Diurnal versus nocturnal pollination success in *Billbergia horrida* Regel (Bromeliaceae) and the first record of chiropterophily for the genus

**JUÇARA S. MARQUES¹, MARCELA C. TAGLIATI¹ and ANA PAULA G. FARIA²**

¹Programa de Pós-Graduação em Ecologia, Universidade Federal de Juiz de Fora, Instituto de Ciências Biológicas, Rua José Lourenço Kelmer, s/n, Campus Universitário, Bairro São Pedro, 36036-900 Juiz de Fora, MG, Brasil

²Universidade Federal de Juiz de Fora, Instituto de Ciências Biológicas, Departamento de Botânica, Rua José Lourenço Kelmer, s/n, Campus Universitário, Bairro São Pedro, 36036-900 Juiz de Fora, MG, Brasil

*Manuscript received on June 30, 2014; accepted for publication on October 24, 2014*

**ABSTRACT**

*Billbergia horrida* is endemic of the Atlantic Forest fragments in southeastern Brazil and characterized by flowers with typical traits for pollination by nocturnal animals. Although the majority of *Billbergia* species rely on diurnal pollination by hummingbirds, *B. horrida* is also visited by bats and this study evidences for the first time the occurrence of chiropterophily within the genus. The role of different groups of pollinators on the reproductive success of *B. horrida* was evaluated, as well as the correlation of nectar features in sustaining these animals during different periods of the day. Bats contributed to 82.1% of fruit set of *B. horrida*. Hummingbirds, in turn, contributed to only 10% of fruit set, and were poorly related to the reproductive success of this species. Amounts of nectar production and sugar concentration were similar to those of other chiropterophilous bromeliads and only the nectar volume changed significantly throughout the period of flower availability. Recurring visits by hummingbirds were probably because the flowers of *B. horrida* were open for 24h, offering energetic rewards for daytime visitors and due to the presence of other attractive bromeliad species growing at the same phorophyte and flowering at the same time.

**Key words:** Brazilian Atlantic Forest, floral biology, hummingbirds pollination, reproductive success.

**INTRODUCTION**

Several fauna function as pollinators of Bromeliaceae, which is among the few families in which vertebrates predominate over insects in this process (Sazima et al. 1989). Ornithophily is the main syndrome (Benzing et al. 2000, Varassin and Sazima 2000, Machado and Semir 2006, Cestari 2009) and especially hummingbirds, attracted by the bright red or pink flowers, inflorescence axis and even leaf tips, use bromeliad species as their main source of food. Besides these avians, other groups of diurnal nectarivorous, such as butterflies and bees, also benefit from the sequential flowering of the species of this family in the same region, which is of extreme importance for the maintenance of pollinators in the area (Varassin and Sazima 2000, Siqueira Filho and Machado 2001, Machado and Semir 2006).
Studies about nocturnal pollination in Bromeliaceae are scarce. Bats form the second main group of vertebrates acting as pollinators of bromeliads (Sazima et al. 1989), which attract these animals with scents, abundant nectar and inflorescences with large flowers with enclosed stamens and petals forming a wide-mouthed gullet or with many flowered brush-type spikes (Sazima et al. 1989, Benzing et al. 2000). Chiropterophily is practically restricted to the Tillandsioideae and Pitcairnioideae subfamilies (infrafamilial delimitation according to Smith and Downs 1974, 1977, 1979). In Bromelioideae, ornithophily predominates among the larger genera, such as Aechmea Ruiz & Pav., Neoregelia L.B.Sm., Billbergia Thunb. and Quesnelia Gaudich. (Benzing et al. 2000). Other floral biology traits also signal entomophily in some bromelioid species with flexible pollination systems involving hummingbirds and butterflies (Hallwachs 1983, apud Benzing et al. 2000) or hummingbirds and bees (Schmid et al. 2011), showing that fewer Bromeliioideae than Pitcairnioideae and Tillandsioideae depend on bats to produce fruits and seeds.

The genus Billbergia Thunb. comprises 64 species (Luther 2008), of which 47 are distributed in Brazil. Billbergia horrida Regel, the focus of this study, occurs in the states of Bahia, Minas Gerais, Rio de Janeiro and Espírito Santo (Forzza et al. 2014). It is endemic to fragments of the Atlantic Forest and grows as an epiphyte or is rupicolous in ombrophile and semi-deciduous forests (Martinelli et al. 2009). No detailed information exists concerning the aspects of floral biology and pollination for this species and the only reproductive study relates to its breeding system, which defines B. horrida as a self-incompatible species (Matallana et al. 2010).

