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## Original Paper / Artigo Original

# Reproductive phenology of *Ditassa burchellii* (Apocynaceae, Asclepiadoideae), a herbaceous vine, in a semi-deciduous forest from Brazil

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**Abstract**

We analyzed the reproductive phenology of *Ditassa burchellii* and the influence of abiotic factors on the species phenophases. The study was conducted on individuals of a natural population from a semi-deciduous forest (Viçosa municipality, Minas Gerais state, southeastern Brazil). We quantified the activity and intensity indices of the following phenophases: flower bud, flower, immature fruit, and dehiscent fruit. To test for seasonality of phenophases, we analyzed each of them using Rayleigh test. We evaluated whether there was any association between abiotic variables (mean temperature, rainfall, and day length) and phenophases, in the month of occurrence and in the three months prior to the occurrence of each phenophase. The analyzed phenophases occurred mainly at the end of the rainy season and during the dry season, with overlapping periods, but with sequential peaks. The periods of occurrence of reproductive phenophases were similar to the ones in other climbing species and were mainly related to the dispersal mode. All phenophases were seasonal and were associated with at least one abiotic variable, either in the month of their occurrence or in the previous months.
**Key words**: anemochory, Atlantic Forest, flowering, fruiting, seasonality.

**Resumo**

Analisamos a fenologia reprodutiva de *Ditassa burchellii* e a influência de fatores abióticos em suas fenofases. O estudo foi realizado em indivíduos de população natural em floresta estacional semidecídua (Viçosa, Minas Gerais, sudeste do Brasil). Quantificamos os índices de atividade e de intensidade das seguintes fenofases: botões florais, flores, frutos imaturos e frutos deiscentes. Para testar a sazonalidade dessas fenofases, analisamos cada uma usando o teste de Rayleigh. Avaliamos se houve associação entre as variáveis abióticas (temperatura média, precipitação e comprimento do dia) e as fenofases, no mês de ocorrência e também nos três meses anteriores à ocorrência de cada fenofase. As fenofases ocorreram principalmente no fim da estação chuvosa e durante a estação seca, com períodos sobrepostos, mas com picos sequenciais. Os períodos de ocorrência das fenofases reprodutivas foram similares aos de outras espécies de lianas e foram relacionados principalmente com o modo de dispersão. Todas as fenofases foram sazonais e foram associadas a pelo menos uma das variáveis abióticas, no mês de sua ocorrência ou nos meses anteriores.
**Palavras-chave**: anemocoria, Floresta Atlântica, floração, frutificação, sazonalidade.

## Introduction

Plant phenology studies provide information on plant growth as well as on the effects of selective pressures imposed by the environment on flowering and fruiting (Fenner 1998). These studies are mainly performed on tree and treelet species (Morellato & Leitão-Filho 1996). Climbing species (woody lianas and herbaceous vines *sensu* Gentry 1991) usually have different phenological patterns from those of tree species, and they may also differ in their phenological responses according to the climber type (Putz & Windsor 1987; Morellato & Leitão-Filho 1996).

In plant communities, reproductive phenophases (flowering and fruiting) are usually associated with the quantity and quality of resources available to pollinators and seed dispersers (Williams *et al.* 1999; Stevenson *et al.*

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2008; Rubim *et al.* 2010; Fonseca *et al.* 2013), besides providing information on the sexual reproductive mechanisms of plant species. In the case of climbing species, flowering can be unimodal (peaking in the dry season) or bimodal (peaking in both wet and dry seasons), while the fruiting peak is commonly associated with the type of fruit dispersal (Putz & Windsor 1987; Morellato & Leitão-Filho 1996; Ramírez & Briceño 2011; Romaniuc Neto *et al.* 2012; Ponnuchamy *et al.* 2013). However, most of the studies addressing the reproductive phenophases of climbing species have focused on woody lianas and did not evaluate the influence of abiotic factors on the phenological responses of the species.

Asclepiadoideae, the largest subfamily in the Apocynaceae, commonly has herbaceous vine representatives in tropical forests (Morellato & Leitão-Filho 1996; Ponnuchamy *et al.* 2013), including *Ditassa burchellii* Hook. & Arn., the species addressed in the present study. This subfamily is represented by approximately 392 species distributed among 32 genera in Brazil (Rapini 2012), where only the exotic shrub *Calotropis procera* (Aiton) W.T. Aiton has been the focus of studies involving reproductive phenophases (Sobrinho *et al.* 2013).

