**TÚLIO GABRIEL SOARES OLIVEIRA**

# ECOFISIOLOGIA DA GERMINAÇÃO DE SEMENTES DE *Xyris* (XYRIDACEAE) DE CAMPOS RUPESTRES

**Tese de doutorado em Biologia Vegetal**

Belo Horizonte
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**UNIVERSIDADE FEDERAL DE MINAS GERAIS**
**INSTITUTO DE CIÊNCIAS BIOLÓGICAS**
Departamento de Botânica
**Programa de Pós-Graduação em Biologia Vegetal**

**UFMG**

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# ECOFISIOLOGIA DA GERMINAÇÃO DE SEMENTES DE *Xyris* (XYRIDACEAE) DE CAMPOS RUPESTRES

Orientadora: Prof.ª Dra. Queila de Souza Garcia
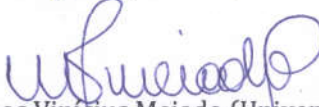
Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Biologia Vegetal.

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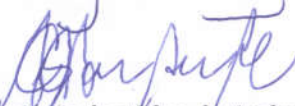
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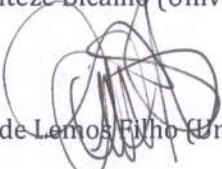
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**DEDICATÓRIA**

*Dedico essa tese aos meus pais Diana Soares Nunes e Sérvio Túlio de Oliveira Silva*

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**Resumo**

O gênero *Xyris* (Xyridaceae) apresenta aproximadamente 100 espécies amplamente distribuídas nos campos rupestres brasileiros. Neste trabalho foram realizados estudos relacionados à ecofisiologia da germinação de espécies de *Xyris* típicas dos campos rupestres. No primeiro capítulo, sementes de três espécies de *Xyris* (*X. asperula, X. subsetigera* e *X. trachyphylla*) foram enterradas em uma área de ocorrência natural com a finalidade de investigar a ocorrência de ciclos sazonais de germinação e dormência, e avaliar longevidade *in situ* das sementes. Para isso, as sementes foram exumadas a cada dois meses e a germinação foi avaliada em uma ampla faixa de temperaturas constantes. No segundo capítulo avaliou-se a variação interanual na germinação de sementes de uma população de *X. longiscapa* durante quatro anos consecutivos (2014-2017), além da longevidade *in situ* e a ocorrência de ciclos sazonais de dormência nas sementes produzidas em 2014. No terceiro capítulo foram determinados os requerimentos para a germinação (luz e temperatura) de seis espécies de *Xyris* (*X. asperula, X. pilosa, X. seubertii, X. subsetigera, X.* sp. e *X. trachyphylla*). A partir dos dados de germinação foram calculadas as temperaturas base ($T_b$) e o requerimento térmico para a germinação de 50% das sementes ($\theta_{50}$) de quatro espécies. Esses dados, em conjunto com a faixa térmica de germinação de cada espécie, foram utilizados para prever o comportamento germinativo das espécies no cenário atual e em cenários de aumento de temperatura. Sementes enterradas de *X. asperula, X. longiscapa, X. subsetigera* e *X. trachyphylla* formam banco de sementes persistente e exibem ciclos sazonais de germinação e dormência. Em todas as espécies, a dormência secundária é adquirida a partir da metade da estação chuvosa e superada durante a estação seca. Sementes de *X. longiscapa* coletadas em 2014 apresentaram um maior grau de dormência em relação aos outros anos possivelmente resultante de efeito do ambiente maternal. Sementes de *Xyris* apresentam requerimento absoluto de luz para germinação. A faixa térmica de

germinação e o requerimento térmico é variável entre as espécies. Demonstrou-se que a germinação de sementes de *X. subsetigera*, *X. pilosa* e *X. seubertii* são mais afetadas pelas temperaturas mais altas, enquanto as sementes de *X. asperula, X.* sp. e *X. trachyphylla* são mais tolerantes ao aumento de temperatura, não apresentando redução no percentual de germinação quando submetidas a temperaturas mais altas.

**Palavras-chave:** ciclos sazonais de dormência, dormência de sementes, dormência secundária, banco de sementes do solo, aquecimento global.

**Abstract**

The genus *Xyris* (Xyridaceae) presents approximately 100 species widely distributed in the Brazilian *campo rupestre*. In this work, studies were carried out related to the ecophysiology of the germination of *Xyris* species typical of *campo rupestre*. In the first chapter, seeds of three species of *Xyris* (*X. asperula, X. subsetigera* and *X. trachyphylla*) were buried in a naturally occurring area to investigate the occurrence of seasonal cycles of germination and dormancy, and to evaluate *in situ* longevity of the seeds. For this, the seeds were exhumed every two months and the germination was evaluated over a wide range of constant temperatures. In the second chapter we evaluated the interannual variation in seed germination of a population of *X. longiscapa* during four consecutive years (2014-2017), in addition to *in situ* longevity and the occurrence of seasonal dormancy cycles in the seeds produced in 2014. In the third chapter the germination requirements (light and temperature) of six species of *Xyris* (*X. asperula, X. pilosa, X. seubertii, X. subsetigera, X.* sp. and *X. trachyphylla*) were determined. From the germination data, the base temperatures ($T_b$) and the thermal requirement for the germination of 50% of the seeds ($\theta_{50}$) of four species were calculated. These data, together with the thermal germination range of each species, were used to predict the germinative behavior of the species in the current scenario and in the temperature increase scenarios. Buried seeds of *X. asperula, X. longiscapa, X. subsetigera* and *X. trachyphylla* form a persistent seed bank and exhibit seasonal cycles of germination and dormancy. In all species, secondary dormancy is acquired from the middle of the rainy season and overcome during the dry season. Seeds of *X. longiscapa* collected in 2014 presented a higher degree of dormancy in relation to the other years possibly resulting from effect of the maternal environment. Seeds of *Xyris* present absolute requirement of light for germination. The thermal germination range and the thermal requirement is

variable among the species. It has been shown that seed germination of *X. subsetigera, X. pilosa* and *X. seubertii* are more affected by the higher temperatures, whereas the seeds of *X. asperula, X.* sp. and *X. trachyphylla* are more tolerant to temperature increase, not presenting reduction in germination percentage when submitted to higher temperatures.

**Introdução Geral**

A família Xyridaceae reúne cinco gêneros predominantemente distribuídos em regiões tropicais (Sajo & Rudall, 1999; Campbell, 2004). O gênero *Xyris* Gronov. ex L. é o maior da família com aproximadamente 300 espécies, em sua maioria herbáceas perenes (Krall, 1988). Os campos rupestres constituem um centro de diversidade para a família Xyridaceae, abrigando cerca de 102 espécies do gênero *Xyris* (Silveira et al., 2016). O termo "campo rupestre" é utilizado para designar a vegetação herbácea-arbustiva associada a afloramentos rochosos predominantemente distribuída em altitudes superiores a 900 m e caracterizada por uma alta biodiversidade e um elevado grau de espécies endêmicas (Alves & Kolbek, 1994; Silveira et al., 2016). Os campos rupestres apresentam um clima sazonal, com estações secas e chuvosas bem definidas (Alves et al., 2014).

A germinação das sementes é uma das fases mais críticas no ciclo de vida da planta, por esse motivo, espécies que habitam ambiente sazonais geralmente apresentam mecanismos para sincronizar a germinação com os períodos favoráveis (Donohue et al., 2010; Baskin & Baskin, 2014). No entanto, as sementes de *Xyris* dos campos rupestres são comumente dispersas sem dormência (Abreu & Garcia, 2005; Dayrell et al., 2017; Giorni et al., 2018). Um estudo com duas espécies de *Xyris* dos campos rupestres brasileiros revelou que sementes enterradas exibem ciclos sazonais de dormência, um mecanismo bem estudado em espécies de ambiente temperado, mas até então inédito para espécies de ambientes tropicais (Garcia et al., 2012). Nos ciclos sazonais de dormência ou dormência cíclica, as sementes adquirem e superam a dormência secundária em resposta às mudanças sazonais na temperatura e umidade do solo (Duarte & Garcia, 2015; Karssen 80/81; Baskin & Baskin, 1985). Esse mecanismo impede que a germinação das sementes ocorra em períodos desfavoráveis para o estabelecimento das

plântulas, sendo, portanto, importante para a regeneração das espécies. Estudos com mais espécies de *Xyris* neotropicais se fazem necessários para determinar se a ocorrência dos ciclos sazonais de dormência é uma característica filogeneticamente conservada em todo o grupo.

Muito além de entender os mecanismos de regeneração das espécies no cenário atual, é importante avaliar como eles serão afetados pelas mudanças climáticas globais. É sabido que a germinação e a dormência em sementes são fortemente influenciadas pelo regime de temperatura (Probert, 2000). Além disso, as projeções de aumento de temperatura são alarmantes (IPCC, 2014), de modo que a identificação de espécies mais vulneráveis é importante em programas de conservação. Apesar da importância dos campos rupestres, devido à sua alta biodiversidade e grau de endemismo, poucos estudos têm abordado o efeito das mudanças climáticas sobre a germinação de sementes de espécies desse ecossistema. Especificamente para espécies de Xyridaceae não há relatos na literatura de estudos com essa abordagem.

Nessa tese, procurou-se estudar aspectos da ecofisiologia da germinação de espécies de *Xyris* dos campos rupestres brasileiros. No primeiro capítulo, avaliou-se a longevidade *in situ* e a ocorrência de ciclos sazonais de dormência em sementes enterradas de três espécies. No segundo capítulo, além das avaliações realizadas no primeiro capítulo, foi também avaliado a variação interanual na germinação/dormência em sementes de uma população de *X. longiscapa*. No terceiro capítulo, a amplitude térmica de germinação e o requerimento térmico das sementes foram utilizados para prever o comportamento das espécies em cenários de aumento de temperatura.

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# Capítulo 1

## Dormancy cycles in buried seeds of three perennial *Xyris* (Xyridaceae) species from the Brazilian *campo rupestre\**

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**Dormancy cycles in buried seeds of three perennial *Xyris* (Xyridaceae) species from the Brazilian *campo rupestre***

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**ABSTRACT**

- Dormancy cycles are an important mechanism for avoiding germination under unfavorable periods for seedling establishment. This mechanism has been scarcely studied in tropical species. Here, we studied three tropical and perennial species of *Xyris*, *X. asperula, X. subsetigera* and *X. trachyphylla*, to investigate *in situ* longevity and the existence of seasonal seed dormancy cycles.

- Seeds of three species of *Xyris* were buried in their natural habitat with samples exhumed bimonthly for 18 months. Germination of exhumed seeds was assessed under a 12h-photoperiod over a broad range of temperatures. Seeds of *X. trachyphylla* were also subjected to treatments to overcome secondary dormancy.

