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Pollination is a major driver of germination in *Comanthera bisulcata* and may improve everlasting flowers (sempre-vivas) conservation in Brazilian campos rupestres

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Abstract: The campos rupestres, ecosystems that are both unique and highly diverse, are home to a variety of endemic species, including *Comanthera bisulcata* (Eriocaulaceae). This species, of economic importance, has its inflorescences used in handicrafts due to the fact that they retain the appearance of living structures even after being harvested and dried. The trade of these inflorescences depends almost exclusively on extraction, an activity of great importance for the traditional communities of the Espinhaço Meridional municipalities, in Minas Gerais. However, research is essential to improve the management of this native species. In this study, we highlight the importance of pollination for the reproductive success of *C. bisulcata*, through the investigation of floral visitors and germination tests. We observed pollinators during the flowering period in cultivated beds in campos rupestres area in Diamantina, Minas Gerais. For the germination test, we collected seeds from two different environments: inside a greenhouse, isolated from pollinating insects, and in a natural habitat, where pollinators were present. Subsequently, we subjected the seeds collected from both locations (no pollination and natural pollination) to treatments with/without gibberellin (0 and 500 mg L^{-1}). The species exhibited a generalist pollination strategy, attracting visitors that collected both pollen and nectar, as well as those that fed exclusively on nectar. We found a greater effect of pollination on germination and only minor effects of gibberellin application. Our results emphasize the importance of pollination in reproductive systems, as gibberellin was not sufficient to overcome the limitation of pollinator absence in *C. bisulcata*. We conclude that natural pollination is a critical factor to consider in management plans for the conservation of *C. bisulcata*.

Key words: Autogamy, Eriocaulaceae, management, reproduction

1. Introduction

The Brazilian campos rupestres (rupestrian grasslands) are among the most diverse environments on the planet and are considered an important biodiversity hotspot (Silveira et al., 2016; Morellato and Silveira, 2018; Fernandes et al., 2020). They present high plant diversity, containing 15% of Brazil's flora within less than 1% of the country's territory, with high levels of endemism (Silveira et al., 2016; Fernandes et al., 2020; Vasconcelos et al., 2020). However, this unique environment faces alarming threats, including global warming and land-use changes (Bitencourt et al., 2016). Given these factors, the importance of campos rupestres has been emphasized in the context of global biodiversity conservation planning (Fernandes et al., 2014; Morellato and Silveira, 2018).

The family Eriocaulaceae stands out as emblematic plants of the campos rupestres, capable of growing on shallow, extremely nutrient-poor soils (Costa et al., 2008; Giulietti et al., 2012). Most species in the family present limited distribution and are classified as microendemic (Costa et al., 2008; Parra et al., 2010; Sano et al., 2013). The Espinhaço mountain range is highlighted as the diversity epicenter of this group, hosting approximately 60% of its genera (Giulietti et al., 2012). Of the Eriocaulaceae species occurring at Serra do Espinhaço, 85% are estimated to be endemic (Costa et al., 2008) and, alarmingly, approximately 30 species are at risk of extinction[1]. There are even records of extinctions in the wild, as seen in Japan (Tanaka et al., 2015). This vulnerability points out

[1]MMA (Ministério do Meio Ambiente) (2022). Lista Nacional de Espécies Ameaçadas de Extinção (Portaria MMA N°. 148, de 07 de Junho de 2022) [online]. (in Portuguese) Website https://www.in.gov.br/en/web/dou/-/portaria-mma-n-148-de-7-de-junho-de-2022-406272733 [accessed 20 June 2023].

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the urgency of studies focused on the production and germination of Eriocaulaceae seeds, aiming to ensure the species conservation.

In addition to their ecological significance, Eriocaulaceae are often referred to as "sempre-vivas" (everlasting flowers) because their inflorescences retain the appearance of living structures for a long time after being collected and dried (Parra et al., 2010). This characteristic makes the genera *Comanthera* and *Syngonanthus* particularly important as ornamental plants (Parra et al., 2010) and an important source of income for many communities in the Espinhaço range (Oliveira et al., 2014). Furthermore, the practice of collecting flowers by traditional communities has gained international recognition from the FAO-UN, being recognized as Brazil's first World Agricultural Heritage site[2],[3].

Currently, the lack of knowledge about reproductive systems of the Eriocaulaceae is considered a major problem for the implementation of effective conservation measures (Horiuchi et al., 2021). Given the economic and social importance of Eriocaulaceae, especially considering its endemic species distribution, conservation measures are necessary. Among these actions are the analysis of the reproductive conditions of Eriocaulaceae species, including their reproductive systems, pollination, and germination (Schmidt et al., 2008; Horiuchi et al., 2020). Such knowledge is essential for improving ex situ cultivation techniques and designing management strategies for native plant populations subjected to extractivism.

