Biotic interactions prior to seed dispersal determine recruitment probability of peyote (*Lophophora diffusa*, Cactaceae), a threatened species pollinator-dependent

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Abstract

Seedlings recruitment is key to maintaining populations and depends on diverse biotic interactions before and after seed dispersal. Cacti recruitment is infrequent and occurs at sporadic pulses associated with rainy years. Furthermore, biotic interactions can limit seeds availability due to scarce or inefficient pollinators, dispersers or to antagonistic interactions with florivores and seed predators, as cactus depend on animals for pollination and dispersal. We identified interactions and traits involved in the regeneration process of *Lophophora diffusa*, during seven-years (2015–2021). We assessed floral rewards, pollination rates, intensity of florivory and seed removal, to estimate the probability of recruitment in a population located in Queretaro, Mexico. We found that pre-dispersal events reduced recruitment as flower–fruit transition has a low probability. High florivory and low pollinator visitation rate due to low nectar availability decreased seed formation in all years. Seed predation was not limiting recruitment, since most seeds escaped predation. Even so, a low proportion of seeds established as seedlings, possibly due to water limitation or seed dormancy. The interactions with pollinators and florivores decreased ovule–seed transition, once the seeds are formed, there is a high probability of evade predation. Hence, pre-dispersal interactions during flowering determine the availability of seeds, and reduce recruitment in peyote, rather than post-dispersal interactions.

Introduction

The recruitment of plant populations, defined as the incorporation of new individuals through the generation of seeds and survival and establishment of seedlings (Eriksson and Ehrlén 2008), has been recognized as the main process limiting population growth for many species (Harper 1977; Rees 1994; Kitajima and Fenner 2000; Silvertown and Charlesworth 2001; Godínez-Álvarez et al. 2003). The recruitment dynamic involves successive steps that can be affected by diverse abiotic and biotic factors (Harper 1977; Houle 1989; Eriksson and Ehrlén 2008; Holland and Molina-Freaner 2012), including limitation of the number of suitable sites for seed germination (Andersen 1989; Franco and Nobel 1989; Eriksson and Ehrlén 1992; Baskin and Baskin 2014), prolonged drought and high temperatures that impede germination and increase the likelihood of death of seedling and juveniles (Nobel 1984; Rey and Alcántara 2000; Traveset et al. 2003; Holland and Molina-Freaner 2012), as well as by the effect of plant–animal interactions that occur during stages of the reproductive cycle, both before and after seed formation; such effect may depend on the type of interaction (mutualist or antagonist), and the frequency with which the interaction occurs (Herrera et al. 1994; Hulme 1997; Herrera 2000; Picó and Retana 2000; Grass et al. 2018).

Population regeneration, as a complex process in the plant demography, requires the simultaneous tracking of a set of plant traits and biotic interactions during flowering, fruiting, germination, and survival of seedlings in order to identify the most critical steps for recruitment in a particular population (Harper and White 1974; Harper 1977; Waloff and Richards 1977; Traveset et al. 2003; Giménez-Benavides et al. 2008). The availability of seeds may be limited by the amount of pollen during a reproductive event as well as by the frequency of pollinators and florivores (Baker et al. 2000; Wilcock and Neiland 2002; McCall

and Irwin 2006). Florivores can limit seed availability by directly consuming ovules and/or pollen, or they can decrease the attractiveness of flowers to pollinators (Kudoh and Whigham 1998; Wilcock and Neiland 2002; McCall and Irwin 2006). In experimental studies, it has been tested whether the exclusion of florivores increases the number of viable seeds for several species (Breedlove and Ehrlich 1968; Louda 1982; Grass et al. 2018).