- Seeds of all species are able to form a persistent seed bank and exhibit seasonal changes in germinability. Secondary dormancy was acquired during the rainy summer and was overcome during the subsequent dry season (autumn/winter). Desiccation partially overcome secondary dormancy in *X. trachyphylla* seeds.

- Soil seed bank persistence and synchronization of seed germination under favorable conditions for seedling establishment contributes to the persistence

and regeneration of *X. asperula, X. subsetigera* and *X. trachyphylla* in their natural environment.

**INTRODUCTION**

Seeds may exhibit cyclic changes in germinability driven by environmental factors, a mechanism called seed dormancy cycles (Baskin and Baskin 1985). This mechanism prevents germination from occurring under unfavorable periods for seedling establishment, enhancing survival of plant populations, especially in annual species (Schütz 1997; Copete et al. 2015). Thus, seed dormancy cycles are an important strategy in species inhabiting seasonal environments, where favorable conditions for germination and seedling survival are restricted to certain times of the year (Baskin & Baskin 1985). The majority of studies involving seed dormancy cycles have been conducted with species from temperate regions (Bouwmeester & Karssen 1989; Schütz 1997; Handley & Davy 2005), however, there are examples of studies in desert regions (Baskin et al. 1993; Cao et al. 2014) and, more recently, in tropical regions (Garcia et al. 2012; 2014).

In seeds with cyclic dormancy, the transition between non-dormant and dormant state (and vice-versa) are not abrupt (Baskin & Baskin 1985). Conditional or relative dormancy is an intermediate stage in which seed germination is restricted to a subset of environmental conditions possible to the species (i.e., the conditions in which non-dormant seeds germinate; Baskin & Baskin 1985; Finch-Savage & Leubner-Metzer 2006). Soil temperature and moisture are the main factors modulating the acquisition

and overcome of secondary dormancy in buried seeds (Karssen 1980/81; Benech-Arnold et al. 2000; Duarte & Garcia 2015), so that the dormancy patterns of a particular species should match the environmental conditions of their natural habitat (Baskin & Baskin 1985; Schütz 1997).

The Brazilian *campo rupestre* is the only tropical ecosystem with reports of species with seasonal dormancy cycles - three species of Eriocaulaceae (*Syngonanthus anthemidiflorus* (Bong) Ruhl., *Syngonanthus verticilatus* (Bong.) Ruhl. and *Comanthera bisulcata* (Körn.) L.R.Parra & Giul.) and two species of Xyridaceae (*Xyris bialata* Malme and *Xyris peregrina* Malme) (Garcia et al. 2012, 2014). *Campo rupestre* is distributed predominantly at high altitudes (900 m a.s.l) and are characterised by high plant diversity and endemism (for more details see Alves et al. 2014 and Silveira et al. 2016). These environments exhibit a marked seasonality, with dry winters and wet summers, and therefore possess the ideal conditions for selection for dormancy cycling and secondary dormancy induction (Garcia et al. 2012; Silveira et al. 2016). *Xyris* Gronov. ex L. (Xyridaceae) is one of the most representative genera in *campo rupestre* with approximately 102 species (Le Stradic et al. 2015a; Silveira et al. 2016), including some endemic and threatened species (Wanderley 2011).

The ability to form a persistent seed bank and the occurrence of seasonal dormancy cycles described for *X. bialata* and *X. peregrina* (Garcia et al. 2012) may represent a pattern for species of *Xyris* from Brazilian *campo rupestre*. The two families in which seasonal dormancy cycles have been described in tropical regions (Eriocaulaceae and Xyridaceae) are phylogenetic sister groups (Linder & Rudall 2005). Thus, this mechanism could be phylogenetically conserved in these families. In addition, seed dormancy cycles were also described for a *Xyris* species (*Xyris tennesseensis*) from a temperate climate (Baskin & Baskin 2003). The previous studies

involving tropical species, however, were conducted at a single temperature, thus preventing identification of conditional dormancy, the key mechanism involved in seed dormancy cycles.

Here, for the first time, we evaluate the changes in germinability in buried seeds of tropical species of *Xyris* over a wide range of temperatures. Our objectives were to: (i) assess *in situ* longevity to determine the ability of species to form a persistent soil seed bank, (ii) examine the existence of seasonal dormancy cycles in buried seeds and if present, (iii) evaluate the efficacy of treatments to overcome secondary dormancy in one of the species.

## MATERIAL AND METHODS

### Seed collection and study area

Mature fruits were collected between July and August 2014 from natural populations occurring in *campo rupestre* areas in the Serra do Cipó National Park, Minas Gerais, Brazil (19º 12'-34'S; 43 27'-38'W). The Serra do Cipó is a geomorphological landform belonging to the Espinhaço mountain range. The region has altitude tropical climate (Cwb) according to the Köppen classification, with marked seasonality - rainy summers and dry winters. For each species, fruits were collected from at least 20 individuals. The fruits were processed manually and the seeds were separated with the aid of sieves. The seeds were then placed in an amber bottle for storage at room temperature (approximately 27 ºC) until the experiments (within 2 months).

### Study species

All studied species are herbaceous and perennial. Of the three species, *Xyris asperula* Mart. has the broadest distribution, occurring from the south region (Paraná State) to the

northeast region of Brazil. *Xyris trachyphylla* Mart. is distributed in *campo rupestre* of the southeast region of Brazil and in Bahia State. Until now, populations of *X. subsetigera* Malme were known only from *campo rupestre* of the Espinhaço mountain range of Minas Gerais State (Wanderley 2011); the species is currently included in the IUCN Red List of Threatened Species as a Near Threatened species (The IUCN Red List of Threatened Species 2016).

**Seed traits and germination tests**

Seed size (length and width) was measured in 100 seeds with digital calipers. Dry mass was determined after drying seeds (four replicates of 150) at 105 °C for 24 hours and subsequent weighing with a digital scale. Dry mass was calculated for a single seed. For determining seed water content, four replicates containing 50 mg of seeds were weighed before (fresh mass) and after drying, as described above. Seed water content was calculated based on fresh mass.

For germination tests, six replicates of 25 seeds were placed in Petri dishes containing a double-layer of filter paper moistened with nystatin solution (0.5%) to prevent fungal contamination. Germination was conducted in germination chambers at constant temperatures and 12h-photoperiod ($\sim$40 $\mu$mol m$^{-2}$ s$^{-1}$). Seed germination were evaluated daily for 30 days with the aid of a stereomicroscope. The requirement for light for germination was previously confirmed for seeds of all three species. The emergence of the vegetative axis was used as the criterion for germination.

**Germination changes in buried seeds**

Seeds (approximately 250) were placed in water-permeable nylon bags of 0.1-mm mesh and buried 5-cm deep in the soil of an area of the natural occurrence of the species in

September 2014 (early spring). Every two months for 18 months, six samples (bags) of each species were exhumed for germination tests at constant temperatures of 15, 20, 25 and 30 ºC under 12h-photoperiod (seed germination of *X. asperula* was also evaluated at 35 ºC). Each exhumed bag corresponded to one replicate at each temperature. Germination was conducted and evaluated as described above. Germinability was used as the indicator of seed viability.

**Climatic data**

Temperature and soil moisture data were monitored with sensors (5TM Soil Moisture and Temperature Sensor) connected to a data-logger (Em50, Decagon, Pullman, WA, USA) and buried in the same place as the seeds. Measurements were taken every hour throughout the experimental period. Monthly precipitation data were obtained from the meteorological station of Conceição do Mato Dentro, Minas Gerais (INMET – Instituto Nacional de Meteorologia 2016), located approximately 30 km from the study site.

**Overcoming secondary dormancy**

Secondary dormant seeds of *X. trachyphylla* (exhumed after 10 months of burial) were stored for one month at ambient room temperature (25±3 ºC) after exhumation and submitted to dormancy-breaking treatments. The treatments were: application of gibberellic acid (GA$_3$) at 100 µM (Cao et al., 2014) and seed drying at 45 °C for 24 h (45 °C/24 h) or 48 h (45 °C/48 h). The seeds stored for one month were considered as control. In the GA$_3$ treatment, seeds were placed in Petri dishes containing a double-layer of filter paper moistened with 5 mL of the solution, and kept in the dark for 96 h in a germination chamber at 20 °C. After this period, the seeds were transferred to new dishes, irrigated with nystatin and returned to the growth chamber at 20 °C and 12h-

photoperiod for the evaluation of germination. Four replicates of 25 seeds were used for each treatment. Germination was assessed after 30 days using the same criteria as described above.

**Statistical analysis**

The data from the experiment to overcome secondary dormancy was tested for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's test). Normal and homoscedastic data were analysed using ANOVA and the means were compared using Tukey test at 5% of probability.

**RESULTS**

**Seed traits**

Seeds of the three studied *Xyris* species were very small and light (Table 1); seeds of *X. trachyphylla* were largest and had the largest dry mass. Seed water content varied between 11.4 and 16.4%, with the highest value being for *X. asperula*.

**Germination changes in buried seeds**

Seeds of studied species remained viable in the soil for at least 14 months and exhibited seasonal changes in germinability across all temperatures tested (Fig. 1). Secondary dormancy was acquired during the rainy season (spring/summer), and alleviated during the subsequent dry season in autumn/winter (Fig. 2). In general, for all species, a decrease in germinability was first observed at the extreme temperatures (15 and 30-35 °C).

Seeds of *X. trachyphylla* exhumed at six and eight months exhibited absolute dormancy (germination below 1% at all temperatures). However, some of the *X.*

*asperula* and *X. subsetigera* seeds exhumed at the same times exhibited germinability of >14% at 20 and 25 ºC. Seeds of *X. trachyphylla* showed a complete cycle: non-dormant – conditionally dormant – absolute dormancy (ND – CD – D), whereas seeds of *X. asperula* and *X. subsetigera* exhibited a ND – CD cycle. No germination was observed in seeds during burial due to their requirement for light to germinate. Periods of lower germination coincided with the months of higher soil moisture, while increasing germination followed a decrease in soil moisture.

**Overcoming secondary dormancy**

Seeds of *X. trachyphylla* buried in the soil for 10 months germinated to <5% at the optimal temperature (20 ºC). After dry storage for a month (control) these seeds showed an increase in germination (28%). Drying at 45 °C for 24h and $GA_3$ application did not promote an increase in germination compared to the control (32 and 45% respectively), whereas drying at 45 °C for 48h increased germination, which reached 61% (Fig. 3).

