

Seasonal changes in germination and dormancy of buried seeds of endemic Brazilian Eriocaulaceae

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Abstract

Seeds can synchronize their germination and dormancy cycles to regular seasonal environmental changes. The present work sought to evaluate the *in-situ* longevity of the buried seeds of two species of *Syngonanthus* and two species of *Comanthera* (Eriocaulaceae) in the region of natural occurrence (Serra do Cipó Range, south-eastern Brazil), and to relate their germination responses to seasonal climatic changes. Samples were exhumed bimonthly and germination was tested under a 12-h photoperiod ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) at optimal germination temperature. The seeds of *Comanthera* showed high deterioration after the first year of burial, especially *C. elegans*, which reflects their incapacity to form viable soil seed banks. The buried seeds of *C. bisulcata*, *S. anthemidiflorus* and *S. verticillatus* acquired secondary dormancy during the rainy season (spring/summer, higher temperatures) which was alleviated during the subsequent dry season (autumn/winter, lower temperatures). It is concluded that *C. bisulcata*, *S. anthemidiflorus*, and *S. verticillatus* form seed banks of the persistent type and demonstrate consecutive cycles of germination/dormancy, accompanying annual seasonal changes for at least 2 years.

Keywords: annual dormancy cycle, *campos rupestres*, *Comanthera*, secondary dormancy, seed longevity, soil seed bank, *Syngonanthus*

Introduction

Buried seeds can demonstrate repeating cycles of dormancy and non-dormancy linked to seasonal

changes in environmental conditions – a well-known phenomenon among seeds showing physiological dormancy (Baskin and Baskin, 1998). Seasonal cycles of dormancy help avoid germination during periods with unfavourable environmental conditions and allow seeds to be ready for germination immediately before periods favourable to plant growth (Vleeshouwers *et al.*, 1995). As such, seasonal dormancy favours the survival of species that are able to limit their germination to periods in which environmental conditions are most favourable to seedling survival and growth (Donohue, 2005).

Seasonal variations in the germination responses of buried seeds are common among annual species (Baskin *et al.*, 2003), and are especially well documented among species in temperate climates (Baskin and Baskin, 1998; Handley and Davy, 2005; Geissler and Gzik, 2010) that are subject to very marked climatic variations. These seasonal variations in germination events, however, have been poorly examined among perennial species, especially those exclusive to tropical climates. A recent study of perennial *Xyris* (Xyridaceae) species endemic to *campos rupestres* (open, rocky, mountainous) vegetation in Brazil (Garcia *et al.*, 2012) identified dormancy cycling during the year, which had not been reported previously for tropical species.

The Eriocaulaceae family has a tropical distribution, and its species are frequently encountered in the Cadeia do Espinhaço mountain range in south-eastern Brazil. This area is considered to be the centre of diversity for this family, and approximately 90% of the species found there are endemic (Giulietti *et al.*, 2005). The regional climate is mesothermic (due to its altitude), with humid summers and dry winters (Cheib and Garcia, 2012). The genera *Comanthera* (recently re-established; Parra *et al.*, 2010) and *Syngonanthus* are typical of the *campos rupestres* vegetation in the Cadeia do Espinhaço Range and comprise perennial species with herbaceous habits that occur in rocky fields or high sandy plains and swampy sites. Their seeds are very small (<25 μg) and spherical, and most species

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have an absolute light requirement for germination (Oliveira and Garcia, 2005, 2011).

The endemism of many Eriocaulaceae species and the annual variations in regional climatic conditions (humid summers and dry winters) where they are found, as well as the indication that they can form persistent seed banks (Garcia and Oliveira, 2007), raise many important questions concerning their germination phenologies. Within this perspective, the present study sought to investigate the dynamics of the germination responses of four tropical perennial species of this family (two from the genus *Comanthera* and two from *Syngonanthus*) to determine: (1) the *in-situ* longevity of buried Eriocaulaceae seeds; (2) if the germination responses of these seeds are related to seasonal changes in the environmental conditions where they occur; and (3) if there are similarities between the germination phenologies of the species investigated.

