



PHENOLOGY, REPRODUCTIVE BIOLOGY AND SPATIAL DISTRIBUTION OF *Chresta scapigera* (LESS.) Gardner (ASTERACEAE)

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Abstract: *Chrestas capigera* (Less.) Gardner is an important medicinal herb which, however, has been poorly studied for its biology and ecology. This study aimed to investigate its phenology, floral biology, reproductive biology (self-pollination tests), spatial distribution and correlations between phenophases and climatic data in two sites (Cerrado *stricto sensu* and *Campo rupestre*) in southern Minas Gerais, Brazil. From August of 2012 to August of 2013, we monitored phenophase occurrence for 70 individuals: emission of new leaves, flowering, production of immature fruits, and mature fruits. Floral anthesis occurred during daytime and remained all day until fruit formation. Peak leaf emergence was observed in April, correlating with minimum monthly temperature and mean monthly precipitation. Flowering and green fruit peaked in May and June, respectively, and correlated negatively with all climatic variables. Mature fruits peaked in June, but did not correlate significantly with any of the climatic variables. However, no difference was found between the two sites regarding the timing of phenophases. The spatial distribution pattern of individuals within sites was random. The self-pollination tests showed that the individuals pollinated and fertilized themselves. Our findings allow us to conclude that the phenology of *C. scapigera* has pronounced phenological seasonality with reproductive peak activities in the drier and colder season, which is congruent with the self-pollination and anemochoric dispersion strategy.

Keywords: adaptation wideness; environmental harshness; phenological strategy; self-compatibility.

INTRODUCTION

Phenological studies (*e.g.* periodicity of events, such as germination, budding of leaves, flowering and fruiting) contribute to the understanding of

species' responses to environmental conditions and their interactions with dispersers and pollinators (Van Schaik 1986, Ghazoul 2005, Hipólito *et al.* 2013). Most of the factors that constrain phenophases of plants are related to

climatic seasonality which manifests in variation of precipitation, day length and temperature (Morellato *et al.* 2000). The phenological timing and floral biology, the reproductive system of the plants and their relationship with dispersers and pollinators (Whellwright 1985, Kuhlmann & Ribeiro 2006) and the capacity of self-pollination are very important determinants of plant strategies, which might be impacted by climatic seasonality and anthropogenic alterations in the environment (Yang & Rudolf 2010, Piao *et al.* 2019). Areas within the Cerrado (Brazilian Savanna) are expected to experience significant changes to temperature and rainfall patterns in the coming century (Marengo *et al.* 2009), potentially leading to dramatic shifts in vegetation dynamics in the regions of this vegetation domain, *e.g.* changes in the seasonality of leaf emission (Bustamante *et al.* 2012). Thus, the increasing impact of global climate change on phenological timing and, consequently, reshaping of community structures (Yang & Rudolf 2010), especially from the 1900s to the present, has stimulated a rapid development and employment of phenology studies (Piao *et al.* 2019).

The high species richness of plants observed in the tropics is largely attributable to the wide range of environmental variation within distinct regions (Townsend *et al.* 2008) which facilitates the assembly of a high diversity of phenological and reproductive strategies (Bawa *et al.* 1985). Cerrado is among the most widespread of vegetation types in Brazil and is the largest Neotropical Savanna. The biome is characterized by high environmental and spatial heterogeneity, which influences species' floral morphology, reproductive strategy and phenology (Gottsberger & Silberbauer-Gottsberger 2006). In the open physiognomies of Cerrado *stricto sensu*, environmental factors such as frequent fires and low water availability, soils with low fertility and high aluminum concentrations and seasonal climate, may limit the phenological cycle of many species (Goodland & Pollard 1973, Batalha & Martins 2004). Environmental constraints such as these impact the pollination strategy of a species, and consequently its typical reproductive cycles, ultimately determining the spatial distribution of its individuals (Pellissier *et al.* 2010).

The Campos rupestres (Rock outcrops;

Rupestrian grasslands) (Silveira *et al.* 2016), a vegetation type associated with Cerrado and considered to host a number of endemic and threatened species, constitute particularly harsh environmental conditions for plants, such as shallow soils confined to cracks between rocks, higher exposure to sunlight, larger variations in temperature during the day-time, limited water storage and higher soil drought during the dry season (Oliveira-Filho & Ratter 2002). Among these conditions, the particular quality and quantity of light radiation that this habitat endures has been found to influence seed germination and leaf flushing (Garcia *et al.* 2007). Besides these factors, the more fragmented nature of the landscape of the Campos rupestres critically influences pollen distribution and plant-pollinator interactions, which in turn regulates the spatial distribution of plant populations (Pellissier *et al.* 2010, Diniz *et al.* 2010b).

Around 2000 species from the Asteraceae family are known to occur in Brazil, of which 65% are endemic (The Brazil Group Flora 2015). The herbs of this family, are among the most abundant plants found in open Cerrado physiognomies and Campos rupestres (Oliveira-Filho & Ratter 2002). Many of its species present brightly colored flowers with diurnal anthesis, as a response to the activity of pollinators, such as bees and beetles, during the day (Willmer 2011). Nevertheless, self-compatibility, *i.e.* the capacity for autonomous self-pollination and fertilization, is common in this family (Cerrana 2004, Grombone-Guaratini, Solferini & Semir 2004) and it represents a reproductive strategy (*e.g.* apomixis and cleistogamy) commonly employed in unfavorable environmental conditions, *e.g.* in fragmented or opened-field landscape, where contact with pollinators may be hampered (Hipólito *et al.* 2013). Moreover, Asteraceae is the family which most commonly deploys anemochoric strategy of seed dispersal in the Cerrado (Kuhlmann & Ribeiro 2006). Thus, such self-pollination and anemochoric strategies exert a crucial influence on the spatial dispersion of the species by enabling expansive distribution, despite the adverse environmental conditions of the ecosystems in which they occur (Gottsberger & Silberbauer-Gottsberger 1983, Kuhlmann & Ribeiro 2016).

