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Frequency and efficiency of nocturnal and diurnal capitulum visitors in the reproduction of *Grazielia intermedia* (Asteraceae)

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ABSTRACT

Nocturnal and diurnal pollination of the same species is rare in Asteraceae. Visitation of nocturnal and diurnal insects has been recorded in florests of *Grazielia intermedia* during preliminary observations. Therefore, our goal was to assess the frequency and efficiency of visitors. We observed capitulum night visitors from 1800 h to 2200 h and daytime visitors from 0700 h to 1800 h and calculated their visitation frequency. We bagged capitula either during the evening or during the day to compare fruit production resulting from diurnal and nocturnal visitors. *Grazielia intermedia* presented capitulum visitors from 149 species (45 nocturnal and 104 diurnal). Lepidoptera were the most abundant visitors (91.9% of species) and the most frequent visitors (90.9%). The foraging peak of nocturnal pollinators was between 1900 h and 2000 h, and diurnal pollinators from 0900 h to 1000 h and 1400 h to 1500 h. There was no statistical difference in fruiting between night- and day-pollinated plants treatments (57.6% ± 20.9 and 32.4% ± 26.2, respectively), and both groups of visitors acted as pollinators. Our results illustrate the possibility of other Asteraceae species exhibiting the same pollination system recorded here, especially those that occur in altered habitats.

Keywords : Lepidoptera, moths, phalaenophily, pollination, psicophily.

Plants have different floral opening and closing mechanisms related to pollinator visitation period (van Doorn and van Meeteren 2003). Species that open their flowers only once and remain open for more than 1 d can be visited by biotic vectors, both during the day and night. Additionally, the pollen deposition efficiency of these vectors may differ (Ne'eman *et al.* 2010). Although Asteraceae species commonly have long-lived capitula (Primack 1985), studies on nocturnal pollination and the efficiency of different groups of visitors are scarce in this plant family.

Grazielia R.M. King & H. Rob. is a polyphyletic genus circumscribed in the subtribe Disynaphiinae (Eupatorieae tribe) and its distribution is restricted to South America (King and Robinson 1987, Pérez Sobrino 2016, Rivera *et al.* 2016, Nakajima *et al.* 2020). The clade to which this genus belongs (CAFE clade) has several endemic species concentrated in Brazilian areas of the Cerrado and Atlantic Forest (Rivera *et al.* 2016). These two phytogeographic domains are biodiversity hotspots (Myers *et al.* 2000), and the risk of species extinction in these domains is high. Additionally, CAFE clade species have great ecological diversification and variation in floral morphology among its members (Rivera *et al.* 2016), highlighting the importance of studies on their reproductive biology as a subsidy for conservation efforts.

Studies on reproductive biology in *Grazielia* have been restricted to two species. Freitas and Sazima (2006) surveyed the pollinators of *G. gaudichaudeana* (DC.) R.M. King & H. Rob in a community study on a tropical high-altitude grassland. The other species, *G. intermedia* (DC.) R. M. King & H. Rob. is psychophilous, and its capitula are considered important tools for monitoring antophilous butterflies (Cruz *et al.* 2012). Additionally, Cruz (unpublished data) recorded nocturnal moths visiting their florets. Other capitulum visitors were registered by Valentin-Silva *et al.* (2016a). In addition to sexual reproduction, apomixis has also been observed in *G. intermedia* (Valentin-Silva *et al.* 2016a).

Our goal was to identify the nocturnal capitulum visitors of *G. intermedia*, determine the frequency of nocturnal and diurnal visitors, and compare the pollination efficiency of both visitor groups.

MATERIAL AND METHODS

Study area and plant species — We conducted the study at the 'Station of Research, Environmental Training and Education Mata do Paraíso' (hereafter, Mata do Paraíso), located in the municipality of Viçosa (20º47'–48'S, 42º50'–52'W), Minas Gerais State, southeastern Brazil. This nature reserve consists in an area of 194 ha with an altitude ranging between 690 and 870 m. Its vegetation is classified as seasonal semi-deciduous montane forest (IBGE 2012), which occurs in the Atlantic Forest domain (Oliveira-Filho and

Fontes 2000). The climate in Viçosa is type Cwa (mesothermal with hot, rainy summers and cold, dry winters) according to Köppen's classification system (Alvares *et al.* 2013).

