Complementary bee pollination maximizes yield and fruit quality in two species of self-pollinating pitaya[1]

Polinização complementar por abelha maximiza produção e qualidade dos frutos de duas espécies de pitaia autopolinizáveis

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ABSTRACT - Large-scale commercial production of pitaya is recent and there is little information on pollination and fruiting in this crop. Therefore, the objective of the present study was to investigate if the Africanized honeybee (*Apis mellifera*), frequent visitor of pitaya flowers (*Hylocereus undatus* and *H. polyrhizus*), plays any relevant role in the pollination of these cacti species, both in terms of fruit quantity and fruit quality. The study consisted of four treatments: natural pollination; restricted pollination; nocturnal pollination and pollination by *A. mellifera*, and all fruits were harvested and analyzed at 30 days after setting. Each treatment was evaluated in the number of fruits produced, total weight of the fruit; skin weight; pulp weight; longitudinal and transverse size; number of seeds; pH; acidity; total soluble solids (TSS); and TSS/total acidity ratio. Results showed that these pitaya species differ in their dependence on biotic pollination for the production, weight and quality of fruits. *H. undatus* did not depend on bees to set fruits but needed the moth *Agrius cingulata* to improve yield quality with larger and heavier fruits. On the other hand, *H. polyrhizus* depended on biotic pollination to maximize fruit production and *Apis mellifera* specifically to increase size and weight of fruits. In addition, the tested types of pollination influenced little the physical-chemical characteristics of fruits, being only relevant in the reduction of the pH in flowers pollinated by *A. mellifera*.

Key words: *Apis mellifera. Hylocereus polyrhizus. Hylocereus undatus*. Pollinator. Semi-arid region.

RESUMO - A produção comercial em grande escala de pitaia é recente e há pouca informação sobre polinização e frutificação nesta cultura. Portanto, o presente estudo teve por objetivo investigar se a abelha africanizada (*Apis mellifera*), visitante frequente das flores de pitaia (*Hylocereus undatus* e *H. polyrhizus*), desempenha algum papel relevante na polinização dessas espécies de cactáceas, tanto no que se refere à quantidade dos frutos, como em suas qualidades. O estudo constou de quatro tratamentos; polinização natural; polinização restrita; polinização noturna e polinização por *A. mellifera*, e todos os frutos foram colhidos e analisados 30 dias após o vingamento inicial. Cada tratamento foi avaliado no número de frutos produzidos, peso total do fruto; peso da casca; peso da polpa; tamanho longitudinal e transversal; número de sementes; pH; acidez; sólidos solúveis totais (SST); e relação SST/acidez total. Os resultados mostraram que essas espécies de pitaia diferem na sua dependência de polinização biótica para a produção, peso e qualidade dos frutos. *Hylocereus undatus* não dependeu de abelhas para vingar frutos, mas precisou da mariposa *Agrius cingulata* para melhorar a qualidade da produção com frutos maiores e mais pesados. Já *H. polyrhizus* dependeu da polinização biótica para maximizar a produção de frutos e de *A. mellifera*, especificamente, para aumentar o tamanho e peso dos frutos. Além disso, o tipo de polinização influenciou pouco as características físico-químicas dos frutos, sendo relevante apenas na redução do pH em flores polinizadas por *A. mellifera*.

Palavras-chave: *Apis mellifera. Hylocereus polyrhizus. Hylocereus undatus*. Polinizador. Região semiárida.

INTRODUCTION

Pitaya, pitahaya or dragon fruit are common names attributed to several species of hemiepiphyte cacti native of tropical and subtropical regions of the globe and that only recently have become commercially cultivated in several countries (FERNANDES *et al*., 2017). Because it is a new crop, and also because it includes different species, little is known about the requirements of pollination of the crop, potential pollinators and about the role of the different types of pollinators (MIZRAHI; NERD; SITRIT, 2002; MUNIZ *et al*., 2019).

