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Floral biology, pollination requirements and behavior of floral visitors in two species of pitaya[1]

Biologia floral, requerimentos de polinização e comportamento dos visitantes florais em duas espécies de pitaia

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ABSTRACT - The present work aimed to study floral biology, pollination requirements and the behavior of floral visitors in two species of pitaya, *Hylocereus undatus* and *H. polyrhizus*, in Northeastern Brazil. The experiment was carried out through diurnal and nocturnal observations and the use of flowers bagged or accessible to visitors. Results showed that flowers of both species are similar both in anatomical and functional traits. They are large, with nocturnal anthesis onset and attract night and daytime flower visitors. The floral visitors found were sphinx moths, ants, wasps and bees, with *Apis mellifera* accounting for 86.1% of visits to flowers. The *H. undatus* species is independent of biotic pollination to set and produce large and well-shaped fruits, but *H. polyrhizus* shows limited self-pollination and requires biotic pollination to set fruits and also to produce larger fruits. In this case, *A. mellifera* appears as the most likely pollinator. It is concluded that biotic pollination deficit is a limiting factor for the productivity of *H. polyrhizus*, but not to *H. undatus* under the conditions studied and that the role of pollinators, especially *A. mellifera*, in the quality of the fruits produced by both pitaya species needs to be investigated.

Key words: *Hylocereus polyrhizus. Hylocereus undatus*. Pitaya flowers. Pollinators.

RESUMO - O presente trabalho objetivou estudar a biologia floral, os requerimentos de polinização e o comportamento de visitantes florais em duas espécies de pitaia, *Hylocereus undatus* e *H. polyrhizus* no Nordeste do Brasil. O experimento foi conduzido com observações diurnas e noturnas e pelo uso de flores ensacadas ou acessíveis aos visitantes. Os resultados mostraram que as flores de ambas as espécies são semelhantes em características anatômicas e funcionais. Elas são grandes, com o início da antese à noite e atraem visitantes florais noturnos e diurnos. Os visitantes florais encontrados foram mariposas, formigas, vespas e abelhas, sendo *Apis mellifera* responsável por 86,1% das visitas às flores. A espécie *H. undatus* é independente da polinização biótica para vingar e produzir frutos grandes e bem formados, mas *H. polyrhizus* apresenta autopolinização limitada e requer polinização biótica para vingar frutos e também para produzir frutos maiores. Neste caso, *A. mellifera* aparece como o polinizador mais provável. Conclui-se que o déficit de polinização biótica é um fator limitante na produtividade de *H. polyrhizus*, mas não em *H. undatus* nas condições estudadas e que o papel dos polinizadores, especialmente *A. mellifera*, na qualidade dos frutos produzidos por ambas as espécies de pitaia precisa ser investigado.

Palavras-chave: *Hylocereus polyrhizus. Hylocereus undatus*. Flores de Pitaia. Polinizadores.

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INTRODUCTION

The pitaya (*Hylocereus* spp.) is a new and promising fruit in the market, and is produced by several related species belonging to the family Cactaceae. Although originating in the tropical and subtropical regions, pitayas are now distributed all over the world, especially because these plants can be grown in soil with low organic matter and nutrient deficit (CÁLIX DE DIOS; CASTILLO MARTÍNEZ; CAAMAL CANCHÉ, 2014). Among the many species of pitayas, four stand out in global cultivation and distribution: *H. undatus*, *H. polyrhizus*, *H. costaricensis* and *Selenicereus megalanthus* (ORTIZ-HERNÁNDEZ; SALAZAR, 2012).

Because it is a new crop, there is little literature on floral biology and pitaya pollination requirements, which may limit its productivity. Because all pitayas are cacti species, several publications point out bats as the natural pollinators of their flowers (LE BELLEC, 2004; WEISS; NERD; MIZRAHI, 1994). However, other studies present bats as the most efficient nocturnal pollinators (VALIENT-BANUET *et al.*, 2007; WEISS; NERD; MIZRAHI, 1994) while bees play this role during daytime pollination, even having a much shorter period of time to visit the flowers (LE BELLEC, 2004; MARQUES *et al.*, 2011a;). A study by Valiente-Banuet *et al.* (2007) carried out in Mexico, where the plant occurs naturally, did not find nectar in the flowers of *Hylocereus undatus*, suggesting that bats probably visit flowers of this species in search of pollen. Bees definitely visit pitaya flowers only for pollen (LE BELLEC, 2004).