Grazielia intermedia (= *Eupatorium intermedium* DC.) occurs in the midwestern, southeastern, and southern regions of Brazil, in addition to Uruguay (Pérez Sobrino 2016, Nakajima *et al.* 2020). It is commonly located in areas with open vegetation, such as savannas, rupestrian field, high-altitude grassland, and close to the edge of forest vegetation (Esteves 2001, Nakajima *et al.* 2020, present study). It is an erect subshrub c. 2.5 m in height; capitula are homogamous, discoid, with five florets having white or lilac corollas. It flowers from January to April (Cruz 2009), and the florets last for 2 d, opening in the morning and remaining open during the night, and are odoriferous and protandrous (Valentin-Silva *et al.* 2016a). The studied population is gynodioecious and presents both sexual reproduction and apomixis (Valentin-Silva *et al.* 2016a). We used 25 individuals in this study,and voucher specimens were deposited in the VIC Herbarium (number 21,530).

Capitulum visitors — We observed and collected nocturnal capitulum visitors from 1800 h to 2200 h (March 2008). Species identification was performed by specialists, and voucher specimens were deposited in the collection of the Regional Museum of Entomology from the Department of Animal Biology of the Federal University of Viçosa.

The diurnal capitulum visitors considered in the present study were butterflies identified according to Cruz *et al.* (2012), and the other visitors were identified according to Valentin-Silva *et al.* (2016a). Both studies were conducted in the same population studied here, during two consecutive flowering seasons (March 2007 and 2008), from 0700 h to 1800 h. In these studies, 104 species of capitulum visitors were recorded, consisting of 88 species of butterflies (Lepidoptera, Rhopalocera), six bee species (Hymenoptera), four moth species (Lepidoptera, Heterocera), four fly species (Diptera), and two wasp species (Hymenoptera).

We calculated the visitation frequency of all visitors (each individual who landed in the capitula), grouping them in family or subfamily, except for the most frequent species. Thus, we observed nocturnal visitors for five non-consecutive days (between March 11 and 19, 2008, from 1800 h to 2100 h, for a total of 10 h) and diurnal visitors for 12 non-consecutive days (between March 3 and 20 2007/08, from 0700 h to 1800 h, for a total of 33 h). For both groups, at every 1 h interval, we quantified all capitulum visitors for 30 min, divided into two blocks of 15 min. Between blocks, we took 15min breaks to prevent changes in the behavior of insects due to human presence (Callaghan 1978). We collected data from different *G. intermedia* individuals per hour.

To compare pollen deposition efficiency (*sensu* Ne' eman *et al.* 2010) between nocturnal and diurnal visitors, we bagged 198 capitula (990 florets, N = 3 individuals) from 0700 h to 1800 h that were exposed to visitation during the night. We bagged other 252 capitula (1,260 florets, N = 3 individuals) from 1800 h to 0700 h, which were exposed to visitors during the day. We conducted tests at the end of flowering (from March 24 to April 10, 2008). Pollination efficiency was calculated as the ratio between the number of fruits formed and the number of bagged florets. We analyzed the data using the Mann-Whitney test (Zar 2010).

RESULTS

Nocturnal visitors totaled 45 species of moths (Lepidoptera, Heterocera) and 120 visits (Tables 1 and 2). Diurnal visitors (104 species) made a total of 1,248 visits (Table 2). Adding night and day visitors (a total of 149 species), lepidopterans were the group with the highest species richness (91.9% of the total species) and the most frequent (90.9% of the total visits).

Nocturnal visitors exhibited peak activity between 1900 and 2000 h (Fig. 1A). The most frequent were moths from the subfamilies Ennominae (Geometridae) and Spilomelinae (Crambidae; Table 2), which accounted for 80% of the visits. The most frequent species were *Eusarca lintearia* (27 visits), *Phrygionis polita* (17), *Eusarca* sp. (7), and *Phrygionis paradoxata* (5) from subfamily Ennominae, and *Phostria* sp. (11) and *Lineodes hieroglyphalis* (6) from subfamily Spilomelinae, which together accounted for 60.8% of the visits.

