Acta Botanica Brasilica - 31(4): 665-676. October-December 2017.
doi: 10.1590/0102-33062017abb0102

Floral and reproductive biology of *Alcantarea nahoumii* (Bromeliaceae), a vulnerable endemic species of the Atlantic Forest

Maria Josirene Souza Moreira Bastos[1], Lucimário Pereira Bastos[1], Everton Hilo de Souza[1,2,3*], Taliane Leila Soares[2], Daniel Vieira Morais[1], Fernanda Vidigal Duarte de Souza[2] and Maria Angélica Pereira de Carvalho Costa[1]

Received: March 16, 2017
Accepted: July 7, 2017

ABSTRACT

Alcantarea nahoumii occurs exclusively in the state of Bahia, Brazil, and is classified as vulnerable due to deforestation and frequent fires in the region. Knowledge of floral and reproductive biology is fundamental to understanding ecological interactions, as well as the reproductive success of plant species. The objective of this study was to evaluate the floral and reproductive biology of *A. nahoumii* in an Atlantic Forest fragment with regard to phenology, pollen viability, stigma receptivity, pollination ecology and reproductive systems, all of which are important parameters for of the development of conservation strategies for the species. Anthesis is diurnal and heterogeneous, starting at 6:30 a.m. and lasting until 8:00 a.m. Highest germination percentages and greatest pollen tube lengths were obtained in BK culture medium. Histochemical tests revealed high pollen viability (89.71 %). Stigma receptivity occurred during anthesis and lasted for up to 24 hours after floral opening. *Alcantarea nahoumii* exhibited preferential allogamy and self-compatibility, and required a pollinator to production of viable seeds. Sixteen species of pollinators were observed visiting *A. nahoumii*, among which were five hummingbird species. Even though its reproductive system is efficient, this bromeliad remains threatened mainly due to habitat fragmentation caused by deforestation, burning and predatory extractivism.

Keywords: Bromeliaceae, floral visitors, nectar, pollen viability, reproductive systems, stigma receptivity

Introduction

The Brazilian Atlantic Forest is one of the main centers of diversity and endemism of the family Bromeliaceae, with a total of 653 species recorded in the last decade (Forzza *et al*. 2013). Fifty-four of these species are classified as critically endangered, 89 as endangered, 182 as vulnerable, 17 as rare and three as extinct in nature (Forzza *et al*. 2013); however, these numbers are probably underestimates due to the lack of knowledge regarding the actual status of wild populations of species of Bromeliaceae. The genus *Alcantarea* is native to Brazil and has approximately 40 species (Versieux & Wanderley 2015). The plants of this genus show various adaptations that enable them to grow in habitats with water stress and high luminosity, such as on granite boulders and other outcrops of the Serra do Espinhaço in the states of Minas Gerais and Bahia (Versieux *et al*. 2012; Versieux & Wanderley 2015). *Alcantarea nahoumii* occurs exclusively in Bahia and is classified as vulnerable (Forzza *et al*. 2013). It is estimated that in recent years the population has declined by 30 % due to deforestation and frequent fires (Versieux & Wanderley 2007; 2015; Forzza *et al*. 2013).

[1] Universidade Federal do Recôncavo da Bahia, Rua Rui Barbosa, s/n, Campos Universitário, 44380-000, Cruz das Almas, BA, Brazil
[2] Embrapa Mandioca e Fruticultura, Rua Embrapa, s/n, Chapadinha, 44380-000, Cruz das Almas, BA Brazil
[3] Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, 70040-031, Brasília, DF, Brazil

* Corresponding author: hilosouza@gmail.com

Because of over-exploitation and ongoing destruction of the Atlantic Forest, combined with the growing interest of traders in ornamental species, bromeliads are at serious risk of disappearing, making it necessary to develop conservation policies (Zanella *et al*. 2012). However, the adoption of conservation strategies requires basic knowledge for the development of correct management practices, *in situ* or *ex situ*.

Knowledge of floral and reproductive biology is fundamental for understanding the interactions among pollen grains, stigma, flowers and pollinators, as well as the reproductive success of plant species through survival strategies and preservation mechanisms (Negrelle & Muraro 2006). Likewise, understanding phenology is important and has been of increasing significance over the past decade due to its relevant role in the management and conservation of native plants (Elmendorf *et al*. 2016). Aspects of the phenology of Bromeliaceae have not been widely studied considering the large number of species. Understanding phenology can shed light on the life cycle of plants, their phases in time, the resources within communities and plant-animal interactions (Tschapka 2004). However, phenological events of plants can be interrupted due to climatic and landscape changes, which together with other anthropic effects can pose threats to local populations (Dai *et al*. 2013).

The family Bromeliaceae exhibits extensive variation in reproductive systems (Benzing 2000). Allogamy and self-compatibility are present in the majority of species (Wendt *et al*. 2001; 2008; Matallana *et al*. 2010; 2016), but there are also records of agamospermy, autogamy, and self-incompatibility (Martinelli 1994; Scrok & Varassin 2011; Paggi *et al*. 2013; Souza *et al*. 2017). Studies of the floral and reproductive biology of *A. nahoumii* are important for formulating management and conservation programs, especially since this species is subjected to pressures from habitat fragmentation, including shortages of specific pollinators and reduced population sizes, which can lead to pollen limitation.

The objective of this study was to investigate the floral and reproductive biology of *Alcantarea nahoumii* in an Atlantic Forest fragment, and acquire novel data related to reproduction forms and pollination mechanisms of this endemic and vulnerable species.

Materials and methods

Species and study area

Plants from a natural population of *Alcantarea nahoumii* (Leme) J. R. Grant, located in Serra da Jibóia in the municipality of Santa Teresinha, Bahia, Brazil (12°51'08.19''S 39°28'34.32''W), were used to analyze phenology, reproductive systems and different aspects of floral and pollination biology. This mountain range encompasses approximately 59 km^2, with a maximum altitude of 850 m and a climate varying from moist tropical to subtropical. A dried voucher specimen was deposited in the HURB herbarium (voucher 9416) (Universidade Federal do Recôncavo da Bahia, UFRB).

Phenology

Monthly observations were conducted between January 2012 and December 2015, for a total of 48 months. Climate data (minimum, maximum and mean temperature and rainfall) were obtained from the National Meteorology Institute (INMET 2016). To determine the timing of floral opening and the months of blooming, 40 randomly chosen individuals were analyzed within the population. Peak blooming was defined as when 50 % of the individuals sampled had flowers. The number of flowers opened per day and the flower availability period (interval when the flower remains open and available to visitors, from anthesis to senescence) were recorded during the entire flowering period. To describe flowering phenology, the number of flowers opened each day and the period of anthesis and of fruit shedding were quantified. Phenological study followed the recommendations of Machado & Semir (2006) and Marques & Lemes Filho (2008).

Pollen viability

Three flowers from three plants were collected at three different times: anthesis (8:00 a.m.) and post-anthesis (noon and 6:00 p.m.). Using a tweezers, pollen grains were uniformly spread on Petri dishes containing 25 mL of one of four culture media: BM (Parton *et al*. 2002); BK (Brewbaker & Kwack 1963); MBK (Brewbaker & Kwack 1963) modified with sucrose (20 %), and SM (Soares *et al*. 2008). The dishes were kept in a dark chamber at a temperature of 27 ± 1 °C.

The experimental design used to study pollen germination was completely randomized in a (4 x 3) factorial scheme, with four culture media (BM, BK, MBK and SM) and three times (8 a.m., 12 noon and 6 p.m.), with 12 repetitions (each repetition being a Petri dish).

The number of germinated pollen grains was counted and the length of the pollen tubes was measured 24 hours after inoculation in the culture medium, as described by Soares *et al*. (2008). Photographs were obtained with a Leica EZ4 D stereomicroscope (Leica, Wetzlar, Germany).