Materials and methods

Study area and botanical material

The seeds of four endemic species of Eriocaulaceae of the *campos rupestres* (rupestrian fields) vegetation of south-eastern Brazil were used. Capitula of *Syngonanthus anthemidiflorus* (Bong.) Ruhland, *Syngonanthus verticillatus* (Bong.) Ruhland and *Comanthera bisulcata* (Korn.) Ruhland (= *Syngonanthus bisulcatus*) were collected in the Serra do Cipó Range in Minas Gerais State (MG), Brazil; the seeds of *Comanthera elegans* (Bong.) Ruhland (= *Syngonanthus elegans*) were collected in the municipality of Diamantina, MG, Brazil. The regional climate where these plants are found is tropical and mesothermic due to the altitude (Cwb in the classification system of Köppen), with two well-defined seasons: a dry period that occurs in the austral autumn/winter; and a rainy period during the austral spring/summer (Garcia *et al.*, 2012). The average precipitation and the minimum and maximum temperatures during the study period are presented in Fig. 1.

In-situ longevity and seed germination

The collected seeds were placed in fine-mesh nylon bags and buried 5 cm below the soil surface for periods that varied from 24 to 34 months. The seeds of *C. bisulcata* (28 months) and *S. verticillatus* (34 months) were buried in a mesic environment where these species occur, which accumulates water during the rainy period, while the seeds of *C. elegans* (24) and *S. anthemidiflorus* (34) were buried in a seasonally dry environment (as described by Oliveira and Garcia,

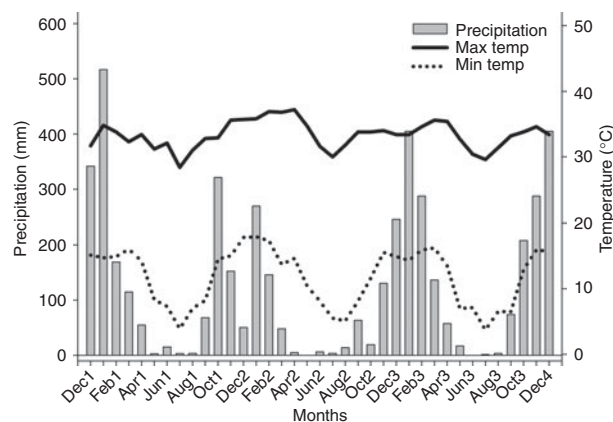


Figure 1. Climate data, monthly precipitation and maximum and minimum air temperature in Serra do Cipó, Brazil, during the period when the experiments were carried out. Environmental conditions were monitored by the weather station of Conceição do Mato Dentro, MG, Brazil.

2011). Seed samples were exhumed every 2 months and tested for their germination responses under a 12-h photoperiod ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 30°C (*C. elegans*), 25°C (*S. anthemidiflorus*), and 20°C (*C. bisulcata* and *S. verticillatus*) – which were previously defined as optimal temperatures for seed germination by Oliveira and Garcia (2005, 2011). The germination tests utilized samples of 200 seeds (four repetitions of 50 seeds each), with the seeds being maintained in Petri dishes lined with two layers of filter paper humidified with a nystatin solution (Oliveira and Garcia, 2005). The criterion adopted for determining germination was the emergence of the vegetative axis.

Statistical analysis

As the germination data did not demonstrate normality (according to the Shapiro–Wilk test) and/or homogeneity (according to the Brown–Forsythe test), they were submitted to non-parametric statistical tests (Kruskal–Wallis), followed by comparisons in pairs using the Conover test, considering a 5% level of significance (Conover, 1999), utilizing Brightstat software (Stricker, 2008).

Results

The germinability of the recently collected seeds of the four species varied from 58 to 80% (Figs 2 and 3). The buried seeds of *C. bisulcata*, *S. anthemidiflorus* and *S. verticillatus* remained viable for more than 24 months after dispersal, although the germinability of *C. bisulcata* seeds decreased by approximately 50% over that period of time. Approximately 80% of