Diurnal visitors displayed two activity peaks: one in the morning from 0900 h to 1000 h, and the other in the afternoon from 1400 h to 1500 h (Fig. 1B). During both peaks, the most frequent visitors were butterflies from the subfamilies Heliconiinae, Ithomiinae, Nymphalinae (Nymphalidae), and Pyrginae (Hesperidae; Table 2), which accounted for 71.4% of the visits. Visitation peaks for these subfamilies corresponded with the peaks for diurnal visitors as a whole (Fig. 1B, C). The most frequent species were *Heliconius sara* (97 visits), *Dryas iulia* (51), *Heliconius ethilla* (23) (Heliconiinae), *Brevioleria aelia plisthenes* (61), *Ithomia drymo* (34), *Hypothyris ninonia* (31), *Episcada carcinia* (25), *Ithomia agnosia* (24) (Ithomiinae), *Tegosa claudina* (105), *Anartia amathea* (89), *Eresia lansdorfi* (31) (Nymphalinae), *Pythonides jovianus* (74), and *Helias phalaenoides* (28) (Pyrginae). *Pompeius pompeius* (71, Hesperiinae) also had a high frequency of visits. These butterflies together made up 59.6% of the diurnal visits.

Table 1 – Nocturnal capitulum visitors to *Grazielia intermedia* in Mata do Paraíso, southeastern Brazil. The list of diurnal capitulum visitors is in Cruz *et al.* (2012) and Valentin-Silva *et al.* (2016a).

Order	Family	Subfamily	Species
Lepidoptera (Heterocera)			Morphospecies 1 Morphospecies 2 Morphospecies 3 Morphospecies 4 Morphospecies 5 Morphospecies 6 Morphospecies 7 Morphospecies 8
	Arctiidae	Arctiinae	*Cosmosoma* sp. *Elysius chimaera* (Druce, 1893) *Eucereon rosa* (Walker, 1854) *Eucereon* sp. *Phaegoptera depicta* (Herrich-Schäffer, [1855])
	Crambidae	Spilomelinae	*Desmia* sp. *Hyalea* sp. *Lineodes hieroglyphalis* Guenée, 1854 *Margaronia australis* Guenée, 1854 *Phostria* sp.
	Geometridae	Ennominae	*Bagodares trilva* (Schaus, 1901) *Eusarca lintearia* (Guenée, [1858]) *Eusarca* sp. *Herbita renipuncta* (Warren, 1895) *Hymenomima* sp. *Ischnopteris miseliata* (Guenée, [1858]) *Ischnopteris* sp. *Macaria crepuscularia* (Warren, 1897) *Macaria regulata* (Fabricius, 1775) *Microgonia rhodaria* Herrich-Schäffer, [1855] *Opisthoxia amphiaria* (Oberthür, 1916) *Paragonia* sp. *Patalene* sp. *Phrygionis paradoxata* (Guenée, 1857) *Phrygionis polita* (Cramer, 1780) *Prochoerodes* sp. *Sericoptera chartaria* (Guenée, [1858]) *Stegotheca* sp.
		Larentiinae	Morphospecies 1
		Sterrhinae	*Semaeopus lunifera* (Warren, 1897) *Semaeopus viridipunctata* (Warren, 1900)
	Noctuidae		Morphospecies 1 Morphospecies 2 Morphospecies 3 Morphospecies 4 Morphospecies 5
		Catocalinae	*Zale* sp.

Table 2 – Number of species and visits to *Grazielia intermedia* by nocturnal and diurnal capitulum visitors in Mata do Paraíso, southeastern Brazil. NI = not identified.

Order	Family	Subfamily	Species	Visits
		Nocturnal visitors		
Lepidoptera	NI		8	8
(Heterocera)	Arctiidae	Arctiinae	5	5
	Crambidae	Spilomelinae	5	20
	Geometridae	Ennominae	18	76
		Larentiinae	1	1
		Sterrhinae	2	3
	Noctuidae	NI	5	6
		Catocalinae	1	1
		Diurnal visitors		
Diptera	Bombyliidae		1	2
	Caliphoridae		1	3
	Sarcophagidae		1	37
	Syrphidae		1	53
Hymenoptera	Apidae	Apinae	3	10
	Halictidae		2	4
	Megachilidae		1	7
	Sphecidae		1	4
	Scoliidae		1	5
Lepidoptera	Arctiidae	NI	1	3
(Heterocera)		Arctiinae	1	4
		Ctenuchinae	1	3
	Notodontidae	Dioptinae	1	1
(Rhopalocera)	Hesperidae	Hesperiinae	10	94
		Pyrginae	22	204
	Nymphalidae	Acraeinae	2	4
		Biblidinae	1	1
		Cyrestinae	1	7
		Danainae	1	14
		Heliconiinae	8	201
		Ithomiinae	16	246
		Nymphalinae	5	240
	Papilionidae	Papilioninae	6	29
	Pieridae	Coliadinae	2	15
		Dismorphiinae	4	25
		Pierinae	2	8
	Riodinidae	Riodininae	8	24

