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The role of nocturnal and diurnal pollinators in the commercial production of Dragon Fruit crops in Costa Rica

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ABSTRACT

Introduction: Dragon fruit (or pitahaya) cultivation is of growing interest in Central America. Several species of the genus *Selenicereus* (Cactaceae) are cultivated around the world, with *S. costaricensis* being the only species native to this region. The pollination syndrome of the species partially matches chiropterophily and phalaenophily, but floral visitors and their effect on commercial fruit production is not well known.

Objective: To analyze the reproductive system, the floral visitors and their effect on fruit size in plantations of *S. costaricensis* in Costa Rica.

Methods: We recorded the anthesis period, stigmatic receptivity, anther dehiscence and nectar production. We conducted pollination experiments (manual self- and cross-pollination) and evaluated the effect of nocturnal and diurnal natural pollination on fruit production and traits related to fruit size and weight. Flower visitors were filmed, identified, quantified, and their behavior was documented.

Results: Plants were self-compatible. All pollination treatments produced viable fruits, but there were differences in fruit weight and size. Natural pollination, nocturnal and diurnal combined, produced the biggest fruits, while self-pollination the smallest. The fruits generated by either nocturnal or diurnal pollinators were of good commercial size. Hawk moths and bees were the main pollinators; no visits by bats were detected. The flowers produced no measurable nectar volume. Mass visitation by bees in periods of 15–20 minutes at dawn was sufficient to produce large fruits.

Conclusions: Diurnal and nocturnal pollinators are equally effective producing highly profitable fruits. The management of *Apis mellifera* and native solitary bee species is recommended to increase production efficiency in these plantations. *Manduca rustica* hawk moths seem the natural pollinators of *S. costaricensis*, and bats played no role in the production of fruits.

Keywords: Pitahaya, reproductive biology, reproductive system, fruit production, hawk moth pollination, bee pollination, *Selenicereus costaricensis.*

RESUMEN

El papel de los polinizadores diurnos y nocturnos en la producción comercial del cultivo de pitahaya en Costa Rica

Introducción: El cultivo de la pitahaya es de creciente interés en Centroamérica. Varias especies del género *Selenicereus* (Cactaceae) se cultivan alrededor del mundo, siendo *S. costaricensis* la única especie nativa de esta región. El síndrome de polinización de la especie coincide parcialmente con la quiropterofilia y la falenofilia, pero los visitantes florales y su efecto en la producción comercial no son bien conocidos.

Objetivo: Analizar el sistema reproductivo, los visitantes florales y su efecto en el tamaño de los frutos en plantaciones de *S. costaricensis* en Costa Rica.

Métodos: Medimos el período de antesis, la receptividad estigmática, la dehiscencia de las anteras y la producción de néctar. También realizamos experimentos de polinización (autopolinización manual y polinización cruzada manual) y polinización natural nocturna y diurna, y evaluamos su efecto en la producción de frutos y en su tamaño y peso. Los visitantes florales fueron filmados, identificados, cuantificados y su comportamiento fue documentado.

Resultados: Las plantas fueron auto compatibles. Todos los tratamientos de polinización produjeron frutos viables, pero hubo diferencias en su tamaño. La polinización natural, nocturna y diurna combinadas, produjo los frutos más grandes y la autopolinización manual los más pequeños. Los frutos generados por polinizadores diurnos o nocturnos fueron de un buen tamaño comercial. Las flores no produjeron un volumen de néctar medible. Las visitas masivas de abejas en periodos de 15-20 min al amanecer fueron suficientes para producir frutos grandes.

Conclusiones: Los polinizadores diurnos y nocturnos son igualmente efectivos produciendo frutos altamente rentables. Se recomienda el manejo de *Apis mellifera* y especies de abejas solitarias nativas para aumentar la eficiencia de producción en estas plantaciones. El esfíngido *Manduca rustica* parece ser el polinizadores natural de *S. costaricensis*. Los murciélagos no jugaron ningún papel en la producción de frutos.

Palabras clave: Pitahaya, biología reproductiva, sistema reproductivo, producción de frutos, esfíngidos, abejas, *Selenicereus costaricensis*.

INTRODUCTION

Animal pollination is highly important at an ecological level (Kearns et al., 1998). The number of flowering plant species pollinated by animals is > 87% worldwide and, in tropical communities, it can reach 95% (Ollerton et al., 2011).