H. undatus and *H. polyrhizus* appear to have total or partial self-incompatibility, requiring hand pollination to achieve commercial yields under cultivation (ORTIZ-HERNÁNDEZ; SALAZAR, 2012; WEISS; NERD; MIZRAHI, 1994). However, some studies suggest that the pollination carried out by bees may produce good results in the development of pitaya fruits (MARQUES *et al.*, 2011a; WEISS; NERD; MIZRAHI, 1994).

The lack of consistent information on the pollination requirements and pollinators of the pitayas, especially away from their native geographic area, has limited the full exploitation of this crop in several regions. Thus, the present work sought to generate knowledge about floral biology, pollination requirements, floral visitors, and their behavior as pollinators in two species of Pitaya, *Hylocereus undatus* and *H. polyrhizus* grown in NE Brazil.

MATERIAL AND METHODS

The research was carried out in the FRUTACOR farm (05º08’11.31”S, 37º59’51.36”W, at 140 m altitude), located at the Apodi plateau, Quixeré county, state of Ceará, NE Brazil. The climate in the region is classified as BSw’h’ according to Köppen-Geiger. In Quixeré, rainfall in 2016 summed 687.8 mm, with 35 ºC and 22 ºC as the maximum and minimum mean annual temperature, respectively, with 62% as the annual mean relative humidity, and 7.5 m/s as the mean wind speed (FUNDAÇÃO CEARENSE DE METEOROLOGIA E RECURSOS HÍDRICOS, 2017).

Pitaya is cultivated in an area of two hectares with two species: *Hylocereus undatus* and *H. polyrhizus*. The planting system is with wooden masts in double rows, and plants are subject to all agricultural practices recommended for pitaya cultivation (CÁLIX DE DIOS; CASTILLO MARTÍNEZ; CAAMAL CANCHÉ, 2014). Irrigation takes place three times a day.

We carried out the experiment for five consecutive flowering periods from February to June 2016, during the rainy season in the region, and investigated floral biology; nocturnal and diurnal flower visitors; and pollination requirements for both pitaya species. To obtain floral biology data, ten flower buds of both pitaya species were randomly selected and observed throughout their entire development, since they first appearance until fruit setting, and also to fruit harvesting. These flower buds were marked with colored ribbons to keep track of their growth, and to measure, take photos and record data every day.

We assumed buds were in pre-anthesis when bracts that covering the tepals began to detach from them. At this stage, we recorded the day and hour and covered buds with Non-Woven Fabric bags. In each pitaya species, five of the selected buds were taken for dissection and the other five left in the plants in order to follow the anthesis pattern. In the five flowers of each species that were dissected we obtained data referring to floral whorls, presence or absence of any flower structures and counting of perianth parts.

Stigma receptivity was tested according to the methodology recommended by Dafni and Maués (1998), as follows. In both species, *H. undatus* and *H. polyrhizus*, we selected 10 pre-anthesis flower buds and covered with Non-Woven Fabric bags. After buds open, one flower of each species was unbagged every two hours, at 20:00h until 6:00h next morning, and its stigma was cut off. Then, we immersed the stigma tip 3 cm deep into a container with hydrogen peroxide. The stigma was submerged for three minutes, time required to visualize the occurrence of bubbles or not. In case they occur, the stigma was considered receptive.

In the two species, we marked other five floral buds with colored bands to observe the anthers dehiscence. We monitored the buds visually and, from 19:00h onwards, at every hour we opened one bud of each species to check for pollen shedding from anthers. In relation to nectar, we bagged other ten floral buds in both species to investigate nectar production throughout the anthesis and if once collected by floral visitors nectar was replenished. Therefore, nectar was collected from the flowers once at 00:00 h, twice in the hours of 21:00 h, 3:00 h and three times from 20:00 h, 1:00 h, 6:00 h during the whole anthesis. Fruiting index was calculated from ten plants we randomly selected in ten rows distributed all over the crop in order to obtain a representative sample of the whole plantation.

The collection of floral visitors was carried out only for *H. polyrhizus* because this was the pitaya species occupying most of the cultivated area. We observed, counted and recorded all diurnal and nocturnal floral visitors during the whole research period. We also collected these floral visitors according to Vaissière, Freitas and Gemmill-Herren (2011). The bees collected were sent for identification by Prof. Dr. Favízia Freitas de Oliveira from the Federal University of Bahia - UFBA and the moths were identified by Prof. Dr. Felipe W. Amorim of the Institute of Biosciences at the Paulista State University - UNESP.

