest vegetation (Franch-Pardo & Cancer-Pomar, 2017). Average, minimum and maximum distance between sites was 2.18 km, 3.25 km and 0.93 km, respectively.

Study species

We selected five species from *Calosphace*, two of them with floral traits corresponding to bee-pollination (*Salvia assurgens*, *S. lavanduloides*), two species corresponding to bird-pollination (*S. elegans, S. iodantha*), and one species with a mixed/intermediate phenotype (*S. mexicana* var. *minor*) according to the proposal of Wester & Claßen-Bockhoff (2011; Fig. 2A). All selected species have diurnal anthesis and produce up to four seeds per fruit.

Salvia assurgens displays white flowers and is visited by small, medium and large bees (Cultid-Medina et al., 2021), bee-flies and butterflies (Tavera et al., 2023; Fig. 2A); *S. lavanduloides* displays blue-violet flowers and is visited by small, medium, and large bees (Dieringer et al., 1991; Fig. 2A). The staminal lever mechanism is active in both melittophilous species.

S. elegans displays red tubular flowers and is visited by several species of hummingbirds, and medium and large bees (Lara, 2006; Cuevas et al., 2018; López-Segoviano et al., 2021; Fig. 2A). *S. iodantha* displays intense-pink tubular flowers and is visited by several species of hummingbirds (Arizmendi, 1994; Lara y Ornelas, 2001; Lopez-Segoviano et al., 2021; Fig. 2A). Both ornitophilous species have exserted stamens and stigma, and lack the staminal lever mechanism.

Finally, *S. mexicana* var. *minor* displays bluish-purple flowers and is visited by several hummingbird species (Arizmendi et al., 1996 and 2007; Strelin et al., 2017; López-Segoviano et al., 2021), large bees (Dieringer et al., 1991; Arizmendi et al., 2007), and diurnal butterflies (Tavera et al., 2023; Fig. 2A). The staminal lever mechanism is active in this species.

Pollinator dependence

To determine whether *Salvia* species depend on pollinators to set fruits and seeds, one inflorescence (with varying number of flowers) per plant from 30 plants per species were used for the following two treatments: (1) *Autonomous self-pollination* – open flowers were removed from each inflorescence leaving only floral buds, then were covered with fine mesh bags and left untreated. For all treatments, floral life span was monitored and approximately three weeks after flower abscission fruit and seed number was quantified. (2) *Open pollination* – inflorescences were tagged and left untreated, then fruits were collected and seeds were quantified. Fruits were only counted if they contained at least one viable seed, and the final seed number included only viable seeds. Nonviable seeds were smaller and had a wrinkled surface.

To determine differences in fruit production between treatments, a generalized linear model (GLM) using a binomial distribution and a logit link function was performed with plant species as independent variable and fruit production as dependent variable. For seed production, a GLM with a Poisson distribution and a log link function was performed with plant species as independent variable and seed number as dependent variable for seed production. All the analyses were performed using the *glm* function built into R version 4.3.0 (R Core Team, 2023).

Floral visitors, pollinators, and floral syndrome matching

Floral visitors

To describe the assemblage of floral visitors, focal observations and videorecordings were performed using 10–50 flowers (depending on plant species) between 0800 and 1500 h (the peak floral visitation period based on previous observations). Observations were conducted two times per week during the entire flowering season of each *Salvia* species in the two years of study. We only recorded the frequency of legitimate floral visitors (i.e., those that contacted anthers and/or stigma), excluding nectar robbers and pollen thieves. Floral visitors were grouped into functional groups (*sensu* Fenster et al., 2004). Because the lenght of flowering season varied among studied species, total observation time was different for each plant species. The total observation time and number of observed flowers per species were 51.25h and 879 flowers, for *Salvia assurgens*, 40.75h and 1787 flowers for *S. lavanduloides*, 46.5h and 2843 flowers for *S. mexicana* var. *minor*, 47h and 1222 flowers for *S. elegans*, and 50h and 3052 flowers for *S. iodantha*.

Pollinator efficiency and effectiveness

To differentiate effective pollinators from non-effective visitors, the fruit and seed set resulting from single visits to virgin flowers in each *Salvia* species were quantified. Using several inflorescences per plant species, we removed all the open flowers leaving only floral buds. These inflorescences were tagged and covered with fine mesh bags until flower anthesis. Given that incomplete protandry has been reported for some *Salvia* species (e. g., *S. verbenaca*, Navarro, 1997; *S. elegans*, Rosas-Guerrero et al., 2017; *S. stachydifolia*, Barrionuevo et al., 2021), only 2- or 3-day-old virgin flowers were exposed to floral visitors to ensure a high stigmatic receptivity. The observer stood between 1 and 3m away from the inflorescence, and after a single visit to a single virgin flower, the flower was marked and the floral visitor was identified. Once all or most of the virgin flowers were visited, the inflorescence was bagged again to prevent any further pollination. Approximately three weeks later, the flowers were collected to quantify fruit and seed number. Non visited inflorescences were discarded. Due to differences in visitation frequency, sample size per floral visitor species differed for this treatment. In the two years of study, the single visit experiments were conducted two times per week, throughout the whole flowering season of the five *Salvia* species.

We employed a per visit basis to assess pollinator efficiency, and considered the joint action of both pollinator efficiency and visitation frequency to assess pollinator effectiveness (Ne'eman et al., 2010; Willmer, 2011; Freitas, 2013; Armbruster, 2014). We used three proxies of pollination efficiency for every floral visitor recorded in our single visit experiments: (1) *Floral efficiency* – measured as the mean number of floral visits per hour; (2) *Fruit efficiency* – measured as the mean number of fruits produced per visit; and (3) *Seed efficiency* – measured as the mean seed number produced per visit. To estimate pollination effectiveness, we used two proxies for each floral visitor recorded in our single visit experiments: 1) *Fruit effectiveness*, measured as the product of the mean number of floral visits per hour and the mean number of fruits produced per visit ; 2) *Seed effectiveness*, measured as the product of the mean number of floral visits per hour and the mean seed number produced per visit. To compare pollination effectiveness among functional groups, we pooled the single visit experiments raw data according to our classification of pollinator functional groups and calculated a standardized pollinator effectiveness index (PEI), by dividing the *seed effectiveness* value of each functional group by the sum of the *seed effectiveness* value of all pollinator functional groups (modified from Martén-Rodríguez et al., 2009). Finally, we calculated visitation rates for each floral visitor species as the mean number of visits per hour and per flower.

Floral morphology and pollinator effectiveness

We used three flowers per plant in 30 individuals (n = 90 flowers per species, except for *S. assurgens*, n = 75 flowers due to population size) to obtain measurements of 11 morphological floral traits, which included corolla length, floral tube length, upper lip length, lower lip length, floral tube width, upper lip-lower lip distance, anthers exsertion, stigma exsertion, upper lip width, floral tube aperture, and lower lip width (Fig. 2B). These floral traits have been suggested to play a relevant role allowing or restricting legitimate floral visitation by bees and hummingbirds (Wester & Claßen-Bockhoff, 2011; Strelin et al., 2017; Benítez-Vieyra et al., 2019; Wester et al., 2020). Fresh flowers were photographed (laterally and frontally) against 1mm graph paper and measured using the ImageJ version 1.54g (Schneider et al., 2012).

We performed two ordination analyses to determine whether floral morphology could group *Salvia* species according to floral syndromes and accurately infer their most effective pollinators. First, we conducted a non-metric multidimensional scaling (NMDS) based on euclidean distances using the log-transformed data of the 11 floral traits measured to test whether *Salvia* species group according to our a priori pollination syndrome classification (that of Wester & Claßen-Bockhoff, 2011). Second, to explore if floral morphology was related to the most effective pollinators, we performed another NMDS using the log-transformed data of the 11 floral traits along with the pollinator effectiveness index (PEI) of each functional group for each plant species, plotting the values of PEI as vectors to the ordination plot. We assessed the significance of the observed clustering patterns by means of analysis of similarities (ANOSIM) with 9999 permutations. Both NMDS ordinations and ANOSIM tests were performed using the functions *metaMDS* and *anosim* from the "Vegan" package in R (Oksanen et al., 2013).