Studies of how a lack of flower-pollinator interactions affects recruitment dynamics continue to be scarce (but see Herrera 2000; Johnson et al. 2004; Gómez et al. 2007; Lundgren et al. 2015; Rering et al. 2020), even though biotic pollination is a necessary interaction for seed formation for many species (Larson and Barrett 2000; Wilcock and Neiland 2002; Mandujano et al. 2010; Ollerton et al. 2011). It is well known that showy flowers with rewards such as pollen and nectar facilitate connections with pollinators (Bell 1985; Kudoh and Whigham 1998; Krupnick et al. 1999; Kariyat et al. 2021), which could increase the probability of incorporating new individuals into the population (Spira 2001; Rering et al. 2020). However, if flowers are unattractive and/or produce few rewards, pollination could be a limitation on population regeneration (Wilcock and Neiland 2002; Flores-Martínez et al. 2013; Briseño-Sánchez et al. 2020, Rering et al. 2020; Kariyat et al. 2021).

Plant-animal interactions that occur during flowering, can impact the number of individuals that can potentially incorporate into a population and therefore represent a demographic bottleneck for recruitment (Breedlove and Ehrlich 1968; Louda 1982; Mandujano et al. 1996; Herrera 2000). Given the current loss of biodiversity, particularly of pollinator insects (Potts et al. 2010; van der Sluijs 2020), a decrease in the frequency of mutualistic flower-insect interactions during one or more flowering periods could affect the conservation of threatened plants, especially those that have a small number of reproductive individuals at the population level or whose flowers are not attractive to pollinators (Spira 2001; Wilcock and Neiland 2002; Johnson et al. 2004; Rering et al. 2020; Kariyat et al. 2021).

On the other side, plant-animal interactions during post-flowering events, such as predation or dispersal of seeds, have been identified as key points in the process of population regeneration (Dirzo and Domínguez 1986; Howe 1989; Herrera et al. 1994; Kiviniemi and Eriksson 1999; Rey and Alcántara 2000; Godínez-Álvarez and Jordano 2007; Schupp et al. 2010). It is common that a large proportion of seeds from desert plants are depredated by ants or rodents shortly after their formation (Brown et al. 1979; Bowers 2000; Mandujano et al. 2001; Ortiz-Martínez et al. 2021). For example, in *Opuntia rastrera* (Cactaceae), seeds losses are more than 90% in a yearly base (Mandujano et al. 2001), which strongly decreases the probability of recruitment. The nurse-protégé interaction is particularly important in desert plants since it reduces the risk of seed predation and increases the probability of recruitment (Flores and Jurado 2003, García-Chávez et al. 2010), and on many occasions, this nurse-protégé interaction requires biotic agents that disperse the seeds to the safe sites (Montiel and Montaña 2000; Nathan and Müller-Landau 2000; Godínez-Álvarez et al. 2002).

Studies of diverse plant species that link the effects of plant-insect interactions during more than one stage of the reproductive cycle have shown the importance of incorporating the interactions that occur

during flowering, in addition to the dispersal and predation of seeds, to determine the probability of seedling recruitment (English-Loeb and Karban 1992; Eriksson 1995; Picó and Retana 2000; Traveset et al. 2003). At the same time, for some species, such as *Rhamnus ludovici-salvatoris* (Rhamnaceae), post-dispersal seed predation continues to represent the most critical biotic interaction for seedling establishment (Traveset et al. 2003), while the pre-dispersal losses in response to plant-insect interactions, such as florivory, have been pointed out as one of the most immediate causes affecting recruitment in species like *Haplopappus squarrosus* (Asteraceae) (Louda 1982). Also, the effect of plant-animal interactions on the population dynamic can depend on spatial variation associated, for example, with the degree of disturbance among sites (Traveset et al. 2003), and on temporal variation, among other causes, in response to resource availability, or the population density and patterns of activity of mutualists and antagonists between periods (Alcántara et al. 1997; Krupnick et al. 1999; Nathan et al. 2000).