The flowers of Billbergia horrida display the typical morphology of pollination by nocturnal animals (e.g. nocturnal anthesis with a sweet fragrance and an inconspicuous color of the calyx and corolla). However, we observed that this species is also visited by hummingbirds during the day. This study discusses aspects related to the morphology, floral biology and pollination of B. horrida in a fragment of the Atlantic Forest in the state of Minas Gerais, southeastern Brazil. The aim was to verify the different pollinators; to establish whether the visits are correlated with nectar production (volume and sugar concentration) and also to assess the effectiveness of diurnal and nocturnal pollinators on the reproductive success of this species.

MATERIALS AND METHODS

STUDY SITE

The studied area is situated in the municipality of Juíz de Fora (coordinates 21°34’–22°05’S and 43°09’–43°45W), state of Minas Gerais, southeastern Brazil. It comprises a fragment of the Atlantic Forest of approximately 370 ha, called Mata do Krambeck and possesses great value for conservation due to its connectivity potential with other fragments in the municipality. The observations and experiments were conducted in a portion of approximately 80 ha of the fragment, acquired by the Universidade Federal de Juiz de Fora, Brazil, to establish a Botanical Garden. According to Köppen (1948), the climate type in the area is Cwa, characterized by hot and rainy summers and dry winters and the vegetation type is classified as montane semi-deciduous forest (Veloso et al. 1991).

POLLENATION AND FLORAL BIOLOGY

Floral visitors were observed in the field during the early morning (7 am) until late afternoon (6 pm) and overnight (from 6 to 11:30 pm), and the total observation time was 65h. Information relating to the time of visitation, pollinator behavior and point of contact of the animal’s body with pollen and the stigma were recorded. The activity of hummingbirds, himenopterans and lepidopterans was monitored with the naked eye or using binoculars and was recorded photographically. Bats were collected using nets placed near the flowering plants, as proposed by Kunz and
Kurta (1988), and were identified posteriorly by specialists. Following capture, pollen that adhered to the head, neck, thorax and limbs was collected for mounting on temporary slides and subsequent analysis of the pollen grains. All procedures related to bat capture are linked to the project “Morcegos urbanos do município de Juiz de Fora – MG”, and were carried out with the approval of the Ethics Committee on Animal Experiments of the Pró-Reitoria de Pesquisa, Universidade Federal de Juiz de Fora, Brazil (under the protocol number 055/2009). Inflorescences were observed in the field and on plants cultivated at the greenhouse of the Federal University of Juiz de Fora, to determine aspects of floral morphology, the number of open flowers/day and features of anthesis. Additionally, stigma receptivity was tested by applying hydrogen peroxide directly to the stigma (Dafni 1992).

Nectar aspects related to variations in sugar concentration and volume were evaluated by measurements of flowers taken every six hours, starting from complete anthesis (10 pm) until 4 pm on the following day. All the flowers from four individuals (n = 268 flowers) were analyzed under the same environmental conditions and with bagged inflorescences and the same number of flowers were sampled at each time-point. The nectar volume was evaluated using a graduated 25 μL microsyringe. The concentration of soluble sugars was determined using a pocket refractometer (Atago Master-T). The means of sugar concentration and nectar volume were compared using one-way ANOVA. Significant differences (P < 0.05) between the measures taken from different hours of the day were assessed with Tukey’s honestly significant difference (HSD) test.

**Effectiveness of Pollination and Reproductive Success**

To understand the role of diurnal and nocturnal pollinators in the reproductive success of *B. horrida*, two experimental groups of plants were labeled in the field, each one corresponding to a period of the day (nocturnal or diurnal) and containing ten individuals per group. The ten inflorescences available for diurnal visitors were bagged between 5 and 6 pm and the paper bags were removed early in the morning, between 7 and 8 am. Inflorescences of the second group were bagged between 7 and 8 am and the paper bags were removed between 5 and 6 pm, and were thus made available to nocturnal visitors. The individuals were chosen at locations where both groups could be sampled under the same environmental conditions. At the end of the flowering period, the paper bags were removed and fruiting occurred in the natural environment for approximately a month. After this period, the fruit set was evaluated and the number of seed produced was analyzed by selecting five fruits from each individual of the two experimental groups.

