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Article

Pollination of *Turnera subulata*: exotic or native bees?

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ABSTRACT. This work analyzed floral visitors of *Turnera subulata* Sm. (Turneraceae) within an anthropized area in Bahia (UEFS Campus), focusing on potential pollinators, fruit and seed production and the influence of climatic factors on this interaction. The study was carried during six months in 2018 and 2019. Aspects of floral biology and visitor behavior were observed. *Turnera subulata* flowers lasted approximately six hours and during this period they were exposed to visitors at different intervals to assess the peak time of fruit formation. The most frequent visitors were the bees *Apis mellifera* Linnaeus, 1758, *Trigona* sp., *Augochlora* sp. and *Protomeliturga turnerae* (Ducke, 1907). The foraging peak occurred in the morning, coinciding with the time of greatest formation of fruits and seeds, and with the time of greatest number of open flowers. Both exotic and native bees acted as potential pollinators, being considered complementary in the flower pollination process.

KEYWORDS. Potential pollinator, *Protomeliturga turnerae*, *Apis mellifera*, *Trigona*, *Augochlora*.

RESUMO. Polinização de *Turnera subulata*: abelhas exóticas ou nativas? Este trabalho analisou visitantes florais de *Turnera subulata* Sm. (Turneraceae) em área antropizada na Bahia (Campus UEFS), com foco nos potenciais polinizadores, na produção de frutos e sementes e na influência dos fatores climáticos nesta interação. O estudo foi realizado durante seis meses em 2018 e 2019. Foram observados aspectos da biologia floral e comportamento do visitante. As flores de *T. subulata* duraram aproximadamente seis horas e durante este período foram expostas aos visitantes em diferentes intervalos para avaliar o pico de formação dos frutos. Os visitantes mais frequentes foram as abelhas *Apis mellifera* Linnaeus, 1758, *Trigona* sp., *Augochlora* sp. e *Protomeliturga turnerae* (Ducke, 1907). O pico de forrageamento ocorreu pela manhã, coincidindo com o momento de maior formação de frutos e sementes, e com o maior número de flores abertas. Tanto as abelhas exóticas quanto as nativas atuaram como potenciais polinizadores, sendo consideradas complementares no processo de polinização das flores.

PALAVRAS-CHAVE. Polinizador potencial, *Protomeliturga turnerae*, *Apis mellifera*, *Trigona*, *Augochlora*.

Biotic pollination is a result of the reciprocal exploration between plant and pollinator (Rech & Brito, 2012), in which the participants are under the effect of the cost-benefit generated by the interaction (Rech & Brito, 2012). Ollerton *et al.* (2011) estimates that 85% of flowering plants are pollinated by animals, highlighting the importance of plant-pollinator interaction. However, classifying a floral visitor as a pollinator is not simple, given that it is necessary to consider the legitimacy of the visit, the frequency of visitation, the touch on reproductive structures, collection behavior, size of the animal, among other factors that influence pollen transfer (Freitas, 2013; Alves-dos-Santos *et al.*, 2016).

In addition to the behavioral and morphological aspects, the temporal aspects of the interaction are also important for the efficiency of pollination involving opening and closing times and other functions of the flowers, and the times of the activities of the floral visitors (Gimenes *et al.*, 1996). In addition, the daily activity of pollinators and plants is regulated by climatic factors in conjunction with intrinsic factors of the species. Van Doorn & Van Meeteren (2003) consider that the process of opening and closing the flowers is related to critical values of temperature, relative humidity and light intensity. Some authors consider that these climatic factors are also important to start and stop the daily activities of foraging bees, in addition to being able to regulate homeostasis inside the colonies of social species (Polatto *et al.*, 2014; Abou-Shaara *et al.*, 2017).

Among the interactions between flowers of angiosperms and pollinating insects, some families of native plants are noteworthy for their occurrence in anthropized environments, such as Turneraceae (Arbo & Silva, 2005; Rocha & Rapini, 2016), which can maintain native pollinators in the environment. *Turnera subulata* Sm. is a native species, classified as a ruderal plant, found in areas of open vegetation and in disturbed environments (Arbo & Silva, 2005; Rocha & Rapini, 2016). It blooms and bears fruit throughout the year contributing to the supply of pollen, nectar and nesting sites for different species of bees in the Caatinga (Brasil & Guimarães-Brasil, 2018), mainly from the Apidae and Andrenidae families (Medeiros

& Schlindwein, 2006). These flowers can be considered melittophilous. However, because the flower is visited by species of different orders of insects it can be considered generalist (Rodríguez *et al.*, 2015).

In view of the importance of some plants in anthropized areas for the maintenance of native pollinator species, this study aimed to understand how the interaction between *Turnera subulata* and floral visitors occurs. This interaction was analyzed especially with a focus on the morphological, behavioral and temporal adaptations of both organisms, thus identifying visitors who can act as efficient pollinators.

MATERIAL AND METHODS

Turnera subulata is characterized by presenting inflorescences uniflorous and solitary or pluriflorous at the apex of the branch; the flowers are hermaphroditic, presenting heterostyly (longistylous and brevistylous) and produce pollen and nectar as floral resources. The color of the petals varies from yellow to cream while the inner side of the flower has a dark brown color at the base of the petal, next to the nectary (Arbo & Silva, 2005; Rocha & Rapini, 2016).