The pitaya flower presents characteristics associated with chiropterophily syndrome, such as large, exposed (accessible to bats) and 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; AVILA JR.; SCHLINDWEIN, 2014). Thus, bats are recognized as the natural pollinators of the pitaya flower (LE BELLEC; VAILLANT; IMBERT, 2006; VALIENTE-BANUET *et al*., 2007; WEISS; NERD; MIZRAHI, 1994). However, some pitaya species do not produce nectar (VALIENTE-BANUET *et al*., 2007) and bats are not usual visitors of these flowers under cultivation (LE BELLEC, 2004; MARQUES *et al*., 2011). When present, they probably are pollenivorous, *i.e.*, feed on pollen (VALIENTE-BANUET *et al*., 2007). Therefore, in crops where the natural pollinator is lacking, pollination is carried out manually. This practice has become frequent for pitaya pollination and produces good results in regard to the weight and quality of the fruits, but entails an increase in production costs (LE BELLEC, 2004; LE BELLEC; VAILLANT; IMBERT, 2006; MENEZES *et al*., 2015a; WEISS; NERD; MIZRAHI, 1994).

Pollination requirements of the many species of pitaya have been little studied. Although the flowers are nocturnal, they remain open and receptive until 7a.m the following morning, sometimes extending until 9 a.m. in cloudy days. Therefore, flowers are frequented by both nocturnal and diurnal visitors that can potentially act as pollinators (LE BELLEC; VAILLANT; IMBERT, 2006; WEISS; NERD; MIZRAHI, 1994). Muniz *et al*. (2019), demonstrated that the species of pitaya *Hylocereus undatus* and *H. polyrhizus* differ on their requirements for biotic pollination. While *H. undatus* is capable of self-pollinating and has a high fruit set rate not depending on floral visitors, in *H. polyrhizus* self-pollinating is limited and the species requires biotic pollinators to maximize its fruit production. However, the role of biotic pollinators in fruit quality remains unknown for both pitaya species.

In the present work, we aimed to investigate whether the honeybee *Apis mellifera*, frequent visitor of the flowers of *Hylocereus undatus* and *H. polyrhizus*, plays any relevant role in the pollination of these cacti species. Our hypothesis is that this bee species can contribute to the pollination of pitaya flowers, increasing fruit production and/or improving its physical and physicochemical characteristics. This would lead to increases in the quantitative and qualitative production of these two species, justifying the use of *A. mellifera* as a managed pollinator of the crop, and avoiding additional costs of manual pollination.

MATERIAL AND METHODS

The study was carried out in the FRUTACOR Farm Ltda, situated in Quixeré, state of Ceará, Brazil. The weather in the region is classified as BSw'h' according to the Köppen-Geiger classification (ALVARES *et al*., 2013), with rainfall in Quixeré reaching 387.5 mm in 2016. The farm is located 140 m above sea level with 35 °C and 22 °C maximum and minimum mean annual temperature, respectively, mean annual relative humidity of 62% and mean wind speed of 7.5 m/s (FERNANDES *et al*., 2005).

The pitaya orchard occupies an area of two hectares (one hectare for each species), surrounded by banana (*Musa paradisiaca*) plantations and 1.28 km far from native forest, with two species (*Hylocereus undatus* and *H. polyrhizus*) grown in the planting system of double-rooted wooden masts, irrigated three times a day and receiving all cultural practices recommended for the cultivation of these cacti species (DE DIOS; MARTÍNEZ; CANCHÉ, 2014).

After flowering, fruits set were marked with colored ribbons to determine their age for harvesting four weeks later, thus obtaining production data on the spot. Fruits for commercialization were classified based on their weight in three categories: large-sized fruit (when it is heavier than 300 g); average-sized fruit (when it is lighter than 300 g and heavier than 200 g); and small-sized fruit (when it is lighter than 200 g and heavier than 150 g). Fruits weighing less than 150 g are not marketed.

Data collection was carried out from February to June 2016, the period of greatest plant production coinciding with the rainy season in the region. In this study, we followed five fruiting cycles, each one comprising an average of three days of blooming and 10 to 15 days between two consecutive flowering episodes, with a 14-18 days period of floral budding until the anthesis in both pitaya species. See also Muniz *et al*. (2019).

A total of ten plants, of each species, distributed along the ten rows of the orchard were randomly selected, in order to obtain a representative sample of the entire area. Among the selected plants, and for each pitaya species and

treatment, we marked six floral buds (only one per plant) in each of the five blooming episodes, totaling 30 floral buds per treatment, and producing a representative sample of all fruits produced during the blooming season. About the fruit set index and post-harvest evaluation, for each species, all floral buds in both species showed the same anthesis pattern, opening at 7 p.m. and closing around 7 a.m. next morning, except in cloudy days when anthesis could be extended up to 9 a.m. See Muniz *et al*. (2019).