Insect counting over night was done every two hours, at 20:00 h until 4:00 h, always following the same track and observing the flowers of each row with the help of two flashlights to find visitors. We used the same procedure for diurnal observations, but they were carried out every hour, at 5:00 h, 6:00 h and 7:00 h, because the flowers spend less time open during daytime than at night. On cloudy days, when flowers took longer to close, observations were carried out up to 9:00h when flowers finally closed.

Tests of pollination requirements were conducted for both pitaya species. For this, we selected 120 floral buds for each species over the five consecutive flowering periods. These floral buds were split into four treatments (Restricted Pollination, Open Pollination, Nocturnal Pollination and Daytime Pollination) with 30 replicates per treatment, being six for each five flowering periods.

The Restricted pollination treatment (flower bagged throughout the anthesis) aimed to observe if the two pitaya species studied are self-compatible and have the ability for self-pollination. The buds were marked with ribbons and bagged with Non-Woven Fabric bags at 17:00 h. The bag had a ribbon to tie it up and prevent any pollinator from reaching the flower. Bags were removed at 13:00 h next day, when the flower has wilted and closed.

The Open pollination treatment (flower open to visitors throughout the anthesis) aimed to know the pollination level which is naturally occurring in the crop, and was carried out as follows: flower buds were first marked at pre-anthesis, and left open to flower visitors during their entire anthesis until the next day at 13:00 h. At this moment, flowers had already wilted, and their locations were marked on the plant with colored clamps to differentiate treatments and to avoid missing any fruit set during data collection or harvesting.

The Nocturnal Pollination treatment (flower open to visitors only at night) aimed to learn if nocturnal visitors contribute to pitaya pollination through yield increment or better fruit formation. Floral buds were marked as described in the previous treatment, and the flowers were left open to nocturnal visitors all night long. Then, at 4:00 h next day, flowers were covered with Non-Woven Fabric bags to prevent any visit from diurnal pollinators that may become active on flowers just before dawn. Flowers remained protected from daytime visitors until 13:00 h when they had wilted and bags were removed.

The Daytime pollination treatment (flower open to visitors only during the day) aimed learning if diurnal floral visitors are pollinators of pitaya flowers and contribute to fruit set. Flower buds were marked as described in the previous treatment and covered with Non-Woven Fabric bags as early as 17:00 h. Then, they spend all night protected from the nocturnal visitors, until 5:00 h next day when bags were finally removed and flowers could receive freely the daytime visitors until they withered.

Data regarding to the floral buds and fruits per plant, and fruiting index per plant were analyzed through Analysis of Variance (ANOVA) and means compared *a posteriori* by Tukey's test ($p<0.05$) using the program R (Version 3.3.1.). The experimental design used to evaluate pollination requirements was completely randomized with four treatments for each species of pitaya, with 30 replicates for each treatment. Due to the binomial character (in which 1 is developed; and 0 is not developed) of fruit setting, data for this parameter were subjected directly to the nonparametric Kruskal-Wallis test, at 5% probability. The R statistical software, version 3.3.1. was also used to perform this analysis.

RESULTS AND DISCUSSION

The flowers in the pitaya species *Hylocereus undatus* and *H. polyrhizus* have identical anatomical forms and the same amount of floral pieces (Figure 1A-B). In this work the flowers in both species had the same size from the presentation of the floral bud to the anthesis. The flowers from both species can be described as actinomorphic, hermaphrodite, bearing all floral parts, pedunculated, with the floral bud originating at the spine base and its axis measuring from the base to the apex 25.2 cm ± 1.3 before pre-anthesis, 27.2 cm ± 1.5 during anthesis, and 28.8 cm ±

2.0 wide when fully open. All flowers are hermaphrodite, with the ovary above the tepals insertion (Figure 1C-D). The stigma is positioned above the stamens (Figure 1E), in a spatial separation of the reproductive parts called approach herkogamy (RECH *et al*., 2014).

The flowers in the two species studied have on average 77.0 ± 1.0 bracts and 23.0 ± 2.3 tepals summing the internal and external whorls. The bracts present size and color variation. When the flowers are dissected, it is observed that the bracts of the base of the floral bud are smaller and with a light green color, and the larger ones of the flower apex are light yellow in color (Figure 1F-G). Those bracts that are at the base of the flower remain during the development of the fruit (Figure 1H-I).

