REPRODUCTIVE BIOLOGY AND FLORAL VISITORS OF *Distimake aegyptius* (CONVOLVULACEAE) IN THE CAATINGA: A COMPARATIVE STUDY

BIOLOGIA REPRODUTIVA E VISITANTES FLORAIS DE *Distimake aegyptius* (CONVOLVULACEAE) NA CAATINGA: UM ESTUDO COMPARATIVO

Original article

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ABSTRACT

Distimake aegyptius, a native species commonly found in the Brazilian semi-arid region, has a rapid growth capacity in anthropized environments and is considered self-compatible. Considering the environmental heterogeneity of the Caatinga, we aimed to conduct a study on the reproductive system and guild of floral visitors of D. aegyptius, comparing the data found with those available for other areas of the domain. We conducted the experiments in Sobral and Frecheirinha, Ceará, between May and August 2019. We confirmed that the flowering of D. aegyptius is directly associated with the occurrence of precipitation, while its fruiting is related to the beginning of the dry season. The flowers exhibit attributes that characterize them as melitophilic with generalist pollination, being *Apis mellifera*, *Ancyloscelis apiformis* and *Polybia ignobilis* their effective pollinators. The guild of floral visitors differed between areas, which may be related to site conservation or competition with other species. Rates higher than 80% were recorded in manual and control cross-pollination experiments (natural conditions), indicating that the species is self-compatible and presents a reproductive strategy similar to that described for other Caatinga environments.

Keywords: Floral biology; Pollination syndrome; Semiarid.

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RESUMO

Distimake aegyptius, espécie nativa comumente encontrada na região semiárida brasileira, apresenta rápida capacidade de crescimento em ambientes antropizados e é considerada autocompatível. Considerando a heterogeneidade ambiental da Caatinga, objetivamos realizar um estudo sobre o sistema reprodutivo e guilda de visitantes florais de D. aegyptius, comparando os dados encontrados com aqueles disponíveis para outras áreas do domínio. Realizamos os experimentos em Sobral e Frecheirinha, Ceará, entre maio e agosto de 2019. Confirmamos que a floração de D. aegyptius está diretamente associada à ocorrência de precipitação, enquanto sua frutificação está atrelada ao início da estação seca. As flores apresentam atributos que as caracterizam como melitófilas de polinização generalista, sendo Apis mellifera, Ancyloscelis apiformis e Polybia ignobilis seus polinizadores efetivos. A guilda de visitantes florais diferiu entre as áreas, aspecto que pode estar relacionado à conservação dos locais ou à competição com outras espécies. Taxas superiores a 80% foram registradas nos experimentosde polinização cruzada manual e controle (condições naturais), indicando que a espécie é autocompatível e apresenta estratégia reprodutiva similar ao descrito para outros ambientes da Caatinga.

Palavras-chave: Biologia floral; Semiárido; Síndrome de polinização.



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INTRODUCTION

Cosmopolitan, but with greater abundance in tropical zones, the Convolvulaceae family comprises about 1900 species, which are distributed in 60 genera and have distinct life-habits (Simões; Staples, 2017). In Brazil, the family is represented by 401 species included in 21 genera, whose presence is seen throughout the national territory. However, the Northeast region receives more emphasis for having the greatest diversity of the group, with 227 species inserted in 16 genera (Simão-Bianchini *et al.*, 2023, constantemente atualizado).

Within the family, there is *Distimake aegyptius* (L.) A.R. Simões & Staples, a widely distributed species commonly found in the Brazilian semiarid region and usually associated with anthropized environments (Kiill; Ranga, 2000a). The first study about the reproductive biology of *D. aegyptius* occurred in Pernambuco and described a self-compatible reproduction system for thespecies, with *Apis mellifera* (Linnaeus, 1758) being its effective pollinator (Kiill; Ranga, 2000a). Subsequently, in a research carried out in the semiarid of Rio Grande do Norte, Pick and Schlindwein (2011) confirmed these data.