RESULTS

Pollinator dependence

Reproductive success was much higher under open pollination than under autonomous self-pollination in the five *Salvia* species (Table 1). Among plant species, the percentage of fruit and seed production resulting from autonomous self-pollination was < 4.5% and < 2.2% among plant species, respectively (Fig. 3A). The seed production percentages were calculated considering the number of seeds produced out of the total possible, multiplying the total number of flowers counted by four, which is the number of ovules per flower in all *Salvia* species.On the other hand, the percentage of fruit and seed production resulting from open pollination ranged from 27.5% to 65.07% and from 11.16% to 39.38%, respectively (Fig. 3B). We found significant differences between the two treatments for both fruit and seed production in all *Salvia* species ($P < 0.001$ in all comparisons; Table 2).

Floral visitors

In total, we conducted 237.5 hours of observations and documented 12,435 floral visits by 72 visitor taxa, including 28 bee (Hymenoptera), three hummingbird (Apodiformes), 25 butterfly (Lepidoptera), eight fly (Diptera), three beetle (Coleoptera), two wasp (Hymenoptera), one bug (Hemiptera), one thrips (Thysanoptera), and one spider (Araneae) species (Table S1). The number of floral visitor guilds per species ranged from four to six, and bees, butterflies and flies were present in the floral visitor assemblages af all the plant species (Fig. 4A). During the two flowering seasons studied, the most frequent visitor guilds were bees for melittophilous species (>60% of visits in *S. assurgens* and >80% in *S. lavanduloides*), hummingbirds for ornitophilous species (>80% of visits in *S. elegans* and >75% in *S. iodantha*), and both bees and hummingbirds in the mixed species *S. mexicana* var. *minor* (>30% and >50% of visits , respectively; Fig. 4B).

For *S. assurgens*, the main floral visitors were two *Deltoptila* bee species and one *Exoprosopa* bee-fly species. For *S. lavanduloides*, two bumblebee species (*Bombus ephippiatus* and *B. weisi*), and *Apis mellifera*. The white-eared hummingbird (*Basilinna leucotis*) and *Selasphorus* sp. were the main floral visitors for *S. elegans* and *S. iodantha*. For *S. mexicana* var. *minor*, *Selasphorus* sp. and *Bombus sonorus* were the most frequent visitors (Table S1).

Except for *S. lavanduloides*, we observed illegitimate floral visitors in all *Salvia* species. Mainly large and medium-sized bees (e. g., *Bombus* spp. and *Xylocopa* spp.) were observed robbing nectar in both ornitophilous species and the mixed species. Different butterfly species were observed robbing nectar in *S. assurgens* (Table S1).

Pollinator efficiency and effectiveness

Pollination performance greatly differed among functional groups, which allowed us to identify primary and secondary pollinators for all *Salvia* species. In *S. assurgens* the butterfly *Lon zabulon* had a fruit efficiency of 0.67 and a seed efficiency of 1.33 (*n* = 3), while the bee *Deltoptila badia* had a fruit efficiency of 0.38 and a seed efficiency of 0.60 (*n* = 48) (Table 3). However, considering the total visitation frequency of both pollinator species, *D. badia* had a fruit effectiveness of 3.83 and a seed effectiveness of 6.18, while *L. zabulon* had a fruit effectiveness of 0.42 and a seed effectiveness of 0.83 (Table 3). Similarly, for *S. lavanduloides* the wasp *Pygodasis ephippium* was the most efficient pollinator, but due to its higher visitation frequency *B. ephippiatus* was more effective (Table 3).

Both ornitophilous species shared the hummingbird *Basilinna leucotis* as their primary pollinator but differed in their secondary pollinators, which were the hummingbird *Selasphorus* sp. for *S. elegans*, and the bee *D. elefas* for *S. iodantha*. The primary and secondary pollinators of *S. elegans* had comparable values of seed effectiveness (8.13 and 8.04, respectively), while those of *S. iodantha* had contrasting values (62.74 and 1.29, respectively; Table 3).

The highest pollinator diversity was recorded for *S. mexicana* var. *minor*, which was pollinated by small, medium, and large-sized bees, hummingbirds, and butterflies (Table 3). The primary and secondary pollinators of this species were *Bombus sonorus* (fruit efficiency = 0.55; seed efficiency = 1.55; fruit effectiveness = 4.82; seed effectiveness = 13.66; *n* = 11) and *Selasphorus* sp. (fruit efficiency = 0.42; seed efficiency = 0.68; fruit effectiveness = 5.24; seed effectiveness = 8.46; *n* = 31), respectively (Table 3).

In all but the mixed *Salvia* species, some other floral visitor species belonging to different functional groups were recorded in the single visit experiments, however, their visits did not contribute to fruit or seed production (Table 3). According to the pollinator effectiveness index, the most effective functional groups were large and medium-sized bees for melittophilous species, hummingbirds for ornitophilous species, and large-sized bees and hummingbirds for the mixed species (Table 4).

Floral morphology and pollinator effectiveness

The NMDS ordination based on the 11 floral traits showed a clear separation between melittophilous and ornitophilous species, and partial overlapping of the mixed species and the ornitophilous species (Fig. 5A). Moreover, *S. elegans* and *S. iodantha* were plotted closer to each other than *S. assurgens* and *S. lavanduloides*, suggesting less variation among ornitophilous species than among melittophilous species (NMDS stress = 0.0448; ANOSIM $R = 0.9012$, $P < 0.001$; Fig. 5A).

According to floral morphology and pollinator effectiveness, the NMDS plot showed a greater separation among syndromes. *S. mexicana* var. *minor* and both ornitophilous species showed no morphological overlap with any other species (Fig. 5B). Vectors indicated that large-sized bee and wasp-pollinated species were clustered along the positive side of the first axis, that small and medium sized bee and butterfly-pollinated species were clustered along the negative side of the second axis, and that hummingbird pollinated species were clustered along the positive side of the second axis (NMDS stress = 0.0473; ANOSIM $R = 0.9879$, $P < 0.001$; Fig. 5B).

DISCUSSION

This study is one of the first to quantitatively assess the contribution of several floral visitors to plant reproductive success and explore the relationship between pollination effectiveness and floral morphology in *Salvia*. Overall, our results showed that plant species depend on specific pollinators to reproduce and support the floral syndrome concept, providing clear examples of melittophily, ornitophily and mixed pollination in *Salvia*. Moreover, our findings highlight the importance of considering different plant fitness surrogates to analyze pollination systems.

Pollinator dependence

According to our first expectation, we found a higher reproductive success under open pollination than under autonomous self-pollination. Our results were congruent with other studies that indicate that *Salvia* species are pollinator-dependent (Haque & Goshal, 1981; Arizmendi et al., 1996; Grases y Ramírez, 1998; Sánchez et al., 2002; Aximoff y Freitas, 2010; Cuevas-García et al., 2013; Jorge et al., 2015; Cairampoma et al., 2020; Barrionuevo et al., 2021; Ling et al., 2022; Xiao et al., 2023a). However, considering the average fruit set and number of seeds produced per flower, reproductive success in natural conditions was rather low for all species. For pollinator-dependent plant species, the number and quality of floral visits have direct consequences for plant fitness (Aizen, 2007). Pollen limitation caused by plant-plant antagonistic interactions such as heterospecific pollen deposition or pollinator competition could explain the low reproductive success observed. To evaluate antagonistic interactions between the five *Salvia* species was beyond the scope of this study, nevertheless, it remains an interesting possibility that deserves further research.

Floral visitors and pollinator performance

Consistent with our second expectation, pollinator assemblages were mainly composed of animals that matched the floral syndrome of each *Salvia* species, however, floral visitor assemblages recorded here were more diverse than expected.