**DISCUSSION**

This study reports seasonal cycles of dormancy in three perennial tropical plant species. For the first time, it was possible to demonstrate conditional dormancy in tropical species with cyclic dormancy. The temporal pattern of acquisition and alleviation/overcoming of secondary dormancy seems to be the same for all tropical species of Xyridaceae and Eriocaulaceae inhabiting Brazilian *campo rupestre*. In these species, including those of the present study, secondary dormancy is acquired during the rainy season (spring/summer), which is then alleviated during the dry season (Garcia et al. 2014; Duarte & Garcia 2015). This mechanism prevents germination from occurring at the end of the rainy season, thus avoiding death of seedlings in the subsequent dry

and unfavorable season (Garcia et al. 2012). Our results, combined with those of Garcia et al. (2012, 2014) and Duarte & Garcia (2015), indicate that seasonal dormancy cycles may be a recurrent mechanism in seeds of plants typical of Brazilian *campo rupestre*.

Seeds of *X. asperula* and *X. subsetigera* presented slightly higher germination after exhumation in certain months than before burial (freshly matured seeds), which suggests that a small fraction of seeds had primary dormancy. Once seeds of all species are dispersed during dry season, seeds dispersed in a non-dormant state are able to germinate in the subsequent rainy season. However, buried seeds (incorporated into the soil seed bank) will not germinate due to the absence of light, since these seeds have an absolute light requirement to germinate (positive photoblastism; Abreu & Garcia, 2005; T. G. S. Oliveira, Q. S. Garcia, unpublished data). Studies on seed germination of species of *Xyris* from the *campo rupestre* were all found to possess a light requirement for germination, but only one has primary dormancy – *Xyris obtusiuscula* (Abreu & Garcia 2005; Garcia et al. 2012; Le Stradic et al. 2015b). The species studied here are herbaceous, produce very small seeds and occur in environments with regular periods of drought. Thus, it was expected that the seeds would exhibit some kind of dormancy (Jurado & Flores 2005; Baskin & Baskin 2014). However, in *campo rupestre,* unlike other seasonal environments, the proportion of species which produce non-dormant seeds is higher than those that produce dormant seeds (Dayrell et al. 2017). Although most seeds do not have primary dormancy, the acquisition of secondary dormancy is an important strategy for the persistence of these species in a seasonal environment (Baskin & Baskin 1985).

Since the Brazilian *campo rupestre* presents adverse conditions for seed germination at certain times of the year, soil seed bank formation can be an important adaptive strategy in these environments. In fact, many species typical of the *campo*

*rupestre* are known to form a persistent soil seed bank (Cheib & Garcia 2011; Munné-Bosch et al. 2011; Garcia et al. 2014), including species of *Xyris* (Garcia et al. 2012). In the present study, all species maintained viability for longer than a year, indicating that they are capable of forming at least a short-term persistent soil seed bank (*sensu* Walck et al. 2005). The small seed size and the requirement for light for germination probably contribute to seed incorporation and persistence in the soil seed bank (Milberg et al. 2000, Honda 2008). Specifically, for *Xyris* seeds, burial prevents seed germination, since the seeds require light for this to occur. As the seeds are dispersed during the dry season in a non-dormant state, untimely rain could promote germination and lead to subsequent seedling death. Thus, seed burial can be important for avoiding periods unfavorable for germination in non-dormant, positive photoblastic seeds.

Seasonal temperature changes have been described as the main environmental factor modulating cyclic dormancy in temperate species (Van Assche & Vanlerberghe 1989; Bouwmeester & Karssen 1993). In tropical regions, on the other hand, the temperature range is substantially narrower than in those environments. The soil temperature data obtained from the sensors demonstrated that at the depth of 5 cm, the seeds are subjected to temperature ranges narrower than 10 ºC, considering all seasons. The average minimum temperature varies between 13.6 and 20 ºC, and the average maximum temperature is between 18.4 and 28.4 ºC. This suggests an important role of soil moisture (which varied widely), and not only temperature, in the acquisition and overcome of secondary dormancy (Duarte & Garcia 2015). In seeds of *Sygonanthus verticilatus* and *Comanthera bisulcata* (Eriocaulaceae), humid soil induces secondary dormancy in seeds regardless of temperature regime, while dormancy alleviation occurs in dry periods (Duarte & Garcia 2015). The same mechanism probably occurs in *Xyris* seeds. Additional studies are required to elucidate the role of these two abiotic factors

(temperature and moisture), alone or together, in the modulation of cyclic dormancy in seeds of *Xyris* species.

Studies with tropical species of Xyridaceae and Eriocaulaceae indicate that the cyclical dormancy is directed through the hormonal balance between abscisic acid (ABA) and gibberellins in the seeds (Garcia et al. 2012; Duarte 2014). Gibberellins and ABA are the main hormones involved in the regulation of seed germination and dormancy (Finch-Savage & Leubner-Metzger 2006). ABA is a positive regulator for inducing dormancy and therefore a negative regulator for germination, while gibberellins are their antagonists (Kucera et al. 2005; Nambara et al. 2010). Thus, treatments that alter the hormonal balance by decreasing ABA levels, increasing GAs levels, or both, contribute to overcoming physiological dormancy (Baskin & Baskin 2004). Exogenous application of gibberellic acid (GA$_3$ 100μM) did not promote the overcome of secondary dormancy in *X. trachyphylla* seeds. The ineffectiveness of GA$_3$ application may be associated with low sensitivity of the seeds to exogenous gibberellin (Derkx & Karssen 1993) or due to the concentration used, which may not have been sufficient.

The drying of the seeds at 45 ºC, on the other hand, may have acted as a heat treatment, reducing ABA levels and promoting the germination of seeds. This hypothesis is supported by some studies that have shown that high temperatures reduce ABA levels in seeds and could act in dormancy alleviation (Jiménez et al. 2008; Vieira et al. 2016). Thus, the slow drying (at soil temperatures <29 ºC) to which the buried seeds are subjected in the natural environment during the dry season, probably causes a gradual alleviation of dormancy, reinforcing the importance of soil moisture in seed dormancy cycles of *Xyris* species. In this sense, drying the seeds at 45 ºC for periods >48 h could be more effective in overcoming secondary dormancy. Seed desiccation

also enhanced germination in exhumed seeds of three temperate weed species (Bouwmeester & Karssen 1989). We suggest that water loss from seeds could be a signal for dormancy release in seeds with cyclical dormancy, especially for those of tropical environments.

Seeds of the studied species demonstrated mechanisms to allow persistence in a seasonal environment: persistence in soil and seasonal dormancy cycles. These species produce a large number of seeds during each reproductive season (T.G.S. Oliveira, personal observation) and seed accumulation in the soil enhances the chance of species regeneration, especially after disturbances (Luzuriaga et al. 2005; Saatkamp et al. 2014), such as fire, which is a recurrent disturbance in *campo rupestre* (Silveira et al. 2016). The ability of seeds to maintain their viability is crucial to the occurrence of repeated dormancy cycles. Synchronization of germination driven by environmental factors (mainly temperature and humidity) ensures that germination occurs under optimum conditions for seedling establishment, hence reducing the risk of reproductive failure. Seed drying (desiccation) partially alleviates seed dormancy and is probably the mechanism modulating dormancy levels under field conditions during the dry season. These traits demonstrate that there is a narrow window of favourable environmental conditions for successful recruitment of *Xyris* species, which requires fine synchrony with the seasonality of the Brazilian *campo rupestre*.