In addition to post-flowering interactions (seed dispersal and seed predation), we hypothesized that the interactions prior to seed dispersal (pollination and florivory) may significantly affect the probability of recruitment of cacti such as peyote, *Lophophora diffusa* (Cactaceae). This species is a perennial plant with low production of pollen and nectar that is pollinated by insects (Díaz-Segura et al. 2017; Briseño-Sánchez et al. 2020). A loss of nearly half of its populations has been reported, mainly due to land use change (Díaz-Segura et al. 2012). It is possible that the low production of floral rewards in *L. diffusa* affects its pollination success and exacerbates the negative effects of florivory and seed predation, limiting seed availability in the population. To identify the most critical stages in the recruitment process, we determined the probability of transition between consecutive events from flowering through seedling survival, for one population over the course of seven years. Specifically, we addressed the following questions: (1) Is the production of seeds limited by plant-pollinator and plant-florivore interactions? (2) What proportion of seeds is removed? (3) What is the probability of transition from ovule to seedling? and, (4) Does the critical process for recruitment differ among years?

Materials And Methods

Study System

Lophophora diffusa is a species that is endemic to the Chihuahuan Desert (Hernández-Magaña et al. 2012). Its populations are restricted to the arid zones of Querétaro, Mexico (Anderson 1969), between 1300 and 1600 m a.s.l. (Gómez-Hinostrosa et al. 2017). The species is xenogamous with facultative intercrossing and requires biotic pollination to form fruits and seeds but suffers from pollen limitation (Briseño-Sánchez et al. 2020). Its flowering period is extensive, with two or more flowering peaks per year (Díaz-Segura et al. 2017; Briseño-Sánchez et al. 2020). The flowers have a one day anthesis, rarely up to three days, the production of floral rewards such as pollen and nectar is low, and the flowers interact with diverse insects such as beetles, orthopterans, and solitary bees, the last of which are considered the main pollinators for the species (Díaz-Segura et al. 2017; Briseño-Sánchez et al. 2020). The fruits are small in size and mature approximately two months after pollination, this species produces few seeds, generally

fewer than 40 seeds per fruit (Díaz-Segura et al. 2017; Briseño-Sánchez et al. 2020). The seeds have an elaiosome and an impermeable coating that allows them to float (Trujillo-Hernández 2002; Ríos-Carrasco 2017; Ríos-Carrasco et al. 2019), such that they may be dispersed by ants or by water during rains. The seeds are also dispersed by gravity close to the mother plant (personal observation). The area occupied by the species has been reduced from 775 km² to 50 km², according to the report by Díaz-Segura and collaborators (2012). Currently it is considered a vulnerable species (VU) (IUCN, Gómez-Hinostra et al. 2017), and a species in danger of extinction (P) on the Mexican red list (NOM-059, SEMARNAT-NOM-ECOL-2010).

This work was carried out between January, 2015 and November, 2021 in a population located at 20°57–21°14' north latitude and 99°42'–100°02' west longitude, at an altitude of 1438 m a.s.l. (locality Peñamiller, Querétaro). The vegetation type is microphyllous xerophytic scrub, dominated by shrubs such as *Larrea tridentata*, *Fouquieria splendens*, *Mimosa* sp., and *Bursera* sp., among others (Zúñiga et al. 2005). The population of *L. diffusa* is composed of seedlings, juveniles, and a percentage of reproductive individuals above 50% with spatially aggregate, distribution with a density of 0.79 individuals/m² (Briseño-Sánchez et al. 2020).

Nectar production and pollinator observations

Nectar production was measured during the reproductive season of 2018 ($n = 70$ flowers, 62 individuals). We excluded floral visitors using tulle bags and recorded the presence of nectar after the opening of each flower. We used 1 µL capillary tubes to measure nectar volume. We obtained the percentage of flowers with the presence of nectar as well as the mean and standard error of the sample of flowers with nectar (Shivanna and Tandon 2014).