Given the widespread distribution of *Distimake aegyptius* in the diverse Caatinga region (Kiill; Ranga, 2000a; Pick; Schlindwein, 2011) and the notable environmental heterogeneity within this domain (Fernandes; Cardoso; Queiroz, 2020), this study aims to investigate the reproductive system of the species and its floral visitors' guild. The objective is to compare the findings with previous studies on the species, addressing the following questions: (i) does the species maintain consistent reproductive strategies across varied environments? (ii) Does the floral visitors' guild exhibit variations across different study sites? (iii) Do the effective pollinators of the species remain the same? (iv) Are there any new insights into the species' life cycle that may impact its reproductive biology?

METHODS

Study area

The studies were performed in two areas of Caatinga located in the cities of Sobral (3° 40′34.22208″S; 40°20′27.86316″W, 68 m) and Frecheirinha (3°45′13.56948″S; 40°48′50.28264″W, 188 m), Ceará state, Brazil, between May and August 2021. The areas have a hot-semi-arid tropical climate, with average rainfall and annual temperature of 821,6 mm and 28 °C for Sobral and 1.139,2 mm and 26 °C for Frecheirinha (IPECE, 2009; 2012).

When the two areas of this study were compared with those chosen by Kiill and Ranga (2000a) and Pick and Schlindwein (2011), it was noticed that the average annual precipitation was the climate data that differed the most, being 5,665.5 mm in the first area and 497 mm in the second. Meanwhile, annual temperatures did not differ much, with values of 26.3 °C in the first area and 28 °C in the second (figure 1).

Other features also differed. Althought all areas are located in the Caatinga domain, with shallow soils and low rainfall, Kill and Ranga (2000a) and Pick and Schlindwein (2011) performed their studies in places with high conservation rates, while the locals chosen in Sobral and Frecheirinha were highly anthropized environments, with many ruderal species.

It was observed that Distimake aegyptius grows as a volatile liana on trees and shrubs, forming dense and extensive branches that made it difficult to identify the individuals. Therefore, aiming to facilitate the conduct of the experiments, each tangle was considered an individual, following the procedure adopted by Kiill and Simão-Bianchini (2011).

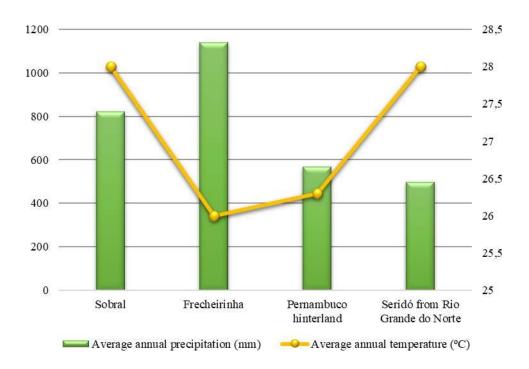
Floral morphology and reproductive system

It were analyzed aspects of the floral morphology, time of the anthesis, flower durability, stigma receptivity, pollen release, floral visitors, number of fruits and number of weeds per fruit. Some aspects of seed predation were also registered.

To assess the reproductive system, 64 flowers, distributed among 20 individuals, were marked. Four experiments were conducted: spontaneous self-pollination (n=24), manual cross-pollination (n=7), agamospermy (n=6), and pollination under natural conditions (n=27), which served as the control group. The agamospermy treatment was exclusively performed in Frecheirinha, as this

experiment was compromised in the first area.

Figure 1. Climatic data (average annual precipitation and temperature) of the compared study sites.



Source: IPECE (2009; 2012); Kiill; Ranga (2000a); Pick; Schlindwein (2011).

The selected inflorescences were marked during the bud phase and, except for the control group, were bagged with tulle. We calculated the success rate based on the proportion of marked flowers and fruits. In the agamospermy experiment, the selected inflorescences had their buds emasculated at the beginning of anthesis using tweezers.