Chresta is a small genus of Asteraceae from the Vernoniae tribe, of which most species are endemic to Brazil (Siniscalchi *et al.* 2016). In the Brazilian territory this genus accounts for 14 species, encompassing herbs, subshrubs and shrubs, and is distributed throughout the Northeast, Center-West and Southeast regions, where it is mostly restricted to the Caatinga and Cerrado, but does also occur in the Atlantic Forest ecosystem (Roque *et al.* 2008, Keeley *et al.* 2009, Moreira & Teles 2014). Of these species, 8 are restricted to the Cerrado and its associated phytophysiognomies, e.g. Campo rupestre (Siniscalchi *et al.* 2016). *Chresta scapigera* (Less.) Gardner is one of the more widely distributed species of this genus in Cerrado, and has been recorded in the States of Goiás, Minas Gerais and São Paulo (Moreira & Teles 2014, Siniscalchi *et al.* 2016), yet there are no studies concerning its population ecology, phenology and reproductive strategies (e.g. self or cross-pollination). Furthermore, *C. scapigera* has medicinal potential due the efficiency of isolated chemical compounds and crude extractions from its roots, stems, leaves and inflorescences, in reducing the viability of the protozoans which cause Leishmaniasis (*Leishmania spp*) and Trypanosomiasis (*Trypanosoma cruzi* Chagas) (Schinor *et al.* 2007).

In accordance with the aforementioned, the investigation of the phenological and reproductive strategies of *C. scapigera* is essential to enable successful modelling of changing vegetation patterns, particularly in light of current predictions of rapid climatic change, and to inform appropriate conservation practices. Thus, this study sought to investigate the phenology and floral biology, relationships of phenophases with climate, reproductive biology and spatial distribution of *C. scapigera*, in two sites (Cerrado *stricto sensu* and Campo rupestre) in the Southeast region of Brazil. In particular, we intended to answer the following questions: a) are there seasonal patterns or peak activities for the phenophases of *C. scapigera*? b) is the phenology of *C. scapigera* correlated with the local climatic conditions? c) are the individuals of *C. scapigera* capable of self-pollination? d) is there a specific pattern of distribution of individuals of this species across the study sites?

MATERIAL AND METHODS

Study site

The study sites were both located in the Unilavras Boqueirão Natural Reserve (21°20'47" S, 44°59'27" W), property of the University Center of Lavras (Unilavras), in the municipality of Ingaí, South Minas Gerais, Brazil. The local altitude ranges between 1 100 and 1 250 m a.s.l. and the climate in the region is classified as Köppen's Cwb type (mesothermic with mild, rainy summers and dry winters) with annual precipitation of 1 411 mm, average annual temperature of 19.3 °C, driest and coldest period between May and August wettest and warmest period is between October and March (Diniz *et al.* 2010a). The most common soil types in the study area and its surroundings encompass Litholic, Cambisol, Red-Yellow Podzolic, Red-Yellow Latosol, Hydromorphic and Alluvial Soils. Most of the vegetation in the reserve is composed of Cerrado (Brazilian savanna) phytophysiognomies, such as the *stricto sensu*, patches of Gallery and Semideciduous Forest and, in the elevated areas, Campos rupestres (Queiroz *et al.* 1980).

Climatic data

The climatic variables (Figure S1 in supplementary material) regarding temperature (minimum, maximum and mean) and precipitation per month were obtained from the database WorldClim 1.4 (Hijmans *et al.* 2005) with a spatial resolution of 30 arc sec (~ 1 km). To extract the climatic data from WorldClim to coordinates of the study site, we used the extension Spatial Analyst from the ArcToolbox of ArcGis 10.6.1.

Phenology and floral biology monitoring

From August of 2012 to August of 2013, 70 randomly selected individuals from two populations (35 located in the *Cerrado stricto sensu* and the other 35 in the *Campo rupestre*) were tagged with red plastic tape and monitored for the occurrence of their phenophases: emergence of new leaves, flowering, production of immature fruits, and mature fruits. Flowering phenology is considered to comprise floral bud initiation and development, blooming (anthesis), and floral persistence, whereas fruiting

encompasses the immature fruit formation following the completion of anthesis, and the growth and ripening of the fruit (Rathcke & Lacey 1985). The emergence of new leaves was evaluated by counting the number of new leaves accrued by each of the monitored individuals at each visit. All individuals were monitored weekly during the flowering period and biweekly otherwise, which allowed us to count the number of individuals in each phenophase at each monitoring time point. Among the 70 monitored individuals, we randomly selected five individuals in pre-anthesis in each site (Cerrado and Campo rupestre) to be monitored daily during the flowering period, until the anthesis was completed and fruits formed. Thus, during flowering, for each inflorescence we analyzed duration of pre-anthesis, anthesis time, end of anthesis and opening of flowers. Then, in the same day that anthesis was completed, we monitored and counted the immature fruits formed. The timing and quantity of these fruits becoming mature following the completion of anthesis was registered in the monitoring visits for the next three to four weeks.

Reproductive Biology

In order to study the reproductive system of *C. scapigera*, we evaluated its inflorescence morphology, autonomous self-pollination and germination. The flower morphology was observed and recorded by taking photos. To investigate pollination and fertilization, ten individuals were randomly selected within each site, totaling 20 individuals. A treatment of self-pollination was carried out by packing their inflorescences in voile and closed with nylon, at pre-anthesis. These inflorescences remained in the voile bags for a week (Darrault & Schlindwein 2005). After one week the voile bags were opened and we counted the number of fruits formed by inflorescences.

Spatial distribution

The intraspecific distribution of *C. scapigera* was investigated in twelve subplots measuring 20 x 20 meters in Cerrado and another twelve of same size in Campo rupestre, where individuals were numbered with aluminum tags, counted and their x and y coordinates within the subplots recorded. With this data we were able to compute the Morisita Dispersion Index (details in Data Analyses section).