Salvia assurgens was primarily visited and pollinated by the bee *Deltoptila badia* (Apidae); similarly, other species from this genus have also been reported as frequent visitors of *S. assurgens*, but their contribution to plant reproduction had only been assessed via pollen load analysis (Cultid-Medina et al., 2021). *Exoprosopa rostrifera* (Bombyliidae) was the second most frequent visitor, but none of its visits resulted in fruit or seed production. Flies have been reported as frequent pollinators for some melittophilous species of *Salvia* (Celep et al., 2014 and 2020; Saravia-Nava et al., 2023), however, our findings suggest that, despite being frequent, flies can not be considered pollinators of this species.

The bumblebee *Bombus sonorus* (Apidae) was the most effective pollinator of *S. lavanduloides*. However, the most efficient pollinator was the wasp *Pygodasis ephippium* (Scoliidae). To our knowledge, this is the first assessment of the contribution of wasps to reproductive success in *Salvia* and one of the few that have reported the interaction between wasps and melittophious sages (e.g., Grant & Grant, 1964; Tavera et al., 2023).

Salvia elegans was mainly visited by hummingbirds, as indicated by previous records for different localities in México (Lara, 2006; Espino-Espino et al., 2014; Cuevas et al., 2018). *Basilinna leucotis* and *Selasphorus* sp. were the main polllinators for this species, and both were almost equally effective (Table 3). Visitation by bees was also recorded, but they represented less than 2.5% of the total visits, and none of their visits resulted in fruit or seed production. This contrasts with the observations of Espino-Espino et al. (2014), who found that up to 23% of the total floral visits during one flowering season of this species were performed by bees. Instead, we found that butterflies were the second most frequent and effective guild, where *Phoebis* philea was the only non-hummingbird pollinator of this species.

For *S. iodantha*, we found that *B. leucotis* was the only hummingbird visitor and pollinator. This result differs from previous studies that have found this species interacts with several hummingbird species (Arizmendi, 1994; Lara & Ornelas, 2001; López-Segoviano et al., 2021). Even when Dieringer et al. (1991) suggested that the bee *Deltoptila elefas* was a nectar robber in *S. iodantha*, our single visit experiments revealed that this bee species slightly contributed to fruit and seed production, and thus should be considered a secondary pollinator.

Although both ornitophilous species have similar floral morphology (tubular shape and exserted stamens and stigma), we found that *B. leucotis* performed better for *S. iodantha* than for *S. elegans*. Morphological matching promotes higher interaction frequencies between plant species and their hummingbird pollinators, and is strongly related to plant fitness (Maruyama et al., 2014; Vizentin-Bugoni et al., 2014; Sonne et al., 2020; Bustos et al., 2023). In this sense, the differences in efficiency and effectiveness of the primary pollinator of *S. elegans* and *S. iodantha* suggest a higher degree of trait matching between *S. iodantha* and *B. leucotis*, however, this needs to be explicitly tested.

As expected, the mixed species was mainly pollinated by bees and hummingbirds, but contrary to previous observations (e. g., Arizmendi et al., 2007; Strelin et al., 2017), bees were the primary pollinators. Furthermore, unlike the other sage species, *S. mexicana* var. *minor* relied on different functional groups for pollination between years. In 2022, its main pollinator was the bumblebee *B. sonorus*, whereas in 2023, it was the hummingbird *Selasphorus* sp. Secondary pollinators may be crucial for plant reproduction if primary pollinators are absent or scarce (Rosas-Guerrero et al., 2014; Ashworth et al., 2015).

It has been suggested that secondary pollinators correspond to the ancestral primary pollinators (Rosas-Guerrero et al., 2014). The secondary pollinators reported here are consistent with some of the major pollinator transitions documented in the literature (e. g., bees for ornitophilous flowers, and butterflies for melittophilous and ornitophilous flowers; Van der Niet & Johnson, 2012; Rosas-Guerrero et al., 2014). Nonetheless, recent studies indicate only pollinator shifts between bee and hummingbird pollination have occurred in *Salvia* (Fragoso-Martínez et al., 2018; Benítez-Vieyra et al., 2019; Kriebel et al., 2019, 2020). Future evolutionary studies aimed to elucidate ancestral pollination systems should include studies like ours for a more insighful view on pollinator transitions.

Floral morphology and pollinator effectiveness

According to our first NMDS, there was a greater morphological affinity among ornitophilous species than among melittophilous species (Fig 5A). Differences in the plotting pattern among syndromes can be explained by the evident differences between *S. assurgens* and *S. lavanduloides* (flower size and corolla aperture), and the similarities between *S. elegans* and *S. iodantha* (e.g., exserted reproductive structures, and long corollas). The first NMDS also showed some overlapping between the mixed and ornitophilous species. Rather than absolute intermediate states, it has been proposed that mixed *Salvia* species are part of a continuum in which floral phenotypes may span from being mainly melittophilous and partially ornitophilous and vice versa (Wester & Claßen-Bockhoff, 2011). In this study, the mixed *S. mexicana* var. *minor* resembled ornitophilous species more than melittophilous species, as other studies have found (Strelin et al., 2017).

In our second NMDS, including pollinator effectiveness apparently increased the separation of melittophilous species while reducing it for ornitophilous species (Fig 5B). This may be explained by the different number of functional groups recorded for each syndrome; while *S. elegans* and *S. iodantha* were pollinated by functionally similar hummingbirds, *S. assurgens* and *S. lavanduloides* were pollinated by different sized bees and other groups representing different vectors in the plot. Likewise, the higher effectiveness of large-sized bees separated *S. mexicana* var. *minor* from the ornitophilous species and brought it closer to melittophilous species. Mixed pollinated species like *S. mexicana* var. *minor* have been regarded as transitional intermediate states in pollinator shifts (Wester et al., 2020). Our results suggest this species could represent an intermediate stable phenotype, however, this needs to be tested.

Overall, our results support the floral syndrome concept and agree with the current syndrome status for the studied *Salvia* species; however, given the existence of secondary pollinators not expected by the syndrome, they also highlight the importance of considering pollinator effectiveness. Finally, as shown by our comparisons of pollinator performance, we suggest that future assessments of the reliability of pollination syndromes should include fruit and seed production as proxies of the pollination service for a better comprehension of plant – pollinator interactions.

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TABLES AND FIGURES

Table 1. Reproductive success of each *Salvia* species under different pollination treatments.

Treatment		Plant species				
		S. assurgens	*S. lavanduloides*	*S. mexicana* var. *minor*	*S. elegans*	*S. iodantha*
autonomous self-pollination	flowers (*n*)	361	822	1090	185	681
	Fruits	0	7	20	8	23
	Seeds	0	19	36	16	49
open pollination	flowers (*n*)	898	2737	2713	458	1051
	Fruits	409	1781	1620	277	289
	Seeds	622	4283	3799	690	469

Table 2. Comparisons of fruit and seed production between the autonomous self-pollination and the open pollination treatments, resulting from the generalized linear models for each *Salvia* species.

Plant species	Fruit production			Seed production		
	X^2	d. f	*P* value	X^2	d. f	*P* value
S. assurgens	359.42	1, 1164	< 0.001	324.89	1, 1164	< 0.001
S. lavanduloides	1399.6	1, 3557	< 0.001	2075.8	1, 3557	< 0.001
S. mexicana var. *minor*	1396.8	1, 3801	< 0.001	2248.3	1, 3801	< 0.001
S. elegans	202.53	1, 641	< 0.001	355.25	1, 641	< 0.001
S. iodantha	196.29	1, 1730	< 0.001	235.73	1, 1730	< 0.001

Table 3. Pollinator performance data for the five *Salvia* species.

