

Strategic Selfing as Reproductive Assurance in Response to Pollinator Instability: The Mixed Mating System of a Dry-Season Flowering Pioneer Shrub, *Wigandia urens*

Haoran Xiu

530028219@qq.com

Universidad Nacional Autónoma de México

Zenón Cano-Santana

Universidad Nacional Autónoma de México

María del Carmen Mandujano-Sánchez

Universidad Nacional Autónoma de México

Research Article

Keywords: auto-fertility, decline of pollinator diversity, flower visit rate, hermaphroditic flowers, reproductive assurance, self-pollen limitation

Posted Date: June 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10048198/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Plant Ecology on August 24th, 2026. See the published version at <https://doi.org/10.1007/s11258-026-01671-w>.

Abstract

In the context of global pollinator decline, understanding reproductive strategies under unpredictable environments is crucial. *Wigandia urens* (Boraginaceae) is a dominant shrub in central Mexico that blooms during the dry season when pollinator activity is significantly diminished. We employed a controlled pollination experiment with eight treatments and 15 replicates to quantify the contributions of autonomous self-pollination, biotic pollination, and abiotic factors. Floral visitation rates were also monitored throughout the flowering phenology. *W. urens* exhibited a mixed mating system predominantly oriented toward self-pollination. The species showed exceptionally high auto-fertility ($AF = 0.699$) and significant reproductive assurance ($RA = 0.372$). While biotic pollinators contributed to 46.5% of total seed set, visitation rates progressively declined (0.016 to 0.009 visits/flower/min) as the dry season peaked. No significant self-pollination limitation was detected ($PLs = -0.020$). Despite marginal seed set in exclusion treatments, field observations suggest that gravity-mediated selfing or secondary pollen presentation, rather than functional anemophily, are the likely abiotic drivers. Our findings demonstrate that *W. urens* prioritizes reproductive certainty through autonomous selfing to buffer against pollinator scarcity in the dry season, while maintaining biotic pathways for potential genetic exchange. This dual strategy provides a vital evolutionary advantage in disturbed habitats. These results offer key insights into plant adaptive evolution under the contemporary crisis of pollinator diversity loss.

Introduction

1. Macro-Evolutionary Context: The Plasticity and Vulnerability of Plant Mating Systems

Within seed plant evolutionary biology, pollination acts as the foundational mechanism governing the encounter and fusion of male and female gametes to successfully yield sporophytes (Roubik, 2000). Across the angiosperm lineage, this reproductive phase operates along a dynamic mating continuum vibrating between self-pollination (selfing) and cross-pollination (outcrossing) (Cronk & Fennessy, 2001). The structural canvas for these strategies is largely dictated by floral sexuality; approximately 80% of angiosperms are monoecious with hermaphroditic flowers, whereas 6–7% are dioecious exhibiting unisexual flowers restricted to separate individuals, and the remaining minor fraction are monoecious but bear distinct unisexual flowers on the same individual (Wang & Hao, 2022). To navigate this landscape and maximize outcrossing, over 80% of flowering plants depend heavily on biotic pollination vectors (Ackerman, 2000). This global mutualism encompasses an intricate web where between 100,000 and 200,000 animal species actively service approximately 250,000 angiosperm species (Ackerman, 2000).

This long-standing reliance on animal vectors has synthesized a 140-million-year historical trajectory of complex, reciprocal plant-pollinator coevolution (Kiestler et al., 1984; Bao et al., 2019). To secure reproductive success, many plants have evolved highly specialized floral syndromes tailored to specific pollinator guilds. For example, vertebrate-pollinated flowers display distinctive diagnostic traits: bird-pollinated systems characteristically produce large quantities of nectar paired with vibrant red petals

(Rodríguez-Gironés & Santamaría, 2004; Cronk & Ojeda, 2008), whereas bat-pollinated flowers are predominantly white or red, featuring elongated, well-developed stamens, emitting intense odors, and restricted to nocturnal anthesis (Fleming et al., 2009). Because vertebrate mutualists are far less abundant than their insect counterparts, vertebrate-pollinated plants typically exhibit prolonged floral persistence, ensuring that slower pollen dispersal rates do not compromise total reproductive output before senescence (Van Doorn, 1997).

Conversely, the vast majority of angiosperms rely on insect vectors (Hoshiba & Sasaki, 2008), deploying brightly colored petals and potent olfactory cues to attract a diverse cohort of Hymenoptera (bees and wasps, and occasionally ants), Coleoptera (beetles), Lepidoptera (moths and butterflies), and Diptera (flies) (Klein et al., 2007). Such biotic partnerships frequently favor high pollinator specialization, which grants a clear selective advantage to plants by preventing pollen loss during interspecific flights and shielding receptive stigmas from being mechanically clogged by heterospecific grains (Harder et al., 2001). Plants actively reinforce this behavioral fidelity through specialized chemical signals that steer vectors toward functionally active flowers (Chittka et al., 1999).

Mechanically, flowers often exhibit precise architectural modifications to compel efficient pollen acquisition; a frequent evolutionary configuration mandates that pollinators must contact the stamens to access concealed nectar rewards (Nocentini et al., 2012). Where mutualistic rewards are absent, plants may even manifest deceptive pollination strategies, utilizing structural traps—such as deep tubular corollas lined with downward-pointing hairs, slippery epidermal surfaces, sticky secretions, or insect sex attractants—to temporarily ensnare and heavily coat visitors with pollen grains (Ferdyn et al., 1998). Furthermore, divergent flowering periods have driven the diversification of sensory traits: day-blooming species predominantly exploit visual cues such as specific hues or shapes, whereas night-blooming species bypass bright pigmentations in favor of white or pale yellow corollas that emit heavy fragrance to guide nocturnal foragers (Jürgens, 2006). However, while these specialized ecological syndromes optimize pollen transfer under stable conditions, they simultaneously expose plants to severe reproductive vulnerability when environmental changes disrupt pollinator availability.