Although Eriocaulaceae are generally poorly studied, the literature suggests that they have remarkably diverse pollination and reproductive systems (Ramos et al., 2005; Del-Claro et al., 2019; Andrino et al., 2022; Martins Junior et al., 2022). Seed studies also indicate a wide variation in germination rates of Eriocaulaceae species, ranging from 19% to 90%, highlighting the importance of investigating the factors underlying the process (Ramos et al., 2005; Simões et al., 2007; Oriani et al., 2009; Oliveira and Garcia, 2011). Among these factors, plant hormones play a significant role in regulating the seed germination process (Nautiyal et al., 2023). Specifically, gibberellins (GAs) emerge as essential activators of germination (Sun and Gubler, 2004; Rajjou et al., 2012; Nautiyal et al., 2023).

The relative contribution of ecological (e.g., pollination) and physiological factors (e.g., hormones) to the germination of everlasting flowers is poorly understood. Especially pollination and its influence on reproduction become essential for the development of wide-ranging conservation strategies. These strategies aim both to conserve natural populations and to stimulate their expansion through reproduction. Therefore, we aim to evaluate the effect of ecological and physiological factors on the germination of the everlasting flowers species *Comanthera bisulcata* (Körn) L.R. Parra & Giul (morphotype "Janeirona carrasqueira"). Initially, we investigated the floral biology and potential pollinators of the species. We also studied the effect of the reproductive system (natural pollination and autogamy) and exogenous application of gibberellin (GA3) on the germination and initial seedling development.

2. Materials and methods

2.1. Study species

Comanthera bisulcata (Körn) L.R. Parra & Giul exhibits variation classified into two distinct morphotypes, generally referred to as "sempre-viva Chapadeira" and "sempre-viva Janeirona Carrasqueira". Both are morphologically characterized by their rosette-shaped growth habits, from which capitulum inflorescences arise. These species are found in campos rupestres, at the Espinhaço Mountain Range, in shallow, sandy soils with low nutrient levels. The "Janeirona Carrasqueira" is among the commercialized everlasting flowers, and often collected at the Diamantina-MG region. It presents inflorescences with two series of white bracts and is collected between January and February. Usually, this collection period coincides with the rainy season in the region, which can lead to the loss of the whitish color of the capitula/bracts due to prolonged exposure to rain. Therefore, the species has a shorter harvesting window to prevent the darkening of the inflorescences. Inflorescences harvested late, which are already darkened, need to undergo a dyeing process before commercialization, resulting in a loss of commercial quality. The flowers are unisexual, remarkably small (on the millimeter scale), with male and female flowers presented in the same flower head. Female flowers are larger and thinner compared to male flowers, which are smaller and have a larger diameter. The inflorescences present alternating series of female and male flowers, with centripetal maturation. Each female flower results in a fruit containing three seeds. Voucher specimens are deposited in the herbarium of the DIAM/UFVJM (Diamantina-MG, Brazil), under registration number 9447.

2.2. Characterization of the study area

The study was conducted inside Campus JK, at the Federal University of the Jequitinhonha and Mucuri Valleys

[2]FAO (Food and agriculture organization of the United Nations) (2020). Apanhadoras e apanhadores de flores sempre-vivas recebem reconhecimento internacional da FAO como o primeiro Patrimônio Agrícola Mundial do Brasil [online]. (in Portuguese) Website http://www.fao.org/brasil/noticias/detail-events/en/c/1265788/ [accessed 15 April 2023].

[3]Borges T, Branford S (2021). Brazil: the flowers of sustainability [online]. Website https://lab.org.uk/brazil-the-flowers-of-sustainability/ [accessed 4 July 2023].

(18°12′3″ S, 43°34′3″ W), located at an altitude of 1296 m a.s.l. (above sea level), with approximately 60 ha of native campos rupestres (rocky outcrops megadiverse grasslands). According to the Köppen classification, the region has a Cwb climate, with rainy summers and dry winters (Alvares et al., 2013). The average annual temperature ranges from 18 to 19 °C, with an average annual minimum of 14.1 and maximum of 23.7 (Gianotti et al., 2013; INMET[4]). The average annual precipitation is 1400 mm, and the rainy season occurs from October to March, representing 88% of the total annual precipitation (Vieira et al., 2010).

The Campus JK area is adjacent to the Biribiri State Park, with more than 16,000 ha of preserved area. This park hosts the greatest diversity of everlasting flower species in the region (Echternacht et al., 2012; Andrino et al., 2015). In this study, we used plants up to 6 years old, grown in outdoor managed beds, and plants grown 15 cm diameter plastic pots containing the same soil as the beds. The potted plants were kept in a greenhouse with a shade screen on the sides and a PVC plastic roof. These potted individuals were originally transplanted from the beds and were already fully established by the time the experiment was conducted. The beds were close to the greenhouse and were therefore subject to similar microclimatic conditions.

2.3. Floral visitors and pollinators

We observed floral visitors for 3 days during the flowering season in December 2019. The observation interval was 8 h (8.00 AM to 4.00 PM), divided into 30-min blocks. The initial 10 min were used to count visitors, and the remaining 20 min were used to describe their behavior. Each observation focused on one bed containing approximately 100 plants. We recorded information on the period and duration of visits, as well as the number of individuals/inflorescences visited at different times throughout the planted bed. Furthermore, during field observations, we carried out a description of floral biology to understand the flowering patterns of the species. This included close observation of the anthesis of male and female flowers over time. Additionally, we captured photographs and videos during the observations to better visualize and understand floral visitor behavior.