To determine the rate of pollinator visitation, we carried out observations during the 2017, 2018, and 2021 reproductive seasons. A pilot study showed that the number of visitors was too low to carry out observations of individual flowers (Briseño-Sánchez et al. 2020), so we observed groups of flowers, each group of flowers had 3 to 6 plants, and 3 to 11 flowers, recording visitors between 10:00 h and 14:00 h for one 15-minute interval each hour (this schedule was established because it was the time of peak activity of the pollinators). During each observation period, we recorded the number of contacts that a pollinator had with the anthers and/or stigma of the flowers, and we recognized the identity of the floral visitors to establish differences in the pollinator guilds among reproductive seasons. The rates of pollinator visits were considered as the number of flowers of *Lophophora diffusa* visited per minute. The pollinator–flower contacts per season were analyzed using a generalized linear model with a Poisson error distribution for the frequencies (Crawley 1993). Given the low number of visits, the observations were grouped into two intervals for the analysis: 1 (10:00–12:00) and 2 (12:00–14:00), with 30 minutes of observation per interval.

Pollination and florivory

The study population is found in an area of approximately 20 × 30 m ($N = 468$ individuals). Each plant was labelled at the beginning of the study, with a total of 340 reproductive individuals (plants > 3 cm in diameter). We carried out censuses throughout the year to recognize the reproductive seasons between January 2015 and November 2021. During each census we recorded the number of buds, open flowers, wilted flowers, and fruits for each plant. *Lophophora diffusa* requires biotic vectors to move pollen between flowers (Briseño-Sánchez et al. 2020), so as a measure of pollination success, we calculated the *fruit set* (proportion of the total flowers that formed fruits), and the *seed set* (the proportion of the total ovules that formed seeds) (Traveset et al. 2003; Giménez-Benavides et al. 2008). In addition, each year we collected at least 10 mature fruits in 4 × 8 cm glassine paper bags. In the laboratory, we counted the seeds per fruit and calculated the average.

To determine the effect of florivory on individual reproductive success, we recorded the proportion of flowers that presented damage during the flowering period in 2021 ($n = 92$ reproductive individuals). The type of florivory was classified according to the extent of the damage as follows: (a) consumption of the perianth and anthers, (b) consumption of the perianth and stigma, or (c) consumption of the perianth, stigma, and anthers. We monitored the flowers until fruit formation to calculate the *fruit set*.

Seed removal experiment

During the spring of 2021, we carried out an experiment under natural conditions to determine the proportion of seeds that is removed (regardless of their fate) as well as the proportion of seeds that is depredated. We collected fruits from at 25 different individuals and established a random block design with three conditions: (a) protected seeds, (b) seeds exposed to ants, and (c) seeds exposed with no protection. We placed 20 replicates with five seeds per experimental unit for each of the three conditions ($n = 60$ experimental units). For the protected seeds condition we used cellulose cones covered with plastic mesh; the seeds exposed to ants condition also consisted of cellulose cones covered with mesh, but the mesh was perforated to allow access to dispersers like ants; the exposed seeds condition consisted of thin strips of plastic mesh to which we glued five seeds using a drop of silicone glue; the strips were anchored to the substrate using nails, ensuring that the seeds were in contact with the substrate (Ortiz-Martínez et al. 2021). The number of seeds remaining on each experimental unit was checked every other day for two weeks. A seed was considered to be depredated if the seed was found empty and with remnants of the testa in the experimental unit. The results of the seeds remaining was compared among the three conditions using a generalized linear model, with a Poisson error distribution for the counts (Crawley 1993).

Seedling emergence and survival

We carried out two censuses each year from 2015 to 2021 to record the emergence of seedlings. We carried out intensive searches in the whole area occupied by the population, paying special attention near adult plants and under shrubs that are considered nurse plants (Zúñiga et al. 2005). We recorded the number of seedlings that emerged per year as well as the proportion of seedlings that survived to the following year.

Transition probabilities (TP)

The probability that an ovule would form a seedling was determined based on a series of sequential stages from pollination through seedling survival for each year. We defined each of these transition probabilities (TP) as the probability that an event would occur:

$$TP = \frac{n}{N}$$

where n represents the number of successful results of an event and N is the total of equally probable results (Gotelli and Ellison 2013). We calculated a total of five transition probabilities:

TP₁: Probability of transition from flower to fruit. That is, the number of fruits in relation to the number of flowers produced each year.