Figure 1 - A and B - *Hylocereus undatus* and *H. polyrhizus* flowers respectively. C and D - Dissected flowers of pitaya *H. polyrhizus* showing bracts and reproductive parts. E - Stigma disposition just above stamens. F and G - Dissected flowers showing the arrangement, size and color of the bracts. H and I - Young fruit and fully-developed fruit of *H. undatus* respectively

At each flowering cycle of *Hylocereus undatus* and *H. polyrhizus*, all plants were blooming on day 1, accounting for about 50% of all flowers produced in the whole flowering cycle. These figuresreached 100% of flowering on the 2 nd day and at the 3 rd day no new flowers were opened and the number of open flowers began to decrease.

In the phase of emergence of the floral bud until pre-anthesis, periods of 14 to 18 days were found. This interval is shorter than any other one recorded in the literature, Marques *et al*. (2011b) observed 19 to 21 days for the budding period until the anthesis, Yah *et al*. (2008) verified periods of 25 to 31 days studying *H. undatus* and Silva *et al*. (2015) found 18 to 23 days with conditions of 50% shading. Perhaps the different conditions of the other studies in relation to the present, such as species, shading conditions and different climatic conditions, can explain the observed differences of results. As for the intervals between flowering, there was variation of 10 to 15 days in the present study. This result was also found by Valiente-Banuet *et al*. (2007).

In the two species studied, the floral bud has a different color. In the flower of the white pitaya (*H. undatus*) it is light green and in the pitaya flower with red (*H. polyrhizus*) it is green with the edges of red developing bracts. The plants have a mass flowering and started always in the same period, lasting three days on average (WEISS; NERD; MIZRAHI, 1994). Flower buds came in pre-anthesis when the bracts that covered the petals began to peel off, which usually started at 14:00 h, with the flowers initiating anthesis around 19:00 h. The anthesis in the two species lasts an average 12 h, with flowers closing completely at 07:00 h of the next morning. Once again, less than that found in the literature, that refers to periods of 15 h hours of anthesis (MARQUES *et al*., 2011b; YAH *et al*., 2008). However, when the day was cloudy both *H. undatus* and *H. polyrhizus* flowers remained open for up to two extra hours until 09:00h. This result suggests a possible influence of luminosity in the duration of anthesis. In this situation flowers continued to receive visits for two more hours. As the cultivation is not totally shaded, with only a few castor bean plants (*Ricinus communis* L.) within the rows, it is possible that the longer anthesis time favors the pollination by floral visitors.

During the anthesis of the two species, the flowers at 00:00 h hours were completely open and remained so until 02:00 h in the morning, when from then on they began to close and diminish the odor emitted. A difference found between the flowers of *H. polyrhizus* and *H. undatus* was in relation to the release of the odors, with the first beginning the production around 20:00 h, one hour before the last one. In both species, the release of odors remained until the flowers closed completely the next morning. Odor is a very useful tool used by plants to attract pollinators, especially by nocturnal plants. Some plants synchronize the maximum odor emissions with the most visited times, favoring pollination by ensuring the presence of the pollinators in the flowers when the flowers are fully receptive (RECH *et al*., 2014).

Observations showed that anthers begin to release pollen when the flowers were still closed, and there were a large quantity already released at 16:00 h, well before the flower opens at 19:00 h. However, due to the length of the stigma and its position above the anthers height, pollen released at that stage reached only the style. When the flower opened, there was already a great amount of pollen available in the anthers, tepals and style, facilitating self-pollination, although this is not evident. According to

Weiss, Nerd and Mizrahi (1994), in the pitaya *Selenicereus megalanthus*, the closure movement of the tepals favors self-pollination and this may be a safety mechanism of the plant in case the flower has not been pollinated until that moment. Also, the presence of large amounts of pollen in various parts of the flower (secondary pollen presentation) would also facilitate floral visitors to acquire and distribute pollen around, favoring pollination.

The stigma was receptive to all times tested, showing stigma from 20:00 h until the flower closes at 7:00 h am the next morning, or until 9:00 h on cloudy days. Apparently, the pitayas studied have evolved mechanisms to facilitate different means of pollination. The long period of stigma receptivity is probably to ensure the deposition of as many pollen grains as possible, necessary to fertilize the large number of ovules present the ovary. Although we have not tested in the present work, it is possible that the stigma is already receptive from the moment of anthers first release pollen when the flower is still closed. This is a common trait in several self-pollinating species (DEPRÁ *et al.*, 2014; OJUEDERIE; BALOGUN; ABBERTON, 2016).

Attempts to collect nectar were unsuccessful, suggesting that the flowers of *H. polyrhizus* and *H. undatus* do not produce nectar, at least under the conditions studied. A study by Valiente-Banuet *et al.* (2007) with the species *H. undatus* in Mexico reached the same conclusion. Apparently, pollen is the only reward for flower visitors.