The study was conducted at the Campus of the State University of Feira de Santana (UEFS), located in the municipality of Feira de Santana, Bahia, Brazil (12°11'S / 38°58'W). According to Santana & Santos (1999), the native vegetation of the study area in Feira de Santana is typical of the Caatinga biome, but currently the area is quite anthropized with the presence of many introduced and invasive species. The average temperature ranges from 20.7 to 26.8 °C, with annual rainfall ranging from 500 to 800 mm, the climate being classified as semi-arid (Köppen classification). The rainy months are mainly from March to July and the dry months, mainly September and October (Costa *et al.*, 2018).

Data collection was carried out between the months of October 2018 and July 2019 in five sample plots with an approximate size of 10 x 10 m each, with a distance between plots of approximately 100 m.

The test of receptivity of stigma and pollen viability was performed before opening and during the duration of the flower, and the flowers used were bagged before opening. These tests were carried out in May 2019, in areas 01, 02 and 03. In the field, these tests were repeated every hour between 5:30 am and 13:00 pm (from pre-anthesis to senescence of the flower).

To check the receptivity of the stigma, ten stigmas were removed from the flower with the aid of scissors and placed in a Petri dish containing 3% Hydrogen Peroxide (H_2O_2) solution. The formation of bubbles on the surface of the stigma indicated the activity of peroxidase enzymes, a positive response to stigmatic receptivity (Dafni & Maués, 1998).

To assess the time of greatest pollen viability ten anthers were removed with the aid of scissors and spread on a Petri dish, where 100 grains were selected to be stained in 1% neutral red solution ($C_{15}H_{17}ClN_4$) and counted (Kearns & Inouye, 1993). The colored pollen grain indicated the presence of cytoplasmic content and, therefore, pollen viability.

The inspection of the opening and closing time of the flowers was carried out in four days, two in October/2018 (one day in area 01 and one day in area 03); one in May/2019 (in area 05) and one in June/2019 (in area 02). The number of open flowers in the sample was counted throughout the flower's duration, at 15-minute intervals (four counts every hour). In each interval, the total number of open flowers was obtained. To define the number of flowers opened in the hour range, the number of flowers in a range was subtracted by the number of flowers in the previous range.

In order to verify the influence of microclimate factors on floral opening and closing, temperature, relative humidity and light intensity, data were collected every 15 minutes after counting the number of open flowers, from the opening to the closing of the flower. The microclimate data referring to the luminous intensity (lux) were measured with a Luxmeter. The temperature and relative humidity of the air were measured through a digital Thermohygrometer fixed at 1.0 m above the ground during field observations.

Data collection of daily foraging activities of floral visitors was carried out in October/2018 in areas 01, 02 and 03, and in November and December/2018 in areas 02, 03 and 04 (areas with the highest number of flowers), during three non-consecutive days of the same month. The first two days were reserved for counting the number of visits to the flowers and collecting the microclimate data, while the third day was intended for collecting the floral visitors. The counts of visits and observation of behavior started 30 minutes before opening until 30 minutes after closing the flower, usually between 5:30 am and 13:00 pm, lasting 15 minutes per hour in each area, so it was possible to monitor three areas in the same hour interval. The same procedure was used for the collection of floral visitors, which were carried out with the aid of an entomological network. In addition, observations of visits and collections were also made in June/2019 (areas 01, 02 and 04). Data were collected at two 15-minute intervals. Both for the data collections from October to December/2018 and in June/2019, there was a total of 30 minutes of observation in each area per month.

The behavior of visitors was described based on some criteria suggested by Alves-dos-Santos *et al.* (2016) to define efficient pollinator. These criteria are: legitimate visit, when the visitor accesses resources through the floral opening and touches the reproductive structures; intrafloral behavior suitable for touching; size and relative frequency. In addition to the efficient pollinator, we can classify the visitors as potential pollinators, taking into account that even if they do not present all the criteria mentioned above, they have some of these, having the potential to pollinate the flowers.

The size of the floral visitor was analyzed for later comparison with the size of the flower. For the analysis of the size of the floral visitors, measurements were made of body length (from the middle ocellus to the apex of the abdomen) and thorax width (distance between the tegulas). Visitors were classified as: robust medium (length 10.0 to 14.0 mm and width 4.0 to 6.0 mm); medium thin (length 10.0 to 14.0 mm and width 3.0 to 4.0 mm); small medium (length 7.0 to 10.0 mm and width 2.5 to 3.0 mm); and small (length under 7.0 mm and width < 2.5 mm) (Viana & Kleinert, 2005).

The relative frequency of visitors was obtained from the frequency of a given species over other species and was estimated using the following ratio (number of visits per species/total number of visits) X 100.

The floral visitors collected were deposited in the Prof. Johann Becker Entomological Collection at the Zoology Museum of the Universidade Estadual de Feira de Santana (MZFS).