Each pitaya species was submitted to four pollination treatments, as follows:

Natural pollination - The objective with this treatment was to know the level of pollination occurring naturally in the orchard and assess the quality of the fruits produced. Flower buds were marked at 4 p.m., when pre-anthesis characteristics were observed, and after opening the flower was left accessible to flower visitors throughout the entire anthesis period. The next day, when the flowers were already completely wilted around 1 p.m., they were marked with colored armbands to accompany fruit development until harvesting.

Restricted pollination - This treatment aimed to verify if the two pitaya species were capable of producing fruits without the action of biotic pollinating agents, and if these fruits were of commercial value. Flower buds were marked and bagged with NWF (Non-Woven Fabric) bags at 5 p.m. The bags had a ribbon to tie it up and prevent any pollinator from getting into the flower. The next day, at 1 p.m., when the flowers were already completely wilted and without the possibility of being pollinated, the NWF bags were removed and flowers were marked as in the previous treatment, but using a different color to allow discriminate between treatments.

Nocturnal pollination - This treatment aimed to investigate the role of nocturnal visitors in pollinating pitaya flowers and the quality of the fruits produced as a consequence of their action. Pre-anthesis flower buds were marked as in the previous treatment but they were not bagged, and flowers spend all night unprotected to be visited by nocturnal floral visitors until 4 a.m. in the morning, when they were bagged with NWF bags to prevent visits from early-foraging diurnal visitors. The flowers remained protected from daytime visitors until the bags were removed at 1 p.m., when they were already withered, then marked as in the previous treatments.

***Apis mellifera* pollination -** In this treatment, it was sought to observe the role of honeybees in the diurnal pollination of pitaya, both regarding fruit set and fruit quality. Pre-anthiesis flower buds were bagged with NWF bags at 5 p.m. and spent all night protected from nighttime visits. Only at 5 a.m. the next morning NWF bags were removed and only visits of *A. mellifera* bees were allowed until the flower was completely wilted. Treatment identification was made as described previously for other treatments.

Fruits of both pitaya species and from all treatments were harvested 30 days after the anthesis and taken to the laboratory for the following analyzes: total fruit weight, skin weight, pulp weight, longitudinal and transversal size of the fruit, number of seeds, pH, acidity, total soluble solids - TSS (ºbrix) and TSS ratio / total acidity. All procedures followed the methodology recommended in the manual of Adolfo Lutz Institute (INSTITUTO ADOLFO LUTZ, 2008).

First, fruits were individually weighed on a digital scale (model Marte BL3200H) to determine the total weight of each fruit. Then, each fruit was measured transversely and longitudinally with a pachymeter. After these measurements, each fruit was cut in half and a spoon was used to separate the skin from the pulp. Then, both the skin and the pulp were weighed separately.

For the seed count, five fruits of each treatment were separated in the two pitaya species. These fruits, after the weighing and measures described above, had their pulps individually bagged and frozen until the time of seed count. At the time of counting, only the pulp of one fruit was thawed at a time, because seed counting is a slow and time-consuming process. As the counting of the seeds of one fruit was finished, another was taken out of the freezer to thaw and the process was repeated one by one until all counts were finished.

Analyses of acidity, pH and TSS (ºbrix) were carried out in triplicates (three samples) taken from each treatment, and each sample contained the pulp of three fruits, which were chosen at random. All samples were homogenized in a blender for two minutes, placed in a plastic becker, and then covered with *pvc* cling film wrap and placed in the refrigerator to be used as the analyses progressed.

The TSS (ºbrix) was measured with refractometer (Abbe OPTRONICS, 0 - 95% brix, with digital reader) and each sample was always measured in duplicates. To do this, a small amount of the sample was collected with a spoon and placed on filter paper that retained the seeds and allowing their removal. Then the filter paper was gently pressed, and three drops of juice were placed in the reader of the refractometer, to then read and write down the results in a spreadsheet.

In the pH and acidity measurements, the equipment used was a Hanna pH 21 pH / mV meter pHmeter, a BEL CAP digital scale, magnetic stirrer, glass becker, glass spatula and burette. The solution used was 0.1 mol / L sodium hydroxide. With respect to pH, the process was simple and direct because it was a liquid sample. A glass spatula was used to stir and homogenize the pitaya juice

in the becker and then the pHmeter was inserted into the juice. After the digital meter stabilizes, the results were read and written in a spreadsheet.