Plant species	Pollinator species	Single visits (n)	Efficiency: Floral efficiency	Efficiency: Fruit efficiency	Efficiency: Seed efficiency	Effectiveness: Fruit effectiveness	Effectiveness: Seed effectiveness
S. assurgens	*Deltoptila badia* (MSB)	48	10.22	0.38	0.6	3.83	6.18
	Deltoptila sp. (MSB)	55	1.46	0.22	0.62	0.32	0.9
	Thygater sp. (MSB)	6	1.09	0.17	0.17	0.18	0.18
	Lasioglossum pharum (SSB)	11	0.82	0.09	0.09	0.07	0.07
	*Exoprosopa rostrifera** (LTF)	20	3.43	0	0	0	0
	Lon zabulon (B)	3	0.62	0.67	1.33	0.42	0.83
	*Thorybes dorantes** (B)	22	3.22	0	0	0	0
S. lavanduloides	*Apis mellifera* (MSB)	170	14.99	0.39	1.12	5.82	16.85
	Bombus ephippiatus (LSB)	133	56.29	0.61	1.59	34.28	89.73
	Bombus weisi (LSB)	131	7.12	0.44	1.29	3.15	9.18
	Lasioglossum sp.* (SSB)	5	0.91	0	0	0	0
	Largus sp.* (BU)	11	0.44	0	0	0	0
	Pygodasis ephippium (W)	11	3.58	1.00	3.64	3.58	13.03
S. mexicana var. *minor*	*Apis mellifera* (MSB)	13	1.61	0.54	0.69	0.87	1.12
	Bombus sonorus (LSB)	11	8.84	0.55	1.55	4.82	13.66
	Deltoptila elefas (LSB)	2	0.39	0.5	0.5	0.19	0.19
	Halictidae 1 (SSB)	10	0.49	0.1	0.3	0.05	0.15
	Halictidae 2 (SSB)	10	0.54	0.3	0.8	0.16	0.43
	Lasioglossum sp. (SB)	4	0.56	0.25	0.25	0.14	0.14
	Megachile sp. (MSB)	4	1.08	1	1.5	1.08	1.61
	Piruna sp. (B)	3	0.32	0.33	0.33	0.11	0.11
	Selasphorus sp. (H)	31	12.49	0.42	0.68	5.24	8.46
S. elegans	*Basilinna leucotis* (H)	29	9.83	0.31	0.83	3.05	8.13
	Selasphorus sp. (H)	19	6.36	0.53	1.26	3.35	8.04
	Phoebis philea (B)	12	2.34	0.17	0.25	0.39	0.59
	Lasioglossum sp.* (SSB)	7	0.15	0	0	0	0
S. iodantha	*Deltoptila elefas* (LSB)	14	9	0.07	0.14	0.64	1.29
	*Lasioglossum costale** (SSB)	5	0.25	0	0	0	0
	Halictidae 2* (SSB)	5	0.13	0	0	0	0

Basilinna leucotis (H)	95	51.83	0.61	1.21	31.64	62.74

Notes: coding for pollinator functional group is shown in parentheses after pollinator species: butterfly, B; bug, BU; hummingbird, H; large-sized bee, LSB; medium-sized bee, MSB; small-sized bee, SSB; long-tongued fly, LTF; wasp, W. The asterisk indicates the floral visitor species failed to produce any fruits or seed.

Table 4. Values of the pollinator effectiveness index (PEI) of each functional group for each *Salvia* species.

Plant species	Functional group	PEI
S. assurgens	MSB	0.92
	SSB	0.01
	B	0.08
S. lavanduloides	LSB	0.75
	MSB	0.14
	W	0.11
S. mexicana var. *minor*	LSB	0.52
	MSB	0.1
	SSB	0.03
	B	0.004
	H	0.35
S. elegans	H	0.98
	B	0.02
S. iodantha	H	0.98
	LSB	0.02

Notes: coding for pollinator functional groups: butterfly, B; hummingbird, H; large-sized bee, LSB; medium-sized bee, MSB; small-sized bee; wasp, W. Only pollinator functional groups that contributed to fruit and seed production are reported.

Figure legends

Figure 1. Geographic location of the five *Salvia* species studied. A) Location in Mexico; B) location in Michoacán; C) location of each plant species in each study site south of Morelia.

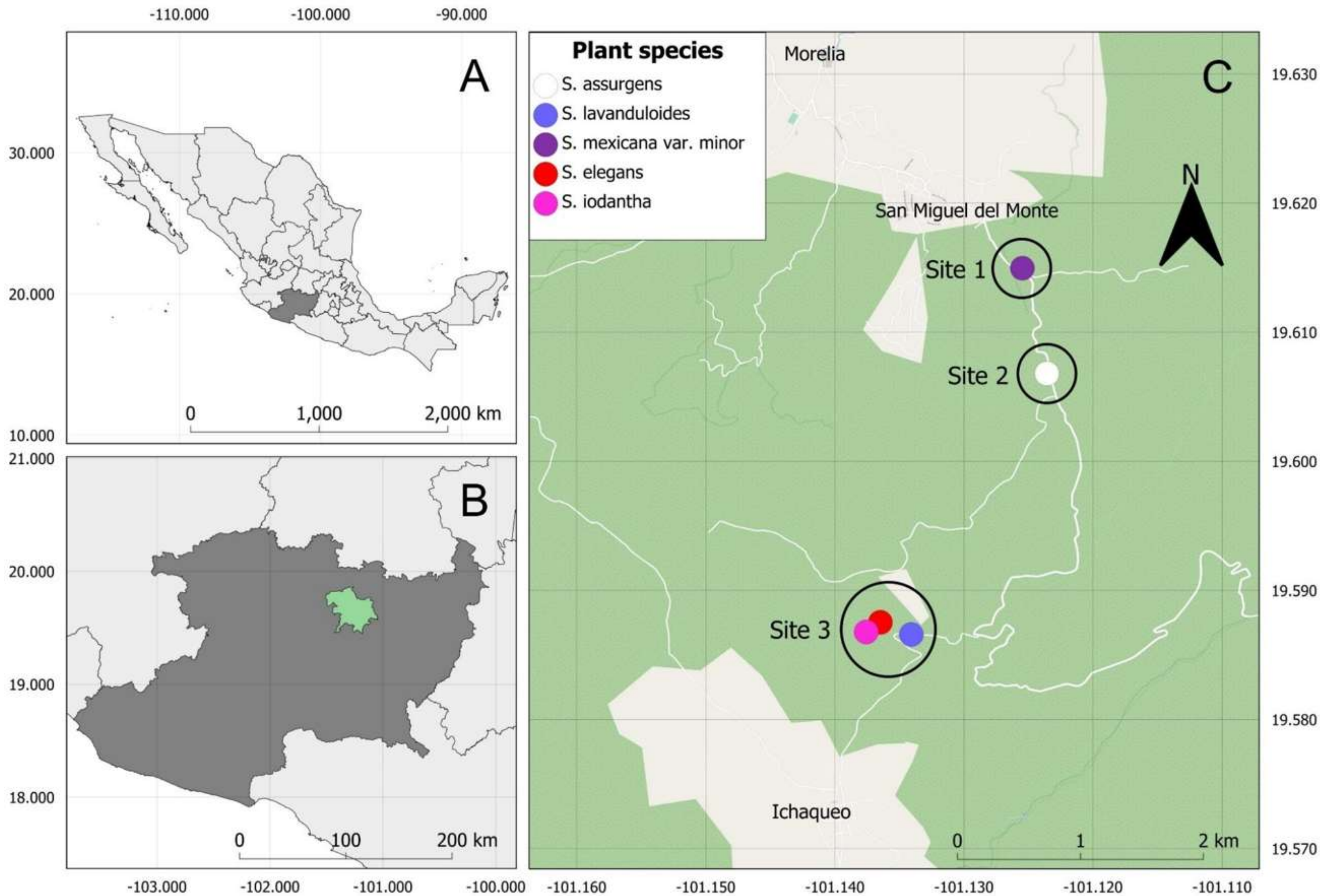
Figure 2. Pollinators and corolla measurements of *Salvia* species with different floral syndromes. A) *Deltoptila* sp.visiting *S. assurgens*, right before activating the lever mechanism; B) *Bombus ephippiatus* inserting it's pollen-dusted tongue into a flower of *S. lavanduloides*; C) *B. sonorus* probing nectar of *S. mexicana* var. *minor* while being dusted with pollen; D) *Selasphorus* sp. visiting flowers of *S. mexicana* var. *minor* with pollen on its bill; E) *Basilinna leucotis* visiting flowers of *S. iodantha*; F) *Selasphorus* sp. visiting flowers of *S. elegans* with pollen on it's head; G) Floral traits measured: AE, anthers exsertion; CA, corolla aperture; CL, corolla length; FTL, floral tube length; FTA, floral tube aperture; FTW, floral tube width; LLL, lower lip length; LLW, lower lip width; SE, stigma exsertion; ULL, upper lip length; ULW, upper lip width. Photos: A. Tavera.