2. Taxonomic Precedent: Reproductive Plasticity vs. Specialization within Boraginaceae

The cosmopolitan family Boraginaceae, encompassing roughly 150 genera and 2,000 species distributed globally, serves as an exceptional taxonomic model for examining the evolutionary tension between complex biotic syndromes and reproductive safeguards (Rzedowski et al., 2005). Morphologically, members of this family are primarily herbs, though shrubs and trees occur and vines remain rare, typically defined by scorpioid cymes, radially or slightly symmetrical hermaphroditic flowers with persistent calyces fused to the fruit, 5-lobed corollas (varying from funnel-shaped and tubular to quadriform, bell-shaped, or nearly whorled), and rough, pubescent leaves embedded with sclereids and calcium carbonate that possess stinging trichomes capable of causing human skin irritation and rashes (Rzedowski et al., 2005). Although some lineages are dioecious, the majority utilize bisexual flowers

heavily reliant on diurnal insects—predominantly bees (Hymenoptera)—for genetic exchange (Trunz et al., 2020).

To orchestrate these insect interactions, Boraginaceae species exhibit sophisticated physiological and mechanical adaptations. In certain taxa, age-dependent anthocyanin dynamics shift flower coloration from red to blue as a visual signal instructing pollinators to bypass older, non-receptive flowers (Hess, 2005). In the Neotropics, species such as *Cordia multispicata* exhibit highly synchronized daily rhythms, blooming around 6:00 a.m. with a nectar sugar concentration calibrated to approximately 16% to specifically target hoverflies (Diptera: Syrphidae) (Machado & Loiola, 2000). A pinnacle of micro-spatial specialization is observed in the Mediterranean *Cerithe major* L., whose dark yellow corollas on dense scorpioid cymes feature green, ring-shaped nectaries at the ovary base; its unique stamen arrangement physically restricts nectar access, forcing insect visitors to load pollen while foraging (Nocentini et al., 2012). This species tightly coordinates a 4-to-5-day floral lifespan wherein the pistils reach full receptivity on the second day of anthesis, perfectly synchronized with anther dehiscence (Nocentini et al., 2012). Moreover, *C. major* initiates abundant nectar production during the bud stage, drastically curtails secretion in senescent flowers, and reabsorbs unconsumed fluids—yielding a reward profile dominated by sucrose ($93.2 \pm 1.4\%$) alongside vital amino acids like proline, taurine, leucine, gamma-aminobutyric acid (GABA), citrulline, and alanine to nourish primary visitors such as *Bombus hortorum* (L.), *Anthophora* sp., and *Eucera* sp. (Nocentini et al., 2012).

Yet, despite these profound mechanical and chemical commitments to diurnal insect vectors (Trunz et al., 2020), environmental instability has simultaneously selected for high reproductive plasticity within the family. A prominent example is *Lithospermum macromeria* J.I. Cohen (= *Macromeria viridiflora*); although its floral architecture suggests a mixed pollination syndrome serviced by both insects and birds, it retains exceptionally high self-compatibility, resulting in natural seed formation rates that suffer no significant reduction in self-pollinated flowers when animal vectors are excluded (Boyd, 2004). This demonstrates a flexible risk-spreading strategy: when pollinators are abundant, outcrossing operates as an exogenous mechanism to augment the genetic diversity of the offspring; however, during periods of acute pollinator scarcity, autonomous self-pollination functions as an indispensable reproductive safeguard to guarantee population persistence (Boyd, 2004). Despite the documentation of these dual pathways across certain lineages, how these fine-scale mechanisms operate under extreme seasonal deficits remains insufficiently characterized for dominant tropical and subtropical shrubs.

3. Study System, Hypotheses, and Objectives

To bridge this critical knowledge gap, we investigated the reproductive biology of *Wigandia urens* (Boraginaceae), a dominant woody pioneer shrub and highly successful colonizer of disturbed habitats and secondary forests across the Valley of Mexico, situated at elevations between 2,250 and 2,600 m above sea level (Gamboa-deBuen et al., 2006). Morphologically, *W. urens* possesses a light brown trunk and substantial oval or round leaves with corrugated margins—measuring 5.5 to 50 cm (or larger) in length and 3.5 to 37 cm in width—that are tomentose to bristly, being slightly pubescent on the upper

surface, densely pubescent on the lower surface, and supported by 2.5 to 10 cm petioles (Rzedowski et al., 2005). Its reproductive structures consist of terminal scorpioid cymes bearing broadly bell-shaped flowers (1.5 to 2.2 cm long) with rod-shaped stigmas and calyx lobes (4 to 15 mm, though more commonly 7 to 10 mm long) heavily covered in purple, violet, blue, or lavender-white bristles (Rzedowski et al., 2005). The stamens are adnate to the corolla, spanning one-quarter of its total length, with the lower three-quarters being densely hairy (1.2 to 1.5 cm long) and bearing oblong anthers (3.5 to 6 mm long) (Rzedowski et al., 2005). Upon successful fertilization, the plant produces densely hairy 10 mm capsules containing thousands of minute (0.7 mm long, 0.5 mm wide), dark brown, polyhedral, triangular, or cylindrical seeds displaying a finely meshed, honeycomb-like matrix when mature (Rzedowski et al., 2005).