Ecosystem services provided by pollinators around the globe have been studied with the aim of quantifying the contributions of animals to human activities (Lautenbach et al., 2012; Porto et al., 2020). Crop pollination has considerable economic value (Ricketts et al., 2004; Giannini et al., 2015; Borges et al., 2020; Porto et al., 2020; Basualdo et al., 2022), and it is estimated that two-thirds of crops require animal-assisted pollination (Nabhan & Buchmann, 1997; Kevan & Phillips, 2001). Moreover, several crops can increase their production when supported by animal-assisted pollination, which is beneficial for the fruit and seed trade (Klein et al., 2007; Garibaldi et al., 2013).

The notion of pollination syndromes is the foundation of multiple studies regarding floral diversity and pollination ecology, and their accuracy in predicting plant pollinators has been recently reviewed (Rosas-Guerrero et al., 2014). However, plants may also benefit from secondary pollinators (Johnson & Steiner, 2000; Fenster et al., 2004), and pollination syndromes have been discussed several times, especially when there is doubt or mismatch between predicted and observed pollinators (Waser et al., 1996; Ollerton et al., 2009; Valverde-Espinoza et al., 2021). How this mismatch affects the economic revenue and management of commercial crops requires further exploration. Different groups of pollinators can benefit from different management strategies in the local landscape in agroecosystems or might depend on the preservation of natural spaces linked to plantations (Klein et al., 2007).

In the Cactaceae family, chiropterophily is commonly reported in species with nocturnal anthesis with records for more than 150 species (Vogel, 1968; Dobat & Peikert-Holle, 1985). There are some cases where species considered ‘bat-pollinated’ have mixed systems, usually involving other animals such as insects from different orders, both diurnal and nocturnal (Muchhala et al., 2009; Queiroz et al., 2015; Queiroz et al., 2016; Muñiz et al., 2020). Phalaenophily (pollination by moths) has also been reported in Cactaceae, and Haber (1983) mentioned that hawk moths (Sphingidae) are pollinators of *S. costaricensis* in natural populations. *Selenicereus costaricensis* flowers present ambiguous traits between chiropterophily and phalaenophily (sensu van der Pijl, 1961). The flowers are well exposed, large and strong, creating good space for landing, with large amounts of pollen, such as those of chiropterophilous species, but are also sweetly perfumed and do not contain large amounts of nectar as sphingophilous flowers. However, the peduncle is wide and does not form a clean tube, such as in sphingophilous flowers, and the bulky floral morphology avoids hovering as a way to reach any reward. Another important aspect to consider is that although anthesis is nocturnal, preliminary observations have shown that these flowers remain open for a brief period after dawn, allowing visitation by diurnal animals in plantations (MFO, personal observations), but this seems not to happen in natural populations (Haber, 1983).

Very few cacti species are commercially planted for their fruits, with dragon fruits or pitahayas (such as *S. undatus, S. megalanthus, and S. costaricensis*) being the most relevant product of this kind. Understanding the pollination mechanisms of these species is important for the management of plantations to increase their productivity.

This work explored the contribution of floral visitors to the pollination of commercially planted individuals of *Selenicereus costaricensis*, the Costa Rican pitahaya, a species of commercial interest typically associated with nocturnal pollinators. We characterize the impact that different groups of pollinators have on crop production and fruit traits related to their commercial value. Our hypothesis is that secondary pollinators (diurnal insects) contribute to the pollination of this dragon fruit species and have an impact on commercial production, measurable through fruit weight and size. This will lead to a better understanding of the pollination dynamics of crop cacti, contributing to the development of better production strategies that will benefit local fruit producers.

MATERIALS AND METHODS

Study site: The study was conducted in three commercial plantations in Costa Rica in the surroundings of Liberia, Guanacaste province: Curubandé (10°43’46.14”N, 85°25’31”W; 322 m asl; 0.5 ha) and El Salto (10°35’01.518”N 85°22’53.46”W; 305 m asl; 0.75 ha), both in the lower premontane forest transitional to dry forest life zone (Holdridge, 1967). The third plantation, Plancillo, is in the borders of the Central Valley (Alajuela province, Atenas: 9°58’54.96”N, 84°25’4.848”W; 970 m asl; 0.5 ha) in the transitional humid premontane forest to dry forest life zone (Holdridge, 1967).

The Curubandé plantation is next to the Rincón de la Vieja National Park and is surrounded by large patches of forest and small towns. El Salto is in a more disturbed area near large farms, cattle ranches, and towns, but still surrounded by small patches of gallery forests. Both plantations consist of evenly distributed wooden or cement poles where the plants climb up to approximately 1.5 m from the ground (Fig. 1). The Curubandé plantation is composed mostly of genetically different plants (plants with different origins according to the farmers) and a small portion of clones; the El Salto plantation is made up mostly of clones. The Plancillo plantation consists of small living trees grouped along several linear paths where the cacti climb, ranging from ground level to approximately 2 m tall. This plantation is in a rural area and is surrounded by small cattle farms, houses, streets and patches of gallery forests. This plantation has a similar proportion of properly identified genetically different plants and vegetatively propagated clones.