TP₂: Probability of transition from ovule to seed. Based on the average number of ovules per flower reported for this species (32.5 ± 1.27 , Briseño-Sánchez et al. 2020), we determined the *seed set* as the proportion of ovules that formed seeds each year.

TP₃: Seed survival. For this stage we considered two possible scenarios:

1. Intense seed predation: under the assumption that all of the seeds that are removed are depredated, the probability that a seed survives and is available to germinate is equal to the number of seeds remaining in relation to the total seeds included in the seed removal experiment.
2. Moderate seed predation: the probability that a seed survives and is available to germinate is equal to the number of seeds without signs of predation in relation to the total seeds included in the seed removal experiment.

TP₄: Probability of transition from seed to seedling. Number of seedlings recorded in relation to the number of seeds produced per year. For this stage we considered two possible scenarios of seed survival (Intense seed predation and Moderate seed predation).

TP₅: Probability of seedling survival. That is, the proportion of seedlings that survived to be recorded the following year.

We estimated the cumulative probability of recruitment for each year (CP) as the product of all of the transition probabilities (TP) involved. Each TP was considered an independent event (Gotelli and Ellison 2013):

$$CP = TP_1 \times TP_2 \times TP_3 \times TP_4 \times TP_5$$

We used the data from the seed removal experiment from 2021 as fixed estimator and used these results to estimate the cumulative probability of recruitment for each year. The CP represented the probability

that an ovule from a flower in anthesis would become a seedling. In addition, we estimated the coefficient of variation (CV) for each of the estimated transition probabilities (Gotelli and Ellison 2013).

Results

Nectar production and pollinator observations

In 78% of the flowers we found the presence of nectar and the remaining flowers did not produce this reward. The average volume of nectar per flower was $0.054 \mu\text{L} \pm 0.007$ ($\bar{x} \pm \text{SE}$), with a maximum value of $0.36 \mu\text{L}$. However, the production of nectar per flower rarely exceeded $0.1 \mu\text{L}$.

With respect to pollinator activity, in 2017 we recorded 36 visits, of which 29 events had behaviors indicating search for pollen and/or nectar by the insects which included at least one flower of *L. diffusa* ($n = 50$ flowers, 29 plants). In 2018, we recorded 13 visits with 10 pollen and/or nectar search events by insects ($n = 18$ flowers, 16 plants), while for 2021, the number of contacts recorded between pollinators and flowers was 28 of a total of 33 visits ($n = 53$ flowers, 33 plants). The pollinator guild consisted of one beetle (*Acmaeodera* sp.) and several species of solitary bees (*Macrotera* sp., *Lasioglossum* (*Dialictus*) sp., *Ashmeadiella* sp., and one species from the subfamily Halictidae). The majority of the visits during 2017 and 2021 were by the bee *Macrotera* sp., with 41% and 95% in each year, respectively, while in 2018 most of the visits were by the beetle *Acmaeodera* sp., with 70% (Fig. 1). The number of flowers visited by pollinators per minute (that is, the visitation rate, $\bar{x} \pm \text{SE}$), was higher in 2017 and 2021, with $0.1 \text{ visits/min} \pm 0.027$ and $0.098 \text{ visits/min} \pm 0.023$, respectively. Meanwhile in 2018 the visitation rate was lower, with $0.039 \text{ visits/min} \pm 0.016$. The generalized linear model showed differences in the number of pollinator–flower contacts among reproductive seasons (years) ($\chi^2 = 11.16$, $\text{df} = 2$, $P < 0.05$). In 2018 we recorded significantly fewer pollinator–flower contacts than in 2017 ($\chi^2 = 9.07$, $\text{df} = 1$, $P < 0.0025$) and 2021 ($\chi^2 = 9.49$, $\text{df} = 1$, $P < 0.002$).