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**TABLES AND FIGURES**

**Table 1.** Size (length and width), dry mass and water content of *Xyris* seeds (mean ± sd).

| Species | Length (mm) | Width (mm) | Dry mass (µg) | Water content (%) |
|---|---|---|---|---|
| *X. asperula* | 0.40 ± 0.05 | 0.24 ± 0.03 | 12.5 ± 0.46 | 16.4 ± 1.41 |
| *X. subsetigera* | 0.48 ± 0.06 | 0.25 ± 0.04 | 9.4 ± 0.56 | 11.4 ± 0.58 |
| *X. trachyphylla* | 0.75 ± 0.06 | 0.31 ± 0.04 | 25.5 ± 0.70 | 11.6 ± 1.05 |
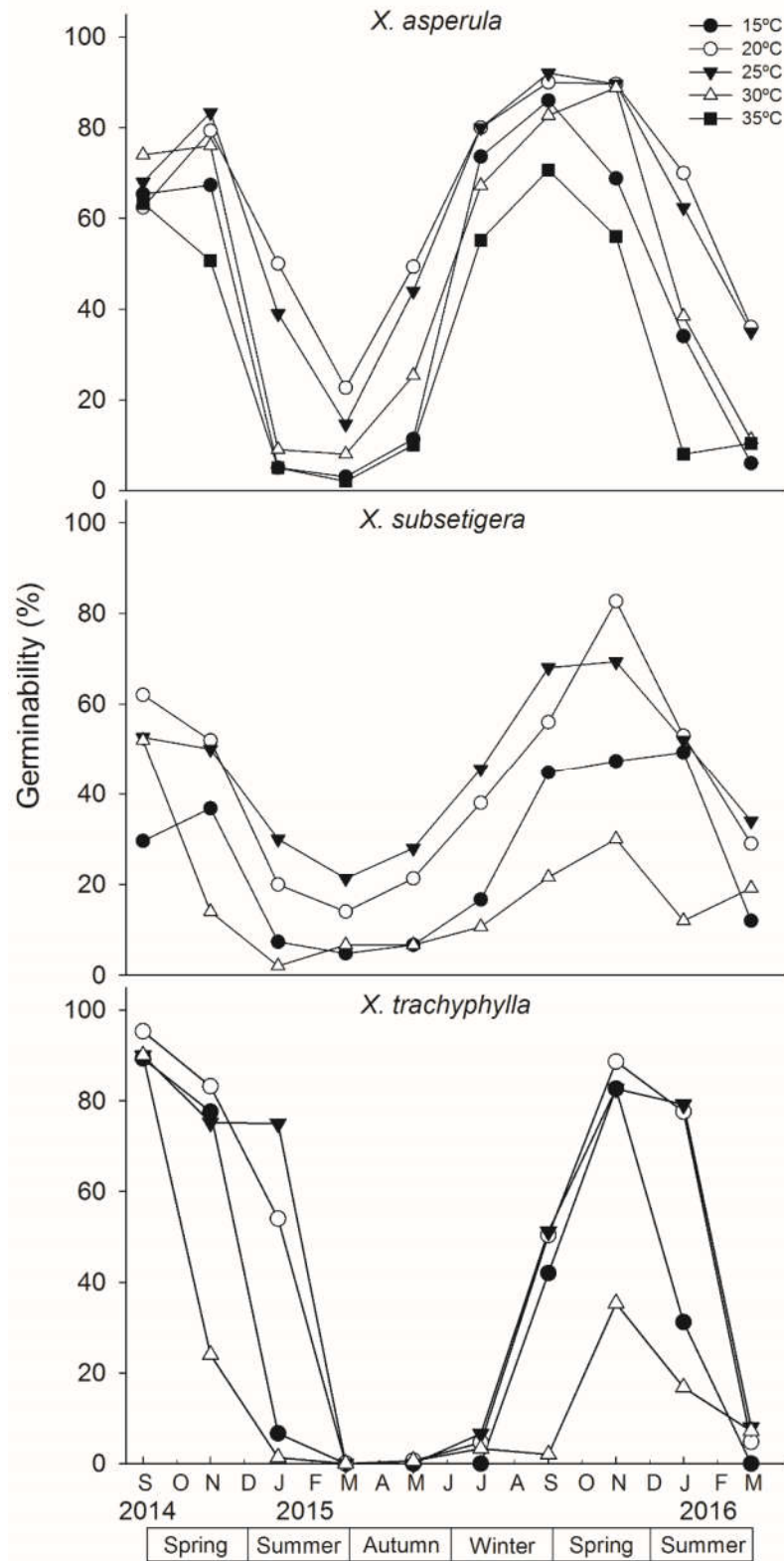
**Fig. 1.** Germination percentages of *X. asperula*, *X. subsetigera* and *X. trachyphylla* seeds at different temperatures under 12h-photoperiod during 18 months of burial in the soil in Serra do Cipó, Brazil.
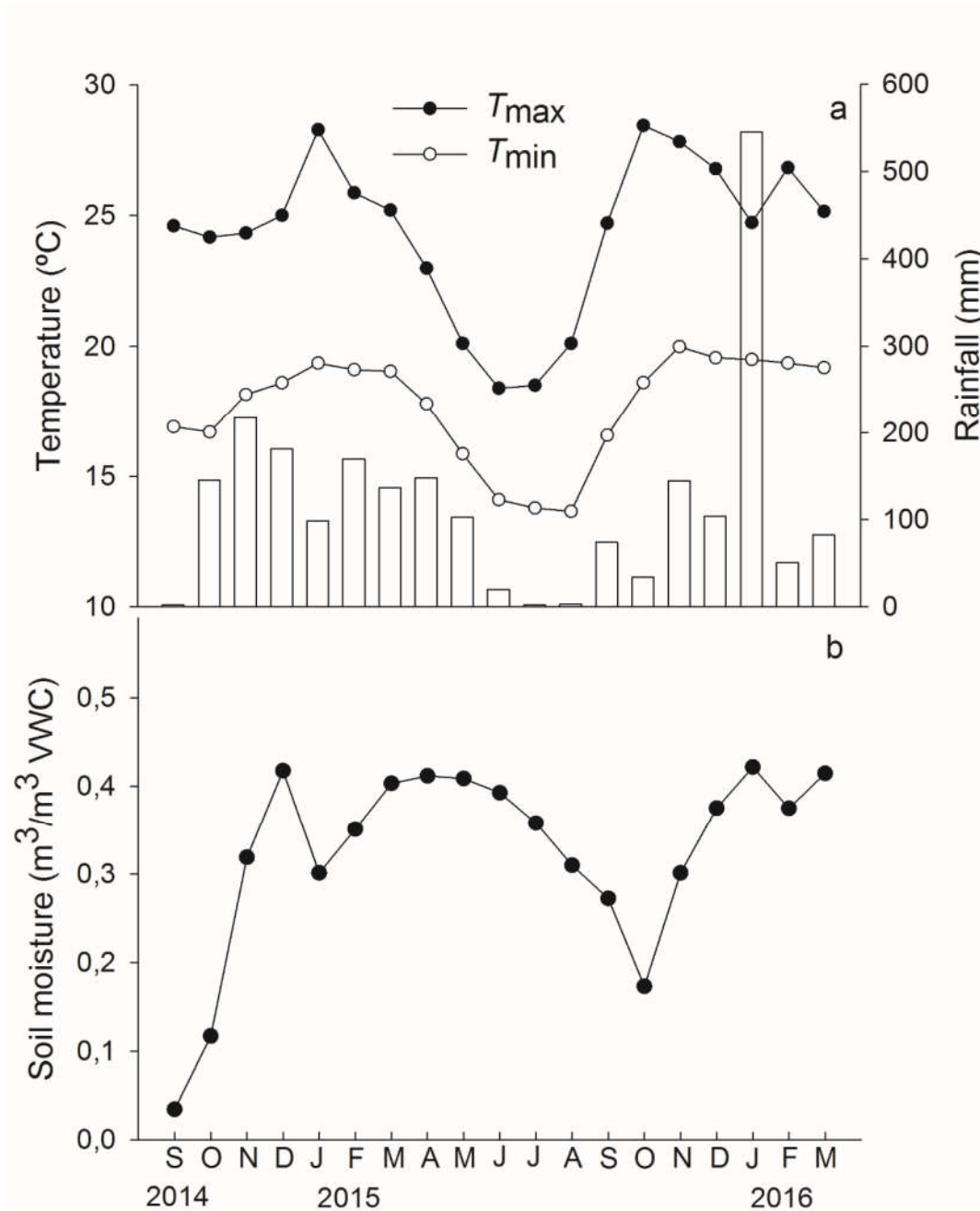
**Fig. 2.** Climate data during the seed burial period in Serra do Cipó, Brazil: monthly precipitation, mean maximum and mean minimum soil temperature (a); changes in soil moisture (b). Monthly precipitation data were obtained from the Conceição do Mato Dentro weather station, MG, Brazil.
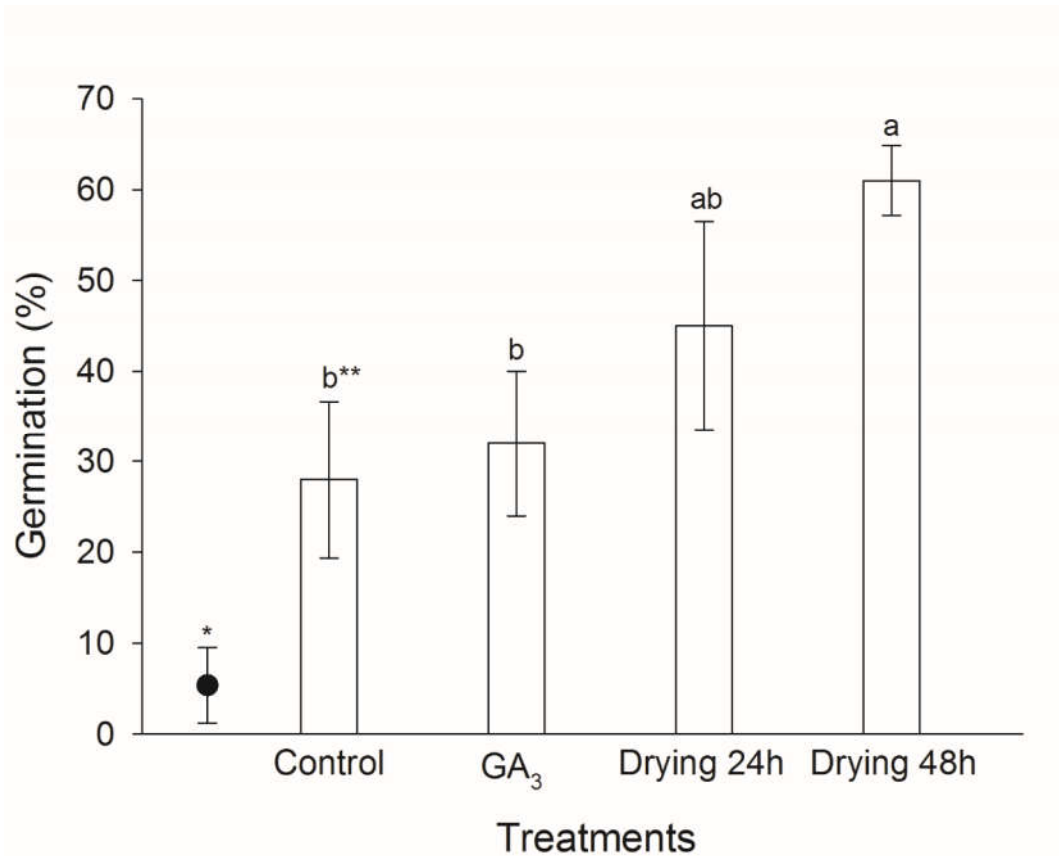
**Fig. 3.** Seed germination of *X. trachyphylla* at 20 ºC after treatments to overcome secondary dormancy. Black circle indicates germination of exhumed seeds buried in soil for 10 months. The control treatment includes exhumed seeds stored at room temperature for 1 month in the laboratory. Same letters represent absence of significant differences by Tukey test at 5% of probability. Asterisks compare only seeds exhumed immediately and after storage for 1 month (control). Bars are mean ± SD.

# Capítulo 2

**Germination ecophysiology of a neotropical herb *Xyris longiscapa*: interannual changes in seed germination and seasonal dormancy cycles\***

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**\* Artigo nas normas do periódico Seed Science Research**

**Germination ecophysiology of a neotropical herb *Xyris longiscapa*: interannual changes in seed germination and seasonal dormancy cycles**

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**Abstract**

Germination ecophysiology was investigated in a natural population of *Xyris longiscapa*, a perennial herbaceous species endemic of the Brazilian *campo rupestre*. Seeds were collected in four consecutive years (2014 to 2017) to evaluated germination responses in a range of temperatures (from 15 to 30ºC). Light requirement was evaluated in the seeds collected in 2014. Seeds collected in 2014 were also buried in soil in the natural habitat of the species to evaluated seed longevity and changes in germinability at different temperatures over the time. Seeds showed an absolute light requirement to germinate. Seed germination is affected by temperature, collection year and interaction between these two factors. Conditional primary dormancy is found in seeds collected in 2014 which germinate only at 15 and 20ºC. Seeds collected in 2015, 2016 and 2017 germinate in a temperature range from 15 to 25ºC. Buried seeds remained viable in soil at least for 14 months and exhibit seasonal dormancy cycles. Secondary dormancy is induced in rainy season and alleviated during dry season in a conditional dormancy/dormancy cycle. The degree of primary dormancy probably is influenced by environmental factors experienced by seeds during maturation. Primary dormancy (when present), seed persistence in soil and seasonal dormancy cycles are

strategies of *X. longiscapa* to enhance its regeneration success in a harsh environment of Brazilian *campo rupestre*.