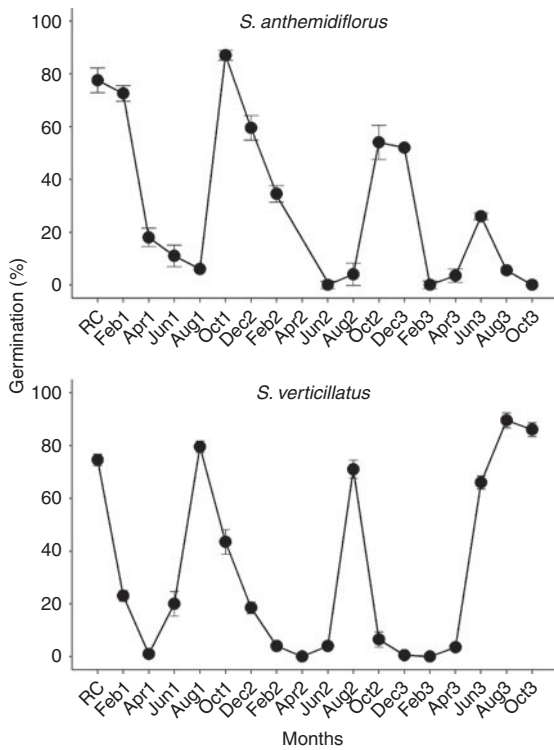


Figure 2. Germinability of *Syngonanthus anthemidiflorus* and *S. verticillatus* seeds during burial in the soil for 34 months under natural conditions in Serra do Cipó, Brazil. Data correspond to the mean \pm SE of four replicates of 50 seeds per data point.

the seeds of *C. elegans* lost their germination capacity after being buried for 20 months (Fig. 3).

All four species demonstrated marked decreases in germinability shortly after burial (with the exception of *S. anthemidiflorus*, which demonstrated an accentuated decrease after 4 months) – but with subsequent returns to high germinability rates in the following months (Fig. 2). The dynamics of the germination responses tracked with seasonal variations, characterized seasonal cycles of germination/dormancy during the study period among the four species.

The buried seeds of *C. bisulcata* acquired dormancy throughout the rainy season (spring/summer, higher temperatures, see Fig. 1). Approximately 50% of the seeds re-acquired their germination capacity at the end of the winter (dry season, lower temperatures), a situation that was repeated in the following year (Fig. 3). Although the seeds of *C. elegans* did not demonstrate any characteristic cycle of dormancy, the seeds that remained viable in the first year overcame the dormancy acquired by burial (during the dry season) and became dormant once again at the beginning of the rainy season (Fig. 3).

The seeds of *S. anthemidiflorus* and *S. verticillatus* demonstrated seasonal cycles of germination and

dormancy that repeated themselves for three consecutive years (Fig. 2). The seeds of both species acquired dormancy after burial and overcame that dormancy at the end of the dry season (*S. verticillatus*) or the beginning of the rainy season (*S. anthemidiflorus*). As the rainy season (accompanied by increase of temperature) became established, the seeds of these species gradually acquired dormancy and remained dormant until the end of the dry season (autumn/winter, lower temperatures) or the beginning of the wet season – when they once again overcame dormancy. The seeds of *S. verticillatus* maintained their viability even after 34 months of burial, while most *S. anthemidiflorus* seeds lost their capacity to germinate (Fig. 2).

Discussion

Soil seed banks provide diaspores for the recruitment of new individuals into plant populations and help maintain long-term ecosystem equilibrium (Doucet and Cavers, 1996; Abella and Springer, 2012). Seed banks therefore constitute an important survival strategy, especially for plants that are exposed to

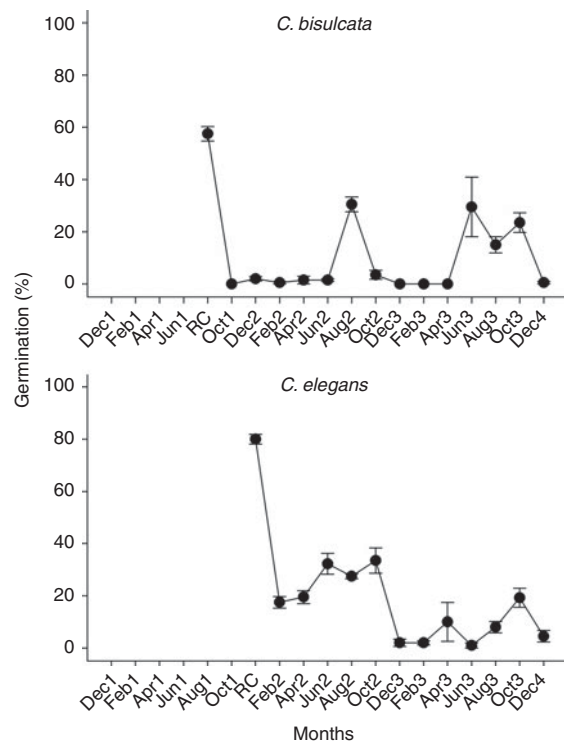


Figure 3. Germinability of *Comanthera bisulcata* and *C. elegans* seeds during burial in the soil for 28 and 24 months, respectively, under natural conditions in Serra do Cipó, Brazil. Data correspond to the mean \pm SE of four replicates of 50 seeds per data point.

adverse environmental conditions during the course of the year (Moles *et al.*, 2003). The formation of a seed bank is dependent on seed longevity in the soil, and will be influenced by environmental (Moles *et al.*, 2003), physical (Mohamed-Yasseen *et al.*, 1994), eco-physiological and biochemical factors (Garcia *et al.*, 2012).