The mean annual precipitation in Liberia is 1 653 mm (range: 1 300-1 700). The mean annual temperature is 27.5 °C (Villalobos & Retana, 2 000). The annual precipitation at Plancillo varies between 1 700 and 2 500 mm per year (mean 2 000 mm). The average annual temperature is 22 °C. The marked dry season lasts from the end of November to mid-May, and the rainy season lasts for the remainder of the year.

Fig. 1. Commercial dragon fruit (pitahaya) plantations in (A) Curubandé, Liberia, Guanacaste (open flowers in the early morning); (B) Plancillo, Atenas, Alajuela (late morning, after flower withering); and (C) El Salto, Liberia, Guanacaste (early morning) in Costa Rica.

The studied species: *Selenicereus costaricensis* (F.A.C. Weber) S. Arias & N. Korotkova (formerly in the genus *Hylocereus*) is a pitahaya or dragon fruit, native from Nicaragua to Panama (Hammel, 2020). This epiphytic or hemiepiphytic cactus develops an elongated triangular stem and is characterized by hermaphroditic, white, aromatic flowers with herkogamy and nocturnal anthesis that lasts for only one night. The large funnel-shaped flowers are approximately 30 cm in length and diameter at the corolla mouth, with a high number of stamens (Fig. 2a). These plants are prone to pollination by nocturnal animals (Haber, 1983).

In plantations from Costa Rica, flowering occurs during the rainy season (in a five-month period between May and September) in three to seven massive bloom events, lasting approximately three nights each. Fruits are ellipsoidal fuchsia false berries with fleshy bracts and numerous small seeds imbedded in a fleshy magenta or reddish purple mesocarp (Mizrahi et al., 2002; Centurión-Yah et al., 2008; Fig. 2b). The fruits of *S. costaricensis* are of commercial interest for human consumption, ranging between 0.1 and > 0.7 kg, and are of growing economic importance in Mesoamerica.

All the following methodologies were implemented in the three plantations. The data were collected during eight mass bloom events between 2019 and 2020 (Supplementary Table S1).

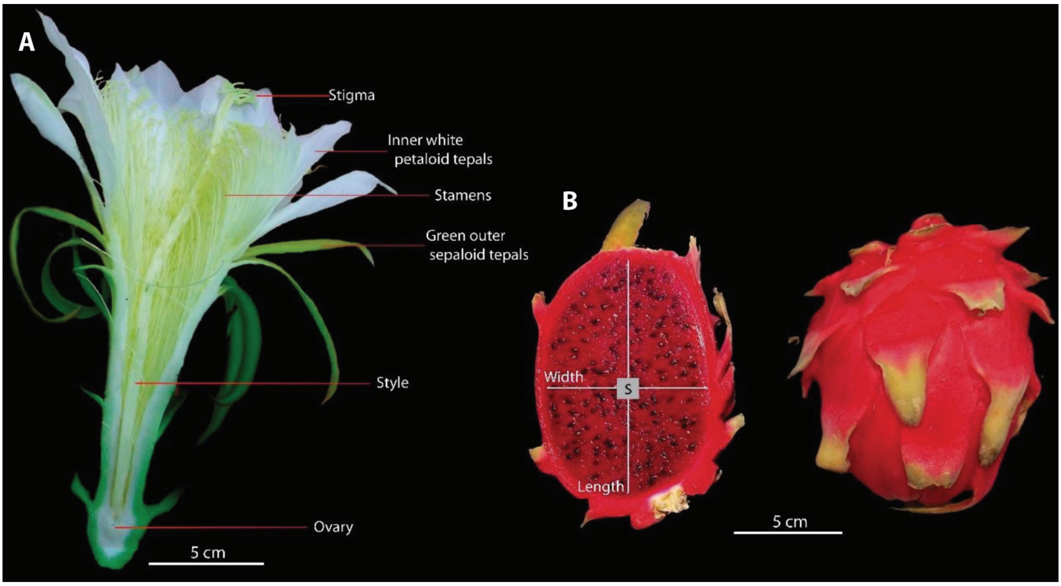

Fig. 2. Flower and fruit of *Selenicereus costaricensis* (Cactaceae). **A.** Longitudinal section of a flower. **B.** Ripe fruit dissected (left) and in its original appearance (right); s= area where the pulp cubes were extracted for seed counting.