There are no studies on the reproductive phenology of *Ditassa* representatives, despite the high endemic-species richness of the genus in Brazil (49 species; BFG 2018). Recent studies have shown that some *Ditassa* species interact with rare and specific groups of pollinators (Domingos-Melo *et al.* 2017; Fontes *et al.* 2018). These findings demonstrate the relevant role played by those plants in maintaining these poorly known mutualistic interactions. Thus, we aimed to describe the reproductive phenology of *D. burchellii*, a herbaceous vine, and determine whether abiotic variables are associated with the species phenological responses.

## Material and Methods

### Study area and species

We carried out the study from March 2013 to December 2014 in a forest fragment at the Mata do Paraíso Station for Research, Environmental Training and Education (hereafter referred to as Mata do Paraíso). Mata do Paraíso is located in Viçosa municipality (20º47'–48'S, 42º50'–52'W), Minas Gerais state, southeastern Brazil, covering an area of 194 ha, at 690–870 m above sea level. This forest fragment is the largest one in Viçosa (Silva *et al.* 2014), and its vegetation is classified as seasonal semi-deciduous montane forest (Veloso *et al.* 1991).

Climate in Viçosa is type Cwa (mesothermal with hot rainy summers and cold dry winters), according to Köppen's classification (Alvares *et al.* 2013). Average annual rainfall is 1,221 mm and average annual temperature is 19.3 °C (averages of meteorological data from 1961–1990; DNMET 1992). The dry season extends from April to September (monthly rainfall < 60 mm; mean minimum and maximum monthly temperatures = 10 °C and 27 °C, respectively; mean photoperiod = 11.3 h) while the rainy season extends from October to March (monthly rainfall > 100 mm; mean minimum and maximum monthly temperatures = 16 °C and 30 °C, respectively; mean photoperiod = 12.9 h). Rainfall and temperature showed a seasonal distribution during the study period (Fig. 1).

*Ditassa burchellii* inflorescences are umbelliform cymes (Fig. 2a) with tiny cream-colored, odoriferous flowers (ca. 3 mm; Fig. 2b); fruits are follicles, each of which contains four comose seeds (Fig. 2c-d). Each flower can produce one (single follicle; Fig. 2c) or two (twin follicles) fruits, as ovaries are apocarpous (Endress & Bruyns 2000). The species occurs commonly in "capoeira" (secondary) vegetation and at forest edges (Konno 2005). In the study site, plants are usually seen along trail edges in partially shaded locations, growing above the surrounding vegetation, which has a low (about 7 m) and discontinuous canopy. A voucher specimen was deposited in the Herbarium of Federal University of Viçosa (VIC Herbarium; no. 43,006).

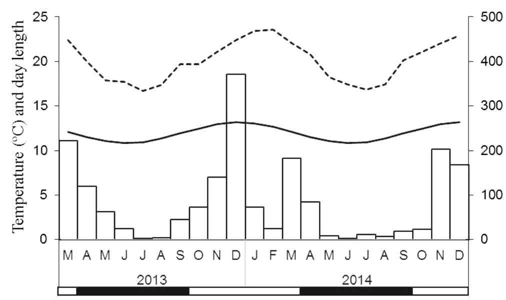


**Figure 1** – Meteorological data on Viçosa municipality, Minas Gerais state, southeastern Brazil (dashed line = mean temperature, solid line = day length, bars = rainfall), during the study period. Black horizontal bar = dry season; white horizontal bar = rainy season.