Percentage data were transformed to arcsin ($\sqrt{x}/100$) prior to statistical analysis. To compare means, the data were submitted to analysis of variance and the Tukey test ($p<0.01$), with the SAS program (SAS Institute 2010).

For histochemical assays pollen grains were collected at 8:00 a.m., distributed on glass slides and submitted to four stains: (i) 2,3,5-triphenyltetrazolium chloride (TTC) at 1 % diluted in Tris HCl 0.15 M buffer at pH 7.8 (Shivanna & Rangaswamy 1992); (ii) Alexander's stain at 1 % in lactic acid (Alexander 1980); (iii) acetic carmine at 1% in water (Kearns & Inouye 1993); and (iv) Lugol's iodine at 1 % (Dafni 1992).

The stained pollen grains were observed by random sampling using the slide scanning method with a Carl Zeiss 77081 optical microscope (Jena, Germany), to count 100 grains per slide, with three repetitions per treatment, for a total of 300 grains for each stain.

Stigma receptivity

Stigma receptivity was assessed using hydrogen peroxide (3 %) (Zeisler 1933) and a solution of α-naphthyl acetate with phosphate buffer, acetone and fast blue B salt (Pearse 1972; Dafni 1992) at three times: pre-anthesis (budding, 6:00 a.m.), anthesis (8:00 a.m.) and post-anthesis (24 h after anthesis), with three repetitions, each one consisting of a stigma from a different plant.

Stigma receptivity was estimated by assigning degrees, as adapted from Dafni & Maués (1998): (-) no reaction; (+) weak positive response; (++) strong positive response; and (+++) very strong positive response.

Reproductive systems

Reproductive systems were investigated in the field using the method described by Kearns & Inouye (1993). The treatments included: a) open pollination - floral buds were only selected and identified; b) manual self-pollination - flowers were protected during pre-anthesis and at anthesis were pollinated with grains from the same plant; c) spontaneous self-pollination - flowers during pre-anthesis were protected and remained that way until fruiting; d) manual cross-pollination - flowers were protected during pre-anthesis and anthesis and were pollinated with grains from other plants; and e) agamospermy - flowers were emasculated (anthers removed) and protected until fruiting. In treatments (b-e), flowers were protected from floral visitors with voile fabric bags (15 x 10 cm) of about 5 cm mesh, from pre-anthesis until fruiting.

Pollination was performed at 8:00 a.m. (anthesis) under the same conditions of temperature (22 ± 3 °C) and relative humidity (88 %). Different numbers of flowers were used for each reproductive treatment, according to their availability (Tab. 1). Fruiting was monitored weekly until ripening, and fruits were collected before falling. The following parameters were determined for each treatment: fruiting percentage, length (cm), diameter (cm), number of seeds, and *in vitro* germination rate (%).

The autogamy index (AI) and self-incompatibility index (SII) were calculated according to Ramirez & Brito (1990). A species is considered autogamous or partially autogamous when the AI is greater than 0.30, and non-autogamous when it is lower than 0.30. In turn, a species is considered compatible or partially compatible with a SII greater than 0.30, while self-incompatible with a value below a 0.30.

The number of pollen grains produced per flower was estimated according to Kearns & Inouye (1993), in three flowers. The number of ovules was counted in each locule of the ovary with a Leica EZ4 D stereomicroscope (Wetzlar, Germany), using three different flowers from each individual. The pollen/ovule ratio was determined according to Cruden (1977).

In vitro germination of seeds

Seeds resulting from the different reproductive treatments were removed from ripe fruits and disinfested in a solution of sodium hypochlorite (2 % active chlorine) and distilled water (2:1) for 20 minutes, followed by washing three times in distilled water and sterilization in a laminar flow chamber. The seeds were distributed in Petri dishes containing 25 mL of MS culture medium (Murashige & Skoog 1962) supplemented with 3 % sucrose and solidified with 2 g L^{-1} of Phytagel®, with the pH adjusted to 5.8. The seeds were kept in a growth room at a temperature of 25 ± 2 °C, and with luminosity of 22 mmol $m^{-2}s^{-1}$ and a 16-hour photoperiod. The experimental design was completely randomized, with 10 repetitions of groups of eight seeds. The germination percentage was determined for each reproductive treatment.

Nectar production

Floral buds were protected on the day before collection and the nectar produced was collected hourly from 8:00 a.m. to 6:00 p.m. The volume of nectar produced was quantified

Table 1. Number, length and diameter of fruits, seeds produced and seed germination percentage of *Alcantarea nahoumii* (Leme) J. R. Grant (Bromeliaceae) in function of different reproductive treatments.

Pollination systems	Fruit formation rate (%)	Fruit length (cm)	Fruit diameter (cm)	Number of seeds	Seed germination (%)
Open pollination	35 (31/86) a	4.25 ± 0.44 a	1.25 ± 0.12 a	446 ± 116 a	82 a
Manual self-pollination	10 (10/100) b	3.26 ± 0.33 b	1.14 ± 0.10 a	199 ± 73 b	52 c
Spontaneous self-pollination	0 (0/104) c	0	0	0	0
Manual cross-pollination	52 (41/76) a	4.46 ± 0.21 a	1.25 ± 0.10 a	454 ± 141 a	75 b
Agamospermy	0 (0/98) c	0	0	0	0
AI	0.00				
SII	0.44				

The number of fruits /number of flowers produced are in parentheses. (AI) Autogamy Index; (SII) Self-Incompatibility Index. The means with different letters in the column differ significantly by the Tukey test at 1 % probability.

by introducing a needle attached to a graduated microsyringe (Hamilton 50 μL) through the corolla and into the floral nectary of each flower in the direction toward the base of the petals. After quantification, the nectar was placed in a Reichert digital refractometer and the refraction index was converted into sugar concentration (sucrose equivalent) according to Bolten *et al*. (1979). To transform the data into Kcal, the total volume of nectar per flower (mg) was multiplied by the factor 4.0 (Galetto & Bernardello 2005).

The experimental design was completely randomized and composed of 20 flowers from different individuals. Pearson correlation coefficients were calculated for the traits and their significance was measured by the t-test at 1 % probability, using Genes 7.0 software (Cruz 2008).

Floral visitors

The floral visitors were observed with a digital infrared camera directly in the field during two time periods (five consecutive days in October and eight alternating days in November), from 6:00 a.m. to 6:00 p.m. and from 6:00 p.m. to 6:00 a.m., for a total of 156 daytime hours and 96 nighttime hours. The following parameters were observed: behavior, floral resource, time of day, visit duration and relative frequency of each floral visit per day. The relative frequency of visits of each species was calculated as the number of visits performed by the species in relation to the total number of visits recorded, and denoted as a percentage. Visitors were photographed with the digital camera from three angles and invertebrates were collected in an insect net and sacrificed in a bottle containing ethyl acetate. The dead invertebrate specimens were sent for identification by the Insect Group of Universidade Federal do Recôncavo da Bahia. All occurrences of agonism between hummingbirds and flowers were recorded, and the birds were identified from the photos by a specialist from the Zoology Museum of Universidade Estadual de Campinas.

Results

Phenology

Flowering occurred from 2013 to 2015, with peak blooming in October through December, and the number of flowered plants in 2013 was lower than in 2014 (Fig. 1). Only two plants bloomed in 2012, in December. This low flowering rate is related to the low rainfall that year (714 mm) and a fire in March that affected most of the experimental area (Figs. 1, 2A).

No flowering was observed for plants in any of the four years during the period from February to July (Fig. 2B). The first bracts from the floral scape were observed in August through mid-September, at the end of the rainy season, with a change in coloration from green to yellow-green (Fig. 2C). The time between emission of the floral scape and the first flower was on average 61 ± 9 days, with a further 87 ± 11 days before opening of the last flower at the apex of the inflorescence, a period covering spring and summer, from October through March (Fig. 1).