We based the distinction between pollinators and nonpollinators on the foraging behavior displayed by visitors during the collection of floral resources and contact with the flowers' reproductive structures (anthers and stigmas). Furthermore, we checked the presence of pollen attached to the body of floral visitors to exclude those that did not carry *C. bisulcata* pollen. Based on the type of resource they were collecting—nectar, pollen, or both—we classified visitors into three categories: nectar collectors, pollen collectors, or both.

We evaluated the collected floral visitors regarding the site of adhesion and the identity of the pollen load adhered to their body surfaces. Based on this assessment, the visitors were classified as either vectors of *C. bisulcata* pollen or not. Thus, the pollen load was considered an indicator of the floral fidelity of the visitors. For this purpose, the collected visitors were placed in individual vials, euthanized with fumes of ethyl acetate. After that, we removed the pollen on their bodies using small cubes of glycerinated gelatin. Knowing the pollen morphology of *C. bisulcata*, all other pollen grains were considered to come from different plant species and quantified as heterospecific.

2.4. Germination test

We randomly selected 20 capitula from each collection site: outdoor beds and the greenhouse. In the outdoor beds, which represented the natural habitat, there were no barriers restricting the access of floral visitors to the inflorescences, thus characterizing the treatment of natural pollination. In the greenhouse, representing the enclosed environment, the plants were kept isolated from any contact with floral visitors, configuring the autogamy treatment. In the lab, we extracted seeds from flower heads by gently rubbing the capitula with tweezers in Petri dishes. This was done after approximately 6 months of storage at room temperature for dormancy-breaking (Baskin and Baskin, 2015; Carta et al., 2015). We examined the seeds under a stereoscopic magnifying glass STEMI 2000-C (Zeiss, Germany) and found no morphological differences in shape, size, or color between the seeds developed through natural pollination or autogamy. We then separated them, eliminating any malformed, damaged, or below-average-sized seeds.

We aseptically treated the seeds with a 2.5% (v/v) commercial sodium hypochlorite solution for 15 min, and two rinses of distilled water. Afterward, we immersed the seeds in a 500 mg L^{-1} gibberellin (GA3) solution for 24 h at room temperature. Plain distilled water was used as the control for seed immersion. Subsequently, we placed the seeds in Petri dishes containing two sheets of filter paper and moistened them daily with distilled water. The Petri dishes were then incubated in a Mangelsdorf germinator at 25 ± 2 °C, without supplemental artificial light. We employed a completely randomized experimental design following a 2 × 2 factorial scheme (two collection environments, with or without GA3). Each treatment contained five replicates of 30 seeds. We conducted evaluations every 5 days using a stereoscopic magnifying glass. We evaluated the number of seeds formed per capitulum, the germination rate, relative frequency of germination (Labouriau and Valadares, 1976) and postseminal development. We conducted the

[4] INMET (Instituto Nacional de Meteorologia)(2020). Normais Climatológicas do Brasil 1991-2020 [online]. (in Portuguese) Website https://portal.inmet.gov.br/normais [accessed 20 March 2023].

experiment for 52 days until seedling senescence and considered germinated seeds as those that presented a protrusion of the embryonic axis.

2.5. Statistical analyses

To estimate the probability of flower visitation associated with resource collection, we classified visitor behaviors. We categorized them as collectors of nectar, pollen, or both and modeled the probability of these behaviors as a function of visiting hours and the number of flowers visited using a Generalized Linear Model (binomial GLM).

We also assessed the difference in the number of seeds produced per capitulum in each environment (no pollination and natural pollination) using an analysis of variance (ANOVA). We tested the effects of treatments on seed germination using generalized linear mixed models (GLMM) with the 'glmer' function from the R package 'lme4' (Bates and Maechler, 2009). Each seed germination outcome (germinated or not) was modeled using a binomial distribution, with replication treated as a random factor. We built models considering combinations: pollination * gibberellin, pollination + gibberellin, only pollination, only gibberellin, and a null model with only the intercept. We selected models based on their Akaike Information Criteria (Burnham and Anderson, 2002). We conducted all statistical analyses using the R statistical environment version 3.2.1 (R Core Team, 2018).

3. Results

3.1. Floral visitors and pollinators

Based on our close observation of the anthesis of male and female flowers over time, we found that the flowering of *C. bisulcata* is synchronized at the population level but sequential at the individual and inflorescence level. This sequence is protandrous, meaning that male flowers open before female flowers. Female flowers offer nectar as the only resource to visitors.