The fruit set under natural conditions were also evaluated in five individuals with unbagged inflorescences exposed to open pollination, and seed production was analyzed by selecting ten fruits from each individual. Differences in fruit set and means of seed production between diurnal, nocturnal and open pollination were analyzed by one-way ANOVA followed by Tukey’s HSD test. Fruit set data percent were arcsine transformed prior to analysis (Zar 1999). All the statistical analyses were performed using the software package Statistica version 12.0.

**RESULTS**

**Pollination and Floral Biology**

The flowers of *B. horrida* are arranged in a spike with a green axis and peduncle bracts and the flowers possess greenish sepal and petals, exposed stamens and a tubular corolla from the middle to the base of their length (Fig. 1A–C). Anthesis is nocturnal, starting around 8:30 pm and continuing until approximately 10 pm and the flowers are fragrant after opening. The individuals produce, on average, 81 ± 40 flowers per inflorescence.
The number of opened flowers each day varied from four to ten and the flowering time for each individual lasted approximately one week to ten days. The flowers remain available to visitors for 24h after anthesis with the receptive stigma, and provide pollen and nectar as floral rewards throughout this period. Although *B. horrida* does not display typical attractiveness of ornithophilous pollination syndrome, individuals within the studied area were visited by hummingbirds from the species *Thalurania glaucopis* (Gmelin, 1788) and *Phaetornis pretrei* (Lesson and Delattre, 1839). Both species acted as pollinators by contacting the beak and the frond with pollen and stigma (Fig. 1C). Pollination also occurred nocturnally, through visits of bats from the species *Glossophaga soricina* (Pallas, 1766) (Fig. 1D).

Moths from the families Noctuidae and Sphingidae were occasionally observed visiting the flowers, but these insects were not collected to confirm pollen deposition during their foraging behavior.

Throughout the period of 24h, the flowers of *B. horrida* provided nectar with a mean volume of $64.8 \pm 22.3$ μL and sugar concentration of $17.7 \pm 2\%$ ($n = 268$ flowers). We recorded a slight increase
in the mean sugar concentration from the anthesis (10 pm) until 4 am, decreasing in the morning with the lowest value (15.7%) at 10 am and increasing again during the afternoon, with the greatest value (19.7%) at 4 pm (Fig. 2). There was no statistically significant difference between means of sugar concentration over the period of flower’s availability ($F = 1.10, P = 0.35$). The mean volume of produced nectar showed increasing values from the anthesis to a maximum recorded at 4 am (93.5 μL) and then decreasing from this period until the lowest value (34.2 μL) at 4 pm (Fig. 2). Statistically significant difference was determined by ANOVA ($F = 5.08, P = 0.002$) and the Tukey test pointed out significant difference between the volume measures taken at 4 am and 4 pm ($P = 0.001$).

**Effective of Pollination and Reproductive Success**

The experiments conducted in the field showed a fruit set of 10% for the group of individuals available to active pollinators in the morning/afternoon. The mean of seed production for the diurnal group was 3.8 ± 6.1 seeds. The values found for individuals available to nocturnal pollinators were higher, with fruit set of 82.1% and mean of seed production of 139.6 ± 58.6 (Table I). Results from open pollination indicated a fruit set of 77.9% and mean of seed production of 73.9 ± 34.8. Statistically significant differences between fruit sets were stipulated by ANOVA ($F =$

![Figure 2 - Means and standard deviation of nectar volume (μL) and sugar concentration (%) produced by flowers of B. horrida according to the hour of day.](image)

**Table I**

<table>
<thead>
<tr>
<th>Pollinators</th>
<th>Fruit set</th>
<th>Number of seeds/fruit (X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diurnal pollination (N = 10)</td>
<td>10% (81/808)</td>
<td>3.8 ± 6.1 (50 fruits)</td>
</tr>
<tr>
<td>Nocturnal pollination (N = 10)</td>
<td>82.1% (647/788)</td>
<td>139.6 ± 58.6 (50 fruits)</td>
</tr>
<tr>
<td>Open pollination (N = 05)</td>
<td>77.9% (327/420)</td>
<td>73.9 ± 34.8 (50 fruits)</td>
</tr>
</tbody>
</table>