Ecologically, *W. urens* presents a fascinating paradox: it exhibits an extensive flowering phenology that peaks during the severe dry season in central Mexico—a harsh period characterized by acute floral scarcity and drastically diminished pollinator activity (César-García, 2002). To resolve how this dominant shrub achieves massive seed output under such environmental constraints, the objective of this study was to evaluate the **Reproductive Assurance Hypothesis** in *W. urens* by rigorously quantifying the relative contributions of autonomous selfing, biotic pollination, and abiotic factors to its total reproductive output. Specifically, we aimed to determine whether this species' high auto-fertility provides a distinct competitive advantage in environments where pollinator services are highly unpredictable. Given that *W. urens* flowers during this critical dry-season deficit, we hypothesized that the species has evolved a highly adaptive mixed mating strategy to maximize reproductive success through three distinct, coordinated pathways:

1. **Autonomous self-pollination** serves as a vital reproductive assurance mechanism to directly counter the high spatial and temporal variability of insect pollinators during the dry season peak.
2. Concurrently, its prolonged flowering phenology allows it to function as a critical resource for surviving pollinators, whereby **occasional outcrossing** via biotic vectors enhances offspring fitness and genetic diversity.
3. While wind pollination is not considered the principal mechanism in Boraginaceae, **abiotic factors** (such as wind or gravity) may provide a supplementary, albeit limited, pollen source under the arid and windy conditions of the dry season.

Methods

Study Site and Species Background

This study was conducted at the Paseo de las Esculturas, situated within the Ciudad Universitaria campus of the National Autonomous University of Mexico (UNAM) and its adjacently integrated ecological reserve, the San Ángel Ecological Reserve (PSAER), in Mexico City, Mexico (19° 19' N, 99° 11' W; 2,300 m a.s.l.). The local climate supports a native vegetation community phytosociologically classified as xerophytic scrubland (Cano-Santana et al., 2008).

Pre-test Validation: Emasculation Resilience

Prior to the implementation of the comprehensive pollination plot, a pilot study was executed to verify whether the structural integrity and reproductive viability of *Wigandia urens* buds were compromised by manual emasculation (E). On two discrete occasions (October 24 and 30, 2023), 10 mature, late-stage flower buds immediately prior to anthesis were strategically marked (n = 20 total buds). For each cohort, all stamens were meticulously excised manually before corolla opening. These manipulated flowers were subsequently monitored to evaluate their capacity for normal anthesis and subsequent fertilization. All 20 experimental flowers successfully expanded, underwent normal pollination, and advanced to mature fruit set, validating that the floral organs of *W. urens* possess robust mechanical resistance to early emasculation.

Manipulative Pollination Experiment

To dissect the exact pollination mechanisms governing *W. urens*, we implemented a factorial manipulative framework adapted from the experimental design of Eckert et al. (2010), comprising eight distinct treatments with 15 replicates each. On January 8, 2024, 15 individual shrubs of homogeneous architecture were selected based on precise phenotypic baselines: a height range of 2.5–3.0 m and a branch density of 5–6 major branches per individual. On each selected shrub, one flower per treatment was marked (totaling 15 replicates per treatment across the population).

This orthogonal design executed pairwise comparisons across two floral integrity states—Intact (FI) and Emasculated (FE)—crossed with four pollen-mediated environments:

1. **Natural Open-Pollination (FIN and FEN):** Fully open flowers exposed directly to the local ambient environment without obstruction, serving as controls for baseline insect mediated pollination.
2. **Wind-Mediated Exclusion (FIC and FEC):** Flowers continuously enclosed in fine tulle netting, a mesh configuration structurally calibrated to exclude all macro-invertebrate pollinators while permitting the unobstructed passage of airborne pollen grains to evaluate potential anemophily.
3. **Controlled Hand-Cross Pollination (FIX and FEX):** Flowers encapsulated within robust paper bags to eliminate any external vector or airborne pollen influx. Receptive stigmas were artificially supplemental-pollinated using outcross pollen harvested from randomly selected, non-nesting conspecific individuals.
4. **Controlled Hand-Self Pollination (FIS and FES):** Flowers similarly isolated via paper bags, where receptive stigmas received self-pollen sourced via geitonogamy from distinct, synchronous flowers residing on the same maternal individual.

For all hand-pollination treatments (FIX, FEX, FIS, FES), artificial pollen deposition was executed via direct flower-to-flower contact, utilizing clean metal clamps to stabilize donor organs. For all exclusion cohorts, the bagging isolation spanned an unbroken duration of one week to encompass the entire

temporal window of individual floral anthesis. Manual emasculations were systematically performed prior to corolla expansion via the manual removal of all un-dehiscid stamens.

The progression of manipulated flowers to mature fruit set was evaluated on February 17, 2024. Subsequently, on April 16, 2024, all developed fruits were harvested to determine total seed yield per fruit across all treatments. To further characterize the mating system, these empirical yields were integrated with the algebraic formulations established by Mandujano et al. (2010), defining the crossover rate in non-emasculated flowers as $FEC/(FEC + FEX)$ and the corresponding rate in emasculated flowers as $FIX/(FIX + FIS)$.