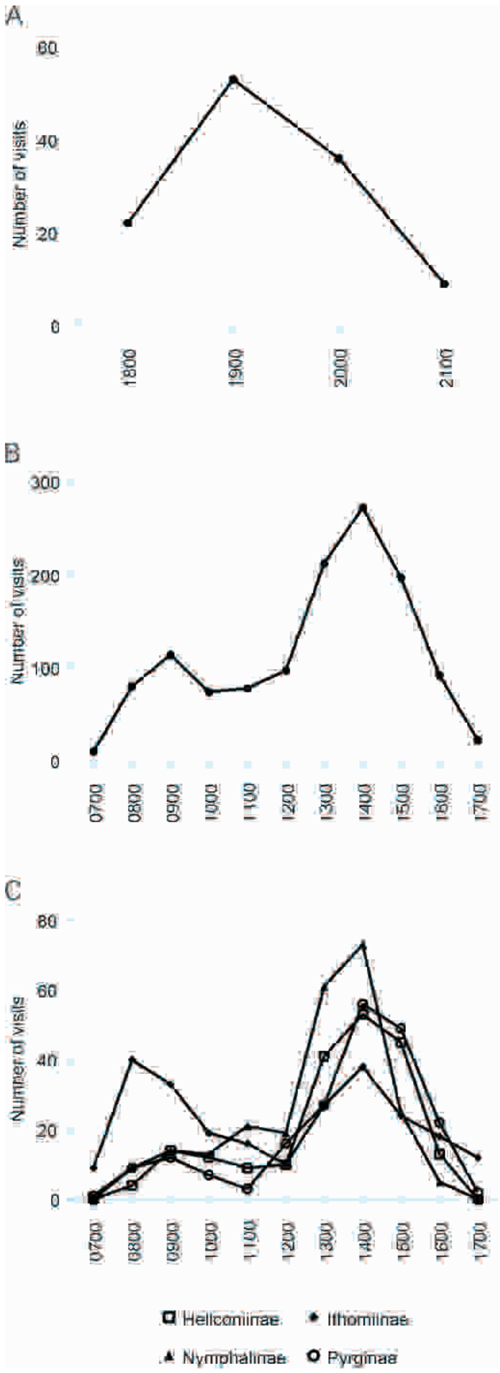

Fig. 1– Frequency of capitulum visitors observed in *Grazielia intermedia* in Mata do Paraíso, southeastern Brazil. A) Nocturnal capitulum visitors. B) Diurnal capitulum visitors. C) Most frequent butterfly subfamilies.

The efficiency test between nocturnal and diurnal visitors revealed that the fruit set was 57.6% ± 20.9 and 32.4% ± 26.2, respectively. Although fruiting was higher in the capitula exposed at night, there was no statistical difference between the treatments.

DISCUSSION

Considering that the florets of *G. intermedia* are diurnal and remain open during the night, the nocturnal moths may have learned to use these floral resources and are considered potential pollinators. This statement is based on the following factors: 1) the florets of *G. intermedia* present residual pollen in the late afternoon of the first day of anthesis (Valentin-Silva *et al.* 2016a); and 2) despite the pollen limitation, 4–6 grains are sufficient to fertilize an ovule, according to Cruden (2000).

Moths of the families Geometridae, Noctuidae, and Pyralidae were recorded in the present study and are commonly observed as nocturnal pollinators in species in different families (Oliveira *et al.* 2004, Oliveira *et al.* 2014, Borges *et al.* 2016). In Asteraceae, these moths were registered as pollinators in *Espeletia grandiflora* Bonpl. (Fagua and Gonzalez 2007) and as capitulum visitors in *Helianthus annuus* L. (Torretta *et al.* 2009) and *Commidendrum robustum* DC. (Paajanen and Cronk 2020).