The maintenance of the germination capacity of the seeds of *C. bisulcata* (50%), *S. anthemidiflorus* (70%) and *S. verticillatus* (100%) in the soil for periods greater than 24 months, as observed in the current study, confirms that at least some species of Eriocaulaceae can form seed banks of the persistent type (*sensu* Thompson, 1993), as was suggested previously by Garcia and Oliveira (2007). Considering the absolute requirement for light for germination of the seeds of the species investigated (observed by Oliveira and Garcia, 2005, 2011), the formation of a seed bank requires the relatively rapid burial of some of the seeds produced in a given year. According to Hölzel and Otte (2004), small, spherical seeds (like those of the Eriocaulaceae) tend to be more easily buried in the soil – thus increasing the probability of their incorporation into soil seed banks.

The seeds of *Comanthera*, especially *C. elegans*, showed high deterioration after the first year of burial, which reflects their incapacity to form viable soil seed banks. This seed mortality may be due to intrinsic factors, such as the chemical composition of the seed coat, the position of the embryo (Mohamed-Yasseen *et al.*, 1994) or the thickness of the seed coat (Gardarin *et al.*, 2010), or to external factors such as their storage environments. The results of the present study suggest that soil conditions were not the primary determinants of seed deterioration among the species evaluated, as the seeds of *S. anthemidiflorus* (buried in the same environment as those of *C. elegans*) maintained their initial capacity to germinate for more than 24 months.

The results of the present work demonstrated the development of secondary dormancy after burial. Secondary dormancy can be induced, lost and re-induced repetitively in response to seasonal changes (Finch-Savage and Leubner-Metzger, 2006), and is usually associated with annual cycles of dormancy (Baskin and Baskin, 1998; Fenner and Thompson, 2005). Dormant seeds wait for appropriate environmental information before breaking dormancy (Mohamed-Yasseen *et al.*, 1994) and adjust the seasonal emergence window to guarantee their reproductive success (Batlla and Benech-Arnold, 2010).

The present study confirmed for the first time that the seeds of tropical perennial species demonstrate marked annual cycles of dormancy that can be repeated consecutively for at least 2 years. With the exception of *C. elegans* (seeds of which demonstrated irregular variations in their germinability over time and lost 80% of their germination capacity during the

first year of burial), the buried seeds of the other species examined here (*C. bisulcata*, *S. anthemidiflorus* and *S. verticillatus*) acquired secondary dormancy during the rainy season (higher temperatures), which was alleviated during the subsequent dry season (lower temperatures). Additionally, our data indicated that seeds that did not germinate at the beginning of the rainy season (such as those that were not exposed to light) developed secondary dormancy and remained in that state until the end of the subsequent dry season. Cycles of germination/dormancy accompanying annual seasonal changes were recently observed in two *Xyris* species (Xyridaceae) endemic to the *campos rupestres* vegetation of Brazil (Garcia *et al.*, 2012), which suggests a recurrent strategy among plants occurring in that environment.

The germination process must be highly responsive to environmental conditions (Donohue, 2002) and seeds have adapted to synchronize their germination/dormancy cycles with the different seasons of the year through environmental signalling (Footitt *et al.*, 2011). Temperature is considered the principal factor regulating dormancy in temperate-climate species (Baskin and Baskin, 1998; Benech-Arnold *et al.*, 2000). The influence of wet–dry cycles on germination phenology is rarely discussed in the literature, although humidity levels (drought) can often be more important than temperature (cold) in terms of breaking dormancy (Wagmann *et al.*, 2012). Species growing in tropical environments are subject to smaller temperature variations during the course of the year but can suffer from more extreme degrees of seasonal water restrictions, which, together with temperature, can modulate the degrees of seed dormancy (Adámoli *et al.*, 1973; De Miguel and Soriano, 1974; Kruk and Benech-Arnold, 1998). Studies under controlled conditions, isolating the factors soil temperature and soil humidity (with either constant or fluctuating levels, simulating rainy and dry seasons of the study region), will be needed to establish the influence of each factor on the induction and alleviation of the secondary dormancy and the dynamics of dormancy/germination in Eriocaulaceae species – which are typical (and exclusive) representatives of tropical regions.

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Conflicts of interest

None.

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