Each inflorescence contained an average of 150.76 ± 64.98 buds, with a range of 34 to 312, of which only 37.50 % produced flowers. Blooming occurred heterogeneously, with an average of 0.64 flowers per plant/day and varying from zero to six flowers from the start to end of blooming.

Anthesis was observed to be diurnal and not homogeneous, starting at 6:30 a.m. and lasting until 8:00 a.m., when the thin yellow petals were fully open and curled downward (Fig. 2C – detail). The flowers remained open 24 hours after anthesis, although exhibiting clear signs of necrosis of the petals, pistil and style, and remained adhered to the inflorescence regardless of the occurrence of pollination.

Fruiting took place from February to April and the fruits fell 178 ± 18 days after pollination, in the period from May to June, with opening of the capsules and dispersion of some of the seeds by wind (Fig. 2D). Some seeds remained adhered to the fruits and germinated in the capsules and inflorescences (Fig. 2D – detail). The seeds dispersed by the wind to form new individuals stood apart from the others by having a feathery appendage, a typical characteristic of the subfamily Tillandsioideae.

After flowering and senescence (February to March), plants formed five sprouts (lateral shoots), which started a new reproductive cycle two years later. Therefore, the reproductive, flowering, fruiting and dispersion period of this species was relatively long (approximately seven months), with reproductive structures forming in the second half of the year and the dispersion period ending in the middle of the next year.

Pollen viability

Alcantarea nahoumii exhibited high pollen viability regardless of the type of test (*in vitro* germination or histochemistry). We observed a significant effect of the interaction between the factors of culture media and collection time for *in vitro* germination percentage of pollen grains and pollen tube length. The BK culture medium was associated with the highest germination percentages during anthesis, with 95.41 % of the grains germinating, while the MBK medium was the least favorable for germination (82.00 %) (Tab. 2, Fig. 2E). However, in all media the highest pollen viability occurred during the 8 hours of anthesis.

With respect to the histochemical tests, the highest pollen viability percentage was obtained with 1 % Alexander's stain, with 94 % viability, followed by 1 % TTC with 91 % (Fig. 2G-H), 1 % Lugol's iodine with 90.40 % and 1 % acetic carmine with 83.46 % (Fig. 2I-J). Also, Alexander's stain and TTC produced the best distinction, by the difference in

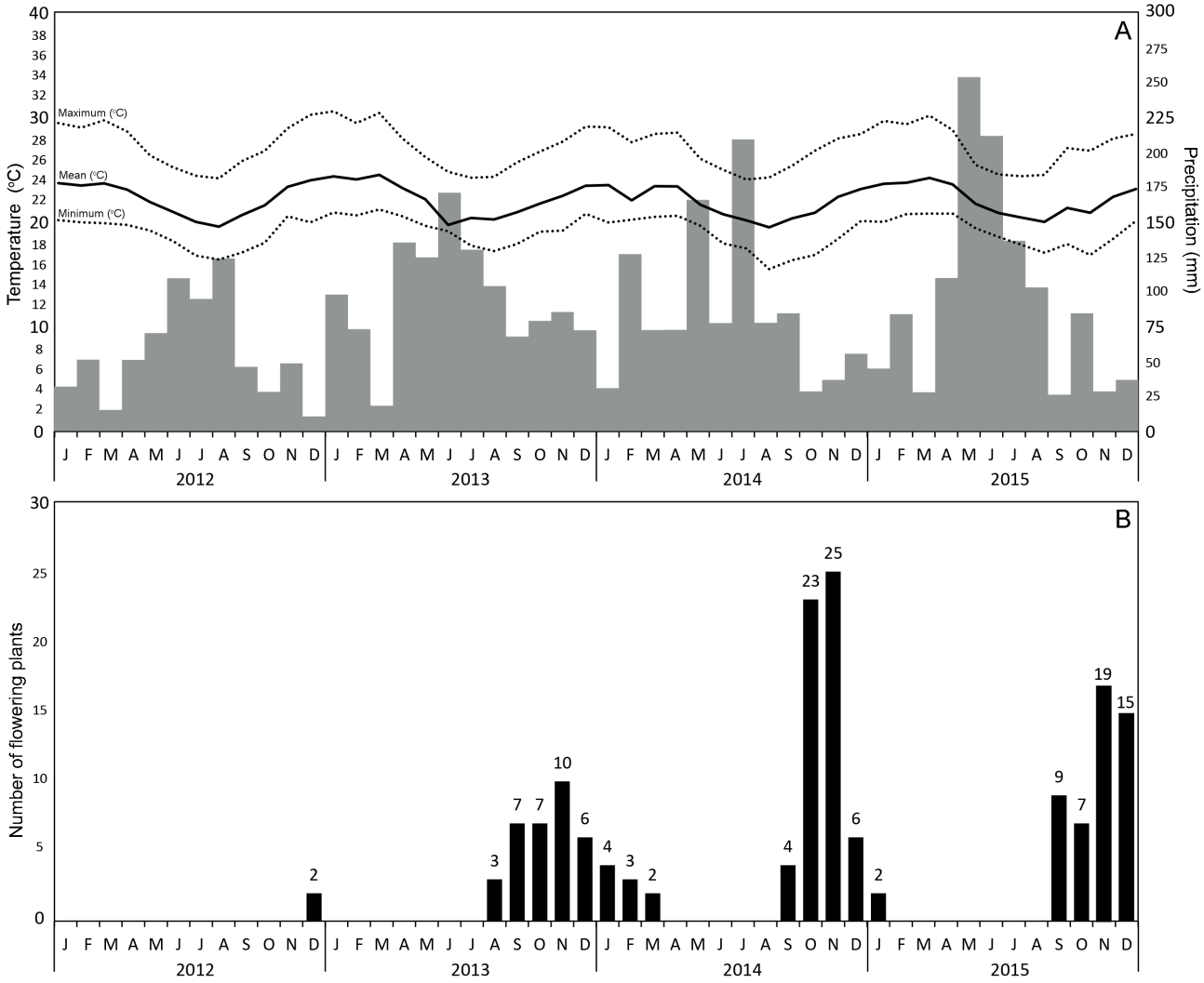

Figure 1. Climate data in the region of Santa Teresinha, Bahia (A) and flowering frequency of *Alcantarea nahoumii* plants (B) from Serra da Jibóia 2012 to 2015.

coloration, between the viable and nonviable pollen grains, in comparison with the other two stains (Fig. 2).

Stigma receptivity

Stigma receptivity started in the beginning of anthesis and continued until 24 hours afterward (Tab. 3). No pre-anthesis receptivity was observed by either of the two methods applied. Both methods were efficient at evaluating stigma receptivity of *A. nahoumii,* producing similar results (Tab. 3). The use of 3 % hydrogen peroxide caused the formation of air bubbles in the stigma area, indicating very strong peroxidase enzyme activity. The solution of α-naphthyl acetate, phosphate buffer, acetone and fast blue B salt conferred a dark brown color when the stigma was receptive.

Reproductive systems

Significant differences were observed among the pollination treatments in the formation of fruits with fertile seeds for manual cross-pollination (52 %, 41/76), open pollination (35 %, 31/86), and manual self-pollination (10 %, 10/100). The treatments of spontaneous self-pollination and agamospermy did not produce any fruits (Tab. 1).