Anthesis period: The anthesis period was divided into three stages: 1. opening of the bud, 2. full extension of the flower tepals, and exposure of the androecium and gynoecium, and 3. corolla decay and wilting. The data from 345 flowers were recorded for flower opening, and those from 325 flowers were recorded for wilting. The flowers were marked in the petiole with flagging tape, and observations were made every 10 minutes from 18:00 h - 21:00 h to record the start time and duration of stages 1 and 2. Observations resumed at 6:00 h and continued until 8:00 h for stage 3. The percentage of flowers in each stage was calculated for each observation time.

Stigmatic receptivity and anther dehiscence: Observations were made every hour on open flowers from 18:00 h until midnight, since few changes were observed after 20:00 h. One more observation was done at 4:00 h to check the late state of the flower. Anthers dehiscence was inspected visually with a magnifier lens (30X) to determine when the pollen was available. To determine receptivity, two small drops of hydrogen peroxide (H_2O_2) were placed on the stigma, and any evidence of bubbling was observed with a magnifying glass (30X) as an indication of enzymatic activity. The stigma is large in *S. costaricensis* (see Fig. 2) and has a lobulated structure that allows placing of small drops at each sampling time in different sections of the stigma and tracking the same flower for a long period. The percentages of receptive flowers and flowers with available pollen during each observation were calculated.

Nectar production: During all the sampling nights (see Table S1), 10 flowers from different plants were covered with voile exclusion bags to prevent nectar extraction by visiting animals. Two-cc syringes with needles and glass capillaries were used for nectar extraction at 20:00 h, 00:00 h, and 4:00 h to measure the nectar volume throughout the night.

Pollination experiments: During four nights in the Plancillo and El Salto plantations, five groups of 8-10 flower buds (depending on availability) were selected for five different pollination treatments.

1. **Manual self-pollination:** To test for self-compatibility, buds were marked and covered with voile fabric bags (applied in all the treatments) at 17:00 h to prevent any visitors from contacting the flower. When the stigma was receptive, anthers with pollen from the same flower were rubbed against the stigmatic surface, and the flower was bagged again.
2. **Manual cross-pollination:** Buds were emasculated using scissors and bagged at 17:00 h. When the stigma was receptive, it was rubbed with anthers from another genetically different plant (one individual) and bagged. The bags were removed the next morning when the flowers were completely withered, and the stigma had lost receptivity.
3. **Nocturnal pollination:** To evaluate the contribution of nocturnal pollination to reproductive success, flowers were marked and kept on display throughout the night to make them available for nocturnal visitors and bagged at 4:00 h to avoid visits by daytime foragers. The bags were removed after the corolla wilted the following morning.
4. **Diurnal pollination:** To evaluate the contribution of pollinators that are active at dawn and early morning, flower buds were marked and bagged at 17:00 h. The next day, bags were removed at 4:30 h to allow the visit of potential pollinators until flowers completely withered.
5. **Control:** Nonmanipulated flowers exposed to nocturnal and diurnal pollination were marked to estimate natural pollination success (nocturnal and diurnal).

All the fruits produced in the treatments were harvested and measured four weeks after anthesis. Fruit size variables (weight, length, width, and seed density) were measured. The fruits were weighed with a Snowrex EQ 1200 balance with a precision of 0.02 g. The fruits were cut longitudinally in two halves, and the maximum length and width of the pulp were measured with a digital caliper (Mitutoyo 500-197-30B, precision of 0.02 mm). To determine the density of the seeds, a 1 cm^3 portion was taken from the center of each fruit, and the seeds were counted (Fig. 2b). This value was not extrapolated to the whole fruit content because the volume of the fruit pulp could not be calculated with confidence. The average value of each fruit trait variable among treatments was compared with one-way ANOVA, and a posteriori Tukey test was used to determine the treatments that differed from each other.

Nocturnal and diurnal visitors: Three digital cameras equipped with night vision (Sony HDR-CX700) aimed at different clusters of flowers (1.5 m apart) continuously video-recorded from 19:00 h to 00:00 h and from 4:30 h to 7:00 h. In total, 87 flowers were recorded (25 at Curubandé, 41 at Plancillo, and 21 at El Salto). For each visit, we recorded the time of occurrence, duration (when possible), and whether the visitors had contacted the reproductive organs. Insect visitors were collected only after the cameras had finished filming. The insects were identified to the lowest taxonomic level possible, and their abundance (visit/hour/flower) at the order level (Hymenoptera, Lepidoptera and Coleoptera; other orders were excluded due to their low abundance) was compared between plantations using a GLM with a negative binomial distribution.