Figure 3. Reproductive success of the five *Salvia* species under different pollination treatments. A) Average fruit set; B) average number of seeds. Data are means + SE, and different letters above columns indicate significant differences between treatments.

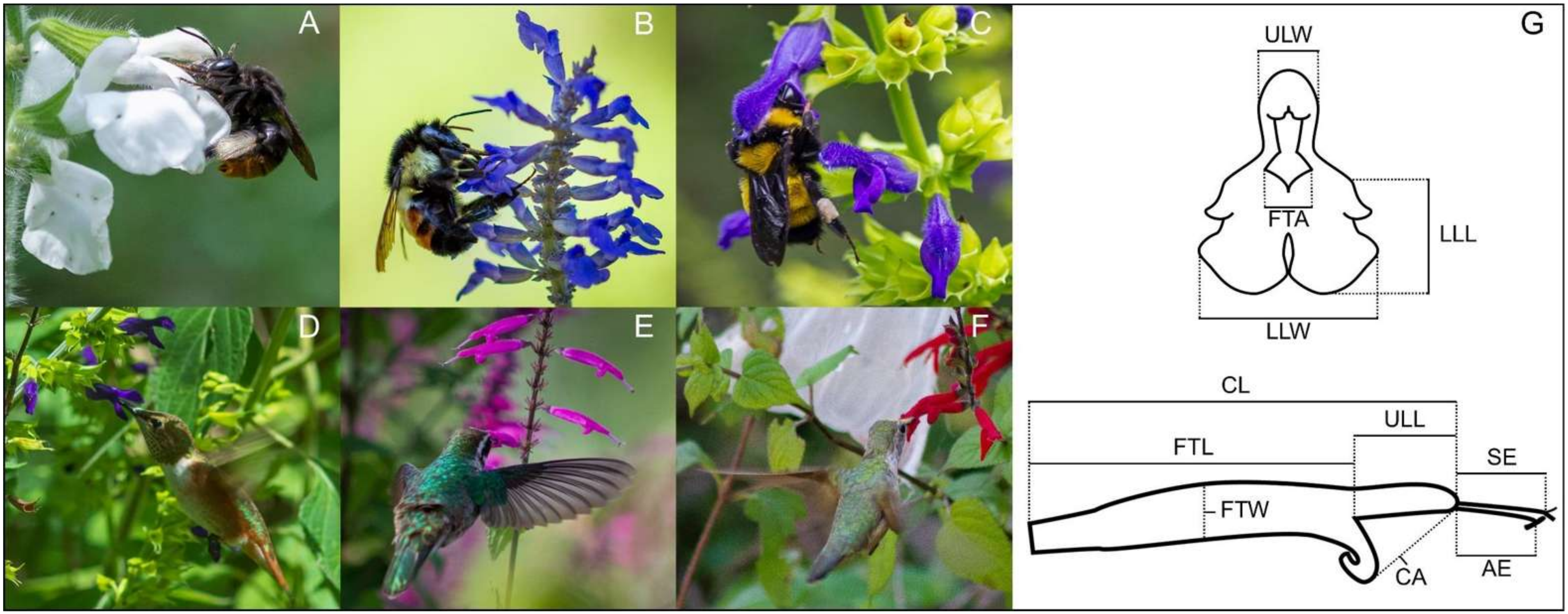
Figure 4. Percentage of floral visits effected by every floral visitor guild recorded in each *Salvia* species.

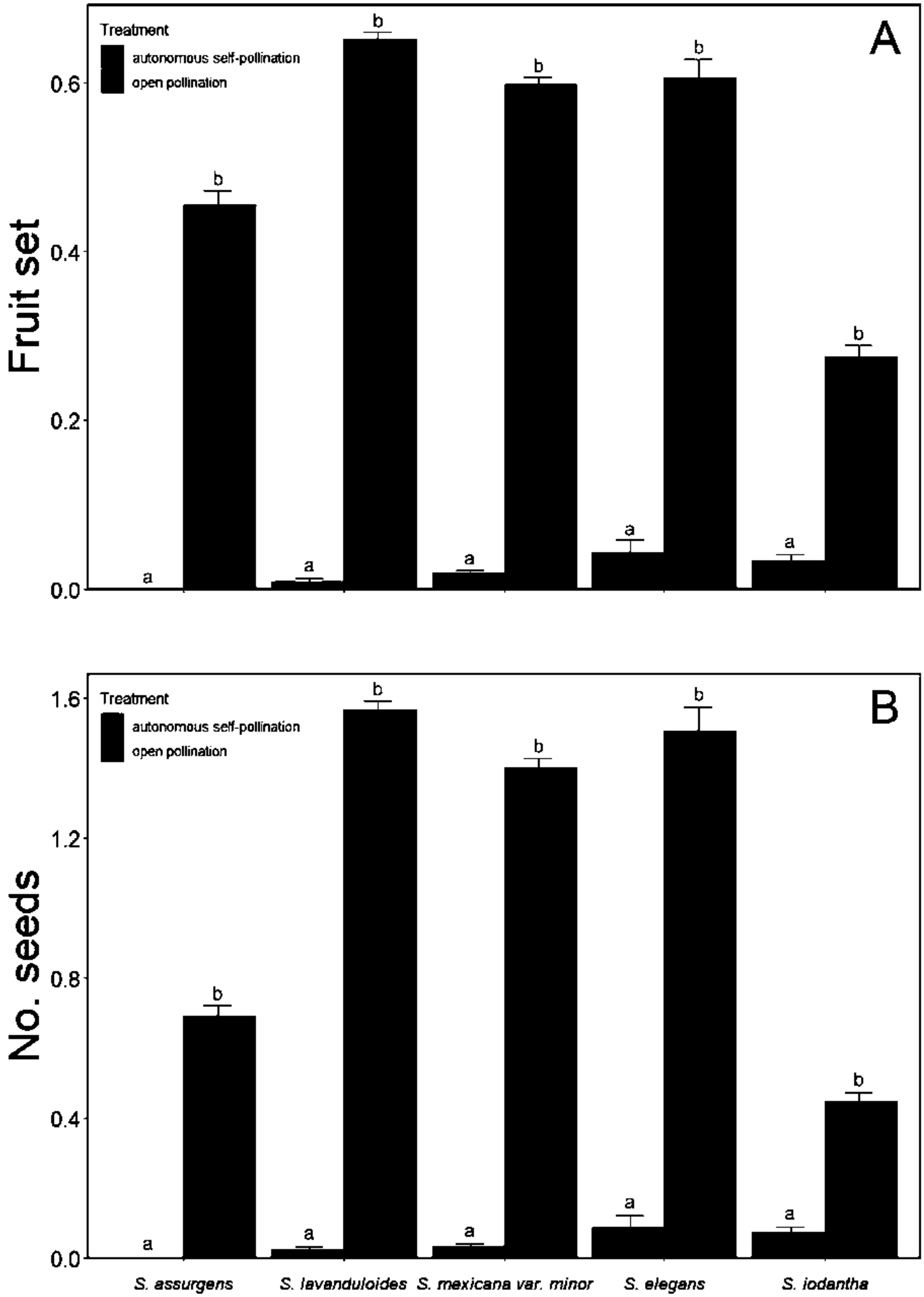
Figure 5. Non-metric multidimensional scaling (NMDS) ordinations of the five *Salvia* species. A) Plotting of the log-transformed data of the 11 floral traits measured; B) plotting of the log-transformed data of the 11 floral traits measured and the pollinator effectiveness index values of each functional group for each plant species. Color coding represents plant