Data analysis

In order to test for seasonality of the phenophases, we calculated descriptive parameters and tested hypothesis based on circular statistics. First, mean date (concentration in a given period of the year) of each phenophase was converted to its corresponding mean angle α (Morellato *et al.* 2000, Zar 2010). Then, each month was converted to angles, ranging from 0 ° (January) to 360 ° (December) at intervals of 30 ° (Zar 2010), which represents the frequency of individuals within the angles of each month. All the above procedures were calculated using three datasets (Cerrado, Campo rupestre and the whole sample, which included the data from both sites) in order to test for the consistency of seasonality of the phenophases.

To test for the significance of the mean angle (*i.e.* seasonality), *i.e.* non-random distribution of the phenophases throughout the year, we applied the Rayleigh test following Morellato *et al.* (2000). The significance of the mean angle (length of mean vector r) represents the central tendency of the data, whereas phenological activity is shown by the median angle and the peak activity by the modal angle presented graphically (Morellato *et al.* 2000, 2010). The phenological patterns of the Cerrado and Campo rupestre sites were compared by performing a two-sample Watson-Williams test (F), which analyses whether the mean angles of each phenological variable exhibited similar seasonality in both ecosystems (Morellato *et al.* 2010). Calculations of all above mentioned circular parameters, tests and the creation of circular histograms were conducted using the software Oriana 4.02 (Kovach Computing Services 2011).

We computed Spearman's rank correlation coefficients (ρ) between the number of individuals in each phenophase per month and the monthly climatic variables using the function "chart.Correlation" from the package PerformanceAnalytics (Peterson & Carl 2019) in R software version 3.5.0. in order to analyze relationships between each phenophase and the climatic variables: minimum, maximum and mean of monthly temperature and mean of monthly precipitation.

The intraspecific dispersion of the individuals was analyzed by calculating the Morisita Dispersion Index - Imor using the function `dispindmorisita` of the package `vegan` (Oksanen

et al. 2017) in R version 3.5.0. When interpreting this index, values less than one represent random dispersion, values close to one indicate uniform dispersion and higher than one clumped dispersion (Horne & Schneider 1995).

RESULTS

We observed a long period of leaf emergence of *C. scapigera* between November 2012 and August 2013. Flowering in the inflorescences occurred between April and August and emission of floral buds from the beginning of March until July. New immature fruits were also produced between April and August which took around three weeks to mature, while mature fruits were observed between March and August.

Floral anthesis took place during the daytime with synchronic opening of the flowers in the inflorescences of the individuals from 9 am to 1 pm. Inflorescences in pre-anthesis (Figure S2a in supplementary material) took two and a half hours to achieve total anthesis (Figure S2b). The opening of the petals (Figure S2c) began first and was completed after the stamens and lastly the stigma was visualized (Figure S2d). Anthesis was then completed and remained until formation of the fruits commenced in the same day.

The individuals of *C. scapigera* had an average height of 1.2 meters (Figure S3 in supplementary material). The inflorescences (Figure S4 in supplementary material) produced monoclone (Figures S5a in supplementary material) and diclamid flowers with purple petals (Figure S5b). Sepals are modified into a pappus located above the seed to assist its dispersal (Figure S5c). The flower produces anthers with obtuse apical appendices (Figure S5d), an ovary with filiform stylus surrounded by hairs below the bifurcation point (Figure S5e) and small achenes (Figure S5f).

All four phenophases (new leaf, flowering, green fruit and mature fruit) were not uniformly distributed throughout the year, as confirmed by the Rayleigh tests (Table S1 in supplementary material). The highest degree of seasonality was observed for immature fruits and flowering in all samples (Cerrado, Campo rupestre and whole sample) according to their high r values (> 0.70 ; Table S1). However, flowering was more reliable when the whole sample (total of 46 flowering

individuals; Table S1) was considered, due to its narrower confidence interval for the mean angle, compared with the wider confidence interval in Cerrado. It is possible that Cerrado was slightly less reliable due the lower number of observations recorded (21 flowering individuals) and consequently, the lower concentration around the mean vector r . Figure 1 (a-d) also shows the concentration of the phenophases.

As indicated by the median angle, the center of emergence of new leaves was in April in Cerrado, Campo rupestre and whole sample (Table S1), but the highest peak activity was in May, as shown by the modal angle (Figure 1a). Flowering activity was centered and peaked in May (Table S1, Figure 1b), while the production of immature fruits occurred in June (Table S1, Figure 1c) in all three samples. Mature fruits were centered in June in all samples (Figure 1d). The timing of all phenophases did not differ between Cerrado and Campo rupestre, as indicated by the Watson-Williams tests (Table S2 in supplementary material). Comparisons within each sample revealed that new leaves and mature fruits did not differ in their mean angles, i.e. they showed similar seasonality (Table S2). The same was found for the comparison between flowering and immature fruits (Table S2). All other pairs of phenological variables showed significant differences in seasonality in all three samples.

New leaves only showed significant positive correlation with minimum monthly temperature and mean monthly precipitation (Table S3 in supplementary material). Flowering and immature fruits were significantly and negatively correlated with all climatic variables, which highlights their concentration in periods of the year with lower precipitation and temperature. Mature fruits did not show any significant relationship with the climatic variables (Table S3).

The self-pollination tests showed that the tested individuals pollinated and fertilized themselves. Each bract formed three fruits and each individual of *C. scapigera* produced 60 fruits on average.