One part of the diurnal visitation was massive and required a different methodology to estimate the number of insects visiting the flowers simultaneously. The mass visits lasted for approximately 20 min. The videos were played, and individual images were extracted at five different times, evenly spaced, during the whole mass visitation event (approximately minutes 1, 5, 10, 15, and 20). The number of insects in every image was counted, and the average number of individuals per minute for each flower was calculated. To measure the average duration of the visits, 15 random insects were registered. The parameters described above were compared for each site using Kruskal-Wallis tests. All analyses were performed in R (R Core Team, 2016).

RESULTS

Floral biology: Flower buds began to open around sunset, on average at 18:31 (±15:49 min), reached full anthesis approximately 1.25 hours later and withered after dawn at 6:41 (±18:29 min). The complete anthesis period spanned approximately 12:09 h (±24:33 min). No pollen was available at 19:00 h, but all flowers had pollen available at 20:00 h. A similar pattern was observed for stigmatic receptivity, with a peak occurring at midnight (Supplementary Fig. S1).

Measurements for nectar production were made on 80 flowers during all the samplings. On no occasion was nectar obtained. Dissections of the flowers during each sampling night did not show appreciable accumulation of nectar in any floral structure.

Pollination experiments: All the flowers used for all the treatments produced viable fruits, and the plants were self-compatible (n=26 per treatment, except for manual cross-pollination, n=16). Fruit weight was highly correlated with fruit length (r=0.93, p<0.001) and width (r=0.90, p<0.001), so we used weight as the variable to measure the effect of pollination treatments on fruit size. The fruits from flowers exposed to natural pollination, that included nocturnal and diurnal visitors, were on average the heaviest of all the treatments, whereas fruits from manually self-pollinated flowers presented the lowest weight ($F_{4,\ 113} = 76.11$; $p<0.001$; Fig. 3a). There was also an effect of the plantation on fruit size ($F_{1.,\ 113} = 4.68$; $p=0.033$).

Seed density showed a weaker correlation with fruit weight (r=0.50, p<0.001) and varied between the different pollination treatments ($F_{4,\ 108} = 15.73$; $p<0.001$). There were more seeds in the natural and nocturnal pollination treatments than in the manual self-pollination and diurnal pollination treatments. Manual cross-pollination did not differ from any other treatment (Fig. 3b). There was no effect of the plantation on the seed density ($F_{1,\ 108} = 0.02$; $p<0.90$).

Floral visitors: A total of 32.88 hours of night video and 9.52 hours of day video were analyzed.

1. **Nocturnal visitors:** A total of 118 individual visits by insects were recorded. Lepidoptera (40%), Coleoptera (36%), and Hymenoptera (18%) were the most frequent visitors (a list of the visitors is provided in supplementary Table S2). Isolated visits by Hemiptera and Diptera were also recorded (6%), but in no case did they contact the reproductive parts of the flowers. The abundance of insects visiting the flowers per hour varied between plantations and between the orders of the

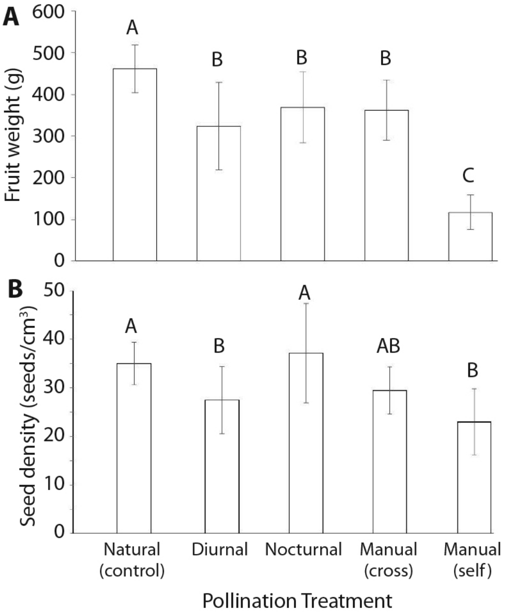

Fig. 3. Fruit traits of *Selenicereus costaricensis* (Cactaceae) from pollination treatments in two crop farms from Costa Rica. Data for: **a.** average weight. **b.** average seed density. The error bars indicate the standard deviation. The treatments with the same letter do not differ significantly (Tukey test with a confidence level of 95%).

insects, and the interaction between these factors was also significant (Table 1 and Table 2).

Beetles generally entered flowers through tepals and stay at the base of the corolla for long periods; contact with reproductive structures was uncommon, but their transit through flowers frequently moves anther filaments and might contribute to self-pollination. Regarding Lepidoptera, almost all the visits were individuals from the Sphingidae family, except for two occasional visits by smaller unidentified moths. Hawk moths flew through the corolla opening, entering and disappearing between the reproductive structures on all occasions (see supplementary video), as described by Haber (1983). The average duration of flower visits did not vary according to the taxonomic group ($\chi^2=1.49$; d.f.=2; $p>0.05$). No visit by bats or any other vertebrate was recorded or observed *in situ*.