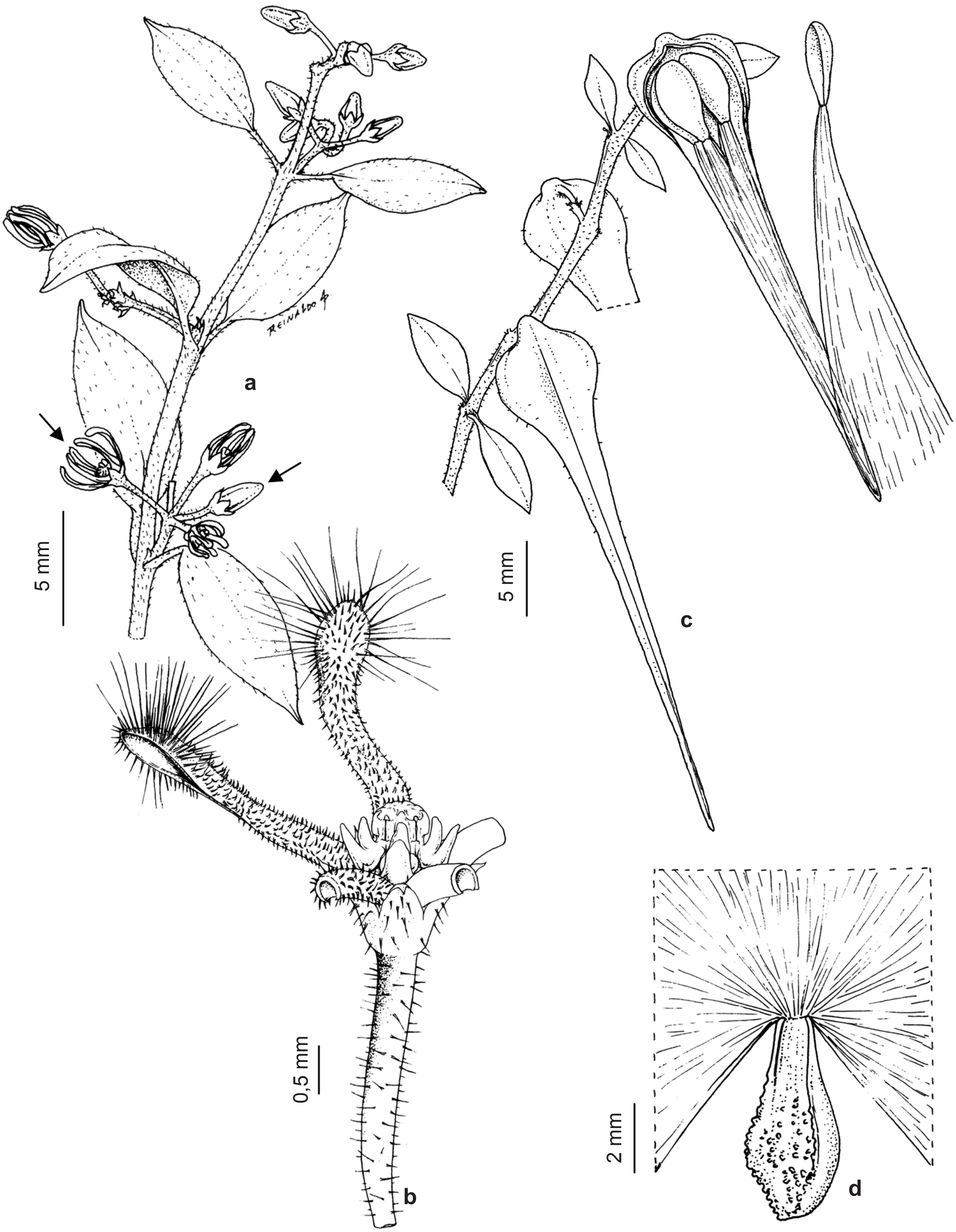


**Figure 2** – *Ditassa burchellii* – a. branch with opposite leaves and one inflorescence per node; notice, on the first inflorescence (from bottom to top), flower bud (right arrow) and flower (left arrow); b. flower, with three petals having been removed; c. immature fruit (single follicle; left side) and dehiscent fruit with exposed comose seeds (right side); notice the occurrence of a single fruit per node; d. seed with the fully expanded coma being partially represented.

### Reproductive phenology

We sampled seven adult plants, respecting a minimum distance of 10 m between each plant to avoid repeated evaluations in a same individual. We recorded the presence of flower buds (Fig. 2a), flowers (Fig. 2a-b), and fruits (immature and dehiscent; Fig. 2c) in two consecutive reproductive periods, with a total 22 months of observation. We performed evaluations on a fortnightly basis during the vegetative period and on a weekly basis in the reproductive period.

We analyzed reproductive phenophases by calculating the activity (percentage of plants at a given phenophase) and intensity indices (Bencke & Morellato 2002). To calculate intensity index, each phenophase was scored on a semi-quantitative scale, as proposed by Fournier (1974). To this end, we used the weekly values of each month for both indices. We classified the frequency of phenophases following Newstrom *et al.* (1994), as continual, subannual, annual, or supra-annual. The annual phenophase was also classified according to its duration, as brief (< 1 month), intermediate (1–5 months), or extended (> 5 months).