In the treatments that produced fruits, the length and diameter of the capsules averaged 3.26 ± 0.33 cm and 4.46 ± 0.21 cm, respectively. The number of seeds produced differed significantly among the treatments, with the greatest number of seeds being formed by manual cross-pollination (454 ± 141) and open pollination (446 ± 116), while manual self-pollination formed 199 ± 73 seeds. The germination percentage of seeds from manual self-pollination was 52 %,

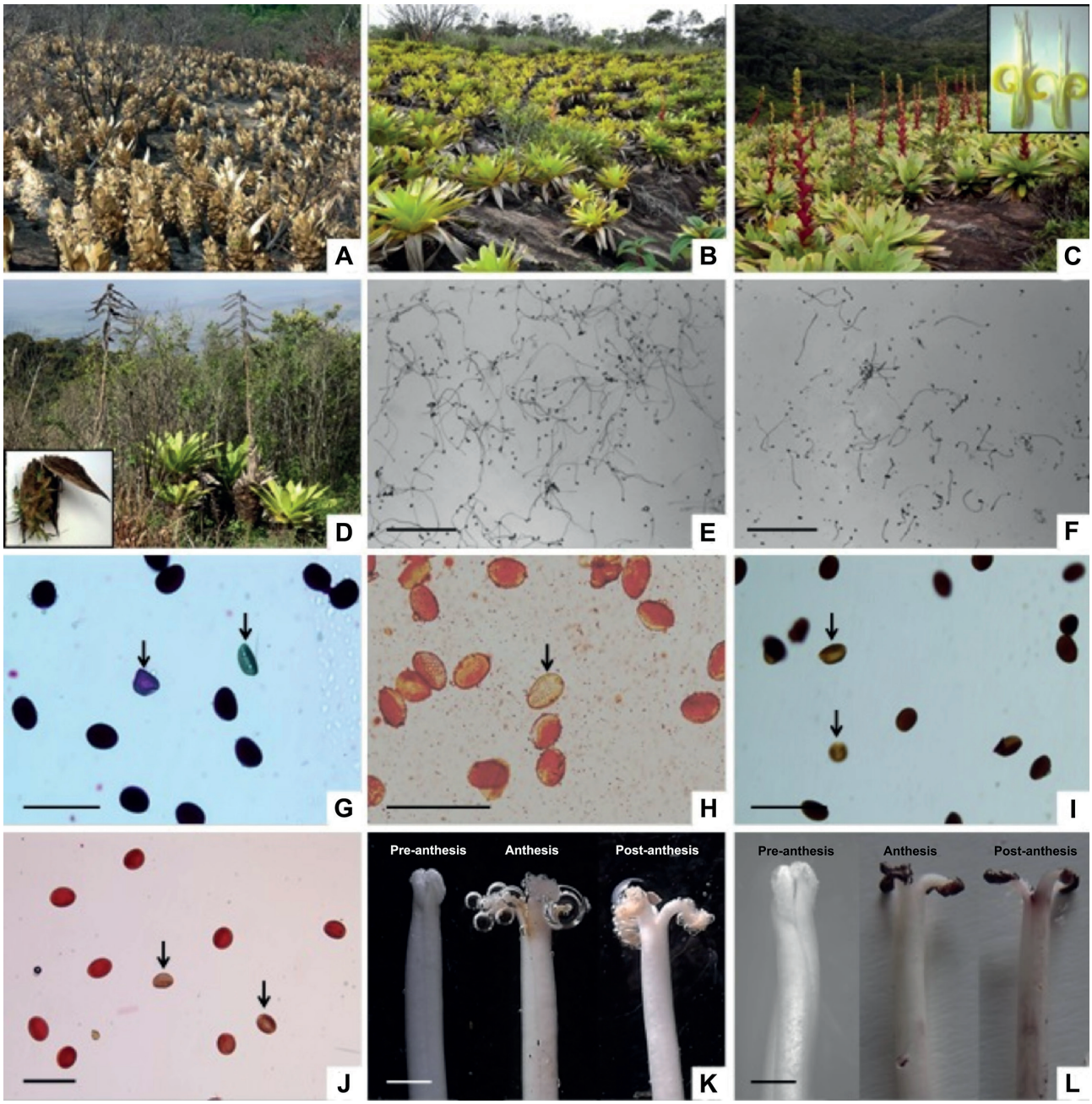

Figure 2. A) Specimens of *Alcantarea nahoumii* (Leme) J. R. Grant (Bromeliaceae) after a forest fire in 2012. B) Plants in the vegetative stage. C) Flowering plants (detail of the flower). D) Fruiting plants (detail of the capsule with germinated seeds). E) *In vitro* germination of pollen grains in BK culture medium (Brewbaker & Kwack 1963). F) *In vitro* germination of pollen grains in BKM culture medium (Brewbaker & Kwack 1963, modified). G-J) Pollen viability with Alexander's stain at 1 %, 2,3,5-triphenyltetrazolium chloride (TTC) at 1 %, Lugol's iodine at 1% and acetic carmine at 1 %, respectively. K-L) Stigma receptivity with hydrogen peroxide and α-naphthyl acetate with phosphate buffer, acetone and fast blue B salt, respectively. Arrows indicate nonviable pollen grains. Bars: E-F) 0.5 mm; g-j) 200 μm; k-l) 1 mm.

versus 82 % germination for open pollination and 75 % for manual cross-pollination (Tab. 1).

Alcantarea nahoumii presented an autogamy index of zero and a self-incompatibility index of 0.44. These results indicate this species is preferably allogamous, self-compatible and requires a pollinator to maintain production of viable seeds.

For *A. nahoumii,* the number of pollen grains per flower in this experiment was 396,300 on six anthers, and the number of ovules per flower was 551 ± 39, or a pollen/ovule ratio of 719.24/1.

Table 2. *In vitro* germination percentage of pollen grains and pollen tube length (mm) of *Alcantarea nahoumii* (Leme) J. R. Grant (Bromeliaceae) in different culture media and collection hours.

Culture media*	Collection hours			
	8:00 a.m.	Noon	6:00 p.m.	
	In vitro germination of the pollen grains (%)			Means
BM	89.91 b A	83.58 a B	82.00 a B	85.16 b
BK	95.41 a A	86.58 a B	85.16 a B	89.05 a
MBK	82.00 c A	75.41 b B	73.91 b B	77.10 c
SM	90.83 b A	86.50 a B	83.16 a B	86.83 b
Means	89.54 A	83.01 B	81.05 C	
CV (%) = 3.88				
	Pollen tube length (mm)			Means
BM	0.96 b A	0.76 b B	0.7.0 b B	0.80 b
BK	1.07 a A	0.94 a B	0.81 a C	0.94 a
MBK	0.94 b A	0.85 b A	0.74 b B	0.84 b
SM	1.14 a A	0.80 b B	0.80 a B	0.91 a
Means	1.02 A	0.83 B	0.76 C	
CV (%) = 24.12				

Means followed by the same lower-case letters in the column and upper-case in the rows, within the same factor, do not differ from each other by the Tukey test at 1 % probability. * Culture media: BM (Parton *et al*. 2002), BK (Brewbaker & Kwack 1963), MBK (Brewbaker & Kwack 1963, modified), SM (Soares *et al*. 2008).

Table 3. Stigma receptivity of *Alcantarea nahoumii* (Leme) J. R. Grant (Bromeliaceae) evaluated between pre-anthesis and post-anthesis, by two methods.

Times of Day	H_2O_2	α-naphthyl acetate
Pre-anthesis (6:00 p.m.)	-	-
Anthesis (8:00 a.m.)	+++	+++
4 hours post-anthesis (noon)	+++	+++
10 hours post-anthesis (6:00 p.m.)	+++	+++
24 hours post-anthesis (8:00 a.m.)	+++	+++

(-) no reaction; (+) weak positive response; (++) strong positive response; (+++) very strong positive response. Methods adapted from Dafni & Maués (1998); 3 % hydrogen peroxide; α- naphthyl = solution of α-naphthyl acetate, phosphate buffer, acetone and fast blue B salt.