Within the grid of the plots sampled to investigate spatial dispersion, 56 individuals of *C. scapigera* were recorded in Cerrado and 79 in Campo rupestre, totaling 135 individuals. The highest percentage of individuals (19 %), were separated from their nearest conspecifics by between six and eight meters. Individuals of *C.*

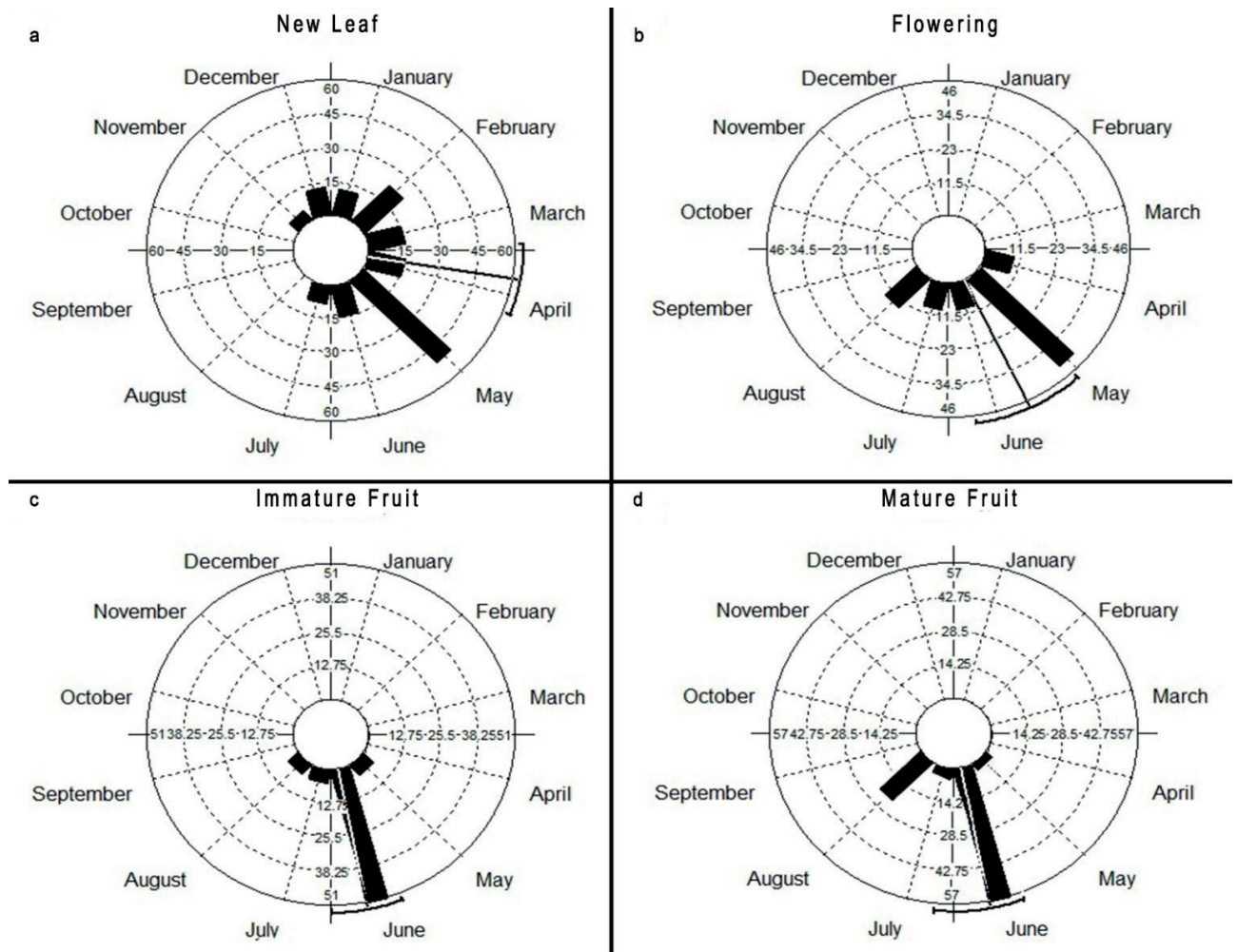


Figure 1. Circular histograms of the average frequencies of individuals per month for each phenological variable of the species *Chresta scapigera* in the whole sample (Cerrado and Campo rupestre; shown in detail in Table S1). **a** peak of new leaves emission, **b** peak of flowering, **c** immature fruits production and **d** peak of mature fruits. The vector line in the circle indicates the mean angle or direction and the sector outside the circle indicates the 95% confidence interval.

scapigera showed a random spatial distribution pattern (Figure S6 in Supplementary material) confirmed by the Morisita Index in Cerrado ($I_{mor} = 0.05$, $p = 0.109$) and Campo rupestre ($I_{mor} = 0.66$, $p = 0.230$).

DISCUSSION

Our findings confirmed seasonal patterns and peak of activities for all studied phenophases of *C. chresta* in both sites (Cerrado and Campo rupestre), as well as a marked correlation of phenology with local climatic conditions. We also confirmed that this species retains the capacity to utilize autonomous and spontaneous self-pollination as the reproductive strategy of the species. However, we found neither a differentiation in the timing of the phenophases

between the two sites, nor a specific spatial distribution pattern. These trends partially concur with common patterns of the ecology and phenological activities of several other Asteraceae species in Cerrado's ecosystem (Gottsberger & Silberbauer-Gottsberger 1986, 2006, Silberbauer-Gottsberger & Gottsberger 1988, Oliveira & Gibbs 2002).

The peak in production of new leaves significantly correlated with the drier and colder season in the Southeast of Brazil, which suggests that *C. scapigera* uses a dual strategy with respect to the emergence of new leaves. Firstly, apparently this species takes advantage of the improved nourishment available during the rainy and warm periods, e.g. longer photoperiod and higher availability of water, minerals and carbon dioxide, to achieve maximum production

of leaves, to enhance its productivity (Chapin III *et al.* 1990, Van Schaik *et al.* 1993). Secondly, this timing allows the species to concentrate the majority of leaf emergence reducing damage by herbivores that are most active during the moist summer (Van Bael & Brawn 2005). Thus, the observed presence of mature leaves in the individuals of *C. scapigera* throughout the drier and colder season with harsher conditions (e.g. strong frosts in Southeast Brazil), as well as all year around, might contribute to optimizing photosynthesis, while leaf emergence commences shortly after the first summer rains, as is the case for many herbs and woody species in Cerrado and its associated vegetation (e.g. Campo rupestre) (Batalha & Martins 2004, Morellato 2013).