2. **Diurnal visitors:** During the samplings at the Plancillo plantation, evening visits by stingless bees (*Trigona* spp.) to unopened flower buds were recorded. Visits by nocturnal bees (*Megalopta amoena*) were also recorded at the two sites in the dry forest between 20:00 h and 22:00 h and one hour before sunrise (~ 4:00 h), when the bees frequently contacted the stigma and anthers. *Xylocopa gualanensis* also visited the flowers before sunrise, starting at ~4:30 h.

   Massive honeybee (*Apis mellifera*) visitation events involving thousands of individuals were registered in the plantations, occurring just before dawn (starting at approximately 4:45 –5:00 h). Groups of bees collectively search for pollen for 15 to 20 minutes (see supplementary video). In each event, once the resource was depleted, the swarm abandoned the plantations. In 66% of the cases, the bees contacted the stigma, and in 34%, they came into contact strictly with stamens or parts of the perianth. The lowest visitation rate during massive visits was recorded in El Salto (22 $^{bees}/_{min}$), which differed significantly from that at the other two sites (75 $^{bees}/_{min}$ in Curubandé and 60 $^{bees}/_{min}$ in

Table 1

Frequency of insect visitors to the flowers of *S. costaricensis* according to the plantation, order, and sampling effort (hours recorded).

Plantation	Order	Visits	Sampled hours	Number of flowers
Plancillo	Coleoptera	6	18.2	41
	Hymenoptera	3		
	Lepidoptera	9		
Curubandé	Coleoptera	35	6.9	25
	Hymenoptera	15		
	Lepidoptera	7		
El Salto	Coleoptera	1	12.5	21
	Hymenoptera	3		
	Lepidoptera	31		

Table 2

Results for a GLM comparing the frequency of visit per hour per flower by insects in the orders Hymenoptera, Lepidoptera and Coleoptera, to El Salto, Plancillo and Curubandé plantations from Costa Rica.

Factor	Df	Deviance	Residual deviance	p
Order	2, 144	8.47	131.50	**0.014**
Plantation	2, 142	20.49	111.01	**<0.001**
Order x Plantation	4, 138	19.59	91.95	**<0.001**

Plancillo; $\chi^2=39.05$; d.f.=2; $p<0.05$). Much lower abundances of other bee genera were also detected during the morning before wilting (Supplementary Table S2).

DISCUSSION

Floral Biology: *Selenicereus costaricensis* presents nocturnal anthesis, as do other species in the genus (Chang et al., 1997; Valiente-Banuet et al., 2007). Weiss et al. (1994) proposed a general anthesis pattern for multiple species of *Selenicereus* (reported as *Hylocereus*; including *S. costaricensis*), and our study revealed that *S. costaricensis* flowers were fully open approximately one hour earlier than previously reported, indicating geographic and population variability.

The flowers of *S. costaricensis* showed no dichogamy and released pollen while their stigmas became receptive, approximately 1 h after anthesis began. Overlap in the maturation of reproductive organs has been described for *S. undatus* in Mexican crops (Valiente-Banuet et al., 2007). However, Weiss et al. (1994) reported that in wild populations of *S. costaricensis*, *S. polyrhizus* and *S. undatus* anthers dehisce before flower opening. The same was reported by Corona (2018) for *S. ocamponis* and *S. purpusii* in Mexico.

Homogamy or adichogamy is an uncommon trait among angiosperms with perfect flowers and is present in only 14.4% of the species (Bertin & Newman, 1993). The effects of adichogamy on commercial production require further investigation; a longer floral opening period maximizes the probability of pollination (Fenster et al., 2004; Flores-Martínez et al., 2013; Xu & Servedio, 2021), however, it may also favor self-pollination which produces the smallest fruits.

Reproductive system: Plants in the plantations were self-compatible, differing from the findings of Weiss et al. (1994), who reported *S. costaricensis* as a self-incompatible species. Compatibility is variable within the genus, with self-compatible species such as *S. undatus* (Weiss et al., 1994; Pushpakumara et al., 2005; Valiente-Banuet et al., 2007) and self-incompatible species such as *S. ocamponis, S. purpusii*, and *S. polyrhizus* (Weiss et al., 1994; Corona, 2018). A self-compatible plant is favorable from the farmer's perspective and can be related to artificial selection during the domestication of this species or to variations in the populations of origin of the plants used in these commercial plantations. However, herkogamy should reduce natural self-pollination in *S. costaricensis*, and fruit production is clearly favored by an animal vector.