Nectar production

The average volume of nectar was 44.71 µL and the average concentration of sugars was 29.7 % (n=20 flowers from 20 individuals) during 10 hours (8:00 a.m. to 6:00 p.m.), but the production rate was not constant during the day. The greatest nectar production occurred at 11:00 a.m., with 73.55 µL, and declined to 11.11 µL at 6:00 p.m. The highest sugar concentration, 45.01 %, was observed at 6:00 p.m. (Fig. 3).

High correlations were observed between the presence of floral visitors (hummingbirds, bees, other birds, butterflies, wasps, beetles and ants) and sugar concentration (r= 0.71**). With respect to time of collection, there was a significant positive correlation with sugar concentration (r=0.77**). No significant correlations were observed between collection hour and nectar volume (r= -0.048^{ns}).

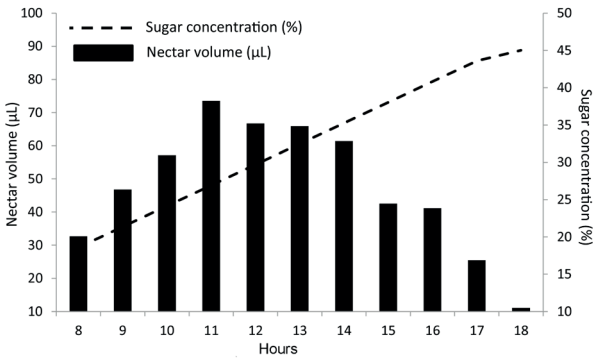

Figure 3. Volume (µL) and sugar concentration (%) of nectar from flowers of *Alcantarea nahoumii* (Leme) J. R. Grant (Bromeliaceae) in Serra da Jibóia, Santa Terezinha, Bahia, Brazil.

Table 4. Floral visitors, floral resource collected, relative frequency and timing of visit to *Alcantarea nahoumii* (Leme) J. R. Grant (Bromeliaceae) in Serra da Jiboia, Santa Teresinha, Bahia, Brazil.

Species	Floral resource	Number of visits	Relative frequency (%)	Period of visit
Birds				
Eupetomena macroura	Nectar	6	3.17	M
Chrysolampis mosquitos	Nectar	27	14.31	M / A
Florisuga fusca	Nectar	23	12.19	M
Anthracothorax nigricollis	Nectar	21	11.00	M
Thalurania glaucopis	Nectar	26	13.75	M / A
Coereba flaveola	Nectar and Pollen	13	6.89	M
Bees				
Apis melífera	Nectar and Pollen	24	12.72	M
Trigona spinipes	Nectar and Pollen	18	9.54	M / A
Oxytrigona tataíra	Nectar	23	12.19	M
Augochlorella sp.	Pollen	3	1.59	M
Butterfly				
Ascia monuste	Nectar	5	2.65	M
		189	100	

M = Morning; A = Afternoon.

Floral visitors

In the 252 hours of observation (96 hours at night and 156 during the day), 189 visits were recorded, all during the daytime, of 11 species. Of these species, five were hummingbirds of the family Trochilidae [*Eupetomena macroura, Chrysolampis mosquitus* (female), *Florisuga fusca, Anthracothorax nigricollis* (female), *Thalurania glaucopis* (female and male)]; one a bird of the family Thraupidae (*Coereba flaveola);* four bee species of Apidae (*Apis mellifera*, *Trigona spinipes*, *Oxytrigona tataira* and *Augocholorella* sp.); and one butterfly of Pieridae (*Ascia manuste*) (Tab. 4, Fig. 4).

Visits by hummingbirds started at 9:00 a.m., extended until 4:00 p.m., and were more frequent in the morning hours, coinciding with the greatest nectar availability, with intervals of 10 to 20 minutes between visits and an average of 15 visits per plant.

Visits were fewer and spaced at longer intervals in the afternoon, (from 30 to 40 minutes, with an average of three visits per inflorescence). The hummingbirds *Chrysolampis mosquitus* (Fig. 4A), *Thalurania glaucopis* (Fig. 4B) and *Florisuga fusca* (Fig. 4C) remained for three to six seconds at each flower and then moved to other inflorescences or rested on nearby stems, without leaving the area, waiting to make new visits. The species *Chrysolampis mosquitus* and *Thalurania glaucopis* were the only visitors that exhibited agonistic interactions, and were the only species to visit flowers in the afternoon. Females of *Anthracothorax nigricollis* (Fig. 4D) were observed with less frequency (11.13 %) than the other hummingbird species, with the exception of *Eupetomena macroura* (Fig. 4E), which exhibited isolated behavior, with frequency of 3.17 %, only landing on plants near wooded areas at the edges of the study areas. Of the five hummingbird species observed in this study, all collected nectar by introducing their long beaks between the petals toward the base of the corolla, where the nectar chamber is located.

Coereba flaveola (bird) (Fig. 4F) paid only one illegitimate visit, collecting exposed pollen and nectar, so this species can be classified as a robber. The bees *Trigona spinipes* and *Apis melifera* (Fig. 4G) visited the flowers individually and remained on the flowers for long periods. In contrast, *Oxytrigona tataira* (Fig. 4H) visited the flowers in groups of 8 to 10 individuals, and only in the mornings, most intensely between 6:30 to 11:30 a.m. All these bee species were present throughout the blooming period and collected nectar. In turn, the bee *Augochlorella* sp. was found only on the anthers collecting pollen, and remained for an average of 10 seconds. The butterfly *Ascia manuste* (Fig. 4I) landed on flowers only to collect nectar and remained for only 8 seconds on average, with a relative frequency of 2.65 %.

In addition to the species mentioned sthuso far, we also observed one species of the order Coleoptera (*Diabrotica septenliturata*) and four of Hymenoptera: *Polybia* sp, *Polistes* sp, *Polybia platycephata* and Formicidae (not identified to species level). None of these visitors showed any interest in floral resources and mainly visited in the morning hours, except for the ants, which were present throughout the day.

Discussion

Phenology

Alcantarea nahoumii displays a two-year cycle after sprouting of lateral shoots and an additional seven months from flowering to seed generation. The long reproductive period appears to be a characteristic of the subfamily Tillandsioideae, as previously reported by Marques & Lemos Filho (2008). According to these authors, in general,