**Keywords:** secondary dormancy, soil seed bank, Xyridaceae, Brazilian *campo rupestre*

**Introduction**

Strategies to minimize the risk of seed germination failure are important to plant regeneration success, especially in seasonal or unpredictable environments (Donohue et al., 2010; Ramos et al., 2017). Seed dormancy is an adaptative trait that matches seed germination to favorable environmental conditions, enhancing the chance of seedling survival in the field (Jurado and Moles, 2003). Seeds may present primary dormancy when they are dispersed in a dormant state and/or secondary dormancy which is acquired after dispersal (Baskin and Baskin, 1985; Bewley, 1997). The degree of primary dormancy of a seed is influenced both by genetic factors and environmental conditions experienced by seeds during the maturation (Fernández-Pascual et al., 2013; Baskin and Baskin, 2014). Due to the heterogeneity of environmental conditions in the field, variation in seed germinative behavior between populations, years of collection and even in individuals are frequently observed (Andersson and Milberg, 1998; Gutterman, 2000). The environment under which the seeds were matured is commonly referred as the maternal environment (Fenner, 1991).

Secondary dormancy is induced by environmental conditions unfavorable to seed germination or by conditions preceding unfavorable periods (Vleeshouwers et al., 1995; Vázquez-Yanes and Orozco-Segovia, 1996). The acquisition and release of secondary dormancy can follow a cyclic pattern driven by environmental seasonality in a mechanism called cyclic dormancy or seed dormancy cycles (Baskin and Baskin,

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1985). Seeds with cyclic dormancy exhibit changes in dormancy status in response to changes in temperature and/or humidity (Karssen 80/81; Baskin and Baskin, 1985; Duarte and Garcia, 2015). Conditional dormancy is a transition between non-dormant and dormant state, which seeds germinate in a limited set of environmental conditions (Baskin and Baskin, 1985). Seed dormancy cycles is observed in buried seeds of some species (mainly annual species) and represents a strategy to synchronize seed germination to favorable periods for seedling establishment (Schütz, 1997; Copete et al., 2015; Oliveira et al., 2017).

In general, seasonal environments present higher proportions of plant species producing dormant seeds (primary dormancy) compared to more climatic stable environments (Jurado and Flores, 2005; Baskin and Baskin, 2014), however, the Brazilian *campo rupestre* is an exception to this rule, since most of the plant species that inhabit this seasonal ecosystem disperse seeds without dormancy (Dayrell et al., 2017). On the other hand, the cyclic dormancy has been reported for some species typical of this environment. Species from Brazilian *campo rupestre* that present cyclic dormancy (belonging to families Xyridaceae and Eriocaulaceae) are dispersed in a non-dormant state and acquire dormancy to avoid seed germination during unfavorable periods, contributing to the successful regeneration of these species (Garcia et al. 2012, 2014; Duarte and Garcia 2015; Oliveira et al., 2017). Further studies are needed to elucidate whether cyclic dormancy is a pattern within these families and whether it can be found in other representative groups of this ecosystem.

*Xyris longiscapa* A. Nilsson (Xyridaceae) is a perennial herb endemic of the Brazilian *campo rupestre*, distributed along the Espinhaço mountain range – Minas Gerais and Bahia States (Wanderley, 2011). This species produces very small seeds which presents an absolute light requirement for germination (Abreu and Garcia, 2005).

In this study, we investigate seed germination ecophysiology of a natural population of *X. longiscapa* to: (i) determine seed germination requirements (light and temperature); (ii) investigate interannual changes in seed germination patterns; (iii) evaluate seed longevity in field and (iv) verify the existence of seasonal changes in germinability in buried seeds.

**Material and Methods**

*Seed collection*

Mature fruits were collected in four consecutive years (2014 to 2017) of the same population of *X. longiscapa* in the Serra do Cipó National Park, Minas Gerais, Brazil (19º 12'-34'S; 43 27'-38'W). Seeds were collected from at least 20 individuals in May/June of each year. Fruits were processed manually, and the seeds were separated with the aid of sieves.

*Germination requirements and interannual changes on seed germination*

Germination tests were conducted immediately after seed collection. Seeds collected in 2014 were placed to germinate at constant temperatures from 10 ºC to 40 ºC (with intervals of 5 ºC), under 12h-photoperiod ($\sim$ 40 $\mu$mol m$^{-2}$ s$^{-1}$) or continuous darkness in germination chambers. Seeds collected in 2015, 2016 and 2017 were placed to germinate at constant temperatures from 15 ºC to 35 ºC (with intervals of 5 ºC) only under 12h-photoperiod. For all years of collecting, six replicates with 25 seeds were placed in Petri dishes containing a double-sheet of filter paper irrigated with nystatin solution (0.5%) to prevent fungal contamination (Oliveira et al., 2017). Germination was evaluated daily for 50 days or until stabilization (three consecutive equal evaluations). In the dark condition, germination was evaluated under safety green light.

Seeds were considered germinated by the protrusion of the vegetative axis (Oliveira et al., 2017).

*Seed longevity and dormancy cycles*

Seeds of *X. longiscapa* (approximately 250) collected in 2014 were placed in nylon bags (0.1 mm mesh) and buried in the soil at 5 cm depth in the same area of studied population. The seeds were buried in September 2014, which coincides with the beginning of spring in the southern hemisphere. For 18 months, every two months, six samples (bags) containing the seeds were exhumed for evaluation of germination in the laboratory. Each exhumed bag corresponded to one replicate in all tested temperatures. The germination of exhumed seeds was evaluated at constant temperatures of 15, 20, 25 and 30 °C under 12h-photoperiod (based on germination requirements of the newly collected seeds). Germination was conducted and evaluated as described above.

*Climatic data*

Changes in soil moisture and temperature were monitored by sensors buried in the same place the seeds (5TM Soil Moisture & Temperature Sensor) connected to a data-logger (EM50, Decagon). Data of average monthly precipitation were obtained from the meteorological station of Conceição do Mato Dentro, Minas Gerais, distant approximately 30 km from the study site (INMET - Instituto Nacional de Meteorologia, 2017). Drought index was calculated by the ratio between monthly precipitation and potential evapotranspiration (Vicente-Serrano et al., 2010). Values below 1 indicate water deficit. The drought index was calculated to assess whether the water availability affects the seed dormancy degree.

*Data analysis*

Seed germination among temperatures and years of collection were analyzed using a two-way ANOVA. The data were previously tested for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's test). Means were compared using Tukey test at 5% probability.

**Results**

*Germination requirements and interannual changes on seed germination*

Seeds of *X. longiscapa* germinate only in the presence of light. Germination temperature and year of seed collection had significant effects on seed germination, with an interaction between factors ($p < 0.0001$ for all). Temperature range for germination was 15-20ºC for seeds collected in 2014 and 15-25ºC for seeds collected in 2015, 2016 and 2017 (Fig. 1). At 15ºC, higher germination was observed in seeds collected in 2016 (71%). No differences were found in germination at 20ºC of seeds collected in 2015, 2016 and 2017 (germination percentages above 70%). At 25ºC, highest germination percentages were found in seeds collected in 2016 and 2017 ($\geq$ 68%). Germination at 30ºC was very low (below 2%). Climate data of four years of collection are presented in Fig. 2.

*Seed longevity and dormancy cycles*

Buried seeds of *X. longiscapa* exhibit dormancy cycles strongly modulated by seasonal changes in water availability in the soil (Fig. 3). Secondary dormancy is induced in rainy season (spring/summer) and alleviated during dry season (autumn/winter). Seeds

exhumed after four months (January) show absolute dormancy (absence of germination at all temperatures). After 12 months of burial, seeds reacquired germinability, reaching the highest germination percentages after 14 months of burial (November). Thus, seeds of *X. longiscapa* remained viable in the soil at least for 14 months. During burial experiment, seed germination was not observed in the soil because of the light requirement. Dormancy cycle found in *X. longiscapa* could be classified as a conditional dormant – dormant cycle (CD – D cycle).

**Discussion**

Seed germination of *X. longiscapa* seeds collected in 2014 presented marked differences in relation to seeds collected in 2015, 2016 and 2017. The absence of germination at 25ºC in seeds collected in 2014 indicate that these seeds were dispersed with conditional dormancy, once seeds collected in the subsequent years germinate in this temperature. The environmental factors that affect the primary dormancy degree in seeds includes temperature, water availability, photoperiod and nutritional status of mother plant (Fenner, 1991). The response of seeds to each environmental factor is variable, for example, seed dormancy increases with increase maturation temperature in some species (Hume, 1994) while in other species, higher temperatures decrease seed dormancy degree (Fenner, 1991; Sharif-Zadeh and Murdoch, 2000). In this study, we were not able to correlate climatic variables (precipitation and temperature) with the highest dormancy degree found in seeds collected in 2014, however we suggest that the extension of water stress experienced by seeds during maturation in this year (evidenced by the drought index) could have contributed to this behavior.

The highest germination percentages during the burial experiment was observed in seeds exhumed at 14 months (spring). Thus, at least 70% of the seeds collected in

2014 were viable at the beginning of the experiment. In this sense, a fraction of seeds was dormant at dispersal. Seed dormancy is not crucial to seed bank formation (Thompson et al., 2003; Honda, 2008), however, it prevents seed germination shortly after dispersal and thus favors seed burial in soil (Schütz, 1997). Once buried in the soil, seeds of *X. longiscapa* will not germinate because of the light requirement. In this way, the proportion of primarily dormant seeds (which could vary between years) probably influence the amount of seeds incorporated into the soil seed bank (seed burial). Seeds produced in 2015, 2016 and 2017 presented less dormancy degree and therefore could germinate after an untimely rain, resulting in lower chance of seedling survival.

Buried seeds of *X. longiscapa* forms at least a short-term persistent soil seed bank (*sensu* Walck et al., 2005), i.e., seeds remained viable in soil for more than a year. The capacity to form a persistent seed bank is found in other *Xyris* species from Brazilian *campo rupestre* (Garcia et al., 2012; Oliveira et al., 2017) and could be associated with high desiccation tolerance of imbibed seeds. It has been demonstrated that imbibed seeds of two tropical *Xyris* species (*X. asperula* Mart. and *X. trachyphylla* Mart.) can be dehydrated without decrease in survival and germination (Oliveira et al., 2018). Seed persistence in the soil is important to spread germination over time and therefore enhance the chance of seedling survival (Long et al., 2014). These trait is particularly important to species inhabiting seasonal environments. Longevity of *Xyris* seeds in field (uncontrolled conditions) indicate that seeds could be easily stored for use in restoration programs.

During the permanence in the soil seed bank, seeds of *X. longiscapa* present seasonal changes in dormancy status. Dormancy cycles patterns in *X. longiscapa* seeds is like other species from Brazilian *campo rupestre* (Garcia et al., 2014; Duarte and Garcia, 2015), including *Xyris* species (Garcia et al., 2012; Oliveira et al., 2017).