The foraging time of these insects in the florets of *G. intermedia* was similar to that recorded for *E. grandiflora* (between 1900 and 2200 h; Fagua and Gonzalez 2007). The foraging peak (between 1900 and 2000 h) corresponds to the period with the highest temperatures at night (between 23 and 25 °C; Cruz 2009), which is associated with the thermoregulatory behavior of moths (Borges *et al.* 2016).

The visitation of the nocturnal Arctiidae moths to the capitula of *G. intermedia* may be caused by the demand for pyrrolizidine alkaloids (PAs) in the floral nectar by these insects. These compounds are synthesized mainly by species from the Cardueae, Eupatorieae, and Senecioneae tribes (Calabria *et al.* 2009). PAs are used in the production of male pheromones and protect adult individuals and eggs against predators (Brown Jr. 1984a, b, 1987). Additional studies are required to confirm this supposition.

Among the diurnal capitulum visitors, all were classified as pollinators, except bees that were pollen thieves (Valentin-Silva *et al.* 2016a). The insects recorded herein are commonly observed as capitulum visitors or pollinators in Asteraceae species (Figueroa-Castro and Cano-Santana 2004, Freitas and Sazima 2006, Diniz *et al.* 2010, Savaris *et al.* 2015, Figueroa-Castro *et al.* 2016). Three Nymphalidae subfamilies were the most frequent capitulum visitors, which may also be related to the search for PAs (Brown Jr. 1984a, b, Trigo *et al.* 1996, Trigo 2000), as observed in *Adenostemma brasilianum* (Pers.) Cass. at the same study site (Valentin-Silva *et al.* 2016b).

The peak foraging times of the diurnal pollinators coincided with two important events in the floral biology of *G. intermedia* (Valentin-Silva *et al.* 2016a):1) the period of secondary pollen presentation, which occurs between 0830 and 1000 h in the florets on the first day of anthesis; and 2) the moment of the pistillate phase in which the stigmatic lines are fully exposed (florets on the second day of anthesis). Additionally, these foraging peaks coincided with the times when temperatures were in the range that favored the activity of butterflies, which exhibit thermoregulatory behavior (Callaghan 1978). This added to the ability of these insects to carry pollen grains on their legs (Cruz 2009), favor the pollination of *G. intermedia*. The large number of species and individuals of butterflies that pollinate this plant (see Cruz *et al.* 2012) highlights its ecological importance in maintaining the diurnal lepidopteran community, as suggested by our work.

In species that have daytime anthesis and are also visited during the night, such as *G. intermedia*, diurnal pollinators tend to be more efficient than nocturnal pollinators (Morse and Fritz 1983, Fagua and Gonzalez 2007, Figueroa-Castro and Cano-Santana 2011, Luo *et al.* 2011), which was not observed here. Valentin-Silva *et al.* (2016a) registered facultative apomixis in *G. intermedia* because there was fruiting in emasculated florets and in the individual with male sterility; moreover, some individuals showed high pollen viability, and fruiting was higher in pollination tests with manual pollen deposition. In addition to those previously presented, these conditions reinforce the potential of the two groups of visitors addressed here to pollinate this asteracean.

The set of reproductive mechanisms recorded in *G. intermedia*, including potential nocturnal and diurnal pollinators (present study) and apomixis (Valentin-Silva *et al.* 2016a), is an unreported feature of Asteraceae. Nocturnal pollination, in turn, is rare (see Borges *et al.* 2016) but has been registered in *E. grandiflora* (Fagua and Gonzalez 2007). The tropical representatives of this family are poorly known in relation to their reproductive mechanisms, although studies have shown that they are diverse (see Fonseca *et al.* 2013), a reality corroborated by our results. Mixed reproductive systems (sexual and asexual) may have evolved as a mechanism of reproductive assurance (Goodwillie *et al.* 2005) because numerous Asteraceae species occur in altered habitats (Funk *et al.* 2009), such as forest edges in the present study.

CONCLUSION

The capitula of *G. intermedia* were intensely visited, mainly by lepidopterans during the day and night, both potential pollinators. The pollination efficiencies of the groups were similar. In altered habitats, such as those studied here, the absence of a group of pollinators can be compensated for by the presence of another.

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