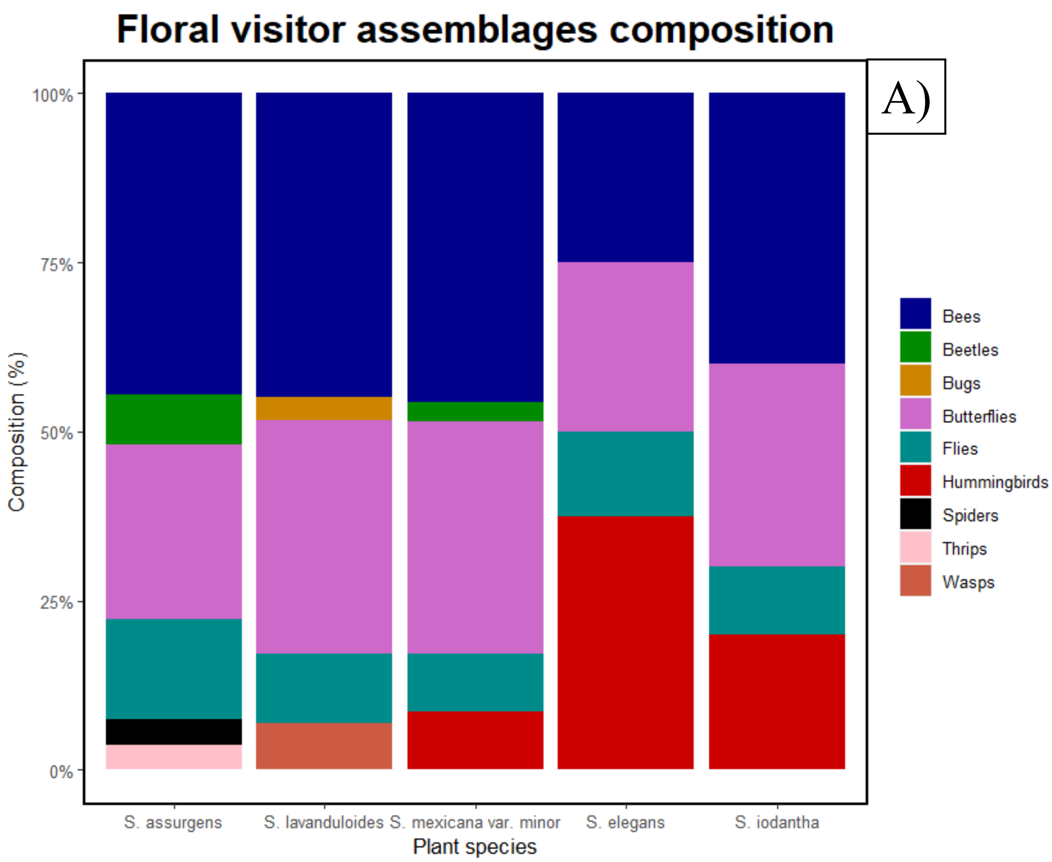
species as follows: *S. assurgens*, grey; *S. lavanduloides*, blue; *S. mexicana* var. *minor*, purple; *S. elegans*, red; *S. iodantha*, pink.