The floral visitors found in *Hylocereus polyrhizus* flowers were sphinx moth, ants, bees and wasps (Table 1). The sphinx moth *Agrius cingulata* with 1.8% of total visits was the nocturnal visitor with greater potential to pollinate pitaya flowers. The pitaya flower is large and well open and it is necessary for the moth to land inside the flower. In this way, they touch the stigma when they arrive or when they leave the flower, the second situation being more frequent.

The sphinxes, as flapping their wings trying to get out of the flowers, they come in contact with the anthers, thus acquiring pollen in the body and wings (Figure 2A). The moths of this species consume nectar, and possibly were being deceived attracted by the strong odor of the flowers, since no nectar was found. In addition, the behavior of proving several times with the spirotromba without setting in a place to suck the nectar as is common among the Lepidoptera, then try to leave the flower, suggests that in fact they did not find any nectar.

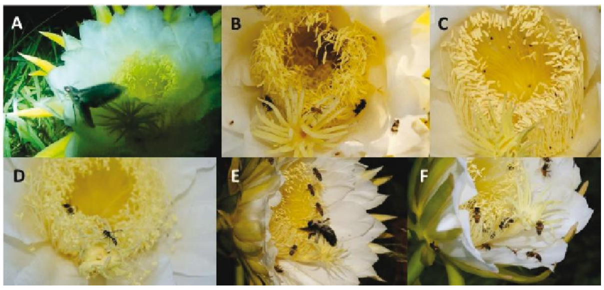
The ants with 1.1% total visits were observed walking through the male and female parts of the flower (Figure 2B). Although they cannot be considered a major pollinator, their behavior suggests that they may act as complementary pollinators, helping to distribute some pollen around. Also during the night, small beetles were found in the flowers, but because they were too numerous and small, it was not possible to count. Although they may potentially carry pollen transfer from anthers to stigma of the same flower (self-pollination), the quasi-static behavior on feeding pollen stamens suggests that they are not pollinators, but opportunistic pollen feeders (Figure 2C).

Bats were not observed visiting the flowers of any of the two pitaya species, although they are considered the natural pollinators of these cacti (VALIENTE-BANUET *et al.*, 2007). In fact, the pitaya flower has characteristics associated with chiropterophilous syndrome, such as large, well open, nighttime flowers with white or light colors, strong nocturnal scent, large amounts of pollen and/or nectar, lasting only one night and closing early the next morning (RECH *et al.*, 2014). Considering that the species studied do not produce nectar bat visits should not be expected, unless they were deceived by the plant or would visit to feed on pollen. According to Valiente-Banuet *et al.* (2007), possibly the bats found in their study visiting the pitaya flowers in Mexico were pollenivores, *i.e.* they fed on pollen.

Table 1 - Relative identity, quantity and abundance of floral visitors of *Hylocereus polyrhizus* flowers, during day and night periods in cultivation in Quixeré - CE

	Floral visitors	Scientific name	Amount	(%)
Nocturnal	Sphinx moth	*Agrius cingulata*	45	1.8
	Ant	-	28	1.1
Daytime	Bee	*Apis mellifera*	2,107	86.1
	Bee	*Xylocopa (Neoxylocopa) grisescens*	21	0.9
	Bee	*Trigona spinipes*	208	8.5
	Wasp	*Polybia (Myrapetra)* sp.	38	1.6
Total			2,447	100

Figure 2 - Floral visitors found in the flowers of the red pulp pitaya in Quixeré - CE. A - Sphinx moths of the species *Agrius cingulata*. B - Ants (Formicidae) and Trigona Spinipes. C - Small beetles on the stamens. D - Wasp *Polybia* (*Myrapetra*) sp.. E - Bee *Xylocopa (Neoxylocopa) grisescens*. F - *Apis mellifera* was the visitor most commonly found in pitaya flowers



Daytime visitors were found in much greater numbers than the nocturnal ones (Table 1), which makes them the potential pollinators of the pitaya in the studied area. In fact, although it is a nocturnal flower, several studies point out that diurnal visitors are the most important for pollination of pitaya flowers under cultivation, especially where the natural pollinator is not present (LE BELLEC, 2004; MARQUES *et al.*, 2011a; WEISS; NERD; MIZRAHI, 1994). Among the daytime visitors, the wasps *Polybia* (*Myrapetra*) sp. comprised 1.6% of total visitors found. But it was observed that these wasps did not feed on the floral resource offered by this plant. Their only purpose in pitaya flowers were prey hunting, and it may have contributed with some minor pollination as in the attempt to catch insects they walked on the reproductive parts of the flower and could also have transferred some pollen grains to the stigma (Figure 2D).