The experiment for analyzing the formation of fruits over the duration of the flowers was carried out in the months of November, December/2018 and February/2019 (areas 02, 03 and 04, respectively), and June/2019 (areas 01, 02 and 04). In the first three months 144 flower buds were used, with 48 buds each month (six every hour and six for the control), which were bagged the day before the experiment. The next day, a set of six flowers was exposed to visitors for just 30 minutes every hour and the rest of the flowers remained bagged. This process repeated every hour as long as there were flowers open. In June/2019, 240 flower buds were used (seventy flowers in each sample plot, ten flowers every hour and ten flowers for the control). This experiment started before the floral opening, at 05:30 am and ended at 13:00 pm. After 15 days, before opening the capsules, the generated fruits were harvested and dissected to count the number of seeds in development.

For statistical analysis, mixed generalized linear models (GLMM) were constructed with Poisson distribution in the IBM SPSS Statistics 20 Data editor, accepting as representative the models with a significance level of 0.05%. To test which predictor variable constituted the most significant model in relation to the abundance of open and closed flowers, two sets were built for the model: one to explain the opening and another to explain the closing of the flowers. The number of flowers opened or closed each hour was used as response variable and temperature, relative humidity and light intensity as predictor variables. In addition, we have included collection dates and time as random variables. Data from the months of October/2018, May and June/2019 were used.

To test which predictor variable constituted the most significant model in relation to the frequency of visitors, the number of visits (abundance) was used as the response variable while temperature, relative humidity and light intensity were used as predictive variables. In addition, we have included collection dates and time as random variables. In the case of bee visits, these visitors were separated into categories, according to the size identified. For these analyzes, data from the months of October, November and December/2018 and June/2019 were used.

RESULTS

Opening and closing hours of flowers. *Turnera subulata* has ephemeral flowers, with an average longevity of 5 to 6 hours. The prefloration is contorted type, thus, during the opening of the flower the petals leave the vertical position (bud phase) and are positioned perpendicularly to the reproductive structures, position in which it receives floral visitors. After senescence, the external whorls return to the vertical position.

All stigmas of the tested flowers were receptive from pre-anthesis to senescence of the flower, indicating activity of the peroxidase enzyme. In addition, all pollen grains submitted to the pollen viability test showed a positive result, indicating that all grains had cytoplasmic content, and thus, they could be viable for pollination from the pre-anthesis phase until the senescence of the flower.

The floral opening period was recorded in the morning, between 6:15 and 9:29 am, and the largest number of open flowers occurred in general between 6:45 and 8:14 am. Small differences occurred between the areas and months of the study, which were between 6:29 and 7:29 am in October (Area 1), between 7:00 and 8:45 am in October (Area 3), between 6:15 and 7:59 am in May (Area 3), and between 6:29 and 8:45 am in June (Area 4). After these intervals, the number of open flowers decreased (Fig. 1). As the opening and closing process occurred during the morning and these are slow processes, sometimes in the same sample and in the same hour interval the flowers could be in different stages of opening: buds, flowers opening, completely open, flowers closing, and completely closed. However, this occurred only three times during the entire fieldwork period. The floral senescence occurred between 7:45 and 12:00 pm. Small differences occurred between the flower peak times closing between the months and the observed areas, which were in October from 9:15 to 10:14 am (Area 1), in October from 9:45 to 10:29 am (Area 3), in May from 9:45 to 11:29 am (Area 3), and in June from 10:00 to 10:14 am (Area 4) (Fig. 1). During the months of October/2018 and May/19 it was observed that both the opening and the floral senescence occurred earlier than in June / 2019.

The flowers of *Turnera subulata* opened when the temperature values varied between 25 and 29°C and began to close with temperature variation between 29 and 35°C (Fig. 1 and 2). Regarding the luminous intensity, the flowers opened at low values, between 18 (x 1000) and 33 (x 1000) lux as the opening hours of the flowers were close to the sunrise times. Conversely, the onset of floral senescence occurred with values of higher luminous intensity, varying between 108 (x 1000) and 549 (x 1000) lux (Figs 1, 2). The tendency for the variation in light intensity followed the same pattern as the temperature, with the values increasing from sunrise, with high values at noon (Fig. 2). In October it was observed that the flowers opened earlier in area 01 than in area 03, and area 03 was located in a more shaded area.