Hymenoptera insects (bees and wasps) were the most frequent flower visitors of *C. bisulcata* (56.22%), followed by Diptera (26.02%), Coleoptera (14.5%), and Lepidoptera (3.26%). Most insects that visited flowers were small relative to the size of the inflorescence (Figure 1), had mouthparts in various sizes and shapes, and displayed a wide range of behaviors. Hymenopterans collected pollen and/or nectar, with visitation lasting 3 to 6 s, moving sequentially to the next flower in the same flower head. Among these, Halictidae bees collected only pollen during visits. For flies, two different behaviors were recorded: in the first group, Syrphidae flies were observed collecting pollen, while in the second group, the other flies exclusively collected nectar. Some Coleoptera were recorded feeding on the pollen. *Pyrausta* sp. (Crambidae) was the only Lepidoptera recorded to feed on floral nectar from *C. bisulcata*.

The probability of visits with pollen collection increased by 25% between 1.00 PM and 2.00 PM and by 50% in the late afternoon; however, it was also related to the number of flowers visited (interaction between time and number of visited flowers: $\chi^2 = 5.82$; $p = 0.015$; Figures 2A and 2B). Pollen collectors, primarily from the family Apidae, carry out sequential visits, tasting more than 12 flowers on each flight to the flowerbeds. The highest richness of visitors (9 species) was observed in the morning when newly opened flowers provided nectar. Five floral visitors were recorded in the afternoon. The average abundances recorded during the 10 min intervals of observation in the morning and afternoon periods were 78 and 100 individuals, respectively. Although both nectar and pollen were available in both periods, exclusive pollen-eating visitors, such as Coleoptera, were recorded only in the afternoon. Nectar was explored by a greater number of visiting species (richness), whereas pollen was used by a greater abundance of visiting insects. A total of 70 floral visitors were collected, with an average of 23 pollen grains per visitor (SD = 36.07), ranging from 1 to 126 pollen grains per visitor. Among the species of flower visitors, 25 did not carry pollen from *C. bisulcata*. In addition to *C. bisulcata*, pollen grains from other 18 plants species were carried by the floral visitors collected.

3.2. Germination test

The seeds of *C. bisulcata* species are small (<1 mm) and reddish-brown. We observed similar seed sets per flower head ($p > 0.05$) from natural (136 seeds per capitulum) and autogamy (144 seeds per capitulum) treatments. Germination was characterized by protrusion of the embryonic axis and began 7 days after sowing for both treatments. During the germination process, leaves develop first, followed by the roots. The maximum number of leaves that emerged during our experiment (52 days) was three, which occurred after 42 days. At day 52, some seedlings showed the beginning of senescence, indicating the depletion of their reserves. The germination process is schematized in Figure 3.

Considering the relative frequency of germination, it was distributed over time regardless of seed origin. We recorded several germination peaks, revealing a polymodal curve (Figure 4). However, the first and highest germination peaks, for both treatments, were observed on the 22nd and 12th days after sowing for natural pollination and autogamy, respectively. Germination stabilization occurred approximately 47 days after sowing (Figure 4).

Germination rates were significantly higher ($p < 0.05$) in the treatments involving seeds from natural pollination, with a median of 80% for seeds without GA3 application and 70% for seeds immersed in GA3 (Figure 5). It highlights the success of seeds from natural pollination, which surpassed those from autogamy by 100%, in which the germination rate was 40% without GA3 and 52% with GA3.