Pollination and florivory

The pollination success, measured as the proportion of flowers that form fruits, was highly variable between study seasons (Table 1). In general, less than half of the flowers produced fruits, with the exception of the 2019 reproductive season, which had a value of 0.62. The same pattern was observed for the *seed set*, with less than half of the ovules forming seeds for most of the reproductive seasons, except for 2019. Although 2019 was the year with the highest *fruit set* and *seed set* values, it was also the year with the lowest total flower production and the second lowest in terms of seedlings.

Table 1

Reproductive and recruitment success of the *Lophophora diffusa* population (Peñamiller, Querétaro, Mexico). Measured as the proportion of flowers that formed fruits (*fruit set*), the proportion of ovules that formed seeds (*seed set*), and the number and survival of seedlings recorded in a 20 × 30 m area during the reproductive seasons from 2015 through 2021 ($N = 468$ individuals). We present the values of $\bar{x} \pm SE$ in parentheses for the number of fruits per individual and the number of seeds per fruit.

Reproductive period	Flower production	<i>Fruit set</i> (Fruits per individual)	<i>Seed set</i> (Seeds per fruit)	Seedlings	Survival of seedlings (Proportion)
2015	462	0.443 (1.79 ± 0.1)	0.36 (26.37 ± 0.95)	21	0.8
2016	562	0.222 (1.62 ± 0.13)	0.191 (27.95 ± 2.55)	21	0.66
2017	783	0.263 (1.73 ± 0.11)	0.229 (28.33 ± 2.31)	31	0.64
2018	835	0.445 (2 ± 0.11)	0.395 (28.88 ± 2.38)	30	0.66
2019	115	0.626 (1.16 ± 0.05)	0.52 (27.1 ± 1.15)	5	1
2020	162	0.259 (1.61 ± 0.14)	0.219 (27.53 ± 0.41)	1	1
2021	423	0.328 (1.73 ± 0.15)	0.268 (26.56 ± 2.69)	18	0.99

With respect to florivory, 23% of the flowers in the 2021 reproductive period showed some degree of florivory ($n = 113$ flowers, 92 reproductive individuals). The highest proportion corresponded to flowers with damage to the perianth and anthers (0.57), while damage that included consumption of the perianth, anthers and stigma was recorded in a lower proportion (Table 2). The reproductive success was null for flowers with the most intense florivory category (Table 2c), while a very small proportion achieved the transition from flower to fruit in the other two florivory categories (Table 2a and b).

Table 2

Type of florivory and frequency recorded at the peak of flowering in June 2021 in the *Lophophora diffusa* population (Peñamiller, Querétaro, Mexico). A total of 113 flowers from 92 individuals were assessed, and 23% of the flowers presented florivory ($n = 26$ flowers, 19 individuals).

Type of florivory	Proportion	Fruit set
(a) Damage to the perianth and anthers	0.57	0.26
(b) Damage to the perianth and stigma	0.23	0.16
(c) Damage to the perianth, anthers and stigma	0.2	0

Seed removal experiments

The probabilities of seed removal and predation were very low; of the total seeds, 13% of the seeds were removed, and only 0.66% presented some sign of predation. Although empty testas (considered a sign of seed predation) were only recorded in the exposed seed treatment, it is possible that seeds that were completely removed were also depredated. The removal of seeds was observed even in the protected seed treatment, possibly by very small ants that were able to pass through the mesh (with 8% of the seeds removed). The rest of the seeds that remained in the experiment (86.33%) would be available to germinate or be removed later. The generalized linear model did not show significant differences in seed removal among the three experimental conditions ($\chi^2 = 0.55$, $df = 2$, $P = 0.75$).

Seedling emergence and survival

We recorded a total of 127 seedlings during the study period with a maximum value of 31 seedlings in 2017, and only one seedling recorded in 2021. In general, more than half of the seedlings recorded in one period were present in the following year (Table 1).