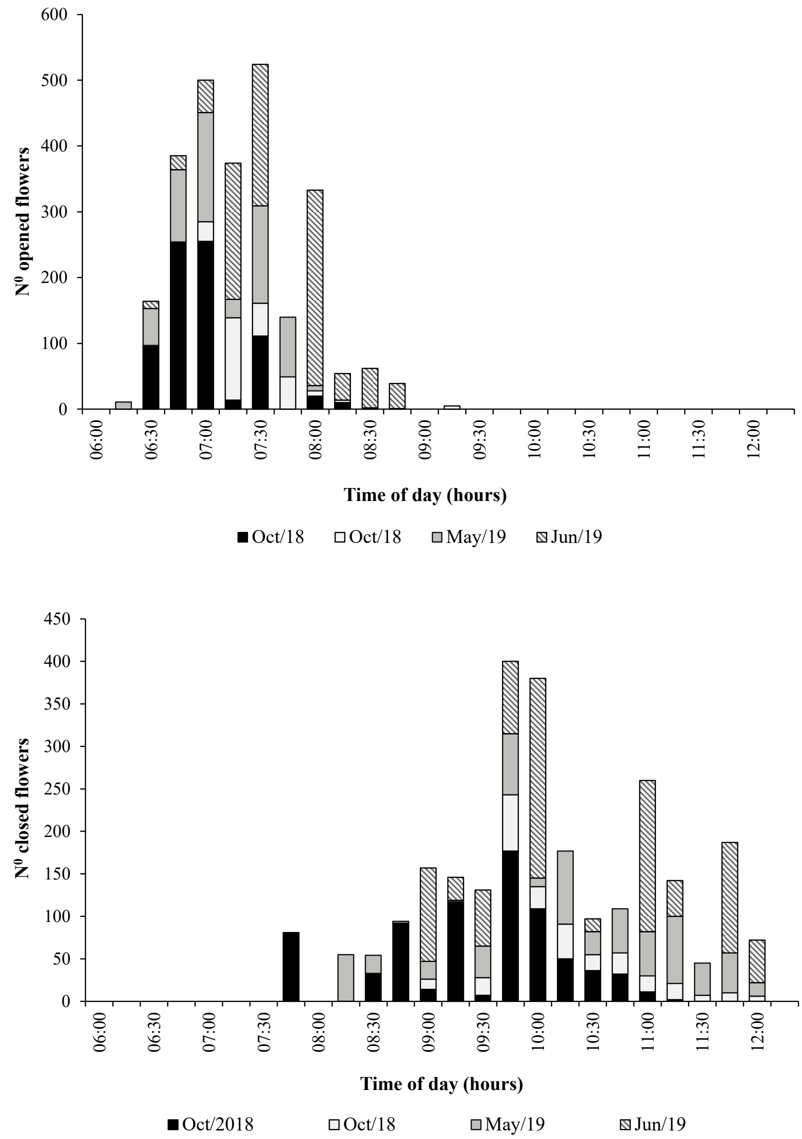

Fig. 1. Opening and senescence time of the flowers of *Turnera subulata* Sm. in October, 2018, May and June, 2019 in the UEFS campus, Feira de Santana, BA, Brazil.

During this month, the highest values of temperature and light intensity and the lowest relative humidity were also observed compared to other months of flower observation.

The result of the statistical analysis for opening the flowers was significant for increasing the luminous intensity [F (1, 96) = 39.947; p = 0.000; t = 6,320], indicating a positive effect. In relation to the closing of the flowers, the result was significant for the reduction in the relative humidity value [F (1.96) = 88.443; p = 0.000; t = -9.404] and for the interaction between relative humidity and light intensity [F (1.96) = 10.017; p = 0.002; t = 3.165]. Indicating that the most important microclimate factor for the closure of *T. subulata* flowers was the reduction of relative humidity, followed by an increase in light intensity.

Floral visitors. Insects of the orders Hymenoptera (95%), Lepidoptera (3.7%) and Diptera (0.2%) were observed in *Turnera subulata* flowers. Bees were the predominant visitors, especially those from Apidae family such as *Apis mellifera* Linnaeus, 1758, *Tetragonisca* sp. and *Trigona* sp.; Andrenidae: Protomeliturgini, mainly *Protomeliturga turnerae* (Ducke, 1907) and Halictidae: Augochlorini, mainly *Augochlora* sp. and some species of Halictini.

During the observations of visits, individuals of Andrenidae were not identified to species level and for this reason they were identified with the family name. However, in the collected specimens the predominance of *P. turnerae* was observed. The same occurred for the species of the Halictidae, which during the visits' observations were identified as Halictini or Augochlorini, nevertheless, among the specimens collected in the flowers there was a predominance of the genus *Augochlora* sp. Regarding the size of the bees that visited the flowers of *Turnera subulata*,