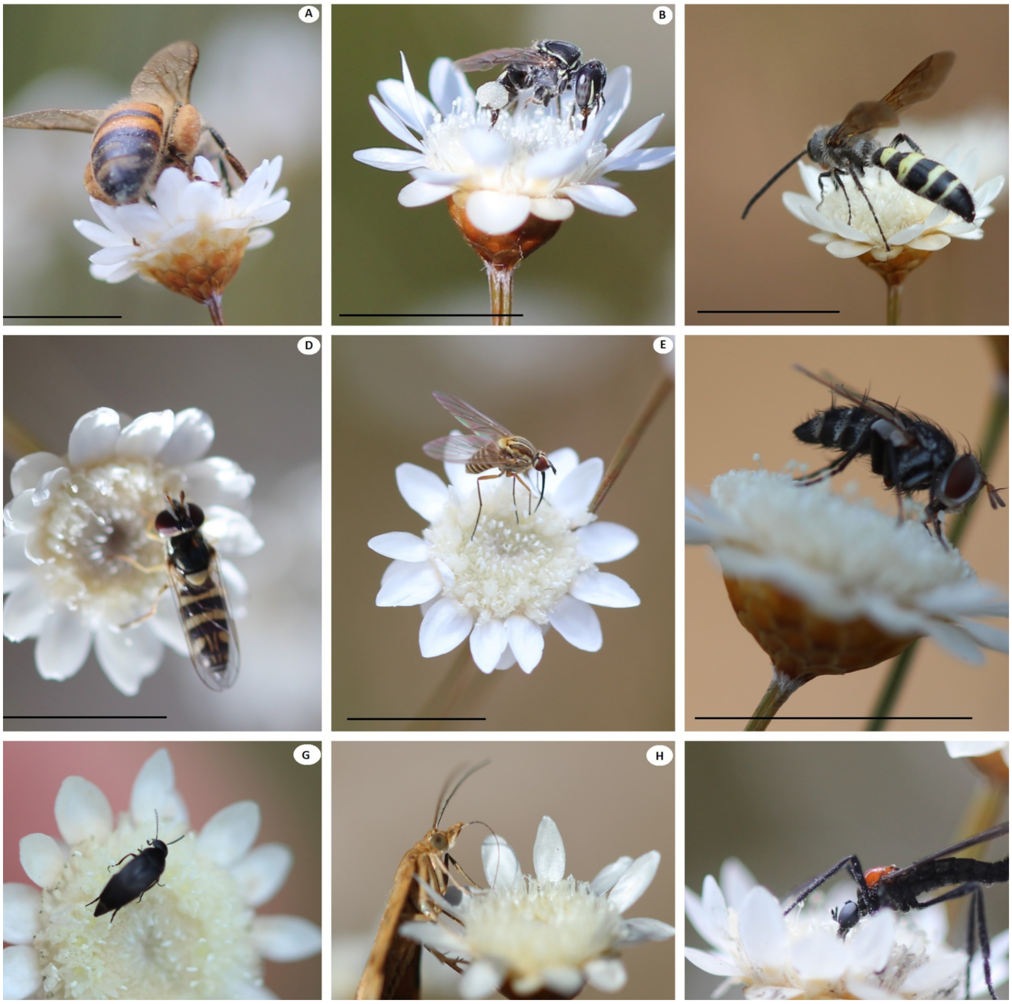

Figure 1. Sampled floral visitors of *Comanthera bisulcata* in Diamantina - MG. Bees: *Apis mellifera* (A); *Plebeia* sp. with pollen (B); wasp (Vespidae) (C); fly eating pollen (Syrphidae: Diptera) (D); fly feeding on nectar (Diptera) (E); fly feeding on nectar (Muscidae: Diptera) (F); beetle (Coleoptera) (G); *Pyrausta* sp. (Crambidae) (H); *Plecia* sp. (Diptera) (I). Bars = 1 cm.

The most effective model for explaining the germination test results exclusively featured the pollination mode as the predictor variable, with the lowest dAIC value (0.0) and the highest weight (0.485) (Table). The inclusion of the hormone in the models did not improve the fit, indicating a weak physiological response to gibberellin stimulation. Therefore, the simpler model, considering only the pollination factor, was deemed the most appropriate for explaining the observed results.

Seeds from natural pollination exhibited an estimated germination rate of 65% (SE = 0.0605, 95% CI: 52.4%–75.7%), while seeds resulting from autogamy showed a predicted germination rate of 41% (SE = 0.0636, 95% CI: 29.7%–54.2%). The Generalized Linear Model highlights the substantial influence of pollination on seed germination (Table S1).

4. Discussion

Our study yielded significant insights into the reproductive dynamics of *C. bisulcata*, a species endemic to campos rupestres. This research uncovered a striking pollination strategy, where a diverse array of visitors, including Hymenoptera, Diptera, Coleoptera, and Lepidoptera, played a key role in the reproductive success of this unisexual-flowered plant. While inflorescences exhibited protandry, with male flowers opening before female ones, female flowers provided nectar as the primary resource to visitors. Notably, the results highlighted the limited effectiveness of gibberellin in promoting germination, underscoring the critical role of natural pollination in the germination process of *C. bisulcata*. Additionally, the study demonstrated that pollination significantly increased the likelihood of pollen collection during visits, with notable

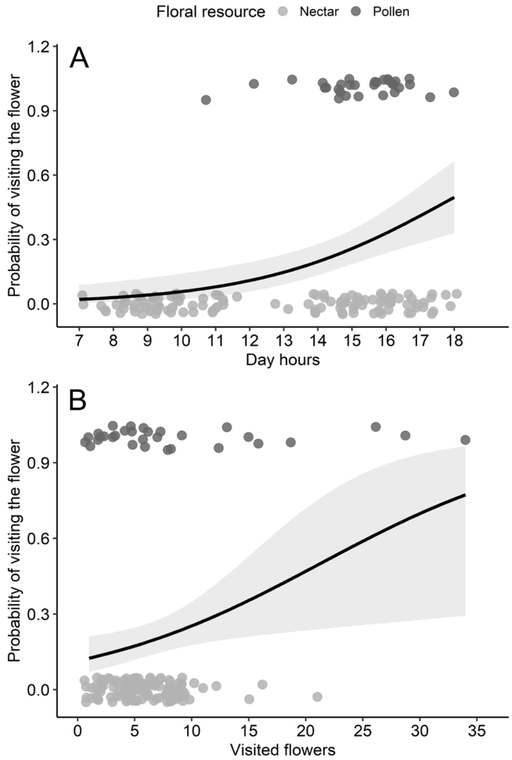

Figure 2. Generalized linear models of the probability of visits with collection of nectar (0) and pollen (1) resources as a function of visiting hours (A), and number of flowers visited (B). The points represent the actual observations, and the solid line represents the values predicted by the model.