For manual pollination, we used pollen from four inflorescences located approximately 100 meters away. Pollen transfer occurred by moving the anthers over the stigma of the receptive flower. To determine stigma receptivity, we followed the method described by Dafni, Kevan, and Husband (2005), applying oxygen peroxide to the stigmatic surface of chosen flowers; those with bubbling stigmas were considered receptive. Pollen liberation was verified by touching the anthers.

Morphological analysis was conducted in the laboratory, where we examined the number of flowers per inflorescence (n=30 inflorescences), length and diameter of the corolla (n=10 flowers) using graph paper, the number of formed seeds per fruit, and the presence of herbivory signs. In the field, we evaluated floral attributes such as color and available resources for pollinators. The specimens from the studied populations were accurately identified and incorporated into the collection of the Herbarium Professor Francisco José de Abreu Matos (HUVA).

Floral visitors

We registered the visiting hours and foraging behavior of the floral visitors by visual remark and photographs. The observations were executed in Sobral, during 10 days, of 04h30min to 13h00min, with a total of 10 hours of sampling effort (one hour per day).

RESULTS AND DISCUSSION

Distimake aegyptis is an annual and flickle liana, whose flowering is directly associated with rain occurrence, while its fruiting period is deeply linked with the beginning of the dry season (Kiill; Ranga, 2000a). Its fruits are subglobular capsules and have four locules that keep one seedeach, which are only relessed after a period of dehydration (figure 2). This pattern is commom between the Convolvulaceae of the Caatinga (Piedade, 1998; Kiill et al., 2017). As an annual species, it has a

terophytic life form, harboring the growth buds in the seeds that remain dormant in the soil during the dry season (Martins; Batalha, 2001), an aspect that ends up helping in themaintenance of its populations.

The species flowers are arranged in a dicasium-type inflorescence (figure 2), with four to 13 buds per inflorescence. We observed an anthesis of only one flower per day, rarely two, agreeing with the pattern described in Pernambuco by Kiill and Ranga (2000a). However, this pattern differs from that observed in *Distimake dissectus*, which exhibits floral anthesis of up to five flowers per day per inflorescence (Paz; Pigozzo, 2013). According to Kiill et al. (2017), the opening of few flowers per inflorescence allow the pollinator movement among individuals of the populations, favoring cross-pollination and increasing the chances of a high genetic variability.

The flowers of *Distimake aegyptius* are dialysépals, gammopetals, actinomorphs, monoclines and white in color. The corolla is infundibuliform (figure 2), with average dimensions of 28,3±1,71 mm in length and 45,5±2,37 mm in diameter, and presents at its base, below the insertion of the stamens, the nectariferous chamber, which is easily accessible and takes place through five channels.

The androceum is composed of five white stamens, epipetals, heterodynamos, with bitecae anthers and rhyming dehiscence, placed in the same level and above the stigma. A superus ovary, containing four eggs, and a white pistil, surrounded by stamens, forms the gynoecium. The reproductive verticils are located in the center of flower, close to each other, an aspect that contributes to self-pollination (Kiill; Ranga, 2003).

Infundibuliform flowers, open and with easily accessible reproductive structures, are characteristics that allow the presence of different types of visitors (oligophiles). The easy access of visitors to floral resources, such as nectar and pollen, proves to be advantageous in species that establish in unstable environments and is a pattern reported in other ruderal Convolvulaceae (Kiill; Ranga, 2003; Neves et al., 2006; Paz; Pigozzo, 2013).

A B C

Figure 2. General aspects of *Distimake aegyptius*. A – Inflorescence; B – Flower; C – Fruits.

Source: Portela, L.H.X.

The anthesis starts gradually around 4:30 am, being fully open around 6:00 am. The stigma is receptive soon after anthesis and pollen is immediately released from the anthers. The flowers remain unchanged until about 11:20 am, when the floral senescence phase begins. This stage is characterized by dehydration of the corolla. The flower lasts approximately nine hours, with the corolla fauce fully closed at 1:30 pm.