Empirical Verification of Wind Pollination Potential

To independently isolate and verify whether *W. urens* exhibits functional aerodynamic capacity for anemophily, a localized observation cohort of 30 flowers was marked on January 7, 2025. Stamen configurations were monitored under natural ambient velocity regimes to directly observe whether ambient wind currents possess sufficient kinetic energy to physically detach pollen grains from dehiscing anthers, and to track whether post-excision pollen grains actively engage in short- or long-distance aerial travel upon exiting the corolla throat.

Seasonal Dynamics of Floral Visitation

To quantify the precise ecological contributions of animal vectors to the reproductive budget of *W. urens*, a continuous monitoring plot was established across 10 distinct individuals. From each maternal shrub, one representative inflorescence was selected, establishing a stable temporal monitoring sample of 120 flowers. Due to the characteristically continuous and synchronous flowering phenology of *W. urens* (Xiu, 2025), this floral sample density remained constant throughout the data collection cycle. Visual observations were conducted over three consecutive 5-day periods chosen to capture distinct seasonal points: October 7–11, 2024; December 6–10, 2024; and January 29 to February 2, 2025.

Daily observations spanned from 07:00 to 18:00 h. Within the first 15 minutes of each hourly interval, observers recorded all faunal visitors to the target inflorescences, identifying each visitor to the finest attainable taxonomic resolution. A discrete interaction was strictly logged as a "functional visit" only when the animal made direct, macroscopically verifiable physical contact with either the receptive stigma or the pollen-bearing anthers. Total visitation velocity was subsequently mathematical-standardized into a formalized floral visit rate, defined as the number of interactions per flower per minute.

Statistical Analyses

All numerical modeling and computational analyses were executed within the R statistical environment version 4.2.0 (R Core Team, 2022). Variations in fruit-set success (the binomial proportion of flowers

successfully transitioning to mature fruits) were analyzed by fitting Generalized Linear Models (GLMs) utilizing a negative binomial distribution framework to accommodate latent variance constraints. Total seed production data per fruit across the discrete manipulative treatments were modeled using Zero-Inflated Poisson (ZIP) GLMs via the glmmTMB package (Brooks et al., 2017) to account for structural zeros resulting from unfertilized ovules. Overdispersion within the foundational Poisson models was systematically evaluated using residual diagnostics; where significant overdispersion was detected, models were mathematically transitioned to a Negative Binomial error distribution framework to ensure statistical robustness.

Results

It was found that *W. urens* can be wind-pollinated, although this vector is not very effective, as only 26.7% of the flowers are pollinated by this mechanism. This method produces an average of $198.7 \pm \text{s.d. } 392.9$ seeds per fruit (range: 0-1102; $=F_{EC}$), which represents 16.3% of the total seeds produced (F_{EC}/F_{IN}). On the other hand, in this species, most seeds are produced by self-fertilization, as the fraction of the maximum potential seed production achieved by autonomous self-fertilization (self-fertility; AF) was 0.699 (F_{IC}/F_{IN}), indicating that 69.9% of the seeds are produced by this mechanism. Additionally, given that the proportional increase in seed production via manual artificial self-pollination [measured as self-pollination limitation; $PLs=(F_{IS}-F_{IN})/F_{IN}$] was -0.020, it is concluded that there was no self-pollination limitation. The fraction of seeds attributable to autonomous self-pollination [reproductive assurance: $RA=(F_{IN}-F_{EN})/F_{IN}$] was also found to be 0.372 (Table 1).

Additionally, both self-pollination and cross-pollination pollen were found to be highly compatible, and pollination can be carried out by animals. The fraction of seeds produced by cross-pollination is estimated to be 0.465 [$(F_{EN} - F_{EC})/F_{IN}$]. Furthermore, no pollen limitation due to cross-pollination [$PLx=(F_{IX}-F_{IN})/F_{IN}$; i.e., the proportional increase in seed production via manual artificial cross-pollination] was found, as it had a value of -0.073. In addition to the design of Eckert et al. (2010), the cross-pollination rate was 0.483 in the case with emasculation and 0.427 in the case with other methods.

Table 1

Results of the pollination mechanisms experiment of *W. urens*. Data from 15 flowers per treatment. F: seed production, N: natural conditions, C: with pollinator exclusion cages, X: artificially pollinated with foreign pollen (cross-pollination), S: artificially pollinated with autogenous pollen (S), I: intact flowers, E: emasculated flowers, Pol. = pollination, emasc.: with emasculation (E). Different letters denote significant differences with $P < 0.05$.

Treatments	No. of seeds/fruit $\pm$ s.d. (range)	Pollinated flowers (%)
Natural conditions, control: F _{IN}	1,222.1 $\pm$ 179.5 (1068-1,608) ^a	100.0 ^{ab}
Natural conditions, emasc.: F _{EN}	767.3 $\pm$ 249.6 (0–1,003) ^e	93.3 ^a
Exclusion of pollinators, control: F _{IC}	854.2 $\pm$ 367.8 (0–1,205) ^d	86.7 ^{ab}
Exclusion of pollinators, emasc.: F _{EC}	198.7 $\pm$ 392.9(0-1102) ^g	26.7 ^b
Manual pollination with allogenic pollen, control: F _{IX}	1133.6 $\pm$ 162.4 (902-1,405) ^b	100.0 ^{ab}
Manual pollination with allogenic pollen, emasc.: F _{EX}	1,001.5 $\pm$ 153.6 (715-1,237) ^c	100.0 ^{ab}
Manual autofertilization, control: F _{IS}	1,198.0 $\pm$ 163.7 (866-1,432) ^a	100.0 ^{ab}
Manual autofertilization, emasc.: F _{ES}	669.1 $\pm$ 460.7(0–1,233) ^f	80.0 ^{ab}

According to GLIM, there were no significant differences in the fruit fractions formed between treatments, except between F_{EC} (exclusion of pollinators with emasculated flowers) and F_{EN} (natural conditions with emasculated flowers), with the latter treatment showing the highest value.