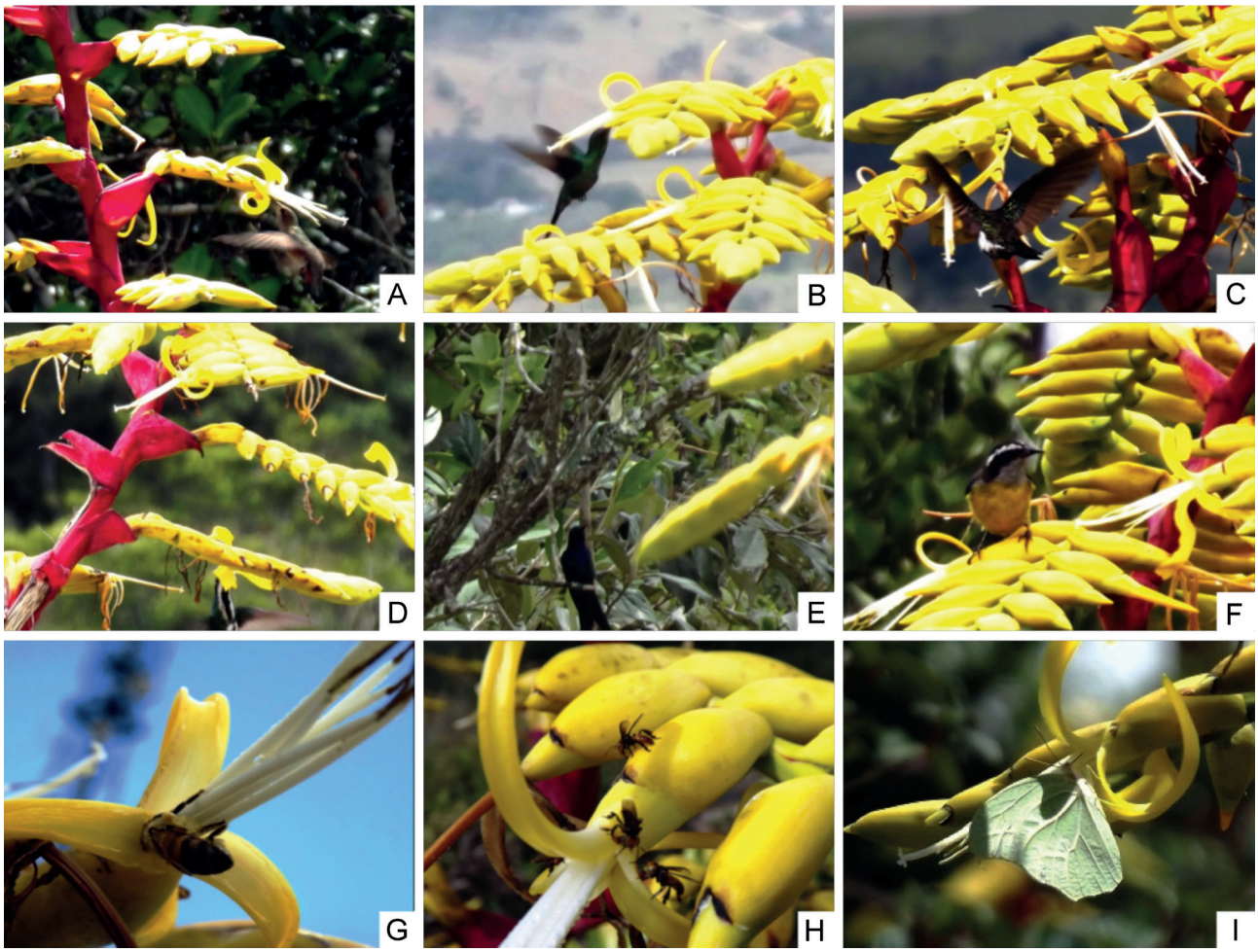

Figure 4. Floral visitors of *Alcantarea nahoumii* (Leme) J. R. Grant (Bromeliaceae) in Serra da Jibóia, Santa Teresinha, Bahia, Brazil. A) *Chrysolampis mosquitus*. B) *Thalurania glaucopis* (male). C) *Florisuga fusca*. D) *Anthracothorax nigricollis* (female). E) *Eupetomena macroura*. F) *Coereba flaveola*. G) *Apis mellifera*. H) *Oxytrigona tataira*. I) *Ascia manuste*. Floral visitors identified by Ivan Sazima.

bromeliads that flower in the rainy season inhabit rocky regions at high elevations (1,400 m), because under these conditions their entire vegetative and reproductive cycle is optimized by the morphological traits of the bromeliads, such as tanks (phytotelmata), absorptive roots and scaly/peltate trichomes. In contrast, in species that develop at elevations lower than 1,300 m, as is the case of *A. nahoumii* (at 850 m), flowering happens in the dry season, which coincides with a greater availability of pollinators, thus avoiding the fog and lower temperatures of higher elevations. The diurnal floral anthesis and prolonged open period of the flowers (24 hours) increased visitation by pollinators and the possibility of fertilization.

Knowledge of floral phenology allows the establishment of the moment, duration and intensity of a species' flowering, as well as the influence of these factors on the behavior of pollinators (Aker 1982; Tschapka 2004). However, the timing of these events is subject to the influence of environmental perturbations and landscape alterations.

Pollen viability

In this study, *A. nahoumii* exhibited high pollen viability regardless of the type of test (*in vitro* germination or histochemistry). *In vitro* germination does not completely reproduce the same pollen tube growth as *in vivo* fertilization, but the results obtained are near those that occur *in vivo*, making it important to perform this technique carefully for each species (Soares *et al*. 2008; Souza *et al*. 2015). *In vitro* germination is the result of interactions between the composition of the culture medium and the peculiarities of each species or genotype. For this reason, it is necessary to adjust conditions to find the best combination for each type of material.

According to Souza *et al*. (2002), pollen viability values above 70 % are considered high and 30 % or below as low. They also stated that pollen viability is considered to be a measure of masculine fertility, and can be assessed by

different techniques, such as *in vitro* or *in vivo* germination, or by indirect methods based on cytological parameters such as coloration, as discussed above (Souza *et al*. 2015). Based on this comment, and according to the results obtained in this study, the pollen grains of *A. nahoumii* exhibited high viability and can be used in controlled pollination.

Among the histochemical methods studied, staining with Alexander's solution produced results closest to those obtained by pollen viability estimation through *in vitro* germination. Similar behavior has been observed for other plant species, such as *Arabidopsis thaliana*, which exhibited pollen viability greater than 90 % when using Alexander's stain, results very close to those obtained with *in vitro* pollen germination (Swanson *et al*. 2016).

Stigma receptivity

Another important aspect for understanding reproductive biology is stigma receptivity. Normally stigma receptivity is associated with floral opening and directly influences the reproductive system, because it is at this moment that the pollen grains should be deposited on the stigma to assure fecundation (Souza *et al*. 2016). Thus, for *A. nahoumii* under the conditions studied, the period from 8:00 a.m. to 6:00 p.m. is the best for performing fertilization, since the pollen grains exhibited the highest viability rates and the stigma was receptive.

These results corroborate those reported by Souza *et al*. (2016) who, when evaluating stigma receptivity of *A. nahoumii* in a controlled environment (greenhouse), also observed that receptivity occurred during anthesis and continued for 24 hours post-anthesis. They also suggested that controlled crosses should be carried out during this interval to attain the highest probability of fecundation and seed production.

Reproductive systems

The *in vivo* pollination tests of *A. nahoumii* revealed greater fruit formation from controlled pollination. This can be partly attributed to variation in the number of pollen grains deposited on the stigma. In manual pollination, the number of grains deposited on stigmas is not only more uniform among flowers, but also likely greater than those deposited by insect visitors in the case of open pollination. This assumption is supported by other studies showing that the number of seeds and quality of fruits are closely related to the number of pollen grains deposited on stigmas (Park *et al*. 2009; Wetzstein *et al*. 2013).

Open pollination led to the formation of fruits, but at only 35% the percentage was low. This result can be attributed to the observed low frequency of floral visits. The fruiting rate was low (10%) for flowers submitted to manual self-pollination, and no fruits were formed in the spontaneous self-pollination and agamospermy treatments. This absence, or low percentage, of fruiting might have been the result of some degree of incompatibility or inbreeding depression of *A. nahoumii*.

Inbreeding depression can occur with manual self-pollination, as reported by Vaughton *et al*. (2010) for the species *Cyrtanthus breviflorus* and by Paggi *et al*. (2013) for *Vriesea friburgensis*, although no studies have been carried out to evaluate the endogamic effects on the progeny of *A. nahoumii*. On the other hand, cross-pollination and/or open pollination promote gene flow between individuals, which can increase the genetic variability within and between populations, both of which are important features for diversification and maintenance of species (Vosgueritchian & Buzato 2006).

With respect to the number of seeds produced, plants submitted to cross-pollination produced the greatest number of seeds, followed by open pollination. The success of open pollination can probably be attributed to greater gene flow between plants due to the action of pollinators, which can travel long distances carrying pollen grains from one plant to another (Barbará *et al*. 2009; Matallana *et al*. 2010).

Another factor that can affect the number of seeds is pollination efficiency, determined by the number of pollen grains deposited on the stigma and the number of ovules per flower (Mione & Anderson 1992). The pollen/ovule ratio observed for *A. nahoumii* was in line with the classification of allogamous species described by Cruden (1977).