Secondary dormancy is acquired in the middle of the rainy season and is alleviated throughout the dry season. This pattern avoids that seed germination occurs very late in the rainy season and allows the seed to germinate at the beginning of the rains. Soil moisture has been suggested as the main factor modulating the cyclical dormancy in tropical species (Duarte and Garcia, 2015; Oliveira et al., 2017). This idea is supported by the small temperature variation of tropical environments compared to temperate or desert environments. Soil moisture, on the other hand, presented large seasonal variations. Periods with high soil moisture coinciding with periods of lower seed germination and periods with lower soil moisture coinciding with higher seed germination. In addition, seed drying (45ºC for 48h) partially overcome secondary dormancy in *X. trachyphylla*, a species with cyclic dormancy (Oliveira et al., 2017), which reinforces the role of soil moisture in the acquisition and overcoming of secondary dormancy.

In summary, seeds of *X. longiscapa* showed different strategies to persist in Brazilian *campo rupestre*. The role of environmental factors on seed dormancy degree (effects of maternal environment) needs to be better investigated. Differences in seed germination behavior provides relevant information to conservation efforts. In studies involving determination of dormancy, we recommend collections in different years and even in different populations of a species, to verify if seed dormancy is innate (genetic factors) or environmentally regulated. Our results show one more *Xyris* species that forms persistent soil seed banks and exhibit seasonal dormancy cycles. Until now, all investigated tropical *Xyris* species inhabiting Brazilian *campo rupestre* have these traits, indicating a pattern of this group (Garcia et al., 2012; Oliveira et al., 2017). Seasonal dormancy cycles match seed germination with the favorable conditions and therefore contributed to regeneration success of *X. longiscapa* in this seasonal environmental.

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**Figures**
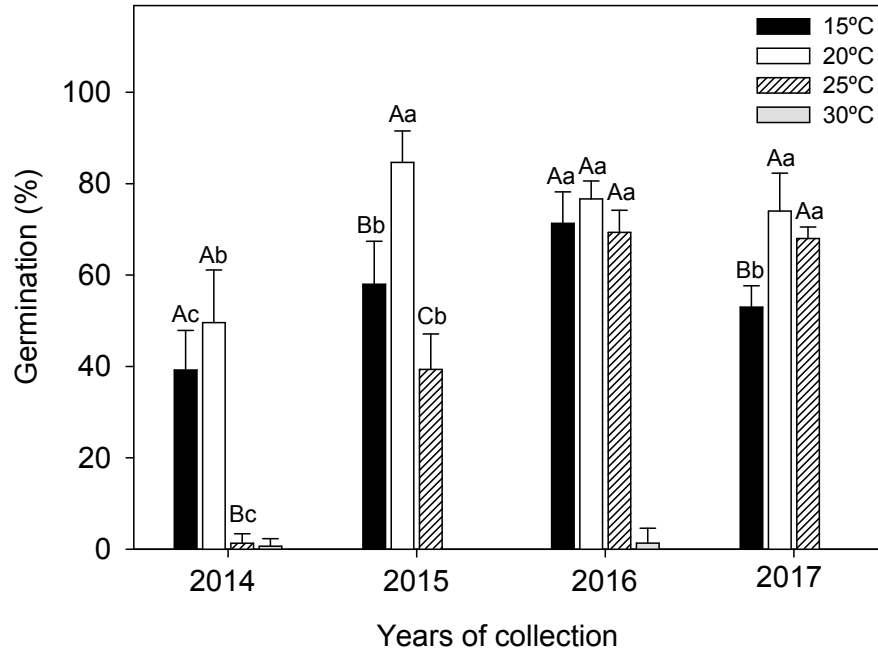


**Figure 1.** Interannual changes in seed germination of *X. longiscapa* seeds in different temperatures. The same uppercase letters indicate no significant differences within each year of collection, while the same lowercase letters indicate no significant differences among germination temperatures in different years of collection, by the Tukey test at a 5% level of probability. Seed germination at 30ºC was not included in the analysis (Mean ± standard error).
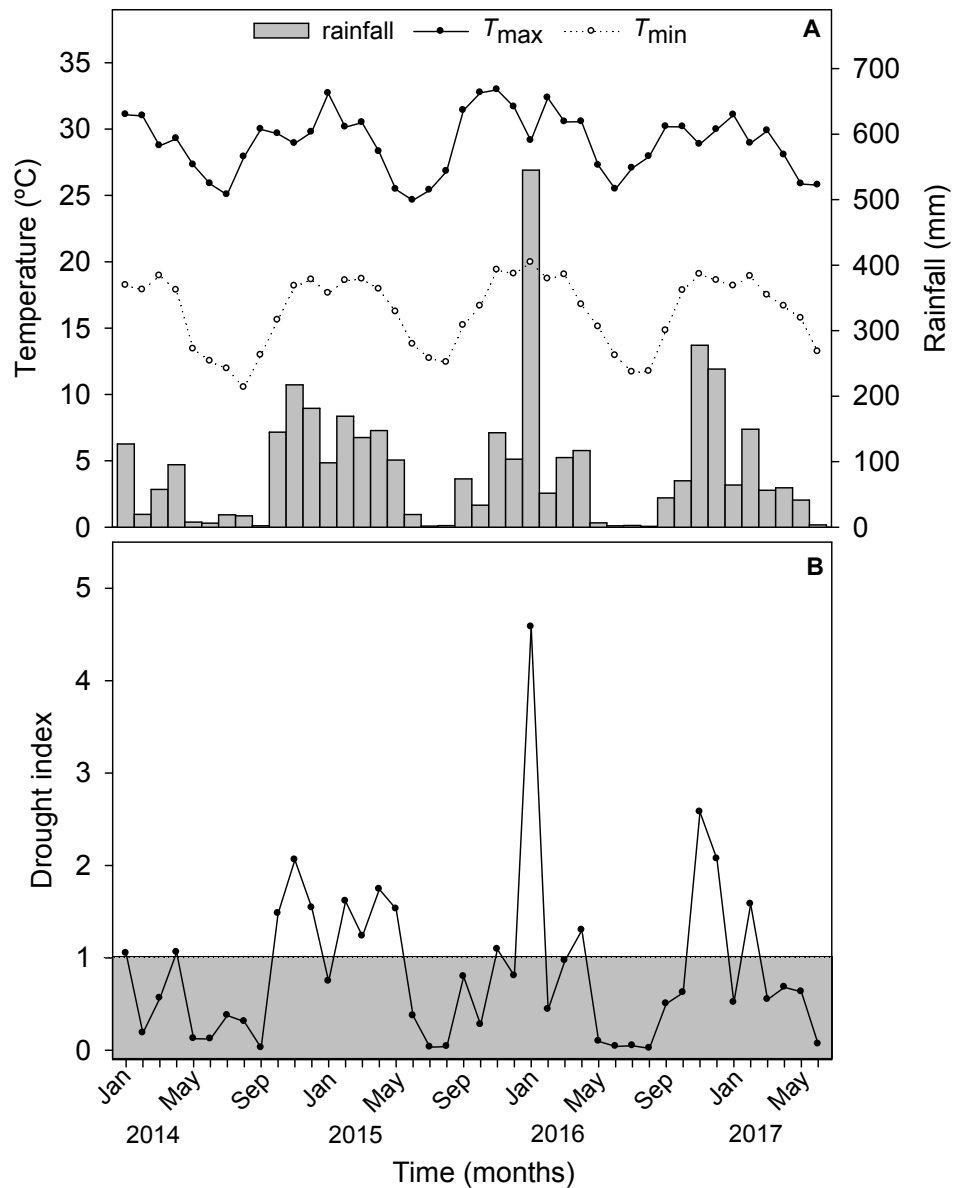
**Figure 2.** Monthly rainfall, mean maximum and mean minimum air temperature (A) and drought index (B) in the period between January 2014 and June 2017. Data were obtained from the Conceição do Mato Dentro weather station, MG, Brazil (INMET, 2017).
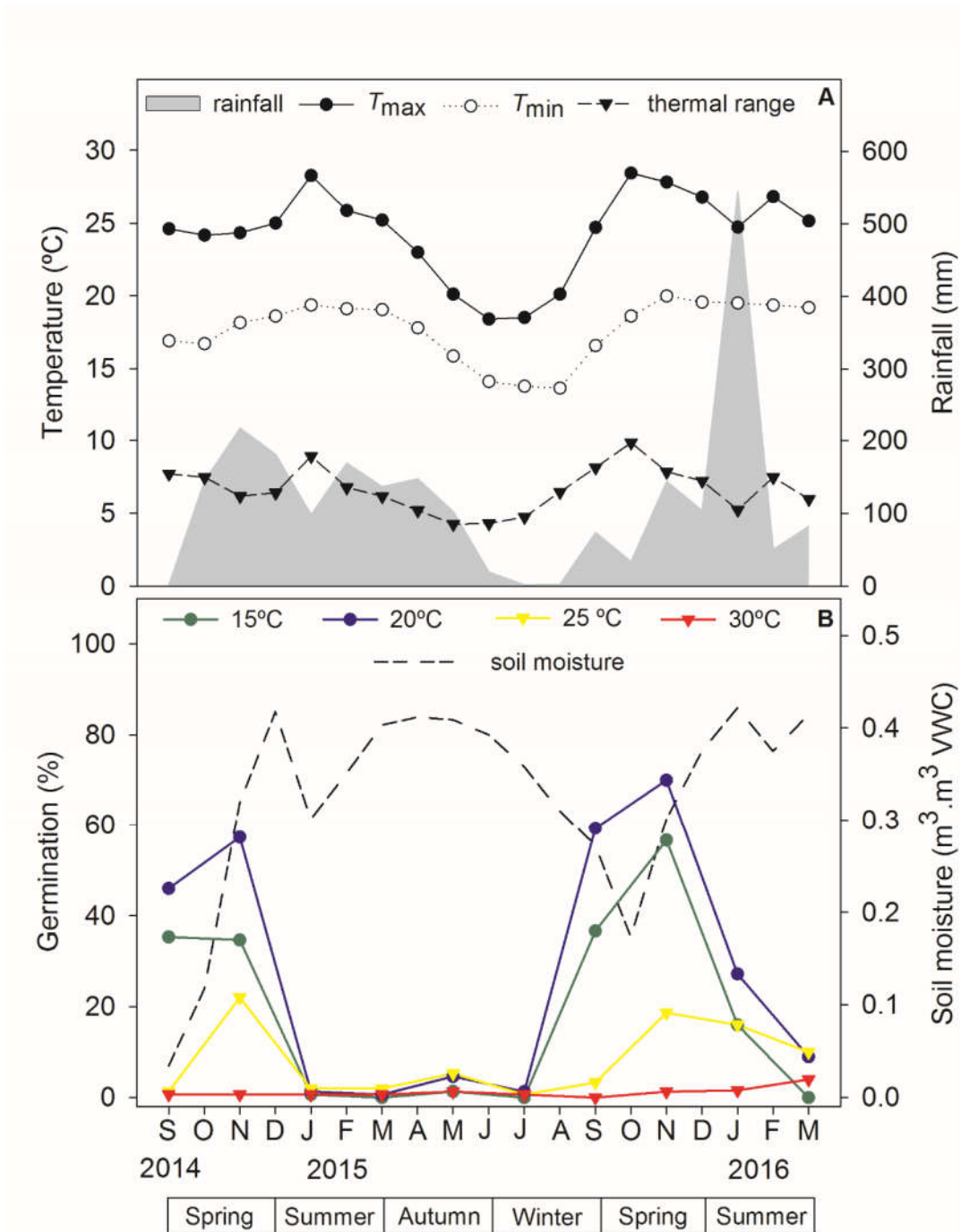
**Figure 3.** Monthly rainfall, mean maximum and mean minimum soil temperature in Serra do Cipó, Brazil, during seed burial experiment (A). Soil moisture and germination percentages of *X. longiscapa* seeds at different temperatures under a 12-h photoperiod and during burial for 18 months (B).