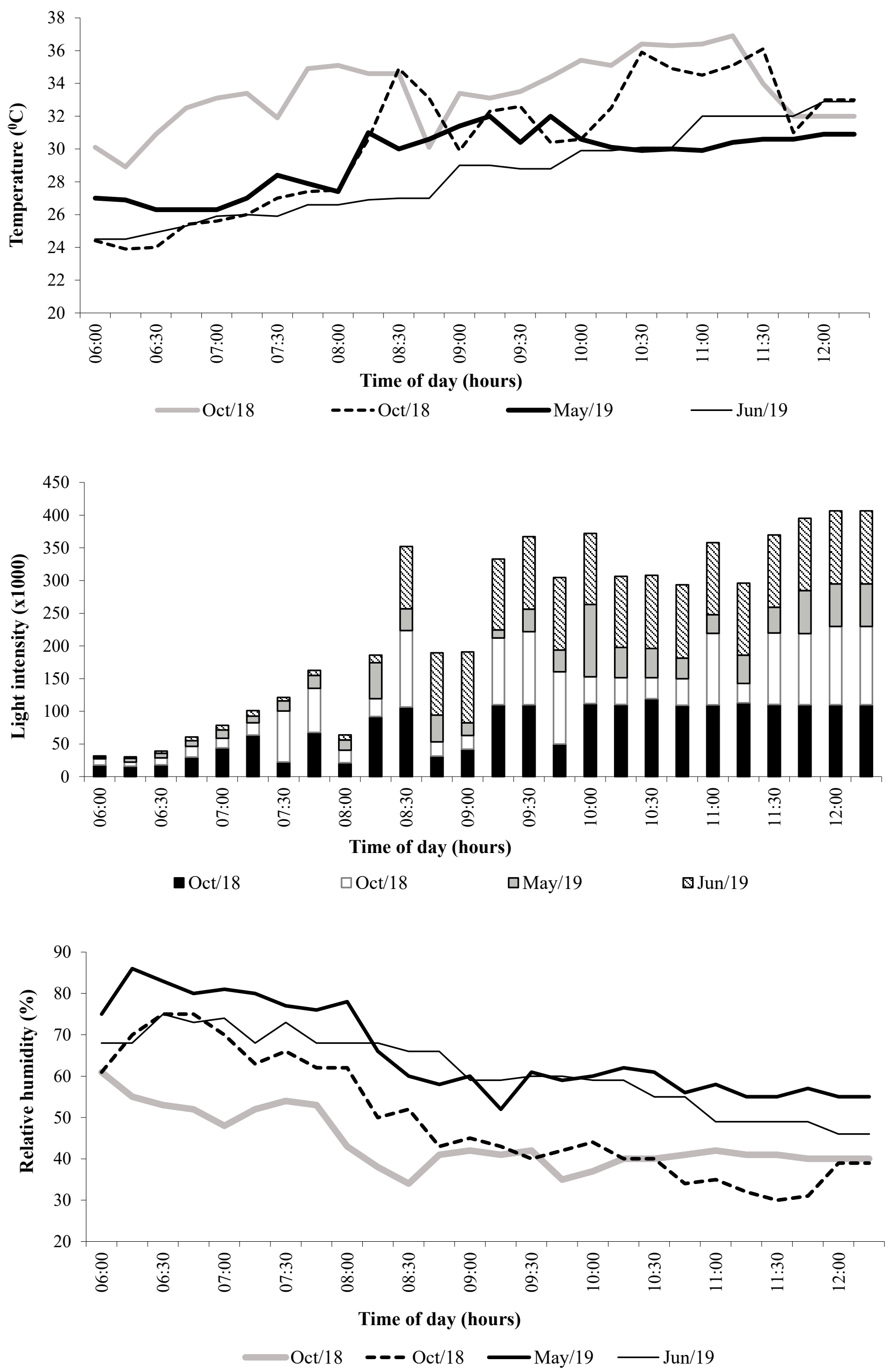

Fig. 2. Climatic factors (temperature, light intensity and relative humidity) along the floral longevity, covering the opening and senescence of the flowers of *Turnera subulata* Sm. in October, 2018, May and June, 2019 within the UEFS campus, Feira de Santana, BA, Brazil.

Apis mellifera was the only bee to have a medium thin size. The other visiting bees were small and among these, *Tetragonisca* sp. presented the smallest size (Tab. I, Fig. 3). The most frequent bees on flowers in number of visits were *Apis mellifera* (65.8%), *Trigona* sp. (18.7%) and Andrenidae species (9.9%) (Tab. I).

In general, the time with the highest number of individuals and species visiting *Turnera subulata* was the hour interval from 8:00 to 10:00 am. In the other intervals there were less visits and species (Fig. 3). Generally, the hours of greatest activity of the bees coincided with those of the greatest number of open flowers, occurring earlier in October and later in June.

Tab. I. Number of visits, relative frequency (FR), length (M+SD, Mean + Standard Deviation), width (M+SD, Mean + Standard Deviation), size and stigmatic contact (SC) of bees visiting *Turnera subulata* Sm in Feira de Santana, Bahia, Brazil from October to December 2018. All the bees observed were collecting pollen and nectar from the flowers. Size: MT = medium thin and P = small. SC: Y = yes, N = no and E = eventually.

Family	Genus/species	Visits (N)	FR (%)	Lenght (mm) (M+SD)	Width (mm) (M+SD)	Size	SC
Halictidae		75	2,8	7,72 ± 2,66	2,26 ± 0,95	P	Y
Andrenidae		269	9,9	6,96 ± 1,90	2,07 ± 0,68	P	Y
Apidae	*Apis mellifera*	1783	65,8	10,12 ± 1,37	3,17 ± 0,16	MT	Y
Apidae	*Tetragonisca* sp.	76	2,8	5,53 ± 1,30	1,34 ± 0,65	P	E
Apidae	*Trigona* sp.	508	18,7	6,87 ± 1,96	2,07± 0,69	P	Y