The pattern we found, whereby flowering and immature fruit production correlated with climatic conditions of the drier and colder season, might represent an anemochoric strategy for fruit and seed dispersion, which is typically associated with open habitats, e.g. field physiognomies of Cerrado (Oliveira & Moreira 1992). In regions of seasonal climate, the reproductive phenophase of species such as *C. scapigera*, attends to coincide with dry periods (Batalha & Martins 2004, Gottsberger & Silberbauer-Gottsberger 2006). The colder and drier season offers advantages for these phenophases peaking at this time, such as, higher intensity of pollination and stronger winds, which aids anemochoric dispersion of both pollen and seeds (Almeida-Cortez 2004, Bendix *et al.* 2006). These processes would be limited in periods of intense and prolonged rains (Van Schaik *et al.* 1993, Kuhlmann & Ribeiro 2006), while in drier and windier periods seeds can be dispersed over considerably longer distances and, subsequently, facilitating germination in the beginning of the rainy season and increasing recruitment success of seedlings (Oliveira 2008).

Among the dispersal strategies that can be found within the Asteraceae family in Brazil, Kuhlmann & Ribeiro (2016) counted 290 species within 83 genera in Cerrado that utilized anemochoric dispersion. Moreira and Teles (2014) found that some species of the tribe Vernoniaceae (e.g. from the genus *Chresta*), which occur in Cerrado sites in Serra Dourada, Goiás state, also concentrate flowering in the driest and windiest season. In another study, Santos *et*

al. (2018) also observed flowering during the dry season in Cerrado for the species *Chresta exsucca* D.C. in Goiás. Thus, the observed concentration of flowering and immature fruit production of *C. scapigera* in the dry season might be related to an anemochoric strategy to increase its pollination and dispersal success, though further study is necessary to confirm this assumption.

Another finding that suggests that *C. scapigera* utilizes an anemochoric strategy, is the observed capability of self-pollination. Despite self-compatibility being considered to be restricted to relatively few species in Cerrado (Oliveira & Gibbs 2002), autonomous and spontaneous self-pollination is found to be common in many herbaceous species of distinct families, including Asteraceae (Saraiva *et al.* 1996; Oliveira & Gibbs 2002). Furthermore, even though self-incompatibility is frequent in Asteraceae, spontaneous self-pollination is also common among the species of this family (Richards 1986, Werpachowski *et al.* 2004). In some species, self-pollination is related to stress caused by harsh environmental conditions, such as those endured in Campos rupestres (Garcia *et al.* 2007), since such conditions limit the availability of pollinators (Girão *et al.* 2007). Thus, by taking advantage of self-pollination, *C. scapigera* reduces its dependence on pollen transfer by pollinators and increases its chance of reproduction, despite being isolated from other individuals, when no suitable pollinator is available (Larson & Barrett 2000, Ghazoul 2005).

Self-pollination combined with an anemochoric dispersion strategy is a plausible explanation for the random spatial distribution we found for the individuals of *C. scapigera*. Although aggregated spatial distribution patterns are more commonly reported in Brazil for species of distinct genera of Asteraceae (e.g. Lima-Ribeiro & Prado 2007, Diniz *et al.* 2010b, Meira-Júnior *et al.* 2017), the non-clumped or random distribution was also observed for some species (e.g. Marques *et al.* 2002, Meira-Júnior *et al.* 2017) including *Vernonia aurea* Mart. ex DC., which also belongs to tribe Vernoniaceae (Lima-Ribeiro 2008). Wind dispersal of the seeds is the most likely explanation for the observed random spatial distribution (Okubo & Levin 1989, Nathan & Muller-Landau 2000). In addition, a non-

clumped distribution of plant individuals is also a possible defense strategy that may also limit damage by herbivores and pathogens (Wright 2002, Schwinning & Kelly 2013). However, various other factors not contemplated in this study, such as resource availability, seed bank formation, microsites, recruitment and competitive strategy might also influence the spatial distribution of a plant species (Chapin III *et al.* 1990, Münzbergová & Herben 2005). To our knowledge, this is the first study investigating spatial distribution patterns of a species of the *Chresta* genus, which emphasizes the necessity for further studies addressing this topic.

This study allowed us to conclude that the phenology of *C. scapigera* has pronounced seasonality and peak activities for all four phenophases (emission of new leaves, flowering, immature fruiting and mature fruiting), mostly concentrated in the drier and colder season. Such peak activities indicate an influence self-pollination and anemochoric dispersion strategies, which in turn plays important role in the spatial distribution pattern of the species.