Nectar production and floral visitors

The lack of a correlation between nectar volume and sugar concentration can be associated with differences in evaporation and nectar secretion rates, since the concentration of sugars in nectar depends on the rate of pollinator visitation and environmental conditions (solar radiation, rain, wind and temperature) (Kajobe 2007). These results corroborate those reported by Aguilar-Rodríguez *et al*. (2014), who observed a non-significant correlation between volume and sugar concentration for the nectar of the bromeliad *Tillandsia macropetala*. On the other hand, Aguilar-Rodríguez *et al*. (2016) observed a negative and significant correlation between collection hour and volume produced, as well as between collection hour and sugar concentration.

Variation in nectar volume and sugar concentration is common among some species, such as *Aechmea pectinata*, *A. nudicaulis*, *A. organensis*, *A. ornata*, *Billbergia amoena*, *Nidularium innocentii*, *N. Rubens*, *Tillandsia geminiflora*, *T. stricta, T. tenuifolia, Vriesea altodaserrae*, *V. carinata*, *V. flammea*, *V. incurvata*, and *V. philippocoburgii*. Canela & Sazima (2003) and Machado & Semir (2006) stated that for these species this variation may be related to the frequency of floral visits: flowers with greater nectar volume and sugar concentration attract more pollinators. On the other hand, Lenzi *et al*. (2006) and Scrok & Varassin (2011), studying

A. lindenii and *A. distichantha*, respectively, observed that nectar volume and sugar concentration remained constant during the day. Krömer *et al*. (2008) studied the sugar composition and concentration of nectar in Bromeliaceae, covering 111 species belonging to all three subfamilies, and concluded that the characteristics of the nectar of Bromeliaceae are predominantly determined by putative adaptations of nectar sugars to preferences of the pollinators rather than by phylogeny.

In the case of bromeliads, nectar is the floral resource that most attracts hummingbirds. It is produced in septal nectaries and stored at the base of the corolla (Sajo *et al*. 2004). This induces hummingbirds to penetrate the flower with their beaks (generally long) while collecting nectar, during which they perform pollination by causing contact of the anthers with the receptive stigma.

Due to the lack of other resources, some bromeliads that grow in rocky fields, such as *Hohenbergia ramageana*, *A. bromeliifolia*, *N. bahiana*, *O. albopictum*, and *Dyckia dissitiflora*, can provide a significant portion of the diet of hummingbirds, with percentages varying from about 30 to 70 %, being influenced by various factors such as competition, resource availability and seasonality (Machado *et al*. 2007).

The nectar robbing behavior observed for *Coereba flaveola* has been reported in relation to a variety of plant species (Navarro 2000; 2001), including some Bromeliaceae (Fumero-Cabán & Meléndez-Ackerman 2007; Santana & Machado 2010; Queiroz *et al*. 2016). Studying floral visitors of *Pitcairnia angustifolia*, Fumero-Cabán & Meléndez-Ackerman (2007) reported that *C. flaveola* was the most frequent species and acted as a primary nectar robber, including piercing the base of the corolla of some flowers, yet they performed pollination incidentally by depositing pollen grains on stigmas.

The results of this study increase knowledge regarding the forms of reproduction and pollination mechanisms of *A. nahoumii*, which is considered endemic and vulnerable in the regions studied. This information can be used in future studies related to programs to preserve genetic resources, since the species is preferentially allogamous and self-compatible and floral visitors are available in the region. Urgent measures are necessary for the conservation and restoration of the entire area to assure that this species habitat is maintained.

Acknowledgements

We acknowledge financial support from Fundação de Amparo a Pesquisa do Estado da Bahia - FAPESB, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PROCAD-2013 n. 88881.068513/2014-01; CAPES-Embrapa n. 15/2014) and Ivan Sazima for identifying the birds from photos.

References

Aguilar-Rodríguez PA, Krömer T, García-Franco JG, Knauer A, Kessler M. 2014. First record of bat-pollination in the species-rich genus *Tillandsia* (Bromeliaceae). Annals of Botany 113: 1047-1055.

Aguilar-Rodríguez PA, Krömer T, García-Franco JG, Macswiney GMC. 2016. From dusk till dawn: nocturnal and diurnall pollination in the epiphyte *Tillandsia heterophylla* (Bromeliaceae). Plant Biology 18: 37-45.

Aker CL. 1982. Spatial and temporal dispersion patterns of pollination and their relationship to the flowering strategy of *Yucca whipplei* (Agavaceae). Oecologia 54: 243-252.

Alexander MP. 1980. A versatile stain for pollen, fungi, yeast and bacteria. Stain Technology 55: 13-18.

Barbará T, Martinelli G, Palma-Silva C, Fay MF, Mayo SJ, Lexer C. 2009. Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae). Annals of Botany 103: 65-77.

Benzing DH. 2000. Bromeliaceae: profile an adaptive radiation. Cambridge, Cambridge University Press.

Bolten AB, Feinsinger P, Baker HG, Baker I. 1979. On the calculation of sugar concentration in flower néctar. Oecologia 41: 301-304.

Brewbaker JL, Kwack BH. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. American Journal Botany 50: 859-865.

Canela MBF, Sazima M. 2003. *Aechmea pectinata*: a hummingbird-dependent bromeliad with incospicuous flowers from the rainforest in south-eastern Brazil. Annals of Botany 92: 731-737.

Cruden RW. 1977. Pollen-ovule ration: a conservative indicator of breading systems in flowering plants. Evolution 31: 32-46.

Cruz CD. 2008. Programa Genes (versão Windows): aplicativo computacional em genética e estatística. Viçosa, UFV.

Dafni A, Maués MM. 1998. A rapid and simple procedure to determine stigma receptivity. Sexual Plant Reproduction 11: 177-180.

Dafni A. 1992. Pollination ecology: a practical approach (the practical approach series). New York, University Press.

Dai J, Wang H, Ge Q. 2013. Multiple phenological responses to climate change among 42 plant species in Xi'an, China. International Journal of Biometeorology 57: 749-758.

Elmendorf SC, Jones KD, Cook BI, *et al.* 2016. The plant phenology monitoring design for The National Ecological Observatory Network. Ecosphere 7: 1-16.

Forzza FC, Costa AF, Leme EMC, *et al.* 2013. Bromeliaceae. In: Martinelli G, Moraes MA. (eds.) Livro vermelho da flora do Brasil. 1st. edn. Rio de Janeiro, Centro Nacional da conservação da flora. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. p. 315-396.

Fumero-Cabán JJ, Meléndez-Ackerman EJ. 2007. Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). American Journal of Botany 94: 419-424.

Galetto L, Bernardello G. 2005. Rewards in flowers – Nectar. In*:* Dafni A, Kevan PG, Husband BC. (eds.) Practical Pollination Biology. Cambridge, Ontario, Enviroquest, Ltd. p. 261-313.

INMET – Instituto Nacional de Meteorologia. 2016. Climatologia. http://www.inmet.gov.br/portal/index.php?r=clima/mesTempo. 21 Jan. 2016.

Kajobe RK. 2007. Botanical sources and sugar concentration of the nectar collected by two stingless bee species in a tropical African rain forest. Apidologie 38: 110-121.

Kearns CA, Inouye D. 1993. Techniques for pollinations biologists. Niwot, University Press of Colorado.

Krömer T, Kessler M, Lohaus G, Schmidt-Lebuhn AN. 2008. Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae. Plant Biology 10: 502-511.

Lenzi M, Matos JZ, Orth AI. 2006. Morphological and reproductive variation of *Aechmea lindenii* (E. Morren) Baker var. *lindenii* (Bromeliaceae). Acta Botanica Brasilica 20: 487-500.

Machado CG, Coelho AG, Santana CS, Rodrigues M. 2007. Hummingbirds e seus recursos florais em uma área de campo rupestre da Chapada Diamantina, Bahia. Revista Brasileira de Ornitologia 15: 267-279.