The determination of acidity was performed in duplicates by weighing on a digital scale 5 g of each sample and adding 50 ml of distilled water for dilution. After dilution, the samples were placed on a magnetic stirrer and a calibrated pHmeter was then inserted into the sample. Then, with the aid of a burette, a sodium hydroxide solution was slowly added until it reached the nearest pH reading of 8.30. At that moment, the volume spent in the titration was recorded and all data inserted in a computer spreadsheet. Calculations to find the acidity by the amount of citric acid / 100g were made according to the following formula:

$$A = (10 \; x \; V \; x \; f \; x \; c) / P$$

Where: A = Acidity; V = mean volume of solutions used in the two titrations of each sample (sodium hydroxide solution 0.1 mol/L); f = Corrector factor of the solution (Sodium hydroxide 0.1 mol/L); c = Corrector factor of the organic acid (0.064); and P = Mean weight of the samples used in the titration (g).

The total soluble solids (°brix) / acidity ratio was calculated to identify the degree of ripeness of the fruits. It is based on the calculation of the ratio of total soluble solids (°brix) by Acidity expressed in organic acid (INSTITUTO ADOLFO LUTZ, 2008). Statistical analyses for comparison of weight and longitudinal and transversal measurement means, as well as the results of the laboratory analyses, were made through analyses of variance followed by the Tukey test at 5% of probability, using the program R (Version 3.3.1.). 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.

RESULT AND DISCUSSION

Results of fruit formation showed that the pitaya *Hylocereus undatus* set 100% of the marked flowers in all treatments, even the one in which flowers were kept bagged and floral visitors, including *Apis mellifera*, had no access to the flowers (Table 1), thus, confirming that this species of pitaya is self-compatible and can self-pollinate. These results show that *A. mellifera* plays no relevant role in the number of fruits set in *H. undatus*, contrasting with Menezes *et al*. (2015b), who found only 7% of fruit set when flowers were kept bagged.

In respect to *H. polyrhizus*, results differed significantly ($p<0.05$; $\chi^2 = 17.75$) among treatments. Only flowers exposed to natural pollination, and those visited by *A. mellifera* set 100% fruits, and did not differ from each other ($p>0.05$; $\chi^2 = 17.75$). Flowers open only to nocturnal visitors set 93%, but despite this lower fructification index they also did not differ significantly ($p>0.05$; $\chi^2 = 17.75$) to treatments of natural pollination and *A. mellifera* visits (Table 1). However, the restricted pollination treatment, which flowers received no floral visitors, set less than 50% flowers and was significantly different ($p<0.05$; $\chi^2 = 17.75$) to all other treatments (Table 1).

Results suggest that although *H. polyrhizus* is self-compatible, this plant species has a reduced capacity for self-pollination, requiring the intervention of biotic pollinators to complement the pollination of flowers and to maximize fruit production.

Table 1 - Total and the percentage of fruits set in two pitaya species, *Hylocereus undatus* and *H. polyrhizus,* under four pollination treatments. Quixeré, state of Ceará, Brazil

Pitaya species	Treatment	Flowers (n)	Fruits set	(%)
Hylocereus undatus	Natural	30	30 a	100
	Restricted	30	30 a	100
	Nocturnal	30	30 a	100
	Apis mellifera	30	30 a	100
Hylocereus polyrhizus	Natural	30	30 a	100
	Restricted	30	14 b	47
	Nocturnal	30	28 a	93
	Apis mellifera	30	30 a	100

Values followed by the same letters in columns, within each plant species, do not differ significantly at 5% of probability (Kruskal-Wallis test)

It is noted, however, that nocturnal pollinators failed to maximize fruit set, whereas *A. mellifera* reached the maximum possible figure. As *A. mellifera* set 100% flowers, the same index observed only in the treatment of natural pollination which also included visits of *A. mellifera*, it is feasible to state that honeybees are important pollinators of *H. polyrhizus*, since, in the other treatments without its presence, fruit setting was lower.

Mean weight of the fruits varied significantly (p<0.05; F = 5.279 and F = 38.7 for *H. undatus* and *H. polyrhizus*, respectively) between the treatments for the two species of pitaya, showing that biotic pollinators play an important role in the final yield weight in these plant species (Table 2). However, the way pollinators affected fruit weight varied among the species of pitaya.