Furthermore, when comparing the number of seeds per fruit, this value varied between treatments in the following order: F_{INa} = F_{ISa} > F_{IXb} > F_{EXc} > F_{ICd} > F_{ENe} > F_{ESf} > F_{ECg}.

After observing the pollen of 30 flowers in the field, it was recorded that in 10 individuals (33.3%) the pollen was not carried by the wind, while it was carried by the other 20 individuals. It was observed that after the pollen detaches from the anthers, it falls to the ground immediately and is not displaced by the wind. The above suggests that the pollen of this plant cannot move through the air, which is a requirement for wind pollination; however, gravity pollination is not ruled out.

Wigandia urens recorded a floral visit rate of 0.016 flower⁻¹ min⁻¹ in October, 0.012 flower⁻¹ min⁻¹ in December, and 0.009 flower⁻¹ min⁻¹ in January-February (data from 5 days and 60 h of observation; $n = 120$ flowers).

Discussion

Field observations and floral morphology indicate that *W. urens* lacks the typical syndromes associated with anemophily, as its pollen grains exhibit limited buoyancy and tend to settle immediately upon release rather than undergoing wind-mediated dispersal. Notably, the residual seed set observed in the FEC treatment (pollinator exclusion with emasculation) likely reflects a highly sensitive reproductive assurance mechanism rather than functional wind pollination. This marginal output may stem from a narrow temporal window for effective emasculation, where trace amounts of self-pollen could contact the stigma during the subtle transition to anthesis. Such a phenomenon underscores the species' robust capacity for autonomous selfing, where even minimal stigmatic contact under bagged conditions can lead to successful fertilization. Furthermore, while the pollen is not "wind-pollinated" in a classical sense, ambient air currents may trigger gravity-mediated self-pollination by dislodging grains from the anthers within the compact, scorpioid cymes. This suggests that *W. urens* may employ secondary pollen presentation—a trait previously reported in Boraginaceae (El Ottra et al., 2024)—wherein pollen is temporarily sequestered within floral structures before being redirected to stigmatic surfaces or vectors. Future investigations into the micro-morphology of the floral chamber are required to confirm whether these abiotic factors function as a supplementary "safety net" for reproduction in pollinator-scarce environments.

Furthermore, this plant was found to have a self-fertility value of 0.699 (F_{IC}/F_{IN}), a very high value among angiosperms (Razanajatovo et al., 2016), and the proportion of seeds produced by self-pollination was 0.372 (reproductive assurance). According to Hu et al. (2021), such reproductive assurance indicates that the reproductive system of *W. urens* is a "self-pollination-dominated" model. This is also confirmed by the fact that there was no significant difference between F_{IN} and F_{IS} (the effect of manual self-pollination is better than manual allopollination, which is similar to the result obtained under natural conditions). At the same time, according to the formula of Mandujano et al. (2010) for calculating the crossing rate, the mating system of *W. urens* is mixed and tends toward self-pollination. All this evidence indicates that this plant possesses a mixed and variable mating system, and that self-pollination is more important, although it is undeniable that cross-pollination by animal means is also relevant. However, it is worth noting that 20% of the flowers failed to be artificially self-pollinated after emasculation. This may indicate differences in self-compatibility among different *W. urens* individuals, or it could be due to problems with the experimental handling, such as the amount of pollen deposited on each flower not being uniform, and the degree of contact with the pistil stigma varying. It is also possible that emasculation damaged some flowers.

So, what is the significance of *W. urens* having a strong capacity for self-pollination? At first, the flowering season of *W. urens* runs from September to June, which means that it starts in rainy season but principally in dry season. Therefore, difference between the two seasons need to be considered. Many studies have indicated a strong negative correlation between flower visitation rate and flowering plant abundance (Dietzsch et al., 2011; Akter et al., 2017). This can be explained by the fact that during seasons when many plants flower together, competition for pollinators among different individuals

increases, thus decreasing the average number of flowers a pollinator can pollinate. This effect is further accentuated when the number of pollinators is limited (Akter et al., 2017). It can also be explained by the fact that, for a given plant, there might be an inverse relationship between the number of flowers and pollination efficiency. However, since experiments found that *W. urens* has no limit to cross-pollination, this should not be the primary reason.

However, in central Mexico, most plants flower during the peak season (César-García, 2002). Therefore, competition among these plant species to attract pollinators is very intense and can result in many specialized pollination strategies or a likely coevolution. (Kiester et al., 1984). However, specialization to reduce temporal competition is very risky, because once the environment changes and the number of pollinators decreases, they will also face the risk of a decline in the number of seeds and the risk of extinction (Ramos-Jiliberto et al., 2020). Therefore, the benefit of self-pollination in *W. urens* is obvious. During the rainy season, the number of flowering plants reaches its peak for the year (César-García, 2002), so competition for pollinators is very high, making a high capacity for self-pollination key to ensuring the number of offspring. In contrast, during the dry season, due to the significant reduction in the number of pollinators (Domínguez-Álvarez et al., 2009; Moyers-Arévalo and Cano-Santana, 2009), insect visitation rates cannot reach high levels even when flowering plant species are scarce. Therefore, self-pollination is key to ensuring seed production during this season, which is the principal flowering season of *W. urens* (César-García, 2002; Xiu, 2025).