Transition probabilities (TP)

A summary of the TP among each of the stages considered in this study is presented in Table 3. The potential recruits decreased to reach just 19% of the original number of ovules by 2016 and around 50% of the original number of ovules by 2019, before the seed dispersal stage due to the low proportion of flowers that result in fruits as well as the low proportion of ovules that form seeds. The mutualistic (pollination) and antagonistic (florivory) interactions during the reproductive season, in conjunction with the transition from seed to seedling, were the most critical stages in the recruitment process in all years analyzed in this population. The survival of the seedlings after one year was generally high in all study seasons ($> 50\%$). The CP was highest for 2019, indicating that an ovule had a 0.0008 probability of surviving as a seedling for one year.

Table 3

Recruitment dynamics in the *Lophophora diffusa* population (Peñamiller, Querétaro, Mexico), in the period from 2015 through 2021. The transition probabilities (TP) between the stages considered were TP₁: Probability of flower–fruit transition, TP₂: Probability ovule–seed transition, TP₃: Seed survival, Scenario a. Intense seed depredation and Scenario b. Moderate seed depredation, TP₄: Probability of seed–seedling transition, TP₅: Seedling survival. We also show the cumulative recruitment for each year (CP): Scenario a. Intense seed depredation and Scenario b. Moderate seed depredation, and the coefficient of variation (CV) for each TP.

Transition probabilities (TP)	2015	2016	2017	2018	2019	2020	2021	CV
TP ₁ Flower-Fruit transition	0.44	0.22	0.26	0.44	0.62	0.25	0.32	39.39
TP ₂ Ovule-Seed transition	0.36	0.19	0.22	0.39	0.52	0.21	0.26	39.41
TP ₃ Seed survival	Scenario a. 0.863 Scenario b. 0.993							
TP ₄ Seed-Seedling transition	4.40E-03	6.90E-03	6.15E-03	4.20E-03	2.96E-03	1.00E-03	5.60E-03	45.14
Scenario a	3.90E-03	6.05E-03	5.34E-03	2.81E-03	2.57E-03	8.70E-04	4.90E-03	47.99
Scenario b								
TP ₅ Seedling survival	0.8	0.66	0.64	0.66	1	1	0.99	20.94
CP	4.80E-04	1.64E-04	1.92E-04	4.10E-04	8.00E-04	4.532 E-05	3.90E-04	73.6
Scenario a.								73.61
Scenario b.	4.90E-04	1.65E-04	1.94E-04	3.10E-04	8.20E-04	4.536E-05	4.00E-04	

Discussion

The probability of transition from ovule to seedling in *Lophophora diffusa* was modulated by mutualistic and antagonistic interactions that were present from flowering to seedling recruitment. The interactions with pollinators and florivores were important for the ovule-seed transition, once the seeds are formed, they were a high probability of remaining and escaping predation. Prior to seed dispersal, the production of floral rewards, pollination, and florivory was factors that influenced the seedling recruitment of *L. diffusa*. The production of nectar was present in more than half of the flowers, however the volume of this reward is very small, which could represent a limitation for pollinators to move pollen among flowers. Dimorphism in nectar production has been reported in the family Cactaceae (*e. g.*, *Mammillaria huitzilopochtli*). As well as *L. diffusa*, some flowers of *M. huitzilopochtli* do not produce this reward and

the amount of nectar in the flowers that do produce it may not be sufficient to ensure flower–pollinator contact (Flores-Martínez et al. 2013).