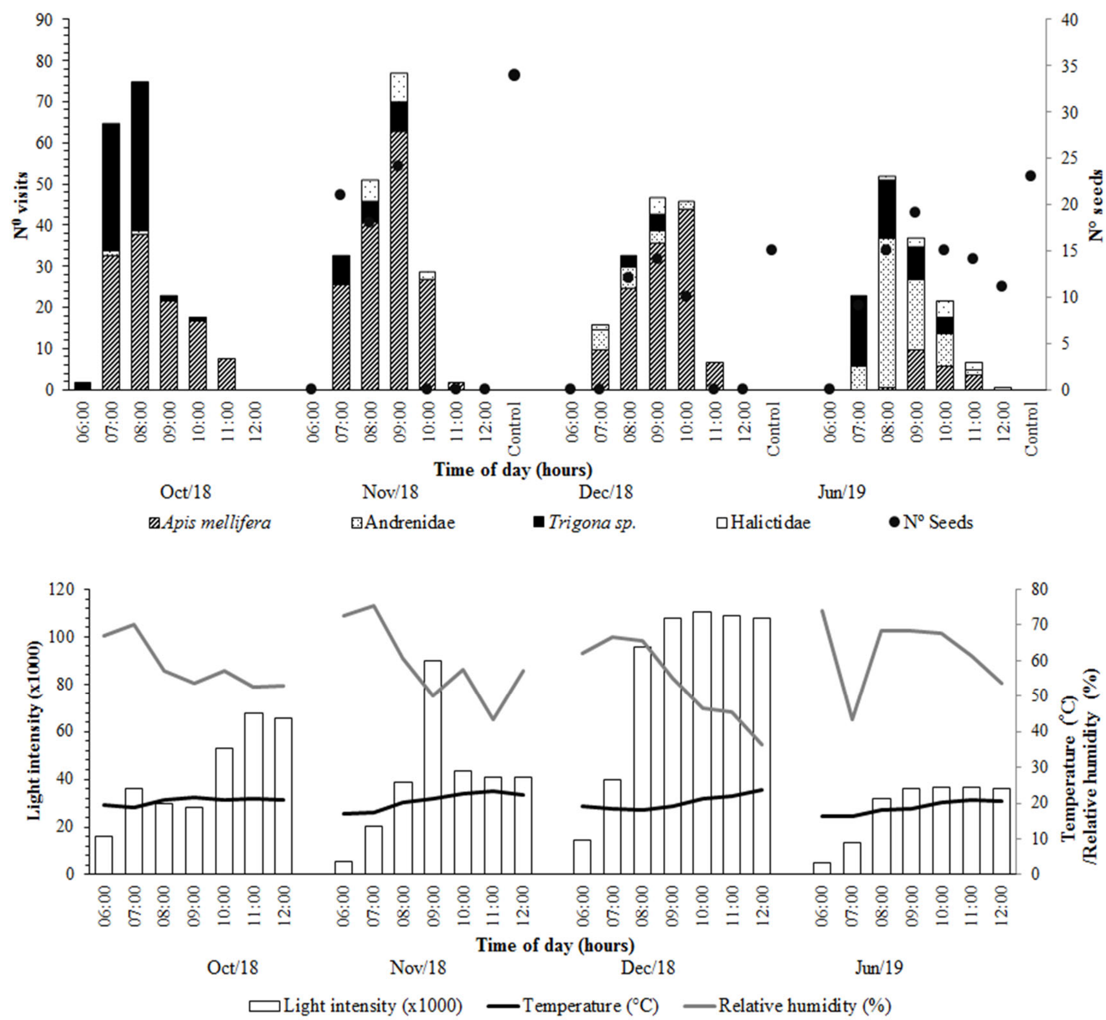

Fig. 3. Frequency of floral visitors during visits to *Turnera subulata* Sm. in October, November and December, 2018 and June, 2019 and average number of seeds at each hour interval in November and December 2018 and June 2019 within the UEFS campus, Feira de Santana, BA.

Apis mellifera was the most frequent visitor in October and *Trigona* sp., a small-sized bee, the second most frequent bee. This month, both bees, had the highest number of visits in the range from 7:00 to 9:00 am. In November and December there was a predominance of *A. mellifera*, especially between 8:00 and 10:00 am, and from 9:00 to 10:00 am, respectively. In June, the month with the highest number of flowers, there was a predominance of small bees (Fig. 3), who visited the flowers between 7:00 and 10:00 am. These were mainly species of Andrenidae, especially *Protomeliturga turnerae*.

The statistical analysis of visits by *Apis mellifera* was significant for light intensity, temperature and relative humidity as predictive variables. Thus, the increase in luminous intensity [F (1,144) = 31,704; p = 0.000; t = 5,631], the increase in temperature [F (1,144) = 50,946; p = 0.000; t = 7,138] and the reduction in relative air humidity [F (1,144) = 25,889; p = 0.000; t = 5,088] promoted a positive effect on the number of visits of this species. There were no significant results for the relationship between the number of visits of small bees and the climatic data.

Visitors behavior. In general, *Apis mellifera*, *Trigona* sp., species of Andrenidae and Halictidae entered the longistylous flowers resting on the stigma with their heads turned downwards, towards the anthers, and collected pollen. In the brevistylous flowers the bees landed directly on the anthers, removing the pollen with the front legs. For the collection of nectar, the bees moved towards the dark spots of the flower (nectar guide) with the head turned downwards. While collecting nectar the bees could touch the stigmas of the flowers. In longistylous flowers, *A. mellifera* and *Trigona* sp. touched the stigmas with the abdomen and thorax, while in brevistylous flowers the bees touched the stigmas with the head and dorsal region of the chest, during nectar collection.