These results indicate a longer flower duration compared to the data found for Pernambuco and Rio Grande do Norte. This extended duration can be advantageous for the species, as it increases the available time for visitation and optimizes the chances of cross-pollination, especially considering that the population in our study area was under strong anthropogenic action. Moreover, the difference in duration may be related to the climate of the studied sites, supporting Kiill and Ranga (2000a), who reported that temperature and humidity are factors that can interfere in the process.

Floral aspects such as diurnal anthesis and roosting platforms indicated that bees would pollinate the flowers of this species (melitophilia), which is a frequent syndrome among representatives of the family (Piedade, 1998; Kiill; Simão-Bianchini, 2011; Paz; Pigozzo, 2013). In fact, bees, wasps, butterflies, beetles and flies visited the flowers of *Distimake aegytpius* (table 1), with *Apis mellifera*, *Ancyloscelis apiformis* and *Polybia ignobilis* being its effective pollinators.

Apis mellifera was the most observed visitor, and the primary effective pollinator of the species, with peak visitation occurring between 7:00 and 10:00 am, in agreement with observations made in the Pernambuco hinterland (Kiill; Ranga, 2000a). During foraging, the bees landed on the corolla and walked towards the floral tube in search of nectar, coming into contact with the reproductive structures (figure 3), and the pollen was deposited on the anterior dorsal region of the insect's body. After collecting the floral resource, the bee took off and visited neighboring flowers.

Ancyloscelis apiformis showed a visiting behavior different from Apis mellifera, having collected only pollen. The individuals of the species entered the floral tube and manipulated the anthers making rotational movements, causing the pollen to concentrate mainly on their legs and the ventral region of their bodies (figure 3). By making these movements, the bees encountered the stigma, pollinating it. Our information on the actions of this floral visitor confirmed what Pick& Schlindwein (2011) described for Rio Grande do Norte.

Meanwhile, the behaviour of *Polybia ignobilis* was similar to that described for *Apis mellifera*, with nectar being the main floral resource collected by both visitors (figure 3). During collection, *P. ignobilis* touched the reproductive structures with the dorsal region of its body. Visitation recordsof this wasp on Convolvulaceae flowers are rare, despite being a species with a wide trophic niche and very abundant in the Brazilian semiarid region (Melo, 2007; Paz et al., 2018).

The fly *Diptera* sp. and the butterflies *Nyctelius* sp. and Hesperiidae sp. 1 collected nectar, but did not touch the reproductive structures, being considered nectar pilers. Ants of the genus *Camponotus* (figure 3) showed intense nectar collection, and may or may not encounter the anthers, being their size a determining factor in the process. Moreover, more than one *Camponotus* sp. was observed collecting nectar from the same flower, an action that may lead to an accidental contact with the stigma, performing pollination. Although not common, ants of this genus have already been reported, in Brazilian territory, as effective pollinators of *Paepalanthus lundii* Korn (Del-Claro et al., 2019).

Among the native visitors, *Ancyloscelis apiformis* and *Polybia ignobilis* were considered secondary pollinators. When compared to *Apis mellifera*, both pollinators showed low regularity of visits, which may be related to the intense activity of the exotic bee. As *Apis mellifera* uses only the nectar it collects, it ends up causing an excessive loss of pollen, compromising the activity of *Ancyloscelis apiformis* (Pick; Schlindwein, 2011).

Ancyloscelis apiformis and Polybia ignobilis had a different visiting schedule. The former pollinator usually visited the flowers in the late morning, between 10:00 and 11:00 am, while the latter had its visits accumulated early in the day, between 7:00 and 8:30 am. Nyctelius sp. and Hesperiidae sp1 concentrated their visits in the early morning, between 6:30 and 8:30 am, while Camponotus sp. showed visiting hours ranging between 6:00 and 10:30 am. Diptera sp. was recorded in the late morning, at 10:30 am.