The evolution of plant pollination strategies is highly complex, but those preserved through natural selection must be able to adapt to specific environmental conditions. Furthermore, it is worth noting that, as *W. urens* is a widely distributed plant, its pollination strategies may vary across different regions. Paseo de las Esculturas represents an environment with relatively strong human disturbance, so the abundance of pollinators may not be as high as it would be in a fully preserved habitat, making it beneficial for them to maintain their self-pollination capacity. If the same research on this species were conducted in other regions in the future, it would provide a better understanding of the differences in pollination strategies when faced with different ecological environments and, at the same time, allow us to better understand the evolution of plant pollination strategies.

Finally, according to the results obtained, the visitation rate to *W. urens* flowers is low compared to typical insect-pollinated plants, whose visitation rates normally reach values of $0.02 \text{ flower}^{-1} \text{ min}^{-1}$ (Dietzsch et al., 2011), while *W. urens* recorded visitation rates of 0.009 to $0.016 \text{ flower}^{-1} \text{ min}^{-1}$. However, according to the observation results from the 2023–2024 breeding season, the pollination rate reached practically 100%. Therefore, it is expected that other means contribute to its pollination, such as autonomous self-pollination. It is important to mention that, although this study used flower visit rate, an indicator commonly used in pollinator-plant interaction research to measure the contribution of animals to pollination, an attempt was made to extend the observation period as much as possible to obtain more robust results (Fijen and Kleijn, 2017). However, some studies have indicated that flower visit rate alone cannot accurately reflect the contribution of animals to pollination because different pollinators have varying efficiencies in transporting and depositing pollen on the stigma (King et al., 2013).

Therefore, some pollinators may visit flowers very frequently but have low pollen transfer efficiency, as might be the case with Lepidoptera (Jennersten, 1984). King et al. (2013), for example, found that, depending on the plant species, approximately 0 to 78% of flower visitors are inconsequential for pollination. However, the importance of a single animal species for the pollination of different plants can vary enormously. For example, butterflies, which are generally considered unimportant, are crucial for the pollination of some plants in northern Europe (Jennersten, 1984). Bees are also not efficient pollinators for plants with many flowers because, despite their high efficiency in transmitting pollen, the pollen deposited by bees for these plants could turn out to be pollen from the same individual, which can lead to a significant reduction in ovules and seed production for plants with low self-compatibility (Diller et al., 2022). Therefore, although in this study the flower visit rate was significantly higher during the rainy season than during the dry season due to the large number of butterflies, no conclusions can be drawn about which animals contribute most to the pollination of *W. urens* during the rainy season.

This study only revealed that *W. urens* has a strong self-pollination capacity because the highest number of seeds in the treatments was obtained with self-pollination without emasculation. Furthermore, according to the GLM results, there were no differences between the control and manual self-pollination without emasculation, which also suggests that self-pollination is the most common in this species. However, cross-pollination is also important, not only because when animals are excluded, the average number of seeds per fruit decreases by 30.1%, but also because cross-pollination is potentially a way to produce genetically diverse offspring. Nevertheless, although animals were found to be very important. Unfortunately, no analysis was performed on which animals were involved.

The effective pollinators of *W. urens* are a key focus of this study. It is recommended that future research quantitatively measure the quantity and quality of pollen dispersed per unit of time by different visitors to *W. urens* flowers. This would reveal the specific contribution of animals to the pollination process and identify the most effective pollinators. Furthermore, a limitation of this study is that it only used the number of seeds to determine the effect of different pollination treatments, without examining the effects on seed quality. To further confirm the mating system of *W. urens*, future research could analyze the quality of seeds obtained under different pollination treatments.

Against the backdrop of a continued decline in global pollinator diversity (Pollinator Decline), the study of *Wigandia urens* not only reveals the survival wisdom of a species but also provides a profound commentary on the resilience of plant evolution. Its self-pollination strategy, characterized by "high fertility and strong protection," reflects how plants mitigate the risk of extinction by balancing "genetic diversity" and "reproductive certainty" in the face of highly uncertain ecological environments. This finding suggests that in future, more volatile ecosystems, species with high reproductive protection capabilities may gain an advantage in species succession, and the assessment framework established in this study provides an important reference model for understanding and predicting global vegetation community succession under climate change.

Declarations

Competing Interests

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

All authors participated in the project planning and methodology design (but María del Carmen Mandujano-Sánchez made the absolute major contribution to the methodology). Haoran Xiu was the only author who conducted fieldwork and data collection; he, along with María del Carmen Mandujano-Sánchez, completed the data analysis. All authors participated in the interpretation of the results. The original text was written by Haoran Xiu, and the other two authors reviewed the entire text and provided feedback.

Acknowledgement

This research is part of the Master's program of Haoran Xiu at Posgrado en Ciencias Biológicas, UNAM, and I would first like to thank my entire team of assessor for their help and support. Special thanks are to Dr. Johnattan Hernández-Cumplido and Iván Castellanos Vargas for their assistance. We employed Gemini to refine and proofread the English text, thanks to UNAM for opening its Master's program in Biological Sciences and the SECIHTI-Mexico for its economic support, which made this research possible.

Data Availability

The original data is available via request.