Individuals of Andrenidae positioned themselves on the petals and with the first pair of legs removed the pollen spread over the head at the end of the collection. Some individuals were observed copulating on the flower petals, others remained immobile on the petals after 11:00 am until the flower was completely closed.

The bees *Apis mellifera*, *Trigona* sp. and those of the Andrenide and Halictidae families flew between *Turnera subulata* co-specific individuals collecting pollen and nectar, contacting the stigma of both floral morphs.

Bees of the genus *Tetragonisca* sp. came into contact with the reproductive structures of the flowers only occasionally, because, when they reached the flower, they always rested on the petals, running through it until reaching the nectary, passing between the stigmas (in brevistylous flowers) and anthers (in longistylous flowers), usually without touching them. In the brevistylous flowers they landed directly on the anthers for the collection of pollen and in the longistylous flowers they positioned themselves on the petals and then collected the pollen.

Insects of other orders were not observed contacting the stigma of flowers. The butterflies (Lepidoptera) landed on the flower petals, introduced the proboscis into the nectary and collected nectar without coming into contact with the reproductive structures. Diptera were also observed collecting nectar; they also did not touch the reproductive structures and made visits only in the early morning, between 6:00 and 8:00 am.

Fruit formation timing. During the fruit formation experiment, microclimate data showed different results between the months studied. In November, December/2018 and February/2019, the flowers generally opened around 6:30 h, when the temperature varied from 26 to 38 °C, luminous intensity between 13390 and 113200 lux, and the relative humidity between 74 and 31%. In June, during three days of observation, the flowers opened from 7:00 am, with temperature varying between 22 and 34 °C, light intensity between 95.6 and 110300 lux and relative humidity between 86 and 43%.

Of the 384 flowers used in the hourly pollination experiment, 135 were pollinated and generated fruit (90 used in the hourly pollination experiments and 45 in the control). Formed fruits were the result of pollination that occurred at different times in the months of observation. In November, fruit and seed formation occurred between 07:00 and 09:59 am, in December and February between 08:00 and 10:59 am and in June from 07:00 am to 12:59 pm. However, in all months of the study, a large number of fruits formed (from 60 to 80% of the total) was observed between 8 and 8:59 am (Tab. II). The number of seeds produced per fruit was higher, generally between 9:00 and 9:59 am. The highest average seed/fruit was obtained in the control experiment in November/2018 (34) (Tab. II).

During November, December and June, the times of greatest production of fruits and seeds coincided with the peak times of visits by bees of both sizes, small and medium thin.

DISCUSSION

Turnera subulata occurs intensively within the UEFS Campus, an anthropized area. This was expected because this species is considered native, ruderal and found in open vegetation areas such as the Caatinga and also in disturbed environments (Arbo & Silva, 2005; Rocha & Rapini, 2016).

Many flowers of *Turnera subulata* opened daily, with the highest number being observed in June, the rainy month in the region. These flowers remained open during the morning, starting to open early and the floral senescence occurred especially between mid-morning and midday, being considered short-lived ephemeral flowers. These flowers had receptive stigma and viable pollen from pre-anthesis to floral senescence, indicating that the viability of reproductive structures allowed the flower to be fertilized at any time during the time it remained open.

Other authors have also observed that *Turnera subulata* flowers were ephemerals, but with differences in opening and closing times, depending on the area of occurrence. Oliveira & Santos (2016) registered that *T. subulata* flower lasted five hours in state of Paraíba, with the floral opening occurring at 06:00 and closing at 11:00 am. However, Oliveira *et al.* (2003) observed that the flowers of *Turnera* sp. lasted approximately six hours in Palmeiras (Chapada Diamantina-BA), but with floral opening and closing occurring later, at 07:30 am and 13:00 pm respectively. Although the flowers were short-lived in the different locations of occurrence, the opening and closing times may vary, which may be the effect of microclimatic differences between areas.

The floral opening generally occurred shortly after sunrise, when the light intensity did not show high values. The effect of light intensity can be observed when the opening of the flowers occurred earlier in areas with greater exposure to the sun and later in shaded areas or on cloudy days.

Tab. II. Formation of fruits and seeds of *Turnera subulata* Sm. by hour interval (HI) in the months of November, December/2018, February and June/2019, in the UEFS Campus, Feira de Santana, Bahia, Brazil.