Carpenter bees of the species *Xylocopa* (*Neoxylocopa*) *grisescens* accounted for only 0.9% of the visitors (Table 1). In the flower, the Carpenter bees collected pollen and presented the behavior of clinging to the anthers and trying to walk on the stamens flapping their wings. In this way, they were able to acquire large amounts of pollen on their bodies and possibly deposited these pollen grains on the stigma, as they usually came into contact with it on arrival and/or departure from the flower (Figure 2E). Comparing only the individual behavior with other visitors of pitaya flowers, carpenter bees apparently perform pollination more efficiently due to their large size and behavior on the flower. In fact, this found corroborates Weiss, Nerd and Mizrahi (1994) which demonstrated the efficiency of large bees as potential pollinators for the genus *Hylocereus* in Israel.

Both the fact that the crop presents large flowers with abundant pollen as well as be cultivated using wooden posts, which are good substrate for nesting of *Xylocopa* (MARCHI; ALVES-DOS-SANTOS, 2013), may have contributed to attract these bees to the pitaya flowers. In the case of the farm where the study was carried out, which has large areas planted with banana (*Musa paradisiaca*) where there are no nesting sites for *Xylocopa*, the wooden posts used in the pitaya crop were good attraction for the bees conveniently close of the food source. However, due to the interval of 10 to 15 days between bloomings and the consequent lack of food resources for the carpenter bees during this period, these bees moved away from the area of the crop and explore the native vegetation far just over a mile away, returning at each blooming cycle.

The stingless bee (*Trigona spinipes*) (Figure 2B) was the second most abundant visitor with 8.5% of the total visits (Table 1). Unlike the other floral visitors, *T. spinipes* foraging in groups and presented three distinct behaviors: i - aggression against others floral visitors: *T. spinipes* bees dominated the pitaya flowers and attacked any other potential visitor approaching it, especially *Apis mellifera* bees, preventing them from visiting the flower; ii - removal of the pollen deposited on the stigma: besides collecting pollen from the stamens, these bees used their mandibles to scrap off the pollen deposited on the stigma; iii - damaging the flowers: every single day, between 16:00 and 17:00 h, *T. spinipes* bees pierced the petals of pre-anthesis buds of *H. polyrhizus*, to collect pollen. These behaviors of the *T. spinipes* foragers are known and described for other crops, although not necessarily all in the same species, as observed here (GIANNINI *et al.*, 2015; JAFFÉ *et al.*, 2016). Although described as an efficient pollinators of other agricultural crops such as pumpkin, coffee, mango, etc. (JAFFÉ *et al.*, 2016), *T. spinipes* bees were not be considered an important pollinator of the pitaya crop due to its small numbers in flowers and distinct behaviors previously presented.

A. mellifera bees were the most abundant floral visitors (86.1%) (Figure 2F). The visits began from 5:00 h and extended until the flower closed completely. The collected resource was only pollen, also observed by Le Bellec (2004); Marques *et al.* (2011a); Weiss, Nerd and Mizrahi (1994). However, in the present study we observed a large number of these bees visiting the flowers, generally reaching more than 30 individuals per flower. The bees collected the pollen in frantic movements, moving rapidly between the stamens, with constant overflows of the flower during which they transferred the pollen to the corbicula before resting again and resuming the collection of more pollen. Both when they took flight and when they landed on the flower again, they commonly touched the stigma. Although Le Bellec (2004) has suggested that pollination by *A. mellifera* bees is inefficient in the pitaya due to the size of the bee in relation to the flower, in our study we noticed that the higher the number of bees *A. mellifera* visiting a flower, the greater the deposition of pollen on stigma. It has been shown that, despite being small, *Apis mellifera* foraging behavior favors pollination, and due of its abundance in the area, it probably constituted the most important pollinator of this crop.

After pollination, the time for total fruit development, since day of anthesis to the fruit skin become completely red, and the time for harvesting was 30 days for both species. In regard to fruit development, from anthesis to harvesting, our results are similar to the 31 days reported by Marques *et al.* (2011b) to *H. undatus* also in Brazil. However, in Israel, periods from 28 to 41 days were necessary for fruit picking (TEL-ZUR *et al.*, 2011). According to Osuna-Enciso *et al.* (2016), the ambient temperature is an important factor in the development of pitaya fruits, and it may explain differences observed in the period of development and maturation of fruits between different locations.