In the case of *H. undatus*, fruits from nocturnal, natural and *Apis mellifera* pollination did not differ among them, but the first two treatments produced significantly (p<0.05; F = 5.279) heavier fruits than the fruits from the restricted pollination treatment, or self-pollination. This means that although *H. undatus* does not rely on biotic pollinators to set fruits by being able to self-pollinate (Table 1), these fruits are smaller and lighter than those from biotic pollination (Table 2). In fact, several crop species capable of self-pollination or wind-pollinated show increases in fruit number or yield weight when submitted to biotic pollination. See Rosa, Blochtein and Lima (2011), Milfont *et al.* (2013), and Rizzardo *et al.* (2012).

Due to the performance of nocturnal pollination and *A. mellifera* treatments, it can be assumed that nocturnal pollinators are responsible for producing heavier fruits in this species of pitaya, and their presence in the area should be stimulated. The study of Muniz *et al.* (2019) conducted simultaneously and in the same orchard of the present work showed that the moth *Agrius cingulata*

is virtually the only nocturnal visitor showing potential to pollinate pitaya flowers. Considering that the pitaya flower presents nocturnal anthesis and sphinx moths are recognized as efficient pollinators of this crop, perhaps the habit of visiting the flowers has favored increases in the percentage of cross-pollination and the effect of heterosis, already proven efficient in increasing the size, weight and/or quantities of substances in fruits and seeds, such as juices, sugars, oils, among others (GIANNINI *et al.*, 2015; MILFONT *et al.*, 2013; RIZZARDO *et al.*, 2012). The *Apis mellifera* treatment, however, produced fruits whose weight did not differ from the heaviest fruits of natural and nocturnal treatments, but also did not differ from the lighter ones from restricted pollination, placing honeybee contribution to the weight of the fruits in an intermediate position. Unlike *Agrius cingulata*, *A. mellifera* moved much within the flower, thus favoring self-pollination, which genetically speaking did not differ much from the pollination already achieved by the flower itself.

The increase in fruit weight of *H. undatus* due to biotic pollinators, especially nocturnal ones, was also important because no significant difference (p>0.05; F = 1.37) was observed in the weight of fruit skin of any treatment (Table 2). Thus, the observed differences in fruit weight of *H. undatus* were due to increases in pulp weight. In fact, pulp weight analysis showed the same significant differences observed for fruit weight, so that the average pulp weight of fruits from nocturnal, natural and *Apis mellifera* pollination treatments did not differ among them, but the pulps of fruits from the first two treatments were significantly (p<0.05; F = 4.97) heavier than the fruit pulp from the restricted pollination treatment (Table 2).

In *H. undatus*, regarding to fruit size represented by its longitudinal (p<0.05; F = 3.675) and transversal (p<0.05; F = 4.845) measures (Table 2), only those fruits originating from nocturnal pollination differed from fruits

Table 2 - Mean weight and measurements (± Standard Deviation - SD) of harvested fruits of pitayas *Hylocereus undatus* and *H. polyrhizus* under four pollination treatments. Quixeré, state of Ceará, Brazil

Pitaya species	Treatment (Type of pollination)	Total mean weights (g)			Total mean measurements (mm)		Number of seeds
		Fruit	Skin	Pulp	Longitudinal	Transversal	
Hylocereus undatus	Natural	487.40 ± 159 a	179.01 ± 71 a	307.44 ± 117 a	95.33 ± 12.4 ab	81.25 ± 9.7 ab	6,041 ± 1,365 a
	Restricted	387.29 ± 87 b	163.95 ± 40 a	225.87 ± 86 b	88.90 ± 9.4 b	75.57 ± 7.8 b	4,837 ± 1,176 a
	Nocturnal	502.56 ± 107 a	190.89 ± 47 a	313.07 ± 86 a	96.81 ± 4.9 a	83.41 ± 7.1 a	6,536 ± 1,102 a
	Apis mellifera	443.13 ± 130 ab	169.99 ± 60 a	274.15 ± 103 ab	93.59 ± 10.9 ab	78.56 ± 8.8 ab	6,678 ± 1,202 a
Hylocereus polyrhizus	Natural	377.22 ± 117 b	123.06 ± 32 b	243.79 ± 95 b	83.90 ± 9.4 a	78.80 ± 10 ab	6,190 ± 1,102 a
	Restricted	195.23 ± 68 d	89.63 ± 42 c	105.78 ± 47 d	67.58 ± 9.0 c	62.38 ± 8.4 c	1,139 ± 202 b
	Nocturnal	290.35 ± 84 c	102.06 ± 25 bc	189.05 ± 62 c	74.57 ± 8.9 b	73,21 ± 9.5 b	2,555 ± 935 b
	Apis mellifera	475.33 ± 138 a	169.45 ± 66 a	303.88 ± 102 a	88.94 ± 11.5 a	83.18 ± 9.8 a	6,829 ± 902 a

Values followed by the same letters in columns, within each plant species, do not differ significantly at 5% of probability (Tukey test)

of restricted pollination. The other treatments did not differ (p>0.05; F = 4.845) to each other.