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REFERENCES

- Almeida-Cortez, J. S. 2004. Dispersão e banco de sementes. In: A. G. Ferreira & F. Borghetti (Eds.), Germinação: do básico ao aplicado. pp. 225–235. Porto Alegre: Artmed.
- Batalha, M. A., & Martins, F. R. 2004. Reproductive phenology of the cerrado plant community in Emas National Park (central Brazil). *Australian Journal of Botany*, 52, 149–161. DOI: 10.1071/BT03098
- Bawa, K. S., Bullock, S. H., Perry, D. R., Coville, R. E., & Grayum, M. H. 1985. Reproductive biology of tropical lowland rain forest trees. II. Pollination systems. *American Journal of Botany*, 72(3), 346–356. DOI: 10.1002/j.1537-2197.1985.tb05358.x
- Bendix, J., Homeier, J., Cueva Ortiz, E., Emck, P., Breckle, S. W., Richter, M., & Beck, E. 2006. Seasonality of weather and tree phenology in a tropical evergreen mountain rain forest. *International Journal of Biometeorology*, 50(6), 370–384. DOI: 10.1007/s00484-006-0029-8
- Bustamante, M. M. C., Nardoto, G. B.; Pinto, A. S., Resende, J. C. F., Takahashi, F. S. C., & Vieira, L. C. G. 2012. Potential impacts of climate change on biogeochemical functioning of Cerrado ecosystems. *Brazilian Journal of Biology*, 72, 655–671. DOI: 10.1590/S1519-69842012000400005
- Cerrana, M. M. 2004. Flower morphology and pollination in *Mikania* (Asteraceae). *Flora*, 199(2), 168–177. DOI: 10.1078/0367-2530-00145
- Chapin III, F. S., Schulze, E. D., & Mooney, H. A. 1990. The Ecology and economics of storage in plants. *Annual Review of Ecology and Systematics*, 21, 423–447. DOI: 10.1146/annurev.es.21.110190.002231
- Darrault, R. O., & Schindwein, C. 2005. Limited fruit production in *Hancornia speciosa* (Apocynaceae) and pollination by nocturnal and diurnal insects. *Biotropica*, 37(3), 381–388. DOI: 10.1111/j.1744-7429.2005.00050.x
- Diniz, E. S., Pavanelli, A. P., & Soares Júnior, F. S. 2010a. A análise da estrutura de uma população de *Lychnophora pinaster* Mart. de um campo rupestre do sul de Minas Gerais, Brasil, por meio da estatística multivariada. *Ecología Aplicada*, 9(2), 141–149.
- Diniz, E. S., Pavanelli, A. P., & Soares Júnior, F. S. 2010b. Estrutura populacional *Lychnophora pinaster* Mart. em um Campo rupestre no sul de Minas Gerais, Brasil. *Pesquisas, Botânica*, 61, 191–204.
- Garcia, Q. S., Jacobi, C. M., & Ribeiro, B. A. 2007. Resposta germinativa de duas espécies de *Vellozia* (Velloziaceae) dos campos rupestres de Minas Gerais, Brasil. *Acta Botanica Brasilica*, 21(2), 451–456. DOI: 10.1590/S0102-33062007000200018
- Ghazoul, J. 2005. Implications of plant spatial distribution for pollination and seed production. In: D. Burslem, M. Pinard, & S. Hartly (Eds.), *Biotic Interactions in the Tropics:*

- Their Role in the Maintenance of Species Diversity. pp. 241–266. Cambridge: Cambridge University Press.
- Girão, L. C., Tabarelli, M., Lopes, A. V., & Bruna, E. M. 2007. Changes in tree reproductive traits reduce functional diversity in a fragmented Atlantic forest landscape. *PLoS ONE*, 2(9), e908. DOI: 10.1371/journal.pone.0000908
- Goodland, R., & Pollard, R. 1973. The Brazilian Cerrado vegetation: a fertility gradient. *Journal of Ecology*, 61(1), 219–224.
- Gottsberger, G., & Silberbauer-Gottsberger, I. 1983. Dispersal and distribution in the cerrado vegetation of Brazil. *Sonderb. Naturwiss. Ver. Hamburg*, 7, 315–352.
- Gottsberger, G., & Silberbauer-Gottsberger, I. 2006. Life in the Cerrado, Volume 1: Origin, Structure, Dynamics and Plant Use: A South American Tropical Seasonal Ecosystem. Origin, Structure, Dynamics and Plant Use, vol. 1. Ulm: RetaVerlag: p. 277.
- Hipólito, J., Roque, N., Galleto, L., Viana, B. F., & Kevan, P. G. 2013. The pollination biology of *Pseudostiffia kingii* H. Rob. (Asteraceae), a rare endemic Brazilian species with uniflorous capitula. *Brazilian Journal of Botany*, 36(3), 247–254. DOI: 10.1007/s40415-013-0023-4
- Keeley, S.C., & Robinson, H. 2009. Vernonieae. In: V.A. Funk (Eds.), *Systematics, Evolution, and Biogeography of Compositae*, pp. 439–469. Austria: IAPT.
- Köppen, W. 1931. *Climatologia. Fundo de Cultura Econômica*.
- Kovach Computing Services. 2011. Oriana—circular statistics for Windows, ver. 4.02. Pentraeth, Wales-UK.
- Kuhlmann, M., & Ribeiro, J. F. 2006. Evolution of seed dispersal in the Cerrado biome: ecological and phylogenetic considerations. *Acta Botanica Brasilica*, 30(2), 271–282. DOI: 10.1590/0102-33062015abb0331
- Larson, B. M. H., & Barrett, S. C. H. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, 69(4), 503–520. DOI: 10.1006/bjpl.1999.0372
- Lima-Ribeiro, M. S., & Prado, E. C. 2007. Distribuição espacial de uma população de *Vernonia aurea* Mart. ex DC. (Asteraceae) em um fragmento de cerrado no município de Caiapônia, GO, Brasil. *Bioscience Journal*, 23(3), 81–89.
- Lima-Ribeiro, M. S. 2008. Efeitos de borda sobre a vegetação e estruturação populacional em fragmentos de Cerradão no Sudoeste Goiano, Brasil. *Acta Botanica Brasilica*, 22(2), 535–545. DOI: 10.1590/S0102-33062008000200020
- Marengo, J. A., Ambrizzi, T., Rocha, R. P., Alves, L. M., Cuadra, S. V., Valverde, M. C., Torres, R. R., & Santos, D. C., Ferraz, S. E. T. 2010. Future change of climate in South America in the late twenty-first century: Intercomparison of scenarios from three regional climate models. *Climate Dynamics*, 35, 1089–1113. DOI: 10.1007/s00382-009-0721-6
- Marques, A. R., Fernandes, G. W., Reis, I. A., & Assunção, R. M. 2002. Distribution of Adult Male and Female *Baccharis concinna* (Asteraceae) in the Rupestrian Fields of Serra Do Cipó, Brazil. *Plant Biology*, 4(1), 94–103. DOI: 10.1055/s-2002-20441
- Meira-Júnior, M. S., Mota, S. L. L., Machado, E. L. M., Pereira, I. M. 2017. Distribuição espacial de *Eremanthus incanus* (Less.) Less. (Asteraceae) em duas áreas com diferentes níveis de conservação. *Revista Brasileira de Biociências*, 15(1), 27–31.
- Moreira, G.L., & Teles, A.M. 2014. Atribo Vernonieae Cass. (Asteraceae) na Serra Dourada, Goiás, Brasil. *Iheringia*, 69(2), 357–385.
- Morellato, L. P. C. 2013. Fenologia reprodutiva e vegetativa de arbustos endêmicos de campo rupestre na Serra do Cipó, Sudeste do Brasil. *Rodriguésia*, 64(4), 817–828. DOI: 10.1590/S2175-78602013000400011
- Morellato, L. P. C., Alberti, L. F., & Hudson, I. L. 2010. Applications of Circular Statistics in Plant Phenology: a Case Studies Approach. In: I. L. Hudson & M. R. Keatley (Eds.), *Phenological Research: Methods for Environmental and Climate Change Analysis*. pp. 339–359. 1 ed. Dordrecht: Springer Netherlands.
- Morellato, L. P. C., Talora, D., Takahasi, A., Bencke, C. C., Romer, E. C., & Zipparro, V. B. 2000. Phenology of Atlantic Rain Forest Trees: A Comparative Study. *Biotropica*, 32(4b), 811–823. DOI: 10.1111/j.1744-7429.2000.tb00620.x
- Münzbergová, Z., & Herben, T. 2005. Seed, dispersal, microsite, habitat and recruitment limitation: Identification of terms and concepts