# Capítulo 3

# Thermal germination niche for neotropical *Xyris* species:

# effects of projected scenarios of temperature increase*

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**Thermal germination niche for neotropical *Xyris* species: effects of projected scenarios of temperature increase**

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- ***Background and Aims*** Seed germination requirements (light and temperature) provide important information on the ecology of species. Understanding the effects of climate changes on regeneration by seeds is important to develop conservation strategies. In this study, we evaluated seed germination requirements for six neotropical *Xyris* species from the Brazilian *campo rupestre.* Thermal time model was calculated for four species to simulate germination behavior under current and predicted temperature scenarios.

- ***Methods*** Seed germination were evaluated at a wide range of constant temperatures (10-40ºC) under light (12h-photoperiod) or dark conditions. Base temperatures ($T_b$) and thermal times for 50% germination ($\theta_{50}$) were calculated for four species. The effect of projected mean temperature increase in the seed germination percentage and velocity were evaluated.

- ***Key results*** All species have absolute light requirement to germinate. The thermal range of germination varied between 15 and 35ºC. Base temperatures for *X. asperula*, *X. pilosa*, *X.* sp*.* and *X. trachyphylla* were respectively 9.0, 12.8, 12.5 and 11.1ºC. Temperature increase would affect more strongly the seed germination of *X. pilosa*, *X. seubertii* and *X. subsetigera* while the other species

are more tolerant. The lowest expected temperature increase (2ºC) is sufficient to decrease in one day the seed germination of *X. asperula* and *X. pilosa*.

- ***Conclusions*** Species of *Xyris* do not present a pattern in relation of thermal germination niche and thermal requirements. In this way, the effects of global warming on regeneration by seeds is variable among the species.

**Key words:** Base temperature, thermal time, climate change, *campo rupestre*, Xyridaceae.

INTRODUCTION

Temperature is a critical factor that influences several processes and at different times in the seed life, including maturation, germination and dormancy (Probert, 2000; Walck *et al*., 2011). Seeds germinate within a specific temperature range, which characterizes its thermal germination niche (Donohue *et al*., 2010; Porceddu *et al*., 2013). Thus, the germination temperature is an important environmental filter which may help to explain the abundance and the distribution of seed-producing plant species (Bykova *et al*., 2012; Jiménez-Alfaro *et al*., 2016). Some studies found a relationship between the thermal germination niche and species distribution (Ranieri *et al*., 2012; Marques *et al*., 2014). In some cases, rare or endemic species (with more restricted distribution) present narrower thermal germination niches, in this way, these species are particularly more vulnerable to expected temperature changes (Cochrane *et al*., 2011).

The Brazilian *campo rupestre* comprises a montane grassland mosaic associated with rocky outcrops, recently classified as an old, climatically buffered, infertile landscape (OCBIL) (Silveira *et al*., 2016). These OCBILs present high species endemism and act as a biodiversity refugia, thus represents priority areas for

conservation (Hopper *et al*., 2016). Predict the impacts of climate changes on regeneration success of plants inhabiting these environments is important to direct conservation efforts. Projections for southeastern Brazil (2081–2090) indicate an increase of temperature of approximately 3 to 7ºC in the most pessimistic scenario (A2) and 2 to 4ºC in the less pessimistic scenario (B2) (Nuñez *et al*., 2009; IPCC, 2014). In general, plants can respond in two ways to a changing climate – 1) adapt to the new changing environment or 2) migrate to track climatically-suitable habitats (Aitken *et al*., 2008). However, plant species inhabiting the top of mountains may not found new suitable habitats to migrate (Bell *et al*., 2014). Moreover, species inhabiting Brazilian *campo rupestre* have reduced dispersability which limits the probability of migration to other sites and increase the extinction risk (Hopper *et al*., 2016).

Determination of germination thresholds and use of thermal time model are important tools to characterize thermal germination niche and have often been used to predict the seed germinative behavior of species in the face of global warming (Orrù *et al*., 2012; Ordoñez-Salanueva *et al*., 2015; Müller *et al*., 2016; Cochrane, 2017). For each plant species, the seeds present a base temperature ($T_b$) below which there is no germination, an optimum temperature (or temperature range) ($T_o$) and a ceiling temperature ($T_c$) above which there is no germination (Hardegree, 2006). Seed responses to accumulated temperature could be predicted using the thermal time model ($\theta$) (Garcia-Huidobro *et al*., 1982; Covell *et al*., 1986; Hardegree, 2006). This model is calculated by the sum of the daily accumulated temperature above $T_b$ (and below $T_o$) required for germination of each seed percentiles, i.e. thermal time predicts the amount of accumulated heat (degree-days) needed to reach a certain germination percentage, usually 50% (Garcia-Huidobro *et al*., 1982). Although the thermal niche characterization is an important measure for the identification of more sensitive species

to increased temperatures (Cochrane *et al*., 2011; 2017; Ooi, 2012; Briceño *et al*., 2015), little attention has been given to this approach (Porceddu *et al*., 2013).

Xyridaceae comprises five genera distributed in the tropical and subtropical regions, occurring mainly in Central and South America, but with representatives in Australia, Africa, India and North America (Sajo and Rudall, 1999; Campbell, 2004). *Xyris* is the largest genus of the family with approximately 300 species (Sajo and Rudall, 1999). Brazilian *campo rupestre* is a centre of diversity for neotropical Xyridaceae with approximately 102 species of *Xyris*, many of them endemic to this ecosystem (Wanderley, 2011; Silveira *et al*., 2016). *Xyris* seeds are characterized by the small size, by the light requirement to germination and by absence of primary dormancy for most species (Abreu and Garcia, 2005; Le Stradic *et al*., 2015; Dayrell *et al*., 2017; Oliveira *et al*., 2017; Giorni *et al*., 2018), thus, these species represent a good study model to evaluate the effect of temperature increase on seed germination. In this study, we evaluated seed germination of six neotropical sympatric *Xyris* species over the constant-temperature range of 10-40ºC. We calculated the base temperatures ($T_b$) and the thermal times required for 50% germination ($\theta_{50}$) for four of studied species. Our objectives were to: i) determine seed germination responses to temperature under light and dark conditions and ii) simulate the germination behavior in field under current and projected temperature increase scenarios. We hope to identify the species most sensitive to temperature increase in relation to seed germination.

MATERIAL AND METHODS

*Seed collection*

Mature seeds were collected of at least 20 individuals from natural populations growing in *campo rupestre* areas in the Serra do Cipó National Park, Minas Gerais, Brazil (19º

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12'-34'S; 43 27'-38'W). Seeds of *X. asperula* Mart., *X. subsetigera* Malme, *X. seubertii* L.A.Nilsson and *X. trachyphylla* Mart. were collected between July and August 2014 and seeds of *X.* sp. and *X. pilosa* Kunth were collected in July 2015. Serra do Cipó has altitude tropical climate (Cwb) according to the Köppen classification and present two well-defined seasons: a rainy season (spring/summer) and a dry season (autumn/winter). The mean annual maximum and minimum temperature of the last five years were respectively 28.6 and 16ºC (mean 22.3ºC). The mean rainfall of the same period was 1226 mm.

*Germination tests*

Germination tests were conducted in germination chambers. For each species, seeds (six replicates of 25) were placed in Petri dishes containing a double layer of filter paper. Seeds were submitted to a range of temperatures from 10ºC to 40ºC (with intervals of 5ºC) under 12-h photoperiod (~40 µmol m$^{-2}$ s$^{-1}$) or darkness. The seeds were irrigated with a nystatin aqueous solution (0.5%) to avoid fungal c the average annual temperature of the last five years was 22.35ºContamination (Oliveira *et al*., 2017). The number of germinated seeds were recorded daily using a stereomicroscope until germination stabilization or until 30 days, when no germination was observed. Germination of seeds kept in the dark were evaluated only at day 30. Germination criterion was the emergence of vegetative axis (Oliveira *et al*., 2017). We calculated the germination percentage (%G) and the mean germination time (MGT) by the following formula: MGT = Σ(GnTn)/ΣGn), where Gn is the number of seeds germinate at time Tn (Heydecker, 1966).

*Determination of base temperatures*

Base temperatures ($T_b$) were determined for *X. asperula*, *X. pilosa*, *X.* sp. and *X. trachyphylla*. Germination data of *X. seubertii* and *X. subsetigera* did not present the necessary requirements to perform the linear regressions. Initially, we considered as optimum temperature ($T_o$) the one that provided the highest percentage of germination in the shortest time. Temperatures below $T_o$ (sub-optimal range) were utilized to calculate the $T_b$. For each species, was plotted the germination rate as a function of temperature for different germination percentiles (every 10% increase in germination) (Covell *et al.*, 1986). The average of x-intercept values of each percentile was used to determine the $T_b$. Linear regressions were recalculated for each percentile, but constrained to pass through $T_b$ (Hardegree, 2006). This last model resulted in the smallest residual variance and highest $R^2$ value, and so was considered as a best estimate of $T_b$ (Covell et al., 1986).

*Thermal time model*

Thermal time ($\theta$, ºCd) estimates for each species were calculated as the inverse of the sub-optimal regression equations (Covell *et al.*, 1986). Germination percentages data were transformed to probits using tabular data from Finney (1971). Linear regression was used to express probit (g) as a function of thermal time ($\theta_g$) and the cumulative germination response of seeds described by the following equation (Covell *et al.*, 1986):

probit(g) = K + log $\theta_g/\sigma$

where g is the germination percentage, K is an intercept constant when $\theta_g$ is zero, $\theta_g$ may be normal or log-normal distributed and the $R^2$ value indicate the best model (Hardegree, 2006), $\sigma$ is the standard deviation of the response to log $\theta_g$ (Covell *et al.*,

1986). Thermal requirements to reach 50% of germination ($\theta_{50}$) was presented for each species.