Machado CG, Semir J. 2006. Phenology da floração e biologia floral de bromeliáceas ornitófilas de uma área de Mata Atlântica do Sudeste brasileiro. Revista Brasileira de Botânica 29: 163-174.

Marques AR, Lemos Filho JP. 2008. Phenology reprodutiva de species de bromeliads na Serra da Piedade, MG, Brasil. Acta Botanica Brasilica 22: 417-424.

Martinelli G. 1994. Reproductive biology of Bromeliaceae in the Atlantic Rainforest of southern Brazil. PhD Thesis, University of St. Andrews, St. Andrews.

Matallana G, Godinho MAS, Guilherme FAG, Belisario M, Coser TS, Wendt T. 2010. Breeding systems of Bromeliaceae species: Evolution of selfing in the context of sympatric occurrence. Plant Systematics and Evolution 289: 57-65.

Matallana G, Oliveira PE, Silva PRR, Wendt T. 2016. Post-pollination barriers in an assemblage of Bromeliaceae in South-Eastern Brazil. Botanical Journal of the Linnean Society 181: 521-531.

Mione T, Anderson GJ. 1992. Pollen-ovule ratios and breeding system evolution in solanum section *Basarthrum* (Solanaceae). American Journal of Botany 79: 279-287.

Murashige T, Skoog FA. 1962. A revised medium for a rapid growth and bioassays with tobacco tissues cultures. Physiologia Plantarum 15: 473-479.

Navarro L. 2000. Pollination ecology of *Anthyllis vulneraria* subsp. *vulgaris* (Fabaceae): nectar robbers as pollinators. American Journal of Botany 87: 980-985.

Navarro L. 2001. Reproductive biology and effects of nectar robbing on fruit production in *Macleania bullata* (Ericaceae). Plant Ecology 152: 59-65.

Negrelle RRB, Muraro D. 2006. Aspectos fenológicos e reprodutivos de *Vriesea incurvata* Gaudich Gaudich (Bromeliaceae). Acta Scientiarum Biological Sciences 28: 95-102.

Paggi GM, Silveira LCTS, Zanella CM, *et al.* 2013. Reproductive system and fitness of *Vriesea friburgensis*, a self-sterile bromeliad species. Plant Species Biology 28: 169-176.

Park NI, Yeung EC, Muench DG. 2009. Mago Nashi is involved in meristem organization, pollen formation, and seed development in *Arabidopsis*. Plant Science 176: 461-469.

Parton E, Vervaeke R, Delen BR, Vandenbussche R, Proft M. 2002. Viability and storage of bromeliad pollen. Euphytica 125: 155-161.

Pearse AGE. 1972. Histochemistry, theoretical and applied. 2. ed. Edinburgh, Churchill Livingstone.

Queiroz JA, Quirino ZGM, Lopes AV, Machado IC. 2016, Vertebrate mixed pollination system in *Encholirium spectabile*: A bromeliad pollinated by bats, opossum and hummingbirds in a tropical dry forest. Journal of Arid Environments 125: 21-30.

Ramirez N, Brito Y. 1990. Reproductive of a tropical palm swamp community in the Venezuelan llanos. American Journal of Botany 77: 1260-1271.

Sajo MG, Rudall PJ, Prychid CJ. 2004. Floral anatomy of Bromeliaceae, with particular reference to the evolution of epigyny and septal nectaries in commelinid monocots. Plant Systematics and Evolution 247: 215-231.

Santana CS, Machado CG. 2010. Fenologia de floração e polinização de espécies ornitófilas de bromeliáceas em uma área de campo rupestre da Chapada Diamantina, BA, Brasil. Revista Brasileira de Botânica 33: 469-477.

Sas Institute Inc. 2010. Sas/Stat user's guide: statistics. Version 9.2. 3rd. edn. Cary, NC.

Scrok GJ, Varassin IG. 2011. Reproductive biology and pollination of *Aechmea distichantha* Lem. (Bromeliaceae). Acta Botanica Brasilica 25: 571-576.

Shivanna KR, Rangaswamy NS. 1992. Pollen biology. A laboratory manual. Berlin, Springer-Verlag.

Soares TL, Silva SO, Costa MAPC, *et al.* 2008. *In vitro* germination and viability of pollen grains of banana diploids. Crop Breeding and Applied Biotechnology 8: 111-118.

Souza EH, Carmello-Guerreiro SM, Souza FVD, Rossi ML, Martinelli AP. 2016. Stigma structure and receptivity in Bromeliaceae. Scientia Horticulturae 203: 118-125.

Souza EH, Souza FVD, Rossi ML, Brancalleao N, Ledo CAS, Martinelli AP. 2015. Viability, storage and ultrastructure analysis of *Aechmea bicolor* (Bromeliaceae) pollen grains, an endemic species to the Atlantic forest. Euphytica 204: 13-28.

Souza EH, Versieux LM, Souza FVD, *et al.* 2017. Interspecific and intergeneric hybridization in Bromeliaceae and their relationships to breeding systems. Scientia Horticulturae 223: 53-61.

Souza MM, Pereira TNS, Martins ER. 2002. Microsporogênese e microgametogênese associadas ao tamanho do botão floral e da antera e viabilidade polínica em maracujazeiro-amarelo (*Passiflora edulis* Sims f.*flavicarpa* Degener). Ciência e Agrotecnologia 26: 1209-1217.

Swanson RJ, Hammond AT, Carlson AL, Gong H, Donovan TK. 2016. Pollen performance traits reveal prezygotic nonrandom mating and interference competition in *Arabidopsis thaliana*. American Journal of Botany 103: 1-16.

Tschapka M. 2004. Energy density patterns of nectar resources permit coexistence within a guild of Neotropical flower-visiting bats. Journal of Zoology 263: 7-21.

Vaughton G, Ramsey M, Johnson SD. 2010. Pollination and late-acting self-incompatibility in *Cyrtanthus breviflorus* (Amaryllidaceae): implications for seed production. Annals of Botany 106: 547-555.

Versieux LM, Vasconcellos N, Martinelli G, Wanderley MGL. 2012. *Alcantarea pataxoana* (Bromeliaceae), a New Species from Bahia, Brazil. Systematic Botany 37: 636-640.

Versieux LM, Wanderley MGL. 2007. Two new species of *Alcantarea* (Bromeliaceae, Tillandsioideae) from Brazil. Brittonia 59: 57-64.

Versieux LM, Wanderley MGL. 2015. Bromeliads-gigantes do Brasil. 1st. edn. Natal, Capim Macio & Offset.

Vosgueritchian SB, Buzato S. 2006. Sexual reproduction of *Dyckia tuberosa* (Vell.) Beer (Bromeliaceae, Pitcairnioideae) and plant-animal interaction. Revista Brasileira de Botânica 29: 433-442.

Wendt T, Canela MBF, Faria APG, Rios RI. 2001. Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). American Journal of Botany 88: 1760-1767.

Wendt T, Coser TS, Matallana G, Guilherme FAG. 2008. An apparent lack of prezygotic reproductive isolation among 42 sympatric species of Bromeliaceae in southeastern Brazil. Plant Systematics and Evolution 275: 31-41.

Wetzstein HY, Yi W, Porter AJ, Ravid N. 2013. Flower position and size impact ovule number per flower, fruit set, and fruit size in pomegranate. Journal of the American Society for Horticultural Science 138: 159-166.

Zanella CM, Janke A, Palma-Silva C, *et al.* 2012. Genetics, evolution and conservation of Bromeliaceae. Genetics and Molecular Biology 35: 1020-1026.

Zeisler M. 1933. Über die abgrenzung des eigentlichen narbenfläche mit hilfe von reaktionen. Beihefte zum Botanischen Centralblatt 58: 308-318.