The unclear syndrome: Although the flowers of *S. costaricensis* present characteristics consistent with the chiropterophily pollination syndrome and bats have been reported as pollinators of *S. undatus* (Valiente-Banuet et al., 2007), there were no bat visits at any of the study sites. The morphology of this flower does not fully agree with phalaenophily; however, hawk moths were the most important nocturnal visitors of *S. costaricensis* (~40% of the visits), which agrees with observations by Haber (1983) in natural populations. Instead of hovering in front of the flowers, as in many hawk moth adapted flowers, the insects entered the flowers, disappearing between the anthers, which allowed them to come into immediate contact with the reproductive organs (see supplementary video). The constant flapping of its wings during this process warrants pollination. An analysis of floral fragrances of *S. costaricensis* is needed to evaluate the presence of specific compounds related to chiropterophily or phalaenophily.

Hawk moths consume nectar, but contrary to what was reported by Weiss et al. (1994), no evidence of nectar production was found during this study. The absence of nectar has also been reported for *S. undatus* in Mexico (Valiente-Banuet et al., 2007). The same result was obtained by Muñiz et al. (2019) for the nectar production of flowers of *S. costaricensis*, *S. undatus*, and *S. polyrhizus*. However, the large visitation of hawk moths indicates that there are either small amounts of nectar these moths can collect or pollination by deceit occurs in commercial plantations. Natural populations bearing nectar-producing flowers around plantations could allow the maintenance of pollination by a deceit mechanism, maintaining large hawk moth visitation to plantations where no nectar is produced. The study of natural populations of *S. costaricensis* is necessary to determine whether the lack of nectar is the original trait.

Only *Manduca rustica* was collected in the plantations, but our sample was small, and it cannot be ruled out that other species or even genera of hawk moths also visited *S. costaricensis*. Moth species cannot be identified in video recordings and Haber (1983) also observed other species of *Manduca* visiting *S. costarricensis*. Landscape management around pitahaya plantations can favor the populations of *Manduca*, e.g. by planting host species.

The role of secondary pollinators: The lack of nectar creates an unexpected scenario in which pollen becomes the main reward for visiting insects in a nocturnal flowering species. However, in this case, nocturnal visitors (excluding the few nocturnal bees) were not observed collecting pollen. The extension of flowering for a short period during the morning could be a safeguard ensuring abundant pollination in cases where nocturnal visitors are rare or absent.

Visits by bees were dominant during the early morning, and bees are important pollinators of *S. costaricensis* that favor cross-pollination. The important visitation by opportunistic diurnal bees, also observed in plantations of other species in the genus by Weiss et al. (1994) and Valiente-Banuet et al. (2007), supports the previously reported discordance between predicted and observed pollinators in angiosperms based on traditional pollination syndromes (Ollerton et al., 2009; Abrahamczyk et al., 2017; Valverde-Espinoza et al., 2021).

Large carpenter bees (*Xylocopa* spp.) carry large amounts of pollen on their bodies and commonly contact the stigma when moving between flowers. Weiss et al. (1994) previously mentioned the potential of large Hymenoptera as pollinators of the genus *Selenicereus*. Similar behaviors were observed during the visits of nocturnal *Megalopta amoena* bees.

Finally, Coleoptera (~36% of the visits) cannot be considered important pollinators of *S. costaricensis*, but they can potentially self-pollinate flowers. For farmers, some beetles are considered pests since they can damage buds and open flowers, but none of the filmed beetles exhibit harmful behavior on any occasion.

The differences between the visitation rates to the flowers at the three study sites highlight the importance of local effects on the frequency and richness of floral visitors. Local effects are also evident in the composition of the visitors at each plantation with most of the collected species being exclusive at every location.

Mass visits: The number of honeybees (*Apis mellifera)* was high in all the samplings. Muñiz et al. (2020) reported honeybees as the main pollinators of *S. polyrhizus* and as a complementary pollinator of *S. undatus*. Diurnal bees have also been reported as the most effective pollinators of *S. ocamponis* and *S. purpusii* (Corona, 2018). The frenetic behavior during visits and the high number of bees visiting the flowers in the early morning result in greater pollen deposition and probably also cross-pollination, becoming one of the main pollinators of this crop. Generalist diurnal pollinators can play an important role in the pollination of cacti with nocturnal anthesis, especially when nocturnal pollinators are low in abundance (Weiss et al., 1994; Molina-Freaner et al., 2004; Le Bellec et al., 2006). In fact, some studies suggest that pollination of various dragon fruit species, which is mediated mainly by bees, can produce good results in terms of the production of commercial quality fruit (Weiss et al., 1994; Marques et al., 2011; Muñiz et al., 2020). In this case, diurnal and nocturnal pollinators have synergistic effects, explaining the larger dimensions of fruits produced by natural pollination.