*An Acad Bras Cienc (2015) 87 (2)*

Within the genus Billbergia, most taxa rely on diurnal pollination by hummingbirds (Benzing et al. 2000). The flowers of Billbergia species are usually odorless and present diurnal anthesis. The petals’ coloration varies from dark violet, yellow-greenish with blue apices to totally green. The scape bracts are mostly red, in fact, they are the most attractive reproductive structure for these avians. Billbergia horrida and B. robert-readii E. Gross & Rauh differ from the norm with their night-blooming, fragrant or odd-smeling flowers. Due to these floral traits, Benzing et al. (2000) assigned to B. horrida the entomophily syndrome and the chiropterophily syndrome to B. robert-readii, but no evidence regarding the observation of these animals visiting these bromeliads species was mentioned. Thus, the present study records the role of bats as pollinators within the genus Billbergia for the first time.

Although pollination by hummingbirds have been reported for B. horrida (Ruschi 1949, apud Benzing et al. 2000), the results of this work indicate that they contribute poorly to the fruit and seed set of this species in the studied area. The reduced reproductive success of B. horrida when it is available only to hummingbirds, compared to bats, reflects the difference in the effectiveness...
of the removal and deposition of pollen from the anthers to the stigma. Since observations of the local deposition of pollen on the body of T. glaucopis and P. pretrei are compatible with what is known about effective pollination for several species of hummingbirds (Siqueira Filho and Machado 2001, Machado and Semir 2006), the reduced fruit and seed set observed might be related to the quantity of pollen deposition. For B. horrida, the insufficient deposition of pollen by hummingbirds might be due to the removal of small amounts of grains and their subsequent transferal (considering that under natural conditions, the flowers were previously visited by nocturnal pollinators). The visitation of B. horrida by hummingbirds can be explained by the availability of flower reward, which extends during daylight, since the supply of nectar during the morning and afternoon is the only attractive resource produced by this bromeliad for these avians. Within the studied area, B. horrida is sympatric with Portea petropolitana (Wawra) Mez, and frequently cohabits the same phorophyte. Both species have coincident flowering periods and the bright pink inflorescences and bracts of P. petropolitana probably increase the probability of attraction of these avians close to B. horrida.

ACKNOWLEDGMENTS

We thank the Pró-Reitoria de Pesquisa da UFJF for the field-collection permits, José Carlos da Silva and Edson Novais Filho for their help during the fieldwork, Pedro Henrique Nobre, Sônia Brugiolo, Laodicéa Lopes and Marco Antônio Manhães for the taxonomic identifications of bats, lepidopterans and hummingbirds, Nádia Somavilla and Honasses David for helpful comments on statistical analysis. Financial support was provided for Ana Paula Gelli de Faria and Marcela Cezar Tagliati by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), through the Programa de Capacitação em Taxonomia (PROTAX), process nº 562218/2010-6.

RESUMO

Billbergia horrida é endêmica dos fragmentos de Floresta Atlântica do sudeste do Brasil e caracterizada pelas flores com atributos típicos da polinização por animais noturnos. Embora a maioria das espécies de Billbergia apresente polinização diurna por beija-flores, B. horrida também é visitada por morcegos, e esse estudo evidencia pela primeira vez a ocorrência de quiropterofilia dentro do gênero. O papel dos diferentes grupos de polinizadores no sucesso reprodutivo de B. horrida foi avaliado, assim como a correlação de características do néctar no sustento desses animais durante os diferentes períodos do dia. Morcegos contribuíram com 82.1% da taxa de frutificação de B. horrida. Beija-flores, por sua vez, contribuíram com apenas 10% da taxa de frutificação e foram fracamente relacionados ao sucesso reprodutivo dessa espécie. A quantidade de néctar produzido e a concentração de açúcares foram similares às de outras bromélias quiropterófilas e apenas o volume de néctar apresentou mudanças significativas ao longo do período de disponibilidade da flor. As visitas recorrentes de beija-flores provavelmente acontecem devido ao fato das flores de B. horrida ficarem abertas por 24h, oferecendo recompenças energéticas aos visitantes durante o dia e devido à presença de outras espécies de bromélias atrativas crescendo sobre o mesmo forófito e florescendo na mesma época.

Palavras-chave: Floresta Atlântica brasileira, biologia floral, polinização por beija-flores, sucesso reprodutivo.

REFERENCES