HI	Flowers (N)	Fruits (N)	Sucess (%)	Seed/fruit (N)
Nov/2018				
07:00	6	3	50	21
08:00	6	4	67	18
09:00	6	1	17	24
Control	6	6	100	34
Dec/2018				
08:00	6	5	83	12
09:00	6	3	50	14
10:00	6	1	17	10
Control	6	6	100	15
Fev/2019				
08:00	6	4	67	15
09:00	6	4	67	7
10:00	6	1	17	14
Control	6	6	100	25
Jun/2019				
07:00	10	1	10	9
08:00	10	6	60	15
09:00	10	5	50	19
10:00	10	4	40	15
11:00	10	4	40	14
12:00	10	1	10	11
Control	10	9	90	23

The influence of light intensity on these flowers can be confirmed by the significance of the statistical analyzes where the positive effect of this factor on the opening moments of the flowers was observed. MEDEIROS & SCHLINDWEIN (2006) observed that on cloudy days (with less light intensity) *T. subulata* flowers opened later. CAJÁ *et al.* (2015) verified in flowers of *Turnera ulmifolia* L., plants that were fully exposed to the sun, opened earlier than plants that were in the most shaded areas. The effects of light in different forms such as light cycles, wavelength and light intensity were considered by BAI & KAWABATA (2015) in the floral opening processes in *Eustoma grandiflorum* (Raf.) Shinners (Gentianaceae).

The influence of climatic factors on senescence occurred differently than on floral opening, considering that the reduction in relative humidity followed by the increase in light intensity influenced the process of closing the flowers.

Flowers of *Turnera subulata* can be classified as melitophilous due to their floral characteristics, the frequency of bees visiting the flowers and the inefficiency of non-bee visitors as pollinators. Among the visitors, bees of medium thin size (*Apis mellifera*) and small size (*Augochlora* sp., *Tetragonisca* sp., *Trigona* sp. and *P. turnerae*) stood out, with *A. mellifera* being the most frequent visitor, especially in the months hotter and drier. According to MEDEIROS & SCHLINDWEIN (2006), the fidelity of *A. mellifera* to flowers of this plant is observed by the low number of pollen grains of other plant species found in the corbicles during foraging in the flowers. According to HUNG *et al.* (2018), different plant species can be pollinated through a single visit by *A. mellifera*, demonstrating how efficient this bee can be. On the other hand, there are also studies demonstrating that *A. mellifera* can negatively influence the pollination of native plants (AIZEN *et al.*, 2008; ABOU-SHAARA, 2014; NABORS *et al.*, 2018).

Small bees also visited flowers of *Turnera subulata*, among them *Trigona* sp. and *Protomeliturga turnerae*. MEDEIROS & SCHLINDWEIN (2006) considered that *Trigona spinipes* (Fabricius, 1793) presented a behavior of fidelity to the flowers of *T. subulata* because they present a high number of pollen grains in the corbicle. Another small bee, *P. turnerae*, was considered an oligolectic species by several researchers, because it collects pollen almost exclusively from *T. subulata* flowers, in addition to using them to establish male territory and as a mating place (MEDEIROS & SCHLINDWEIN, 2003, 2006; MILET-PINHEIRO *et al.*, 2013).

The reduced number of bees species that visited the flowers observed in the study area can be attributed to the fact that the area is constantly modified, undergoing an intense process of anthropization. In works carried out in natural areas, the number species visiting this plant was higher (LOCATELLI *et al.*, 2004; MEDEIROS & SCHLINDWEIN, 2006; VILHENA *et al.*, 2010). P. P. Carlos (pers. observ.)

identified that the reduction of green areas and the increase in urbanization provides the large number of individuals of *A. mellifera* and the decrease of native bee individuals visiting the flowers of *T. subulata*. However, in the rainy month (June) a small number of *A. mellifera* was observed in the flowers, with small bees predominating, especially females and males of *P. turnerae*. This bee is probably associated with rainy periods, because in another area of Caatinga (in Paraíba, Northeast of Brazil) Medeiros & Schlindwein (2003) observed that *P. turnerae* was present in the *T. subulata* flowers during the rainy period but absent in the driest periods of the year.

The influence of climatic factors in daily foraging activities was only statistically verified for *A. mellifera*, in which the positive effect of light intensity and temperature, and the negative effects of relative humidity were verified. This relationship between higher values of temperature and light intensity with the increased visit of bees to flowers was also reported by Polatto *et al.* (2012): observation based on 19 plants species.

Medium thin (*A. mellifera*) and small bees foraged throughout the duration of the flowers, showing peak activity at similar times throughout the day in the months of observation, concentrated in the interval from 7:00 to 11:00 am. In October, November and December, *A. mellifera* dominated visits in flowers and in June there were a greater number of small bees, especially *Protomeliturga turnerae*.

In general, the daily bee activity time coincided with time of the highest fruit production, being earlier in the driest and hottest months and later in rainiest and coldest month, regardless of the species and the size of the bees. Even with the viability of reproductive structures during the entire duration of the flower, fruit production and seed occurred at defined hour intervals, demonstrating that the flowers in this period received enough pollen for fruit and seed formation.

In general, bees of different sizes are expected to have different performances in terms of resource collection, both related to activity time and to resource collection behavior in flowers. However, this was not observed in this study. *Turnera subulata* was pollinated and produced fruits, both in months when *A. mellifera* was abundant, and in June, when this species visited few flowers, but small bees were frequent visitors. Both pollinators groups may be considered as redundant in flowers of *T. subulata* and thus keep fruit production of this plant. In addition, these two groups of bees can be considered as complementary in different months of the year, thus guaranteeing pollination in this species of plant, when one group or another is in dominance in the flower.

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