The *H. polyrhizus* pitaya, however, presented quite different results from *H. undatus* in relation to the characteristics of the fruit as a function of the type of pollination (Table 2). The weight of the fruits, particularly, in this species of pitaya differed (p<0.05; F = 38.7) in all treatments (Table 2). Pollination by *A. mellifera* produced the heaviest fruits, followed by natural, then nocturnal and finally restricted pollination. This result clearly shows that *H. polyrhizus* not only depends on biotic pollinators to set most of its fruits, but that *A. mellifera* is the main pollinator under the conditions prevailing in the present study, since it maximizes fruit production (Table 1). Increasing in the weight of fruits of crops dependent on biotic pollination has already been demonstrated for other plant species. See Abrol *et al.* (2019), Giannini *et al.* (2015), and Vinícius-Silva *et al.* (2017). In the case of *H. polyrhizus*, the use of hand pollination has been recommended to ensure fruit set and production of heavier fruits (LE BELLEC; VAILLANT; IMBERT, 2006; TRAN; YEN; CHEN, 2015; WEISS; NERD; MIZRAHI, 1994).

Unlike the pitaya *H. undatus*, in *H. polyrhizus* fruits, the weight of the skin varied significantly (p<0.05; F = 19.21) with the pollination treatments. The fruit skins from *A. mellifera* treatment were the heaviest and differed significantly (p<0.05; F = 19.21) from the others (Table 2). The natural pollination treatment produced skins as heavy as the ones from nocturnal pollination treatment, but significantly (p<0.05; F = 19.21) heavier than those from the restricted pollination treatment. On the other hand, the restricted and nocturnal pollination treatments did not differ among themselves (p>0.05; F = 19.21) in terms of skin weight (Table 2). These results are important, because the fruit of the pitaya usually has 22-44% of skin that, in many cases, is discarded (ESQUIVEL; STINTZING; CARLE, 2007).

Despite the differences in the skin weights, pulp weights presented the same pattern observed for the weight of the fruits and discussed previously. In *H. polyrhizus*, fruits from pollination by *A. mellifera* presented pulp significantly (p<0.05; F = 33.19) heavier than the other treatments, followed by natural, then nocturnal, and finally restricted pollination (Table 2).

Regarding *H. polyrhizus* fruit size, those from *A. mellifera* and natural pollination treatments were significantly (p<0.05; F = 28.42 and F = 27.00 for longitudinal and transversal length, respectively) larger (longer and wider) than those from restricted and nocturnal pollination treatments, except for the transverse diameter between natural and nocturnal pollination, which were not significantly (p>0.05; F = 27.00) different

(Table 2). Nocturnal pollination produced significantly longer and wider fruits (p<0.05; F = 28.42 and F = 27.00, respectively) than restricted pollination (Table 2). The size and shape of the fruits have aspects of commercial importance because they can determine value gains or losses to agricultural production (BASHIR *et al.*, 2018; CALVETE *et al.*, 2010; GARRATT *et al.*, 2014; KLATT *et al.*, 2013). Finally, the same significantly different (p<0.05; F = 51.96) pattern among the four treatments observed for fruit weight and pulp weight was also proven for the number of seeds. Again, unlike *H. undatus*, in which the pollination type did not affect (p>0.05; F = 2.371) the average number of seeds per fruit, in *H. polyrhizus* fruits resulting from *A. mellifera* visits and natural pollination treatments presented a significant higher number of seeds (p<0.05; F = 51.96), followed by nocturnal and restricted pollination treatments, which did not differ (p>0.05; F = 51.96) between them (Table 2). This positive correlation between the weights of fruits and pulp, and the number of seeds has already been observed by other authors in conditions different from that studied here (OSUNA-ENCISO *et al.*, 2016; VALIENTE-BANUET *et al.*, 2007; WEISS; NERD; MIZRAHI, 1994). In this study, however, we demonstrated an association between these factors to flower visitation by *A. mellifera*.