In addition, we labeled 40 inflorescences (n = six individuals) having flower buds and monitored them on a daily basis from anthesis of the first flowers to fruiting. Thus, we recorded the duration of flowers and inflorescences, the number of flowers per inflorescence, and the number of open flowers per day per inflorescence. We monitored 40 other flowers (n = 40 inflorescences, six individuals) from ovary swelling to seed dispersal, to record the duration of fruit development stage. We determined the natural fruit set (open pollination) of 5655 flowers and made non-systematic observations on 650 inflorescences, throughout the two reproductive periods, to record the number of fruits per inflorescence and per flower.

### Seasonality and association with abiotic variables

To test for seasonality of reproductive phenophases, we analyzed each phenophase using Rayleigh test (circular statistics; Zar 1999; Morellato *et al.* 2010). For that, we converted months to angles and calculated mean angle, mean date (for phenophases with significant mean angles), and vector length, considering the result significant when $p < 0.01$ and $r > 0.5$ (Morellato *et al.* 2010). We carried out these analyses using BioEstat (Ayres *et al.* 2007), based on the weekly values of number of individuals at each phenophase.

We evaluated whether there was any association between abiotic variables (mean temperature, rainfall, and day length) and phenophases, in the month of occurrence and also in the three months prior to the occurrence of each phenophase. To this end, we obtained meteorological data from the Meteorological Station of Federal University of Viçosa, which is 10 km away from the study area, and calculated the mean weekly values of mean temperature and day length and the sum of weekly rainfall values. We used generalized linear models with Poisson distribution and logarithmic link function, selecting the best model using the Akaike information criterion (AIC; Crawley 2007). We carried out these analyses in R (R Core Team 2016), considering the weekly values of number of individuals at each phenophase as the response variable and temperature, rainfall, and day length as explanatory variables.

## Results

All individuals reproduced once a year during the study period. The reproductive phenophases occurred mainly at the end of the rainy season and during the dry season, with overlapping periods of occurrence, but with sequential peaks (Fig. 3a-d). Peaks of activity and intensity were similar for all phenophases (Fig. 3a-d).

Flower buds were observed in March and April 2013 and 2014, during the transition from the rainy to the dry season, peaking in March (Fig. 3a). In both years, flowers were observed in the same period as flowers buds (about 35 days), but peaking in April (Fig. 3b). Both phenophases were annual with an intermediate duration.

Flowers opened early in the morning and lasted an average 6.4 days (with a range of 2–11 days). The mean number of flowers per inflorescence and mean inflorescence duration were, respectively, 8.7 ± 1.8 flowers and 21.1 ± 4.4 days. The mean number of open flowers per day per inflorescence was 4.1 ± 1.6, depending on the number of flowers per inflorescence and on the dynamics of flower opening, which occurred gradually.

In both years, immature fruits were mainly observed in the dry season (Fig. 3c), and such phenophase was annual with extended duration. Peaks occurred from May to September 2013 and in May 2014 for activity index and in May of both years for intensity index (Fig. 3c). The mean time of fruit development, from ovary swelling to seed dispersal, was 139.3 ± 24.2 days. Dehiscent fruits were observed from August to October 2013 and