Regarding to fruiting, the mean number of flowers per plant, number of fruits and fruiting index were not significantly (p>0.05) different between the studied pitaya species (Table 2). Both species presented a great variation in the number of flower buds emitted per plant, with values ranging from 2 to 34, but with a similar mean number around 10 flower buds. The mean number of flowers per plant found in the present study was generally much lower than those observed in other studies, ranging from 12.6 to 65 flowers per plant (OSUNA-ENCISO *et al.*, 2016; TRAN; YEN, 2014; VALIENTE-BANUET *et al.*, 2007). However, these values seem to vary greatly from year to year, since Osuna-Enciso *et al.* (2016) obtained averages of 20.7; 36.5 and 12.6 flowers per plant in consecutive years, with the lowest result of 0.3 flowers per plant. There is a need for further investigation into the factors influencing such variations in flowering and consequent fruiting of the pitaya.

As for the quantity of fruits harvested per plant, the *H. polyrhizus* species produced from 0 to 30 fruits, while the *H. undatus* species from 2 to 20 fruits. Means did not differ significantly (p>0.05). Regarding the fruiting index, pitaya *H. undatus* averaged 94.25% fruits, while *H. polyrhizus* presented an average of 88.66%, and no significant difference (p> 0.05) was observed between species. However, the species differed as to the amplitude of the fruit set index. While the red pulp pitaya *H. polyrhizus* presented plants whose flowers did not set a single fruit (zero fruiting) to plants in which all the flowers turned into fruits (100% of fruiting), the white pulp pitaya *H. undatus* produced a fruiting index that ranged from a minimum of 72% to a maximum of 100%. These results suggest that the latter species is much less dependent on biotic pollinators than the former one.

The evaluation of pollination requirements for the four treatments tested presented different results regarding the role of pollinating biotic agents in the two pitaya species *Hylocereus undatus* and *H. polyrhizus* (Table 3). Both species presented high fruit setting under all treatments, except *H. polyrhizus* when submitted to the restricted pollination treatment (p<0.05). The fact that *H. undatus* presented a maximum fruit setting when submitted to the restricted pollination treatment and *H. polyrhizus* 47% indicates that both can self-pollinate with no autoincompatibility. The difference between these two species is the fact that the former is totally independent of the action of biotic pollinators for fruit production, and the latter have only a partial ability to self-pollinate, leaving it often dependent on the action of biotic pollinators to complement pollination and ensure fruit setting. In addition, the fruits from *H. undatus* of this species were large and uniform, suggesting efficient pollination.

One explanation for the *H. undatus* independence of the action of biotic pollinators would be that the flower has the ability to auto-pollinate and the species to accept self-pollination. Actually, Tran and Yen (2014) alleged that the white pitaya, *H. undatus*, is totally self-compatible.

H. polyrhizus dependence on the action of biotic pollinators to complement pollination and ensure fruit setting is plausible in view of when nocturnal pollinators were allowed to visit the flowers the fruiting index rose from 50%, in the case of restricted pollination treatment,

Table 2 - Number of floral buds and fruits per plant, and fruiting index of the pitayas *Hylocereus polyrhizus* and *H. undatus* in Quixeré, Ceará 2016

Species	N	Floral Buds/ plant	Minimum and maximum	Fruits/ plant	Minimum and maximum	% fruiting	Minimum and maximum
H. polyrhizus	80	10.5 ± 7.1	2 - 34	9.1 ± 6.5	0 - 30	86.6 ± 21.3	0 - 100
H. undatus	20	10.0 ± 6.9	2 - 27	9.1 ± 5.8	2 - 20	94.2 ± 10	72 - 100