The results obtained in the treatment of pollination with *Apis mellifera* for the pitaya *H. polyrhizus* are surprising. This treatment produced significantly better results (p<0.05) for fruit, pulp and skin weights than the natural pollination treatment, in which *A. mellifera* bees were also present. One would expect that these treatments, at the very least, would not differ due to the presence of honeybees in both treatments. One explanation, however, may be the fact that flowers of the *A. mellifera* pollination treatment remained covered all night long and were made available for visitation only at 5 a.m. in the morning. This procedure preserved the resources of these flowers, while in the natural pollinating treatment the flowers were accessible to visitors during the whole night, and were no longer so attractive the following morning. We observed that after removing the cover of the flowers in the *A. mellifera* pollination treatment, the number of bees visiting these flowers was always very large, up to more than 30 bees per flower (Figure 1A), and always greater than flowers of the natural pollination treatment and other flowers of the orchard that were not part of the experiment.

The relatively small size of *A. mellifera* in relation to the pitaya flower suggests that this bee is not an efficient pollinator because it could easily enter and leave the flower without touching the stigma. However, what we observed in the conditions described above was that the frenzy of many honeybees visiting the flower at the same time

Figure 1 - Flowers of pitaya (*Hylocereus polyrhizus*) in the experimental orchard in Quixeré, state of Ceará, Brazil. A - Many honeybees visiting a flower in the pollination treatment allowing only *Apis mellifera* visits. B - A stigma loaded with pollen grains following a great number of honeybee visits in the pollination treatment allowing only *Apis mellifera* visits

promoted contact with the stigma of the flower frequently, leading to a large deposition of its pollen (Figure 1B). But the smaller number of bees in the flowers of the natural treatment made them get in and out of the flower having less contact with the stigma. The result found contradicts Le Bellec (2004) when he argues that bees have limited effectiveness in pollinating *Hylocereus* flowers due to flower size. In the present study, we noticed for the pitaya *H. polyrhizus* that a large number of *A. mellifera* bees per flower produced more and heavier fruits, which also contained more pulp. In fact, in pitaya, fruit weight and size depend on several factors, such as management, pollination and genotype (CISNEROS; TEL-ZUR, 2012; MIZRAHI; NERD; SITRIT, 2002; TRAN; YEN; CHEN, 2015; WEISS; NERD; MIZRAHI, 1994).

When weeds were not cleaned out, the number of bees fell very much in the pitaya flowers of all the treatments, because they preferred to visit flowers of *Commelina benghalensis* L., a common weed growing in the orchard. Even though many of the still-open pitaya flowers were available early in the morning, *C. benghalensis* flowers attracted the bees even in cloudy days when the pitaya flowers remained open beyond 7 a.m.. This behavior clearly demonstrates that *C. benghalensis* flowers are more attractive for *A. mellifera* than pitaya flowers. Indeed, wild plants often compete for pollinating agents with cultivated plants and generally take advantage (MORANDIN; KREMEN, 2013). The higher number of honeybees in the flowers protected from nocturnal visitors, with consequent increase in the weight of the fruits, and the competition for pollination imposed by weeds, mainly, *C. benghalensis* are important factors that must be considered in the management of this crop aiming at yield increment due to pollination.

The type of pollination also influenced some of the physical-chemical characteristics for both pitaya species

(Table 3). Fruit pH was significantly lower (p<0.05; F = 6.29 and F = 9.91 for *H. undatus* and *H. polyrhizus*, respectively) in the treatment of *A. mellifera* visits in both pitaya species compared to other treatments, except in the case of restricted pollination in *H. undatus*. The other treatments did not differ (p>0.05; F = 9.914) from each other (Table 3).

There was no significant difference (p>0.05; F = 1.32 and F = 3.55 for *H. undatus* and *H. polyrhizus*, respectively) between the fruits of all treatments and both species of pitaya for total soluble solids - TSS (°brix). Also, no significant difference was found for acidity (p>0.05; F = 2.21) and the ratio of total soluble solids (°brix)/acidity (p>0.05; F = 3.16) for *H. undatus* (Table 3). However, in the case of *H. polyrhizus* there were significant differences for acidity (p<0.05; F = 7.08) and for the ratio of total soluble solids (°brix/acidity) (p<0.05; F = 5.73). Fruits from nocturnal, *Apis mellifera* and natural pollination treatments did not differ among them, but the first two treatments produced fruits with significant (p<0.05; F = 7.08) higher values of acidity compared to fruits from the restricted pollination treatment (Table 3). Regarding total soluble solids (°brix)/acidity ratio, fruits from restricted, nocturnal and natural pollination treatments did not differ among them, but the restricted treatment produced fruits with significant (p<0.05; F = 5.73) higher values of total soluble solids (°brix)/acidity ratio compared to fruits from the *A. mellifera* pollination treatment (Table 3).