Table 1. Floral visitors of *Distimake aegyptius* and its foraged resources compared to other studies in the Caatinga. FP = floral parts; N = nectar; Pol = pollen; Pre = predator; Pi = piler; Pep = Primary effective pollinator; Sep = Secondary effective pollinator.

				Other studies	
ORDER/Family	Species	Foraged resource	Visitor classification	Kiill and Ranga (2000a)	Pick and Schlindwein (2011)
COLEOPTERA					
Chrysomelidae	<i>Megacerus</i> sp. (Fåhraeus, 1839)	FP	Pre	-	-
DIPTERA	Diptera sp.	N	Pi	-	-
HYMENOPTERA					
	Apis mellifera (Linneaus, 1758)	N	Рер	×	×
Apidae	Ancyloscelis apiformis (Fabricius, 1793)	Ро	Sep	-	×
Formicidae	Camponotus sp. (Mayr, 1861)	N	Pi	×	
Vespidae	Polybia ignobilis (Haliday, 1836)	N	Sep	-	-
LEPIDOPTERA					
Hesperiidae	<i>Nyctelius</i> sp. (Hayward, 1948)	N	Pi	-	-
	Hesperiidae sp1	N	Pi	-	-

Source: the authors.

Apis mellifera and Polybia ignobilis are generalist pollinators, having adaptive attributes that favor their dominance in disturbed environments and being generally associated to populations of ruderal species (Leal et al., 2018). On the other hand, *Ancyloscelis apiformis* is considered an oligolectic bee, with a clear preference for the Convolvulaceae family (Neves et al., 2006; Pick; Schlindwein, 2011). The visitors' easy access to floral resources and their non-specific association with only one pollinating agent makes the species a "generalist pollinator", a resultthat confirms what was described by Kill and Ranga (2000a).

When comparing the guild of floral visitors, it was found that the number of species found here (n=8) is higher than that reported in the sertão of Pernambuco (n=6) but lower than that observed in the Seridó of Rio Grande do Norte (n=14). This difference may be related to two factors: (i) the conservation status of the study sites, which, in the case of Pick and Schlindwein (2011), was a Conservation Unit; (ii) and, in the case of Kiill & Ranga (2000a), the overlap of the peak flowering of Distimake aegyptius with other species, leading to competition for visitors (Piedade, 1998).

Although all the studies were conducted in the Brazilian semiarid region, the populations observed in this research were located in highly anthropized sites, justifying the presence of pollinators with generalist behavior. This fact could explain the difference between the occasional pollinators of *Distimake aegyptius* documented in this study and those observed by Kiill and Ranga (2000a).

The results of the pollination experiments are presented in Table 2, while comparative data on success rates are displayed in Table 3. They reveal rates higher than 80% in pollination under natural conditions, consistent with findings by Kiill & Ranga (2000a) and Pick & Schlindwein (2011), demonstrating the efficiency of pollinators. The success rate of spontaneous self-pollination experiments was 58.3%, higher than that recorded for this species in the Pernambuco hinterland and lower than the rates reported for the Seridó of Rio Grande do Norte. Despite the differences between the values, they are sufficient to confirm the self-compatibility of *Distimake aegyptius*.

This feature explains the potential permanence of the species populations in diverse types of environments, as it does not solely depend on visitors to ensure the next generations. In all three studies, manual cross-pollination rates were higher than 60%, while apomictic fruit formation was nonexistent, reinforcing what has already been observed for the family in the Caatinga (Kiill; Ranga, 2003; Kiill; Simão-Bianchini, 2011; Kiill et al., 2017).

Table 2. Results of pollination experiments of *Distimake aegyptius* in the disturbed environments of Sobral/Frecheirinha, Ceará, Brazil.