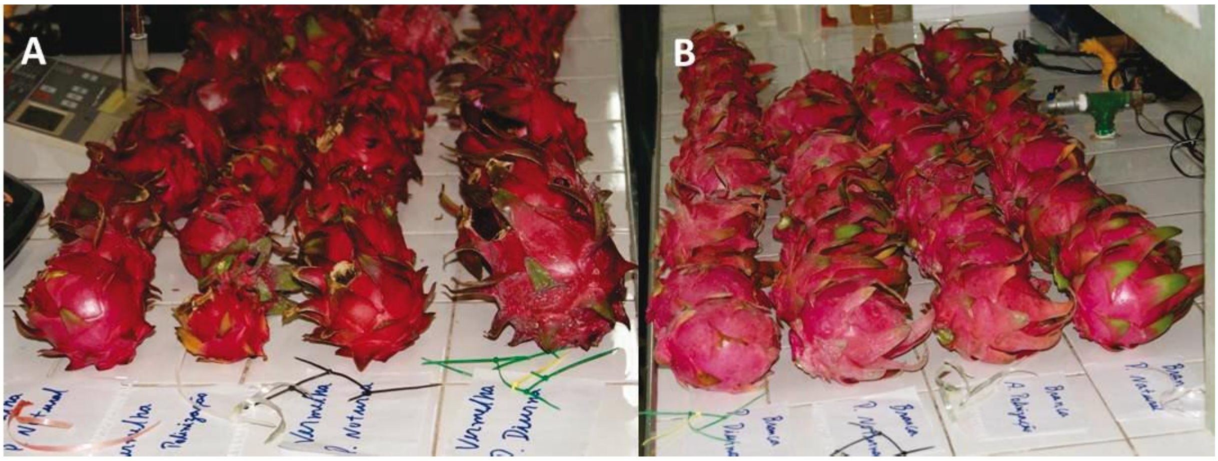
to 93% ($p<0.05$), and when the daytime pollinators, which were more abundant, visited the index reached the maximum possible value of 100% ($p<0.05$). Therefore, although *H. polyrhizus* is self-compatible and able to auto-pollinate, the flower has a large number of ovules and the amount of pollen grains deposited on the stigma by auto-pollination may not always reach the minimum number necessary to ensure fruit setting. Therefore, the flower benefits from floral visitors that searching for pollen end up transferring the necessary amount of grains to the stigma, thus acting as pollinators of the species. This is further evidenced by the fact that fruits set in the treatment of restricted visits were generally smaller and lighter (unpublished data) than those from the treatments with flowers open to visitors (Figure 3A).

Auto-pollination and self-compatibility seem to be characteristics common to various species of pitaya. However, it seems that in most species self-pollination produces pollination deficits, not enough to ensure the potential production of plants, both in terms of quantity and quality of fruits. In fact, most authors recommend the use of manual pollination to maximize crop yields of the various pitaya species (LE BELLEC, 2004; MENEZES *et al*., 2015, TRAN; YEN, 2014; WEISS; NERD; MIZRAHI, 1994). The ability of auto-pollination in pitaya can be economically advantageous by reducing production costs, eliminating manual pollination and increasing profitability of fruit production (VALIENTE-BANUET *et al*., 2007). Cases such as that observed with *H. undatus* in this study in which self-pollination ensured 100% setting of well-formed and uniform fruits in size (Figure 3B) appear to be exception among the species of this fruit, and studies on the quality of fruits originated from this type of pollination need to be carried out.

Table 3 - Total number of fruits set and harvested, and fruit weight in two species of pitaya, *Hylocereus undatus* and *H. polyrhizus*, under cultivation in Quixeré - CE and submitted to four pollination treatments

Species of pitaya	Treatment	Flowers	Fruits set	(%)	Harvested fruits	(%)
H. polyrhizus	Open	30	30 a	100%	30 a	100%
	Restricted	30	14 b	47%	14 b	47%
	Nocturnal	30	28 a	93%	28 a	93%
	Daytime	30	30 a	100%	30 a	100%
H. undatus	Open	30	30 a	100%	30 a	100%
	Restricted	30	30 a	100%	30 a	100%
	Nocturnal	30	30 a	100%	30 a	100%
	Daytime	30	30 a	100%	30 a	100%

Figure 3 - A - Fruits harvested from the treatments performed in *Hylocereus polyrhizus*, visually showing the difference in size between treatments. B - Fruits harvested from treatments on *H. undatus*, visually show little difference between treatments



CONCLUSIONS

1. The flowers of *Hylocereus undatus* and *H. polyrhizus* present nocturnal anthesis onset and chiropterophilous syndrome, but in this study bats played no role in the crop pollination;
2. The stigma is receptive throughout the anthesis, the flower is self-compatible and can auto-pollinate. *Hylocereus undatus* is independent of biotic pollination to set fruits. *Hylocereus polyrhizus*, however, has limited auto-pollination capacity and requires biotic pollination for fruit formation;
3. *Apis mellifera* is the main floral visitor and potential pollinator of *Hylocereus polyrhizus* and biotic pollination deficit is a limiting factor for the productivity of *H. polyrhizus*;
4. The role of pollinators, especially *Apis mellifera*, on the quality of fruits produced by *Hylocereus polyrhizus* and *H. undatus* needs further investigation.

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