The literature presents few studies for the physical-chemical characteristics of pitaya fruits, especially regarding differences as a function of types of pollination. In the present work, all treatments presented TSS (ºbrix) within or very close to acceptable values between 12 and 13 (MERTEN, 2003) and similar to the value 12.6 found by Centurión Yah *et al*. (2008) in Yucatán, Mexico with fruits harvested at 29 and 31 days after anthesis. However,

Table 3 - Mean values (± Standard Deviation - SD) for physycal-chemical characteristics of harvested fruits of pitayas *Hylocereus undatus* e *H. polyrhizus* under four pollination treatments. Quixeré, state of Ceará, Brazil

Pitaya species	Treatment (Type of pollination)	pH	TSS* (°Brix)	Acidity	TSS*/Acidity
Hylocereus undatus	Natural	5.48 ± 0.30 a	13.45 ± 0.90 a	0.15 ± 0.00 a	91.17 ± 19.70 a
	Restricted	4.92 ± 0.10 ab	13.47 ± 0.10 a	0.23 ± 0.00 a	59.42 ± 1.20 a
	Nocturnal	5.53 ± 0.50 a	12.92 ± 0.00 a	0.23 ± 0.10 a	63.37 ± 26.80 a
	Apis mellifera	4.67 ± 0.10 b	12.75 ± 0.50 a	0.26 ± 0.00 a	48.51 ± 3.40 a
Hylocereus polyrhizus	Natural	5.22 ± 0.20 a	13.87 ± 0.30 a	0.22 ± 0.00 ab	62.83 ± 8.30 ab
	Restricted	5.03 ± 0.20 a	12.78 ± 0.80 a	0.19 ± 0.00 b	68.94 ± 9.10 a
	Nocturnal	5.21 ± 0.00 a	13.57 ± 0.20 a	0.27 ± 0.00 a	51.72 ± 8.40 ab
	Apis mellifera	4.60 ± 0.20 b	13.15 ± 0.10 a	0.29 ± 0.00 a	45.02 ± 5.50 b

Values followed by the same letters in columns, within each plant species, do not differ significantly at 5% of probability (Tukey test); TSS - Total Soluble Solids

these values are well below those observed by Tran and Yen (2014), who found values of TSS (°brix) 19.4 and 18.2 in the self-pollination and natural pollination treatments, respectively. As for acidity, the means of the present study were exceptionally low, not higher than 0.29, while values around 0.4 are more usually found, as observed by Centurión Yah *et al*. (2008). This certainly explains why the value of the TSS (°brix)/acidity ratio was always above the desired value for consumption, which is below 40 (WEISS; NERD; MIZRAHI, 1994). In the present study, the treatments that presented values closer to these values were *A. mellifera* pollination in *H. undatus* (48.51) and *H. polyrhizus* (45.02), although no significant differences (p>0.05; F = 3.585 and F = 3.797 for *H. undatus* and *H. polyrhizus*, respectively) were found in respect to the other treatments, except between the *Apis mellifera* and restricted treatments for *H. polyrhizus* (Table 3).

CONCLUSIONS

1. The pitaya species studied here differ in their dependence on biotic pollination for fruit production, fruit weight and fruit quality. Although *Hylocereus undatus* is independent of floral visitors to set fruits by self-pollination, it requires complementary biotic pollination to produce larger and heavier fruits, increasing the quality of yield. *H. polyrhizus*, in turn, depends on biotic pollination both to maximize fruit production and to increase fruit size and weight;

2. *Apis mellifera* should be managed in the cultivation of *H. polyrhizus* as an essential pollinator. This bee species is capable of producing significantly heavier fruits than all other types of pollination tested and can supply pollination deficits without the need to resort to hand-pollination practices;

3. Protection of *Hylocereus polyrhizus* flowers from nocturnal visitors and control of flowering by weeds competing for pollination, such as *Commelina benghalensis*, are management techniques that can be used to increase the number of visits of *Apis mellifera* to flowers with consequent increases in the number of fruits set and the quality of fruit yield;

4. The type of pollination has little influence on the physical-chemical characteristics of the fruits of *Hylocereus undatus* and *H. polyrhizus*, being only relevant in the reduction of pH in flowers pollinated by *A. mellifera*.

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