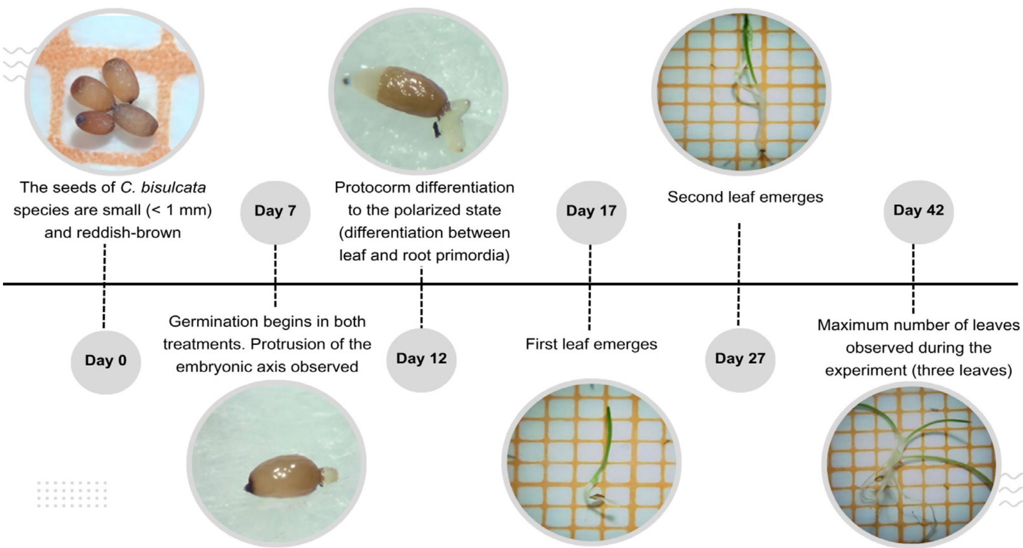

Figure 3. Schematic of the germination process of *Comanthera bisulcata*. The germination process initiates with the protrusion of the embryonic axis at 7 days after sowing, followed by the differentiation of the protocorm to the polarized state at 12 days. Subsequently, the first leaf emerges at 17 days after sowing, succeeded by the second leaf at 27 days. After 42 days, a maximum of three emerging leaves were observed during the experiment. Background squares = 1 mm^2.

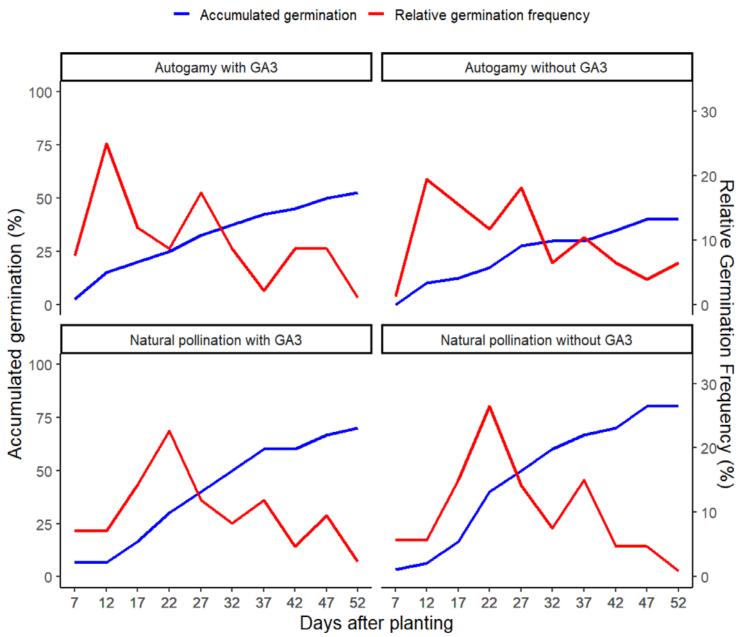

Figure 4. Accumulated germination rate and relative germination frequency of *Comanthera bisulcata* under treatments of pollination (natural × autogamy) and hormonal induction (with and without GA3 application).

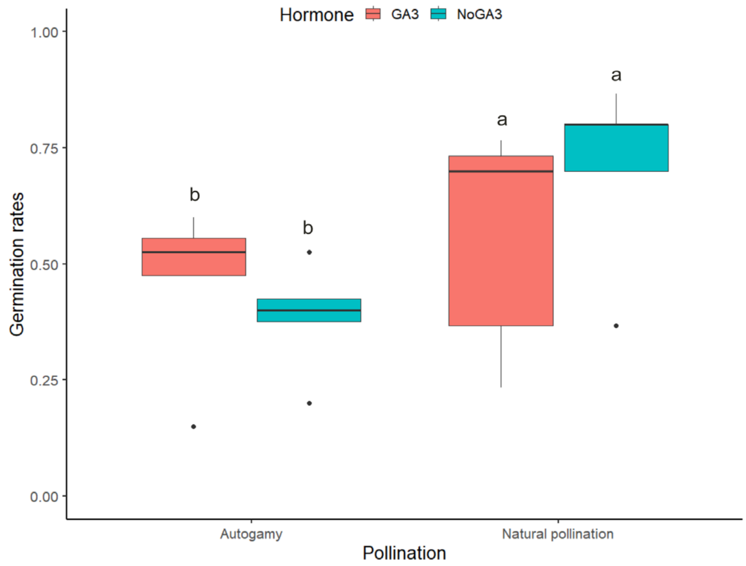

Figure 5. Germination rates in seeds of *Comanthera bisulcata* subjected to pollination (natural vs autogamy) and hormonal induction (with and without GA3 application). Different letters indicate a significant difference ($p < 0.05$) by the Kruskal–Wallis test.

variations in pollinator behavior between different insect orders. Finally, the findings emphasized the essential role of pollination in the conservation of this endemic species and offered practical insights for bridging economic and ecological considerations in preserving these precious harvested plants.