References

1. Ackerman JD (2000) Abiotic pollen and pollination: Ecological, functional, and evolutionary perspectives. *Plant Syst Evol* 222:167–185
2. Akter A, Biella P, Klecka J (2017) Effects of small-scale clustering of flowers on pollinator foraging behaviour and flower visitation rate. *PLoS ONE* 12(11):e0187976
3. Bao T, Wang B, Li J, Dilcher D (2019) Pollination of Cretaceous flowers. *Proceedings of the National Academy of Sciences*, 116(49), 24707–24711
4. Boyd AE (2004) Breeding system of *Macromerta viridiflora* (Boraginaceae) and geographic variation in pollinator assemblage. *Am J Bot* 91:1809–1813
5. Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften* 86:361–377

6. Cronk JK, Fennessy MS (2001) Wetland plants: Biology and ecology. Lewis, Boca Raton
7. Cronk Q, Ojeda I (2008) Bird-pollinated flowers in an evolutionary and molecular context. *J Exp Bot* 59(4):715–727
8. César-García SF (2002) Análisis de algunos factores que afectan la fenología reproductiva de la comunidad vegetal de la Reserva del Pedregal de San Ángel, D.F. (México). Bs. Sc. thesis. Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City
9. Dietzsch AC, Stanley DA, Stout JC (2011) Relative abundance of an invasive alien plant affects native pollination processes. *Oecologia* 167:469–479
10. Diller C, Castañeda-Zárate M, Johnson SD (2022) Why honeybees are poor pollinators of a mass-flowering plant: Experimental support for the low pollen quality hypothesis. *Am J Bot* 109(8):1305–1312
11. Domínguez-Álvarez LA, Cano-Santana Z, Ayala-Barajas R (2009) Estructura y fenología de la comunidad de abejas nativas (Hymenoptera: Apoidea). In: Lot, A. y Z. Cano-Santana (eds.). *Biodiversidad del ecosistema del Pedregal de San Ángel*. UNAM, Mexico City, pp. 421–432
12. Eckert CG, Kalisz S, Geber MA, Sargent R, Elle E, Cheptou PO, Goodwillie C, Johnston MO, Kelly JK, Moeller DA, Porcher E (2010) Plant mating systems in a changing world. *Trends Ecol Evol* 25(1):35–43
13. El Ottra JHL, Toni JFG, Thaowetsuwan P, Santos PD, Jeiter J, De Craene LR, Claßen-Bockhoff R (2024) Pollen transfer within flowers: how pollen is secondarily presented. *Int J Plant Sci* 185(1):15–31
14. Ferdy JB, Gouyon PH, Moret J, Godelle B (1998) Pollinator behavior and deceptive pollination: learning process and floral evolution. *Am Nat* 152(5):696–705
15. Fijen TP, Kleijn D (2017) How to efficiently obtain accurate estimates of flower visitation rates by pollinators. *Basic Appl Ecol* 19:11–18
16. Gamboa-de Buen A, Cruz-Ortega R, Martínez-Barajas E, Sánchez-Coronado ME, Orozco-Segovia A (2006) Natural priming as an important metabolic event in the life history of *Wigandia urens* (Hydrophyllaceae) seeds. *Physiol Plant* 128:520–530
17. Harder LD, Williams NM, Jordan CY, Nelson WA (2001) The effects of floral design and display on pollinator economics and pollen dispersal. *Cognitive ecology of pollination: Animal behavior and floral evolution*. Cambridge University Press, Cambridge
18. Hess D (2005) Systematische botanik. UTB GmbH, Stuttgart
19. Hoshiba H, Sasaki M (2008) Perspectives of multi-modal contribution of honeybee resources to our life. *Entomol Res* 38:15–21
20. Jennersten O (1984) Flower visitation and pollination efficiency of some North European butterflies. *Oecologia* 63:80–89
21. Jürgens A (2006) Comparative floral morphometrics in day-flowering, night-flowering and self-pollinated Caryophylloideae (*Agrostemma*, *Dianthus*, *Saponaria*, *Silene*, and *Vaccaria*). *Plant*

22. Kiester AR, Lande R, Schemske DW (1984) Models of coevolution and speciation in plants and their pollinators. *Am Nat* 124(2):220–243
23. King C, Ballantyne G, Willmer PG (2013) Why flower visitation is a poor proxy for pollination? measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods Ecol Evol* 4(9):811–818
24. Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274(1608), 303–313
25. Machado IC, Loiola MI (2000) Fly pollination and pollinator sharing in two synchronopatric species: *Cordia multispicata* (Boraginaceae) and *Borreria alata* (Rubiaceae). *Brazilian J Bot* 23:305–311
26. Mandujano MDC, Carrillo-Angeles I, Martínez-Peralta C, Golubov J (2010) Reproductive biology of Cactaceae. *Desert plants: Biology and Biotechnology*. Springer Berlin, Heidelberg, pp 197–230
27. Moyers-Arévalo RL (2009) Fenología de mariposas diurnas de la Reserva del Pedregal de San Ángel, D.F. y su relación con la fenología floral y otros factores ambientales. Bs. Sc. thesis. Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City
28. Nocentini D, Pacini E, Guarnieri M, Nepi M (2012) Flower morphology, nectar traits and pollinators of *Cerithe major* (Boraginaceae-Lithospermeae). *Flora-Morphology Distribution Funct Ecol Plants* 207(3):186–196
29. Ramos-Jiliberto R, de Espanés M, P, Vázquez DP (2020) Pollinator declines and the stability of plant–pollinator networks. *Ecosphere*, 11(4), e03069
30. Razanajatovo M, Maurel N, Dawson W, Essl F, Kreft H, Pergl J, Van Kleunen M (2016) Plants capable of selfing are more likely to become naturalized. *Nat Commun* 7(1):1–9
31. Rodríguez-Gironés MA, Santamaría L (2004) Why are so many bird flowers red? *PLoS Biol* 2(10):350
32. Roubik DW (2001) Ups and downs in pollinator populations: When is there a decline? *Conserv Ecol* 5(1):2
33. de Rzedowski GC, Rzedowski J (2005) *Flora fanerogámica del Valle de México*. 2nd. ed. Instituto de Ecología, A.C. and Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Pátzcuaro, Michoacán
34. Trunz V, Lucchetti MA, Bénon D, Dorchin A, Desurmont GA, Kast C, Praz CJ (2020) To bee or not to bee: The ‘raison d’être’ of toxic secondary compounds in the pollen of Boraginaceae. *Funct Ecol* 34(7):1345–1357
35. Van Doorn WG (1997) Effects of pollination on floral attraction and longevity. *J Exp Bot* 48(9):1615–1622
36. Wang Y, Hao Z (2022) Angiosperm sexual systems-Concepts, evolution, ecology, and future directions. *Biodivers Sci* 30(7):22–65