- in studies of limitations. *Oecologia*, 145(1), 1–8. DOI: 10.1007/s00442-005-0052-1
- Nathan, R., & Muller-Landau, H. C. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution*, 15(7), 278–285. DOI: 10.1016/S0169-5347(00)01874-7
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. 2017. *vegan: Community Ecology Package*. Version R package version 2.4-3.
- Okubo, A., & Levin, S. A. 1989. A theoretical framework for data analysis of wind dispersal of seeds and pollen. *Ecology*, 70(2), 329–338. DOI: 10.2307/1937537
- Oliveira-Filho, A. T., & Ratter, J. A. 2002. Vegetation Physiognomies and Woody Flora of the. In: P. S. Oliveira & R. J. Marquis (Eds.), *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna*. pp. 91–120. New York: Columbia University Press.
- Oliveira P. E., & Moreira, A. G. 1992. Anemocoria em espécies de cerrado e mata de galeria de Brasília- DF. *Revista Brasileira de Botânica*, 15(2), 163–174.
- Oliveira, P. E., & Gibbs, P.E. 2002. Pollination and Reproductive Biology in Cerrado Plant Communities. In: P. E. Oliveira & R. J. Marques (Eds.), *The Cerrados of Brazil – Ecology and natural history of a Neotropical Savanna*. pp. 329–347. New York: Columbia University Press.
- Oliveira, P.E. 2008. Fenologia e biologia reprodutiva das espécies de cerrado. In: S. M. Sano, S. P. Almeida, & J. F. Ribeiro (Eds.), *Cerrado: ecologia e flora*. pp. 273–290. Planaltina: Embrapa.
- Pellissier, L., Pottier, J., Vittoz, P., Dubuis, A., & Guisan, A. 2010. Spatial pattern of floral morphology: possible insight into the effects of pollinators on plant distributions. *Oikos*, 119(11), 1805–1813. DOI: 10.1111/j.1600-0706.2010.18560.x
- Peterson, B. G., & Carl, P. 2019. *PerformanceAnalytics: Econometric tools for performance and risk analysis*. Version R package version 1.5.3.
- Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., Liu, L., Lian, X., Shen, M., & Zhu, X. 2019. Plant phenology and global climate change: Current progresses and challenges. *Global Change Biology*, 25(3), 1922–1940. DOI: 10.1111/gcb.14619
- Queiroz, R., Souza, A. G., Santana, P., Antunes, F. Z., & Fontes, M. 1980. *Zoneamento agroclimático do Estado de Minas Gerais*. Belo Horizonte: Secretaria da Agricultura.
- Rathcke, B., & Lacey, P. 1985. Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, 16, 179–214. DOI: 10.1146/annurev.es.16.110185.001143
- Richards, A. J. 1986. *Plant breeding systems*. London: George Allen and Unwin.
- Roque, N., Gonçalves, J.M., & Dematteis, M. 2008. A new species of the Brazilian genus *Chresta* (Asteraceae, Vernonieae) from Bahia. *Botanical Journal of the Linnean Society*, 157(3), 587–590. DOI: 10.1111/j.1095-8339.2008.00834.x
- Santos, C. S., Abraão, C. F., & Moraes, M. G. 2018. Fructan dynamics in the underground organs of *Chresta exsucca* (Asteraceae), a dry season flowering species. *Acta Botanica Brasilica*, 32(1), 70–79. DOI: 10.1590/0102-33062017abb0214
- Saraiva, L., Cesar, O., & Monteiro, R. 1996. Breeding systems of shrubs and trees of a Brazilian savanna. *Arquivos de Biologia E Tecnologia*, 39, 751–763.
- Schinor, E. S., Salvador, M. J., Pral, E. M. F., Alfieri, S. C., Albuquerque, S., & Dias, D. A. 2007. Effect of extracts and isolated compounds from *Chresta scapigera* on viability of *Leishmania amazonensis* and *Trypanosoma cruzi*. *Revista Brasileira de Ciências Farmacêuticas*, 43(2), 295–300. DOI: 10.1590/S1516-93322007000200016
- Schwinning, S., & Kelly, C. K. 2013. Plant competition, temporal niches and implications for productivity and adaptability to climate change in water-limited environments. *Functional Ecology*, 27(4), 886–897. DOI: 10.1111/1365-2435.12115
- Silberbauer-Gottsberger, I., & Gottsberger, G. 1988. A polinização de plantas do cerrado. *Revista Brasileira de Biologia*, 48, 651–663.
- Siniscalchi, C. M., C., Loeuille, B. F. P., & Pirani, J. R. 2016. A new species of *Chresta* (Vernonieae, Asteraceae) endemic to the Mata Atlântica Domain, Brazil. *Phytotaxa*, 244(1), 79–88. DOI: 10.11646/phytotaxa.244.1.6
- Siniscalchi, C. M., Souza-Souza, R. M. B., Loeuille, B., Pirani, J. R., & Gonçalves-Esteves, V. 2017.