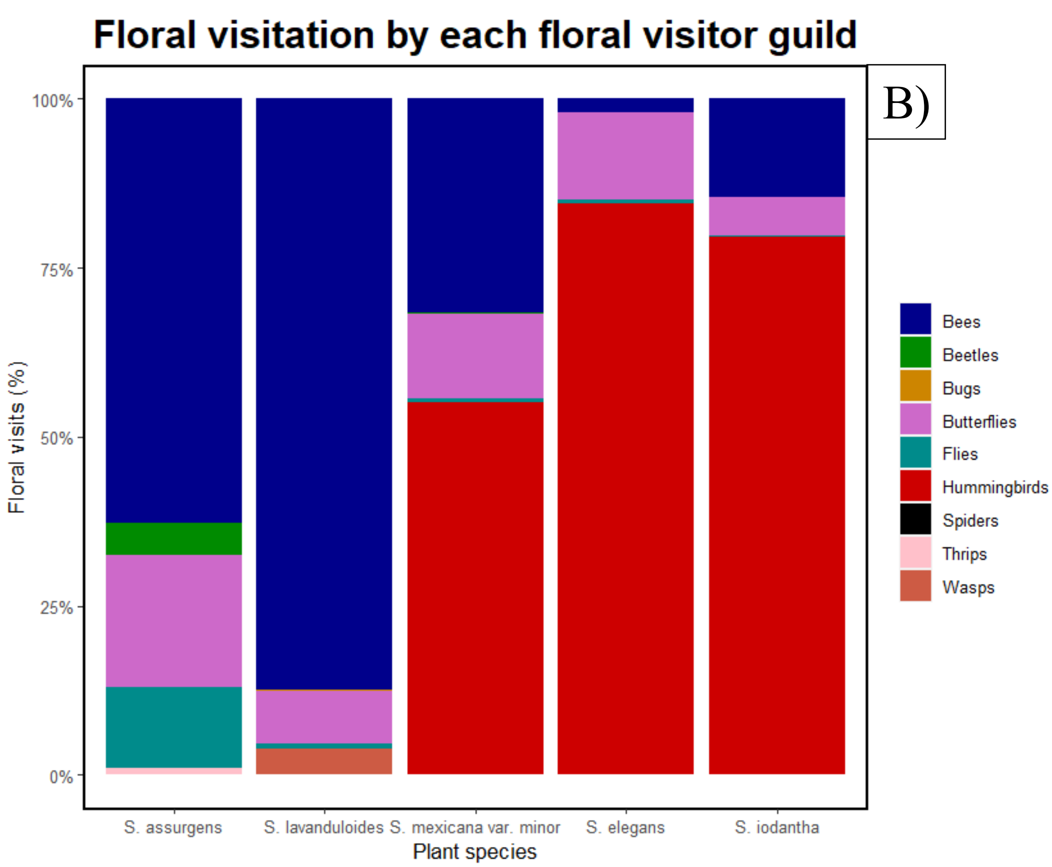


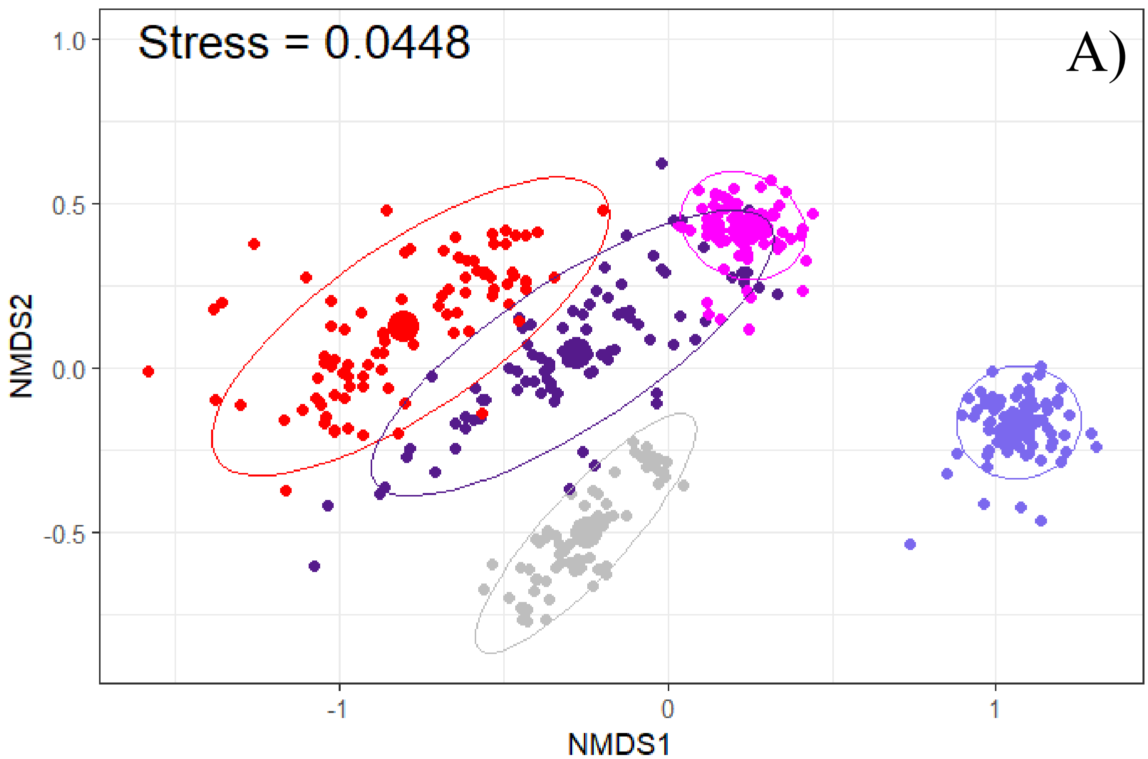
-110.000
-100.000
-90.000
30.000
20.000
10.000
A
0
1,000
2,000 km
21.000
20.000
19.000
18.000
B
0
100
200 km
-103.000
-102.000
-101.000
-100.000
Plant species
S. assurgens
S. lavanduloides
S. mexicana var. minor
S. elegans
S. iodantha
Morelia
C
San Miguel del Monte
N
Site 1
Site 2
Site 3
Ichaqueo
0
1
2 km
19.630
19.620
19.610
19.600
19.590
19.580
19.570
-101.160
-101.150
-101.140
-101.130
-101.120
-101.110

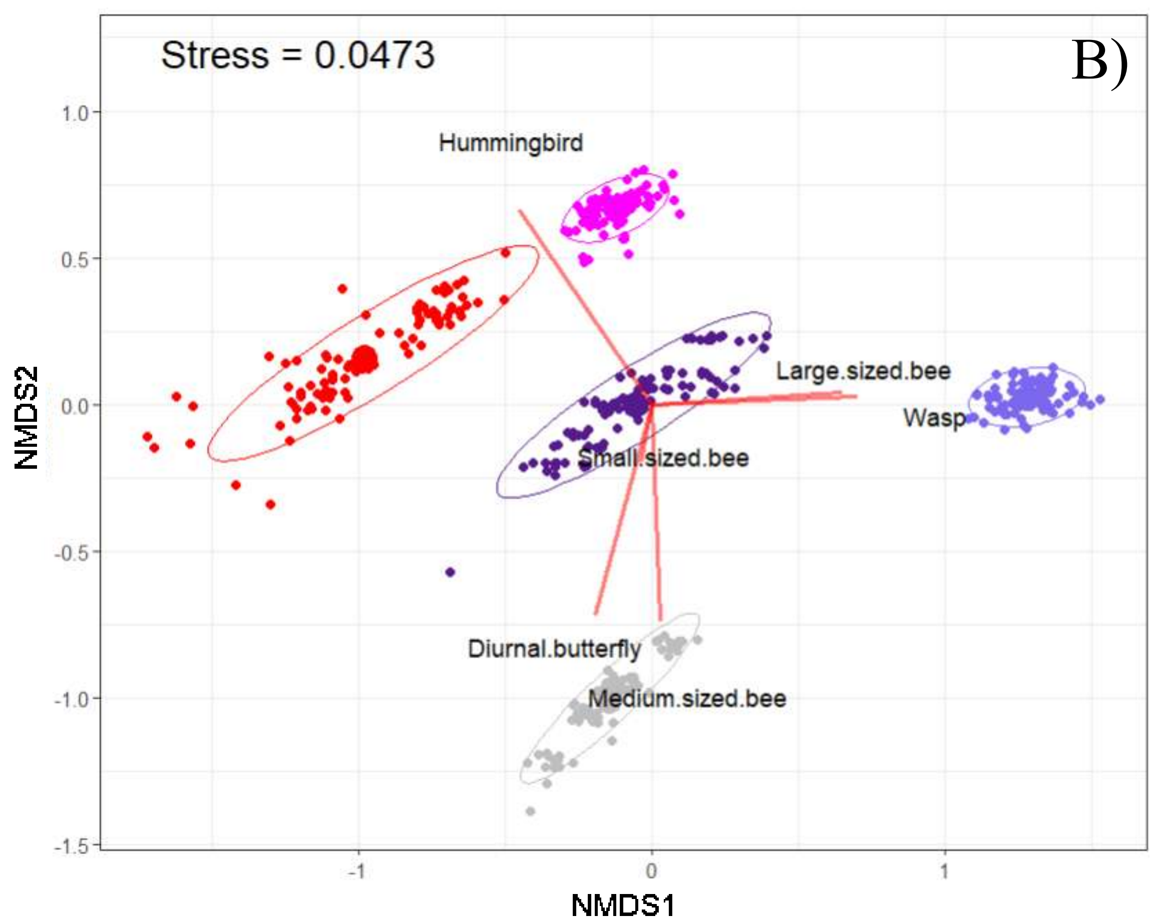

A
B
C
D
E
F
G
ULW
FTA
LLL
LLW
CL
ULL
FTL
SE
FTW
CA
AE


Treatment
autonomous self-pollination
open pollination
A
Fruit set
0.0
0.2
0.4
0.6
Treatment
autonomous self-pollination
open pollination
B
No. seeds
0.0
0.4
0.8
1.2
1.6
S. assurgens
S. lavanduloides
S. mexicana var. minor
S. elegans
S. iodantha


Floral visitor assemblages composition
A)
Composition (%)
100%
75%
50%
25%
0%
S. assurgens
S. lavanduloides
S. mexicana var. minor
S. elegans
S. iodantha
Plant species
Bees
Beetles
Bugs
Butterflies
Flies
Hummingbirds
Spiders
Thrips
Wasps


Floral visitation by each floral visitor guild
B)
Floral visits (%)
100%
75%
50%
25%
0%
S. assurgens
S. lavanduloides
S. mexicana var. minor
S. elegans
S. iodantha
Plant species
Bees
Beetles
Bugs
Butterflies
Flies
Hummingbirds
Spiders
Thrips
Wasps


Stress = 0.0448
A)
1.0
0.5
0.0
-0.5
NMDS2
-1
0
1
NMDS1


Stress = 0.0473
B)
Hummingbird
Large.sized.bee
Wasp
Small.sized.bee
Diurnal.butterfly
Medium.sized.bee
1.0
0.5
0.0
-0.5
-1.0
-1.5
NMDS2
-1
0
1
NMDS1

SUPPLEMENTARY MATERIAL

Table S1. Visitation rate of each floral visitor for each plant species.