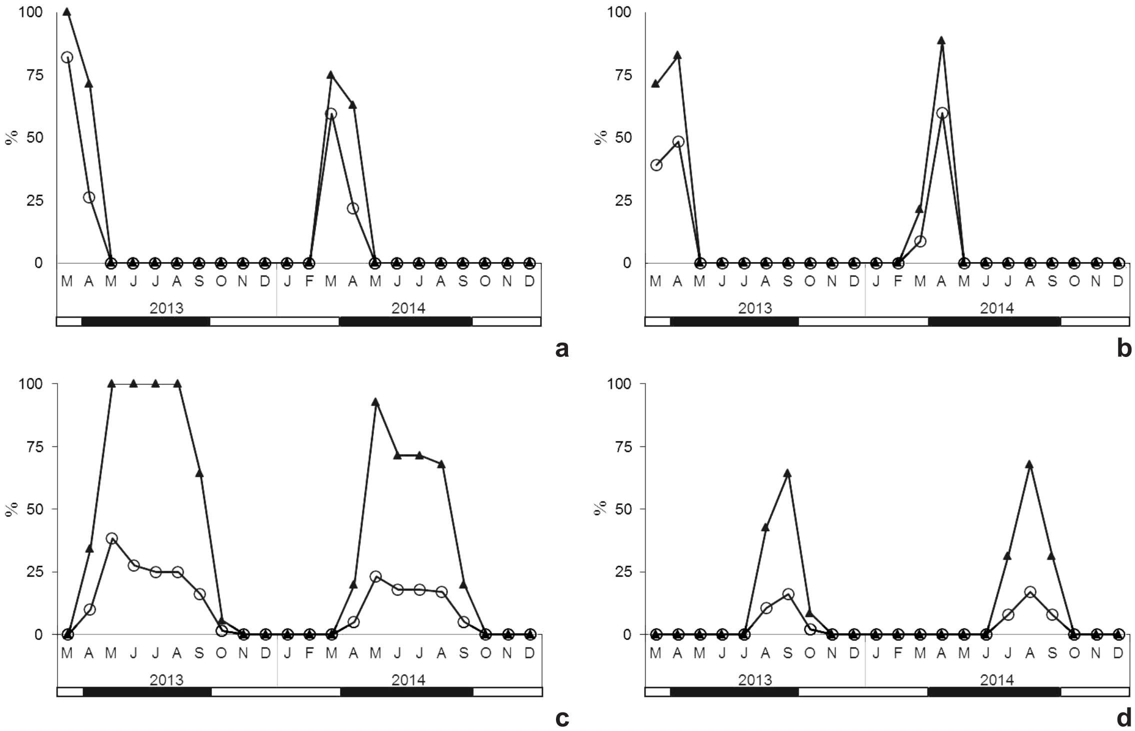


**Figure 3** – a-d. Reproductive phenology of *Ditassa burchellii* at Mata do Paraíso, southeastern Brazi – a. activity (▲) and intensity (○) indices of phenophases flower buds; b. flowers; c. immature fruits; d. dehiscent fruits. Black horizontal bar = dry season; white horizontal bar = rainy season.

from July to September 2014, peaking in September 2013 and August 2014 (Fig. 3d), at the end of the dry season. This phenophase was annual with intermediate duration.

Natural fruit set was low (4.4%). Fruits were observed in 36.9% of the 650 inflorescences monitored over two reproductive episodes. There was a tendency of one flower per inflorescence to result in fruit (97.5%; Fig. 2c) and of one fruit to be produced per flower (single follicle, 96.8%; Fig. 2c).

All phenophases were seasonal, but the immature fruit phenophase was less concentrated and less synchronous around the mean data than the other ones (Tab. 1) due to the presence of immature fruits throughout the dry season. Phenophases were associated with at least one of the abiotic variables, either in the month of their occurrence or in the previous months (Tab. 2). The flower bud phenophase was associated with all abiotic variables, while flowering duration (the period with flowers at anthesis) was best explained by temperature and day length. Fruiting phenology was best explained by day length.

## Discussion

Occurrence of the flowering stage in the transition from the rainy to the dry season was also observed in herbaceous vines from other seasonal forests (Morellato & Leitão-Filho 1996; Romaniuc Neto *et al.* 2012). This period is the most favorable one for fruit ripening and diaspore dispersal by wind (Morellato & Leitão-Filho 1996; Rossatto & Kolb 2011). Flowering in some climbing species is stimulated by decrease in temperature and day length (Gentry 1974; Rossatto & Kolb 2011), similarly to the observed in *D. burchellii*.

Long-lived flowers such as those observed here are common in the Asclepiadoideae (Wyatt 1981; Skutch 1988; Lumer & Yost 1995; Vieira & Shepherd 1999), similarly to the observed in Orchidaceae (Primack 1985); pollen grains of plants from these two groups are clustered in pollinia and have complex pollination mechanisms in common (Endress 1994, 2016). Floral longevity may be associated with pollinator visitation rates (Schoen & Ashman 1995). Thereby, the rates of removal and insertion of pollinia must be related to floral longevity, as unpollinated flowers tend to

**Table 1** – Results of circular statistical analysis on the occurrence of seasonality of reproductive phenophases of *Ditassa burchellii* at Mata do Paraíso, southeastern Brazil.

| Variable | Flower bud | Flower | Immature fruit | Dehiscent fruit |
|---|---|---|---|---|
| Angular mean | 91.1º | 99.0º | 180.9º | 233.5º |
| Mean data | 03/Apr | 11/Apr | 02/Jul | 25/Aug |
| Mean vector length (*r*) | 0.98 | 0.98 | 0.74 | 0.94 |
| Rayleigh test (*p*) | < 0.01 | < 0.01 | < 0.01 | < 0.01 |