The management of *Apis mellifera* in the vicinity or in conjunction with dragon fruit plantations would clearly increase pollination in this species due to its high abundance and behavior in flowers, favoring commercial production. Additionally, the maintenance of dead logs of soft wood near plantations could ensure the presence of nesting sites for carpenter bees (Hanson et al., 2021). The traditional management of *Melipona beecheii* for helping pollination in this cactus requires further study, given that its presence in the studied plantations was very low.

However, not all bees have a positive impact. For example, bees of the genus *Trigona* spp. were abundant visitors to the flowers of the Plancillo plantation, but they are improbable pollinators. These bees reached the flowers prior to anthesis (16:00 h) and pierced the petals to steal pollen from the anthers, damaging them before the stigmas became receptive.

Effect of pollinators on fruit traits: A significant variation in fruit size and mass was found between treatments. Naturally pollinated flowers produced the largest fruits, supporting the additive effects of nocturnal and diurnal pollination. A greater diversity of visitors guarantees bigger fruits and greater profit for farmers. Diverse pollinator assemblages improve fruit quality in some important commercial crops, such as coffee (Ricketts et al., 2004). Furthermore, reports indicate that the quality of some commercial fruits increased when assisted by native pollinators (Hoehn et al., 2008; Garibaldi et al., 2013). In agreement with this rationale, we found that exclusive self-pollination, the outcome expected in a defaunated environment, produces the smallest fruits. The effect of spontaneous self-pollination should be explored to determine management strategies under these conditions.

Diurnal pollination alone produces large fruits. Weiss et al. (1994) obtained similar results with a much larger diurnal visitation period. Here, we demonstrate that 15–20 minutes of massive diurnal visitation is sufficient for successful fruit production.

This finding confirms that pollinators play an important role in the production of fruits of higher commercial value for this species since the movement of pollen and interbreeding increases crop production. Muñiz et al. (2020) reported a similar effect when conducting experiments with *S. polyrhizus* and *S. undatus* in plantations in northeastern Brazil. In fact, pollination mediated by animal vectors can increase the production of various crops, either by increasing the quantity or weight of fruits (Klein et al., 2003; Ricketts et al., 2004; Rosa et al., 2011; Rizzardo et al., 2012; Milfont et al., 2013; Taber & Olmstead, 2016).

The reduced size of self-pollinated fruit, as observed here, is a common result of inbreeding depression (Charkesworth & Charkesworth, 1999; Waser & Williams, 2001; Chautá-Mellizo et al., 2012); however, more studies are needed to confirm this hypothesis. Self-fertilization can affect the quality of export crops by disfavoring their desirable characteristics, such as size, shape, or quantity of seeds (Chautá-Mellizo et al., 2012). Clonal production is the main way to increase the number of plants in dragon fruit plantations in Costa Rica, but this practice can reduce the size of the fruits. The creation of genetically diverse plantations is recommended to ensure larger fruits with the greatest commercial value.

Finally, the variation in seed density between the applied treatments was more irregular than that of the other metrics. It has been reported that for *S. undatus*, the type of pollination does not influence the number of seeds (Muñiz et al., 2020). The same study indicated that, in the case of *S. polyrhizus*, the number of seeds is greater when the flowers are pollinated by morning visitors such as honeybees, which contrasts with our results. Differences in external conditions and the abundance of visitors may explain the differences between the results of both studies.

Conclusions: In this study, the self-compatibility of *S. costaricensis* was reported for the first time. Fruit traits are favored by cross-pollination, resulting in fruits of higher commercial size. An additive effect of nocturnal and diurnal pollinators produced fruits with the greatest commercial value.

This study documented the pollinators of *S. costaricensis* and their importance in commercial production in the original distribution of the species. The participation of bats in the pollination process is ruled out, while hawk moths and morning swarms of bees (*Apis mellifera*) appear to be the main pollinators. Other organisms, such as bees from the genera *Xylocopa* and *Megalopta*, as well as various beetles, appear to be secondary or complementary pollinators. This result demonstrates the contribution of morning pollinators, which could easily be overlooked since many cacti are traditionally associated with nocturnal pollination syndromes, with moths and bats as the main pollinators. Enrichment of the landscape with hawk moth host plants near plantations might benefit their populations and the pollination services they provide. Management of honeybees can complement natural pollinators, and their use is a promising strategy for increasing fruit set in defaunated environments. Finally, increasing the genetic diversity of pitahaya plants within plantations would favor cross-pollination and its positive effects on fruit size.

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See supplementary material
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See supplementary video
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