Plant species	Floral visitor species	Visitation rate (visits/hour/flower)
S. assurgens	**Hymenoptera**	
	Apidae	
	Bombus ephippiatus	0.00224
	Bombus sonorus	0.00038
	Ceratina sp.	0.00016
	Deltoptila badia	0.01163
	Deltoptila sp.	0.00166
	Peponapis pruinosa	0.00064
	Thygater sp.	0.00124
	Xylocopa tabaniformis azteca	0.00160
	Halictidae	
	Halictidae 1	0.00027
	Lasioglossum pharum	0.00093
	Lasioglossum sp.	0.00087
	Megachilidae	
	Megachile sp.	0.00036
	Lepidoptera	
	Hesperiidae	
	Chioides zilpa	0.00013
	Erynnis sp.	0.00007
	Hesperiidae 1	0.00004
	Lon zabulon	0.00071
	Telegonus cellus	0.00215
	Thorybes dorantes	0.00366
	Riodinidae	
	Apodemia zela	0.00004
	Coleoptera	
	Curculionidae	
	Curculionidae 1	0.00078
	Melyridae	
	Listrus sp.	0.00091
	Diptera	
	Bombyliidae	
	Exoprosopa rostrifera	0.00391
	Systropus sp.	0.00013
	Empididae	
	Empididae 1	0.00007
	Tachinidae	
	Tachinidae 1	0.00007

	Araneae	
	Thomisidae	
	Thomisidae 1	0.00004
	Thysanoptera	
	Phlaeothripidae	
	Gynaikothrips sp.	0.00033
S. lavanduloides	**Hymenoptera**	
	Apidae	
	Apis mellifera	0.00839
	Bombus ephippiatus	0.03150
	Bombus sonorus	0.00019
	Bombus weisi	0.00398
	Xylocopa sp.	0.00113
	Halictidae	
	Augochlora sp.	0.00010
	Dinagapostemon sicheli	0.00052
	Dinagapostemon sp.	0.00114
	Lasioglossum costale	0.00016
	Lasioglossum sp.	0.00051
	Paragapostemon coelestinus	0.00012
	Megachilidae	
	Anthidiellum notatum	0.00003
	Megachile sp.	0.00008
	Vespoidea	
	Vespoidea 1	0.00010
	Scoliidae	
	Pygodasis ephippium	0.00200
	Diptera	
	Diptera 1	0.00022
	Syrphidae	
	Asemosyrphus sp.	0.00008
	Palpada mexicana	0.00012
	Hemiptera	
	Largidae	
	Largus sp.	0.00025
	Lepidoptera	
	Hesperiidae	
	Burnsius oileus	0.00033
	Thorybes cincta	0.00016
	Thorybes dorantes	0.00298
	Lycaenidae	
	Leptotes marina	0.00012
	Nymphalidae	
	Dione moneta	0.00005
	Junonia coenia	0.00026

	Vanessa sp.	0.00005
	Pieridae	
	Abaeis mexicana	0.00016
	Phoebis philea	0.00005
S. mexicana var. *minor*	**Apodiformes**	
	Trochilidae	
	Basilinna leucotis	0.00241
	Selasphorus sp.	0.00439
	Saucerottia beryllina	0.00316
	Hymenoptera	
	Apidae	
	*Apis mellifera**	0.00057
	*Bombus ephippiatus**	0.00031
	Bombus sonorus	0.00311
	*Bombus weisi**	0.00022
	Ceratina sp.	0.00004
	Deltoptila elefas	0.00014
	Eucerini 1	0.00005
	Tetraloniella sp.*	
	Thygater sp.	0.00008
	Xylocopa guatemalensis	0.00015
	Xylocopa sp.*	
	*Xylocopa tabaniformis azteca**	0.00005
	Halictidae	
	Augochlorini 1	0.00006
	Halictidae 1	0.00017
	Halictidae 2	0.00019
	Lasioglossum sp.	0.00020
	Megachilidae	
	Megachile sp.	0.00038
	Coleoptera	
	Scarabaeidae	
	Euphoria sp.	0.00003
	Diptera	
	Bombyliidae	
	Systropus sp.	0.00007
	Empididae	
	Empididae 1	0.00002
	Syrphidae	
	Syrphidae 1	0.00002
	Lepidoptera	
	Hesperiidae	
	Astraptes fulgerator	0.00021
	Hesperiidae 2	0.00005
	Hesperiidae 3	0.00002

	Lon zabulon	0.00064
	Piruna sp.	0.00011
	Thorybes dorantes	0.00022
	Urbanus proteus	0.00017
	Nymphalidae	
	Dione moneta	0.00014
	Nymphalidae 1	0.00007
	Pieridae	
	Abaeis mexicana	0.00042
	Phoebis philea	0.00017
	Riodinidae	
	Apodemia sp.	0.00007
S. elegans	**Hymenoptera**	
	Apidae	
	*Xylocopa tabaniformis azteca**	
	Halictidae	
	Halictidae 1	0.00003
	Lasioglossum costale	0.00017
	Lasioglossum sp.	0.00012
	Apodiformes	
	Trochilidae	
	Basilinna leucotis	0.00804
	Saucerottia berylinna	0.00017
	Selasphorus platycercus	0.00521
	Diptera	
	Syrphidae	
	Syrphidae 1	0.00010
	Lepidoptera	
	Papilionidae	
	Papilio sp.	0.00012
	Pieridae	
	Phoebis philea	0.00192
S. iodantha	**Hymenoptera**	
	Apidae	
	*Apis mellifera**	0.00029
	*Bombus ephippiatus**	
	Deltoptila elefas	0.00295
	*Xylocopa tabaniformis azteca**	
	Xylocopa sp.*	
	Halictidae	
	Halictidae 1	0.00004
	Lasioglossum costale	0.00008
	Apodiformes	
	Trochilidae	
	Basilinna leucotis	0.01698

Saucerottia berylinna	0.00045
Selasphorus sp.	0.00004
Diptera	
Syrphidae	
Syrphidae 1	0.00005
Lepidoptera	
Nymphalidae	
Dione moneta	0.00023
Papilionidae	
Papilio sp.	0.00002
Sphingidae	
Aellopos clavipes	0.00101

Notes: Floral visitors are listed according to order, family, genus and species. The asterisk indicates the floral visitor species behaved as a nectar robber. Only the visitation rate of legitimate floral visits is reported.

Table S2. Measurements of the 11 floral morphological traits for all the plant species.

	Plant species				
Floral trait	*S. assurgens*	*S. lavanduloides*	*S. mexicana* var. *minor*	*S. iodantha*	*S. elegans*
CL	13.41 ± 0.82	6.16 ± 0.47	22.92 ± 2.64	20.83 ± 2.19	33.62 ± 2.75
FTL	8.03 ± 0.49	4.89 ± 0.46	15.67 ± 1.80	15.75 ± 1.78	21.83 ± 2.20
ULL	5.92 ± 0.45	1.43 ± 0.20	7.35 ± 1.05	5.16 ± 0.69	11.91 ± 1.12
LLL	10.61 ± 1.11	4.27 ± 0.46	7.88 ± 1.19	3.76 ± 0.58	9.69 ± 1.33
FTW	4.47 ± 0.31	1.51 ± 0.15	4.97 ± 0.78	3.36 ± 0.33	4.54 ± 0.50
CA	4.23 ± 0.53	1.33 ± 0.24	5.71 ± 1.25	5.62 ± 0.90	11.30 ± 1.80
AE	/	/	/	5.50 ± 1.10	0.55 ± 0.73
SE	/	/	/	5.61 ± 1.49	2.16 ± 1.10
ULW	3.08 ± 0.26	1.76 ± 0.25	3.45 ± 0.64	2.64 ± 0.42	3.60 ± 0.54
FTA	1.98 ± 0.2	1.15 ± 0.15	2.35 ± 0.44	2.42 ± 0.40	2.88 ± 0.35
LLW	8.89 ± 1.43	3.69 ± 0.68	4.51 ± 1.09	4.45 ± 0.57	5.39 ± 0.60

Notes: Data are means ± SD. Floral trait coding: corolla length, CL; floral tube length, FTL; upper lip length, ULL; lower lip length, LLL; floral tube width, FTW; corolla aperture, CA; anthers exsertion, AE; stigma exsertion, SE; upper lip width, ULW; floral tube aperture, FTA; lower lip width, LLW.