**Table 2** – Models used to evaluate the association between abiotic variables and the reproductive phenophases of *Ditassa burchellii*. Numbers after each variable refer to the time lag used in the analyses. AIC = Akaike information criterion; temp = mean temperature; rain = rainfall; dayl = day length.

| | Full model | AIC | Selected model | AIC |
|---|---|---|---|---|
| Flower bud | bud ~ temp0+temp1+temp2+temp3+rain0+rain1+rain2+rain3+dayl0+dayl1+dayl2+dayl3 | 110.4 | bud ~ temp1+temp2+temp3+rain1+rain2+rain3+dayl0+dayl1+dayl2+dayl3 | 109.3 |
| Flower | flo ~ temp0+temp1+temp2+temp3+rain0+rain1+rain2+rain3+dayl0+dayl1+dayl2+dayl3 | 117.6 | flo ~ temp0+temp1+temp3+dayl0+dayl1+dayl2 | 108.7 |
| Immature fruit | im_fr ~ temp0+temp1+temp2+temp3+rain0+rain1+rain2+rain3+dayl0+dayl1+dayl2+dayl3 | 258.5 | im_fr ~ dayl1 | 245.5 |
| Dehiscent fruit | de_fr ~ temp0+temp1+temp2+temp3+rain0+rain1+rain2+rain3+dayl0+dayl1+dayl2+dayl3 | 109.4 | de_fr ~ dayl3 | 97.4 |

live longer (Devlin & Stephenson 1984; Richardson & Stephenson 1989).

The occurrence of fruiting in *D. burchellii* during the dry season differed from what is common for herbaceous vines from seasonal semi-deciduous forests (with a slight peak at the end of the rainy season; Morellato & Leitão-Filho 1996). This difference may also be related to the dispersal mode (Romaniuc Neto *et al.* 2012). In fact, seed dispersal occurred at the end of the dry season, a period which might not only favor anemochorous dispersal (Morellato & Leitão-Filho 1996; Rossatto & Kolb 2011) of the species comose seeds but also be conducive to seed germination (Morellato *et al.* 1989; Rossatto & Kolb 2011). Thus, new plants could use the entire rainy season to develop their root system (Fournier & Salas 1966), which may have been additionally favored by the higher rates of decomposition and nutrient release from the litter accumulated in the dry season (Smythe 1970; Morellato 1992). This strategy has also been described for other climbing species with anemochorous dispersal (Putz & Windsor 1987; Morellato & Leitão-Filho 1996; Spina *et al.* 2001; Rossatto & Kolb 2011; Romaniuc Neto *et al.* 2012).

The association between fruiting and day length has also been observed in *Pyrostegia venusta* (Ker Gawl.) Miers (Bignoniaceae), a liana species (Rossatto & Kolb 2011). Day length decreases along the dry season, when wind speed is more intense, which in turn favors anemochorous dispersal. Besides, fruiting of species from seasonal forests usually occurs on shorter days (lower temperature and precipitation; Stevenson *et al.* 2008; Rubim *et al.* 2010). We observed a similar pattern in this study, as fruiting peaked in the dry season.

The low fruit set observed was similar to values assessed in other Asclepiadoideae species (1–5%; Wyatt & Broyles 1994). This result was a consequence of the abortion of immature fruits, a common phenomenon in this plant group, which may be induced by extrinsic (pollinator-regulated) or intrinsic factors (plant-regulated) (Whyatt 1981; Queller 1985; Wyatt & Broyles 1994). The tendency of one flower per inflorescence to result in fruit and of one fruit to be produced per flower is a characteristic commonly reported to Asclepiadoideae species (Liede & Whitehead 1991; Vieira & Shepherd 1999, 2002), and it also contributed to the occurrence of a low fruit set.

This is the first study on the reproductive phenology of a native species of the Asclepiadoideae, a plant group with complex floral morphology and pollination mechanism. The periods of occurrence of reproductive phenophases in *D. burchellii* were similar to the ones in other climbing species and were mainly related to the species dispersal mode. Phenophases were adjusted to climatic seasonality, thus being influenced by abiotic factors, which is a remarkable feature of seasonal tropical forests.

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