- The systematic value of pollen morphology in *Chresta* Vell. ex DC. (Vernonieae, Asteraceae). *Review of Palaeobotany and Palynology*, 244, 182–191. DOI: 10.1016/j.revpalbo.2017.05.003
- Silveira, F. A. O., Negreiros, D., Barbosa, N. P. U., Buisson, E., Carmo, F. F., Carstensen, D. W., Conceição, A. A., Cornelissen, T. G., Echternacht, L., Fernandes, G. W., Garcia, Q. S., Guerra, T. J., Jacobi, C. M., Lemos-Filho, J. P., Le Stradic, S., Morellato, L. P. C., Neves, F. S., Oliveira, R. S., Schaefer, C. E., Viana, P. L., & Lambers, H. 2016. Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant and Soil*, 403, 129–152. DOI: 10.1007/s11104-015-2637-8
- The Brazil Group Flora. 2015. Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguésia*, 66(4), 1085–1113. DOI: 10.1590/2175-7860201566411
- Townsend, A. R., Asner, G. P., & Cleveland, C. C. 2008. The biogeochemical heterogeneity of tropical forests. *Trends in Ecology and Evolution*, 23(8), 424–431. DOI: 10.1016/j.tree.2008.04.009
- Van Bael, S. A., & Brawn, J. D. 2005. The direct and indirect effects of insectivory by birds in two contrasting Neotropical forests. *Oecologia*, 143(1), 106–116. DOI: 10.1007/s00442-004-1774-1
- Van Schaik, C. P. 1986. Phenological changes in a Sumatran rain forest. *Journal of Tropical Ecology*, 2(4), 327–347. DOI: 10.1017/S0266467400000973
- Van Schaik, C. P., Terborgh, J. W., & Wright, S. J. 1993. The phenology of tropical forests: adaptive significance and consequences for primary consumers. *Annual Review of Ecology and Systematics*, 24, 353–377. DOI: 10.1146/annurev.es.24.110193.002033
- Werpachowski, J. S., Varassin, I. G., & Goldenberg, R. 2004. Ocorrência de apomixia e partenocarpia em algumas espécies subtropicais de Asteraceae. *Brazilian Journal of Botany*, 27(3), 607–613. DOI: 10.1590/S0100-84042004000300020
- Whellwright, N. T. 1985. Competition for dispersers, and the time of flowering and fruiting in a guild of tropical trees. *Oikos*, 44(3), 465–477. DOI: 10.2307/3565788
- Willmer, P. 2011. Syndromes and webs: specialists and generalists. In: P. Willmer (Ed.), *Pollination and Floral Ecology*. pp. 434–480. Princeton: Princeton University Press.
- Wright, S. J. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia*, 130(1), 1–14. DOI: 10.1007/s004420100809
- Yang, L. H., & Rudolf, V. 2010. Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecology Letters*, 13, 1–10. DOI: 10.1111/j.1461-0248.2009.01402.x
- Zar, J. H. 2010. *Biostatistical analysis*. 5 ed. New Jersey: Prentice-Hall: p. 944.
- Supplementary Material:**
- Figure S1.** Monthly climatic data for the location of the study site and surrounding region in the Municipality of Lavras, Minas Gerais State, Southeast of Brazil. Tmean, Tmax and Tmin are respectively mean, maximum and minimum monthly temperature; P mean means monthly precipitation. Figure created with R version 3.5.0.
- Figure S2.** Inflorescence of *Chresta scapigera* in pre and total anthesis. **a** stamens in pre-anthesis, **b** stamens in total anthesis, **c** Total opening of the petals, **d** female reproductive system.
- Figure S3.** Height (cm) of the individuals of *Chresta scapigera* and the percentage of them percentage per height classes.
- Figure S4.** Inflorescence of *Chresta scapigera*. **a**) entire peduncle of the individual with the inflorescence at the top, **b**) inflorescence with all flowers opened and fruits formed, **c**) inflorescences with flowers partially opened.
- Figure S5.** Inflorescence of *Chresta scapigera*. **a** arrow 1 indicating the stigma and arrow 2 the stamens, **b** petals, **c** sepals modified into pappus, **d** anthers of apical appendix, **e** filiform stylus surrounded by hairs, **f** achenes.
- Figure S6.** Spatial distribution of the individuals of *Chresta scapigera* based on their distances in meters in the x and y coordinates in five amongst the sampled subplots in Cerrado (**a**) and five *Campo rupestre* (**b**) taken as example. In both **a** and **b** the letter “S” followed by number means the number of the subplot.
- Table S1.** Circular statistics summarizing the vegetative and reproductive phenology of

individuals of *Chresta scapigera* growing in the Cerrado (C), Campo rupestre (CR) and both (Whole sample) in the South of Minas Gerais State, Southeast Brazil. CR - C: Cerrado and Campo rupestre, respectively. All mean angles (α) are significant according to the Rayleigh test ($P < 0.05$). The mean vector value (mean angle α) is the average angle of each phenophase for each site (Cerrado and Campo rupestre) and for the whole sample. The length of the mean vector (r) represents the relative synchrony of each phenophase, that is, if there is unimodality indicating seasonality. The Watson-Williams test compared the mean vector values between the two sites.

Table S2. Comparisons among the four phenophases within each sampling (Cerrado, *Campo rupestre* and Whole sample) at the South of Minas Gerais State, Southeast of Brazil. F is the test statistic of the two-sample Watson-Williams test and P its significance level.

Table S3. Correlation between the phenophases from the whole sample and climatic variables. ρ is Spearman's coefficient and p the significance of the correlation. T_{mean} , T_{max} and T_{min} are respectively mean, maximum and minimum monthly temperature; Prec means mean monthly precipitation.

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