Figures

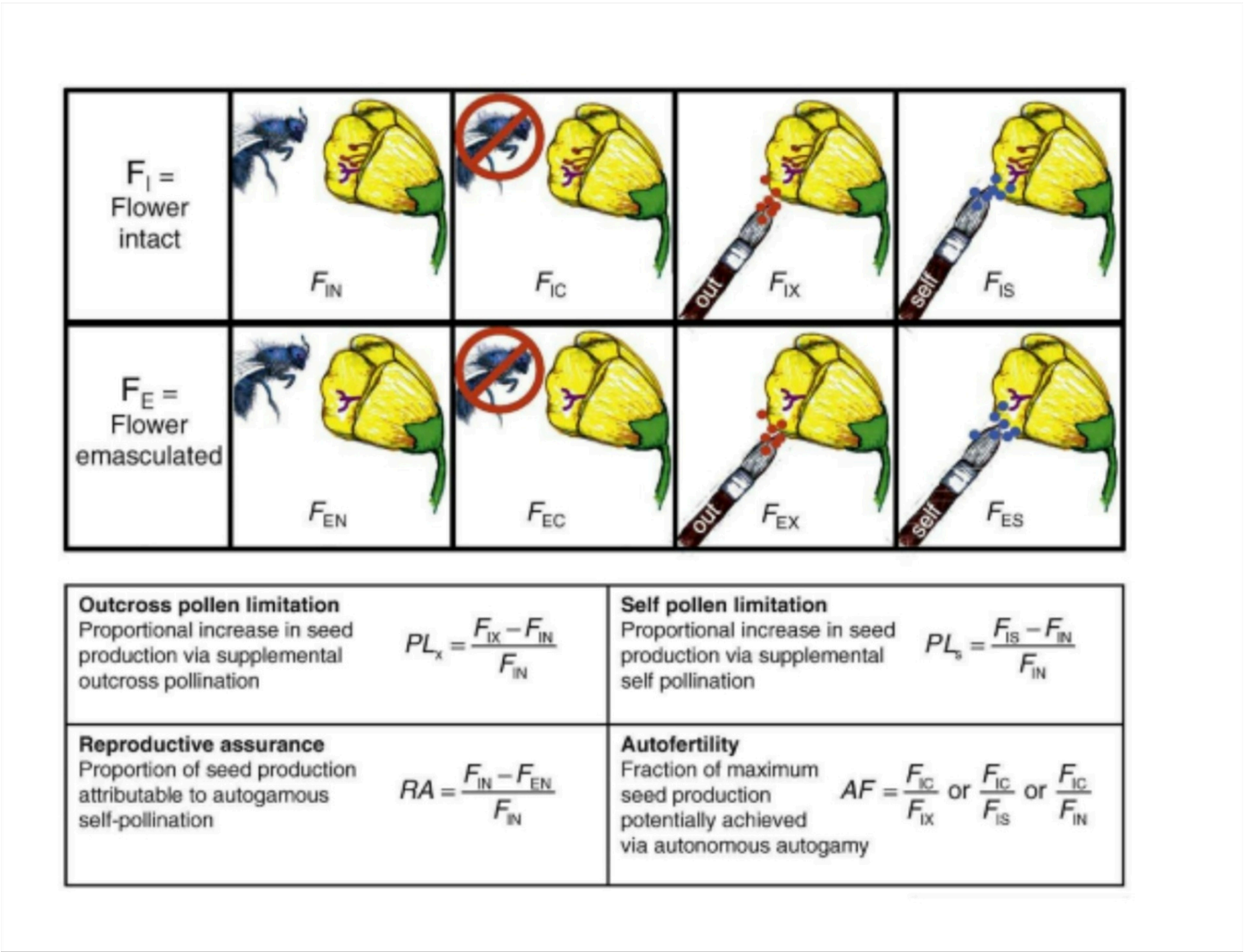


Figure 1

Experimental design to determine the main pollination mechanism. Natural conditions, control (without emasculación): F_{IN} ; natural conditions with emasculación: F_{EN} ; pollinator exclusion, control: F_{IC} ; pollinator exclusion with emasculación: F_{EC} ; manual pollination with cross-pollination, control: F_{IX} ; manual pollination with cross-pollination and emasculación: F_{EX} ; manual self-pollination, control: F_{IS} ; manual self-pollination with emasculación: F_{ES} . Taken from Eckert et al. (2010).