4.1. Floral visitors and pollinators

C. bisulcata produces nectar and pollen as floral resources and attracts a wide diversity of floral visitors. Visits by Lepidoptera were unexpected, as the floral morphology of Eriocaulaceae generally does not match insects with elongated mouthparts, such as moths and butterflies.

Table. Results of model selection analysis for germination success in seeds of *Comanthera bisulcata* subjected to two pollination (natural × autogamy) and hormone induction (with and without gibberellin application) treatments.

Model	dAIC	DF	Weight
Complete model with interaction	1.7	5	0.212
Complete model without interaction	1.7	4	0.203
Pollination mode only	0.0	3	0.485
Hormone only	5.6	3	0.029
Null model	3.8	2	0.071

Other *Comanthera* species studied in campos rupestres, such as *C. curralensis*, *C. mucugensis*, and *C. elegans*, also exhibited pollination by small insects of the orders Hymenoptera, Diptera, and Coleoptera, reaffirming the generalist pollination system in this genus (Ramos et al., 2005; Oriani et al., 2009). Generalist pollination systems can be advantageous for endemic species, playing a pivotal role in enhancing their reproductive success and long-term viability (Krushelnycky, 2014; Senapathi et al., 2015). In *C. elegans*, pollinator visitation peaks coincided with periods of high temperature and low relative humidity, corresponding to the complete opening of the capitula (Oriani et al., 2009). The full opening of the capitula is initiated by the motion of the involucral bracts, driven by hygroscopic cells (Oriani and Scatena, 2009). These specialized cells respond to high humidity conditions, prompting the closure of the capitula (Oriani and Scatena, 2009).

Nectar availability at the beginning of anthesis may be a pollen-saving mechanism. This way, the consumption pressure on this resource is reduced, resulting in a lower probability of visits by insects that collect pollen at the beginning of anthesis (Figure 2A). This strategy may also maximize pollen transport between flowers and, consequently, pollination, once the pollen-collection visits are more likely when more flowers are visited. Moreover, when pollen is dispersed by nectar-feeding visitors, this strategy enhances the likelihood of successful pollen transfer to another flower, reducing the risk of it being consumed by adult flies or bee larvae, who are the primary collectors of pollen. This is especially important considering that, while the pollen quantity within a flower is limited, the nectar supply can be replenished by continuous production throughout the day (Brito et al., 2017). In addition, female flowers exclusively offer nectar, which diminishes the potential for floral visitors who exclusively collect pollen to act as pollinators since they lack motivation to visit female flowers for nectar (Brito et al., 2017).

4.2. Germination test

The germination rate of this species was greater than 40% in both treatments, indicating that seeds had the potential to germinate under the experimental conditions. However, seeds from the natural pollination treatments showed remarkable performance, with germination rates consistently exceeding 70%. These results emphasize the importance of the pollination process for the species' reproductive success, and highlight the need for the protection of pollinators and the environments that support this interaction.

The small-sized seeds of *C. bisulcata* can favor their dispersal, besides facilitating transportation by wind or dispersing organisms. This increases the chance of finding favorable locations for species establishment (Echternacht et al., 2014). The species follows an important adaptative strategy of development in which leaves are formed before roots, classifying it as photoblastic positive. Positive photoblastism inhibits the germination of small buried seeds because their energy reserves would be insufficient to reach the surface (Garcia et al., 2020). During the early stages following germination, the development of leaves plays a vital role in enabling the species to generate the energy required for its growth through photosynthesis (Mascarenhas and Scatena, 2021). This allowed *C. bisulcata* to produce three leaves within 42 days after sowing, even with a relatively modest seed reserve.

Germination for both treatments was distributed over time, presenting germination peaks and a polymodal curve. This pattern can be attributed to the genetic variability inherent in native species seeds (Gonçalves-Magalhães et al., 2021). By allowing germination in different moments, it is possible to take advantage of favorable ecological windows for establishment; thus, genetic diversity represents an adaptive strategy for survival in dynamic ecosystems subject to seasonal and climatic fluctuations (Gonçalves-Magalhães et al., 2021). These results reinforce the possibility of *C. bisulcata* establishing seed banks in the soil, a phenomenon already described for this species (Garcia et al., 2020), which demonstrated its ability to maintain seed banks in the soil for at least 2 years.