Experiments	Flowers (n)	Fruits (n)	Success (fruits/flowers %)	Total number of seeds produced (%)
Natural conditions (control)	27	22	81,5%	82 (75,9%)
Manual cross-polination	7	6	87,8%	18 (64,2%)
Spontaneous self-polination	24	14	58,3%	48 (50,0%)
Agamospermy/Apomixis	6	-	-	-

Source: the authors.

Table 3. Comparison of reproductive success rates of *Distimake aegyptius* with other studies performed in the Brazilian Caatinga.

Even anima anta	Success rates (fruits/flowers %)					
Experiments	Kiill e Ranga (2000a)	Pick e Schlindwein (2011)	This study			
Natural conditions (control)	83%	73%	81,5%			
Manual cross-polination	63%	77%	87,8%			
Spontaneous self-polination	33%	80%	58,3%			
Agamospermy/Apomixis	-	-	-			

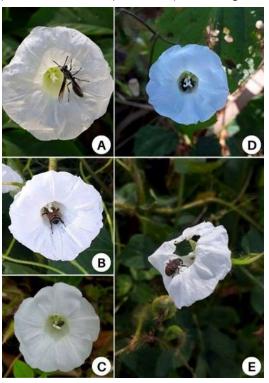
Source: the authors.

Regarding seed production per fruit, we obtained rates higher than 60% in fruits derived from cross-pollination and in the control group, similar to those described for the species in other regions (Kill; Ranga, 2000a; Pick; Schlindwein, 2011). The fact that the species has four ovules per flower reduces the need for many pollen grains to reach the stigma, increasing the chances of fertilization. This characteristic is quite advantageous for species that colonize anthropic environments, since the instability of these sites also affects the frequency of pollinators.

In this study, it was documented a beetle of the genus *Megacerus* feeding on the species' flowers inthe early morning (figure 3). In the predated flowers, it weren't observed visitors, which is harmful to the species' reproduction. Coleoptera larvae were also found inside the fruits, feeding off the endosperm of the seeds.

Canto, Rodriguez and Reyez-Novelo (2019) described the close relationship of *Megacerus* sp. with the Convolvulaceae family, whose larvae depend exclusively on the nutrients contained in the seeds of some species to complete their cycle. The authors also highlight *M. porosus* larvae as the main predators of *Distimake aegyptius* seeds. The antagonistic and specific interaction reported above contrasts with the generalist aspect of *D. aegyptius* pollination, which exemplifies the richness and complexity of the multiple ecological interactions that occur in Caatinga species.

Figure 3. Floral visitors of *Distimake aegyptius*. A – *Polybia ignobilis*; B – *Apis mellifera*; C – *Ancyloscelis apiformis*; D – *Camponotus* sp.; E – *Megacerus* sp.



Photos: Portela, L.H.X.

CONCLUSIONS

The introduction raises questions about the species' reproductive strategy, highlighting its self-compatibility and independence from floral visitors for fruit production. However, the experimentation rates vary across different areas. Despite this, the natural conditions reveal a high fruit set, showcasing the efficiency of pollination agents.

In terms of floral visitors, observations indicate variations in richness among study areas, with Apis mellifera being the primary effective pollinator, influencing the behavior of Ancyloscelis apiformis and Polybia ignobilis, the native effective pollinators. Apis mellifera was consistently present in both studies for comparison, Ancyloscelis apiformis only in the second study, and Polybia ignobilis was reported here for the first time. Visitor differences may be linked to conservation and interspecific competition.

Regarding fourth question, the presence of *Megacerus* sp., a beetle damaging floral parts and laying eggs in immature fruits, was noted. Larvae feeding on seed endosperm poses a threat to future generations. Flowers damaged by the beetle showed no visitors, confirming its negative impact on the reproductive ecology of *Distimake aegyptius*.

The study of reproductive biology in ruderal species is crucial for understanding the insectplant relationship in disturbed environments. Beyond contributing to local biodiversity knowledge, such research is instrumental in developing conservation and population control strategies.

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