In arid environments, hydric stress can affect nectar production, decrease flower-pollinator interactions, and reduce the reproductive success of plants (Rering et al. 2020). In addition, in species that have suffered reductions in their populations, the presence of inbreeding may affect floral traits such as nectar production, and affect negatively pollinator visits (Kariyat et al. 2021). In species pollinated by insects, the formation of fruits may depend on the presence of pollinators, their population densities, and the patterns in which they search for food (Herrera 2000; Ollerton et al. 2011; Lundgren et al. 2015; Grass et al. 2018). *L. diffusa* showed pollinator visitation rates well below those reported for other insect-pollinated species (e. g., Fijen and Kleijn 2017; Latif et al. 2019). Overall, less than half of the *L. diffusa* flowers formed fruits, which leads us to consider that low production of floral rewards and lack of pollination may be limiting seed formation. An average of 60% of the ovules are not pollinated or are aborted in intact flowers, that is, without damage caused by florivores, which strongly reduces the probability of ovule-seed transition. A previous study showed that the addition of pollen in the flowers of *L. diffusa* can increase seed formation by 20% due to the pollen limitation suffered by the species (Briseño-Sánchez et al. 2020). Although it is not the main factor limiting the flower–fruit transition, florivory also affects the reproductive success of *L. diffusa* individuals, probably exacerbating the low attraction of pollinators, which frequently occurs in insect-pollinated species that present damage to reproductive tissues (Schemske and Horvitz 1988; Krupnick et al. 1999; Herrera 2000; McCall e Irwin 2006; Grass et al. 2018).

During the post-flowering events, seed predation is recognized as one of the main antagonistic interactions that impedes plants populations growth (Louda 1982; Godínez-Álvarez and Jordano 2007; Holland and Molina-Freaner 2012). Contrary to expectations, for *L. diffusa*, seed predation after dispersal is apparently low; the results of the present study suggest that seed predation does not represent a strong limitation on seedling recruitment, since the majority of the seeds escaped depredation, unlike other species that develop in arid environments in which high proportions of seeds are reported to be depredated (Mandujano et al. 2001; Traveset et al. 2003; Godínez-Álvarez and Jordano 2007; Ortiz-Martínez et al. 2021). Notwithstanding, seed predation could be higher in other years, since it has been described that the dynamics of predators like ants and rodents are not consistent over time (Hulme 1997; Picó and Retana 2000; Traveset et al. 2003). It is therefore necessary to continue to carry out these experiments under natural conditions to better understand the effects of temporal variation.

In addition to biotic interactions like pollination that determine the availability of seeds, the recruitment probability may also depend on precipitation patterns. It has been reported that the lack of rain mainly affects germination and could lead to the death of seedlings (Rey and Alcántara 2000; Traveset et al. 2003). Water stress has been recognized as an important limiting factor determining the emergence and survival of seedlings in arid zones (Nobel 1984; Godínez-Álvarez et al. 2003; Holland and Molina-Freaner 2012). Although the survival of the seedlings from one year to the next was above 50%, the transition from seed to seedling was very low, probably due to a lack of sites with the moisture and requirements

necessary for germination. The processes after seed dispersal also impacted the recruitment probability, especially the seed–seedling transition, which was below germination rates reported in the laboratory (0.0045 compared to > 70%, Rojas-Aréchiga et al. 2013), which suggests that abiotic factors such as water stress also play a determining role in the growth of this population.

Conclusion

This study supports the idea that mutualistic and antagonistic plant–insect interactions, can limit the probability of recruitment from flowering to after seed dispersal. Our results suggest that interactions that occur during the flowering season are decisive for the availability of seeds, and could more strongly reduce the chances of recruitment in *Lophophora diffusa*, than post-dispersal interactions. In threatened species with restricted populations that have suffered a decrease in the number of adult individuals over recent years, the limitation of pre-dispersal transition probabilities may represent the most critical events for population conservation. In particular, low production of rewards and low rates of visits from pollinators before dispersion, combined with the low transition from seed to seedling due to water stress in arid zones, represent critical stages in the recruitment dynamic of *L. diffusa* over the 7 years of the study.

Declarations

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Figures

Figure 1

Pollinator guild for the *Lophophora diffusa* population (Peñamiller, Querétaro, México) during the reproductive periods of 2017, 2018 and 2021. The proportion of visits of each of the pollinators recorded in each period is presented.