Autogamy was confirmed by seed production inside the greenhouse without pollinators presence, which can be explained by the spatial proximity and temporal overlap between male and female anthesis within inflorescences (Oriani et al., 2009; Horiuchi et al., 2020). Species that spontaneously self-pollinate and are self-compatible can produce seeds without the need for a pollination vector, which is advantageous for adapting to certain environments, especially degraded ones (Proctor et al., 1996). Thus, mixed mating strategies allow reproduction by outcrossing when pollinators are abundant, or by geitonogamy/apomixis when pollinators are scarce or absent (Martins Junior et al., 2022). This way, this species can reproduce even in years that are unfavorable to pollinators.

In *Comanthera elegans*, a lower percentage of germination was observed for seeds resulting from autogamy, which may be a consequence of inbreeding depression (Oriani et al., 2009; Horiuchi et al., 2021). Ramos et al. (2005) also considered entomophily as the main mode of pollination in *Comanthera mucugensis* and *Comanthera curralensis* based on research on floral visitors and insect activities in flowers, although autogamy also occurred in those species. Mixed breeding systems have also been reported for *Paepalanthus bifidus* and *P. tortilis* (Martins Junior et al., 2022). For these everlasting flower species, wind-based pollination systems proved ineffective in their reproductive processes. Consequently, insect pollination emerged as the most efficient means of reproduction for these species.

Autogamy can lead to a lower germination rate due to lower genetic diversity and a higher probability of expressing harmful recessive alleles (Dudash and Carr, 1998; Baskin and Baskin, 2015; Busch et al., 2022). To improve seed germination efficiency, growth regulators have often been used, with gibberellins standing out (Huang et al., 2014). Gibberellins are widely known as promoters of germination, as they are hormones that enhance the growth potential of the embryo (Kucera et al., 2005). Gibberellins induce the expression of genes that encode enzymes, facilitating the mobilization of stored nutrient reserves within the seed. This mechanism is essential for providing the energy and nutrients required for successful germination (Peng and Harberd, 2002; Kucera et al., 2005; Taiz and Zeiger, 2009). Furthermore, they trigger the weakening of the tissues surrounding the radicle to overcome the mechanical constraints imposed by the seed cover layers (Debeaujon and Koornneef, 2000). However, despite all these physiological stimuli for germination, gibberellin alone was not sufficient to overcome the limitation of the absence of pollinators.

4.3. Practices for the conservation of Eriocaulaceae

Pollinator-friendly management practices are essential for ensuring effective pollination during the the in situ and ex situ conservation of Eriocaulaceae (Horiuchi et al., 2021). Studies reporting the occurrence of insect pollination in everlasting flower species are very important for conservation management, given the practice of recurrent burning in areas where these species occur (Ramos et al., 2005). According to Ramos et al. (2005), recurrent burning affects the reproduction of Eriocaulaceae species, which is especially concerning because many of their floral visitors are resident species with limited flight capabilities. Thus, fires may potentially lead to a decrease in the number of potential pollinators. Therefore, controlled burning for the management of everlasting flower species should be limited to their specific habitats and avoided during the pollination phase. Thus, potential links between fire management and its impact on pollinators should be cautiously established and further studied.

Our results contribute to understand the reproductive ecology of *C. bisulcata* and may have significant implications for the conservation and management of natural populations. The relevance of pollination for seed germination emphasized its importance as an ecosystem service. Our results suggest that conservation and promotion of pollinator populations play a very important role in Eriocaulaceae conservation. Therefore, it is crucial to direct conservation strategies aimed at protecting both plants and their pollinators. By identifying these pollinators, we can take specific measures to attract them and conserve their nesting sites. Thus, our results provide relevant contributions to the understanding of the reproductive biology of *C. bisulcata*, which is essential for developing effective management strategies for the conservation of these plants of great ecological and economic significance.

5. Conclusion

In conclusion, our study on *C. bisulcata*, an endemic species in campos rupestres, has revealed valuable insights into its reproductive dynamics. This unisexual-flowered plant exhibited a remarkable pollination strategy, involving a diverse range of visitors, such as Hymenoptera, Diptera, Coleoptera, and even Lepidoptera. The findings underscore the pivotal role of natural pollination in the germination process of this species, emphasizing the limited effectiveness of gibberellin in promoting germination. Our study also highlighted the importance of pollinators in enhancing the reproductive success of *C. bisulcata*. Our findings carry significant implications for the conservation and management of natural populations, particularly concerning the need to protect pollinators and their habitats. It is essential to understand the key role of pollination services in the reproductive success of *C. bisulcata* and, by extension, its ecological and economic significance. Considering the importance of the reproductive system in germination, together with the generalist pollination system and the dynamics of seedling birth, we emphasize the need to incorporate plant reproduction into management plans for traditional flower picking. This action is fundamental both in the implementation of appropriate measures for in situ conservation, and in the cultivation of evergreens, which may contribute to reducing collection pressure in areas where the species naturally occurs.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Supplementary data

Table S1. Summary of the selected model for estimating germination success, with emphasis on the coefficients of fixed and random effects.

Fixed effects				
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-0.34	0.26	-1.32	0.18531
Natural pollination	0.96	0.37	2.57	0.00998*
Random effects				
Groups	**Name**	**Variance**	**Std. Dev.**	
Replication	Intercept	0.53	0.73	