*Temperature increase scenarios*

Projections of temperature increase for south-eastern Brazil were obtained from Nuñez et al. (2009). The mean temperature at the time of the first rains (ten days between the end of October and the beginning of November) in Serra do Cipó was calculated using as reference the year 2014 from data obtained from a meteorological station (INMET - Instituto Nacional de Meteorologia, 2017). This temperature was used because all *Xyris* species are dispersed at the dry season, thus, the first rains represent the first opportunity for germination. Using the germination percentages of the six species studied here, we evaluated how the increase in the average temperature may influence the germinative behavior of the seeds. Two scenarios of temperature increase were considered, one more pessimistic estimating an increase between 3 and 7ºC (A2) and a less pessimistic one that estimates an increase between 2 and 4ºC (B2) in average temperature for the period 2081–2090 (Nuñez *et al*., 2009). Germination timing in the field under current condition was estimated using as reference the average daily temperature of the first days of rain. Days required to reach $\theta_{50}$ ($t_{50}$) was then calculated through the amount of degrees accumulated in each day (above $T_b$). The same calculations were made to estimate the effect of temperature increase in the germination timing by the addition of 2ºC (minimum projected temperature increase) in the local daily temperature. This analysis was possible because an increase of 2ºC in the average daily temperature does not exceed the $T_o$ of none of the studied species.

*Statistical analysis*

The effects of temperature on seed germination percentage and mean germination time (MGT) for each species were analyzed using analysis of variance (ANOVA). Differences in $T_b$ among the species were also analyzed by ANOVA. The data were previously tested for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's test). In all cases, means were compared using Tukey test at 5% probability.

RESULTS

*Seed germination requirements*

Seeds of all studied species germinated only in presence of light. The germination range varied between 15 and 35°C (Fig. 1). The optimal temperature range for germination was 30°C for *X. asperula*, 20-25°C for *X. pilosa*, *X. seubertii*, *X. subsetigera* and *X.* sp., and 20-30°C for *X. trachyphylla*. No germination was observed at 10°C and 40°C. Seeds of *X. seubertii* germinated above 50% only at two temperatures (20 and 25°C). In general, the lowest values of MGT were observed between 20-30°C (Fig. 2).

*Thermal time models*

Base temperatures ($T_b$) varied between 9.0 and 12.8 (Table 1, Fig. 3). The highest base temperatures were observed for *X. pilosa* and *X.* sp., which did not differ from each other, while the smallest values were observed for *X. trachyphylla* and *X. asperula*. In relation of thermal requirement ($\theta_{50}$), the highest value was found for *X. asperula* (127.35 °Cd), which was approximately two times higher than the other species (Fig. 4).

*Temperature increase effects on seed germination percentage and velocity*

The current mean temperature in the germination season of Brazilian *campo rupestre* (first rains) is 22.7ºC. In the most pessimistic temperature increase scenario (A2 scenario, + 7ºC), the seeds can be divided into two groups in relation to germinability – less sensitive (*X. asperula*, *X.* sp. and *X. trachyphylla*) and most sensitive to temperature increase (*X. pilosa*, *X. seubertii* and *X. subsetigera*) (Fig. 5).

Under current temperature regime, seeds of *X. asperula* require more days to reach the $\theta_{50}$ (10 days), while the other species require 6 days. An increase of 2ºC in the current temperature decreased in one day the needed time for *X. asperula* and *X. pilosa* seeds to reach $\theta_{50}$ but would not change for *X.* sp. and *X. trachyphylla* (Table 1).

DISCUSSION

All species germinated at least 60% considering all tested temperatures. Seeds of *Xyris* are dispersed during the dry season, thus, at the time of first rains the temperature will modulate the germination percentage and velocity of seeds exposed to light. Mean air temperature in Brazilian *campo rupestre* at the time of first rains in 2014 was 22.7ºC. In this temperature all species can germinate, once the temperatures of 20 and 25ºC promoted the best germination percentages. However, in some hours of the day the air maximum temperature could exceed the optimum germination temperature. Furthermore, the Brazilian *campo rupestre* has several microenvironments with different microclimates (Silveira *et al*., 2016). For example, rock temperature could exceed 35ºC (Marques *et al*., 2014) while temperature tends to be milder in regions of wet or flooded soil (Oliveira and Garcia, 2011). Seeds of *X. seubertii* presented the narrowest temperature range for germination (20-25ºC) suggesting that this species is habitat-specialist. On the other hand, seeds of *X. asperula* germinated above 60% in

wider range of temperatures (15-35ºC) suggesting a habitat-generalist behavior. However, both species have a broad geographic distribution; *X. seubertii* occurs in Venezuela, French Guiana and Brazil (from the North to the Southeast) while *X. asperula* is endemic of Brazil, occurring from the Northeast to the Paraná (Wanderley, 2011). Seed germination cannot always explain the patterns of species distribution and specifically for other *Xyris* species from Brazilian *campo rupestre* it was not possible to establish a relationship between these two variables (Giorni *et al*., 2018). All species studied here can be found growing in the same area (not subject to flood), thus it was expected that seed germination by itself did not explain the species distribution.

Thermal germination niche could be a good predictor to species sensitivity to climate warming (Cochrane *et al*., 2011). In general, it was expected that species with narrower germination niches to be more vulnerable to temperature changes. In tropical environments, we consider that the ability to germinate under high temperatures (30 and 35ºC) to be very important to identify more tolerant species. Here, all species germinated above 50% at 20 and 25ºC. Current annual mean temperature of Serra do Cipó is approximately 20.6ºC; considering only the rainy season (October to March) this value is approximately 22ºC (INMET - Instituto Nacional de Meteorologia 2017). Thus, in the more pessimistic scenario for south-eastern Brazil (A2) according to Nuñez et al. (2009), the mean temperature can reach 30ºC. Seeds of *X. seubertii, X. subsetigera* and *X. pilosa* showed the highest reductions in germination percentages at 30ºC while *X. asperula*, *X.* sp. and *X. trachyphylla* do not presented differences in germination percentages in interval between 20 and 30ºC (until 35ºC for *X. asperula*). These results have great relevance for the species conservation. For example, *X. subsetigera* which seeds were characterized as more sensitive to climate warming is included in the IUCN Red List of Threatened Species as a Near Threatened species (IUCN Red List of

Threatened Species, 2016). Thus, *ex situ* seed conservation strategies may be necessary for this species.

Data on $T_b$ and thermal requirements is scarce for species of Brazilian *campo rupestre* (Duarte *et al.*, 2018). To our knowledge, this is the first study that brings these information for Xyridaceae species. Determination of thermal requirements improve the discussion about seed germination niche. Here, it was possible to use the example of *X. asperula* that presented the wider temperature range for germination and at the same time have the higher thermal requirement ($\theta_{50}$). In other words, this species can germinate in more diverse temperature conditions but take more time than the other studied species. Species with fast germination (lower thermal requirement) have a competitive advantage if subsequent conditions remain favorable, on the other hand, species with slow germination could be favored in catastrophic events (Kos and Poschlod, 2010) such as the fire passage which is recurrent in *campo rupestre* (Silveira *et al.*, 2016). In relation to expected temperature increase, two scenarios could be considered in relation of germination velocity. If temperature increase does not surpass the optimum temperature range for each species, the time required for germination will decrease, as observed for some species (Ordoñez-Salanueva *et al.*, 2015; Duarte *et al.*, 2018). On the other hand, if temperature increase surpasses optimum temperature range, seed germination would be slower. Here, seeds need 6 to 10 days to germinate and an increase of 2ºC is sufficient to reduce (in one day) the germination timing of *X. asperula* and *X. pilosa* seeds. In this way, thermal time is useful to predict seed germination behavior under different temperature regimes.

It is important to highlight that heat accumulation for seed germination in *Xyris* only occur under light presence. Some trials have demonstrated that *Xyris* seeds requires light throughout the entire germinative process to achieve maximum germination

(T.G.S Oliveira; Q.S Garcia, unpublished data). Thus, the thermal time approach is only applicable to seeds exposed to light. Seeds of several *Xyris* species can form a persistent soil seed bank, i.e., seeds remain viable for more than one year (Garcia *et al.*, 2012; Oliveira *et al.*, 2017). Probably, seeds with slower germination (high thermal requirement) are more likely to be buried in soil. Once buried, seeds will not germinate unless they are exposed again to the light. The increase of air temperatures will increase soil temperatures, especially in open habitats (Ooi, 2012) like the Brazilian *campo rupestre*. Seed longevity in soil is an important trait for plant regeneration, thus, understanding the effects of increase temperature on seed longevity and seed bank dynamics needs to be better studied in this ecosystem.

Species of Brazilian *campo rupestre* and of other OCBILs worldwide evolved under a climatic buffer (Hopper *et al.*, 2016; Silveira *et al*, 2016). This characteristic probably explains the high degree of endemism and the creation of refugia in these areas (Harrison and Noss, 2017). Understanding how plant species will respond under a climate change scenario is crucial to develop strategies to conserve this high biodiversity. Species inhabiting OCBILs have reduced dispersability (Hopper *et al.*, 2016), thus, migration capacity of these species is low. Although some species were more sensitive in the germination phase, we are not able to predict whether species will adjust in a changing environment. For example, plasticity in seed germination traits could improve the chance of persistence under climate warming (Cochrane *et al.*, 2015). In this study, it was evidenced that thermal germination niche and thermal requirement ($\theta_{50}$) varies between *Xyris* species, and from these data is possible to predict the sensitivity to global warming in the germination phase. To increase knowledge on the effect of global warming on the regeneration niche of species inhabiting Brazilian *campo rupestre* it is necessary to evaluate how the seedlings will respond to the

temperature increase and verify if there is a relation between the sensitivity of seed and seedlings. This information together with the ecology and species distribution data are important to identify the most susceptible species and/or populations that will require more urgent conservation efforts.

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TABLE AND FIGURES

TABLE 1. *Base temperatures (T$_b$), optimum temperature range (T$_o$), time required for 50% germination under current mean temperature in Brazilian campo rupestre (t$_{50}$) and under predicted increase of 2°C (t$_{50}$ + 2°C) for seeds of four Xyris species.*

| Species | T$_b$ (°C) | T$_o$* (°C) | t$_{50}$ (days) | t$_{50}$ + 2°C (days) |
|---|---|---|---|---|
| *X. asperula* | 9.0 ± 0.1 c | 25-30 | 10 | 9 |
| *X. pilosa* | 12.8 ± 0.5 a | 25 | 6 | 5 |
| *X.* sp. | 12.5 ± 0.2 a | 25-30 | 6 | 6 |
| *X. trachyphylla* | 11.13 ± 0.1 b | 25-30 | 6 | 6 |

For T$_b$, equal letters indicate absence of significant differences by the Tukey test at 5% probability. [*] temperatures with highest germination percentages and lowest mean germination time.
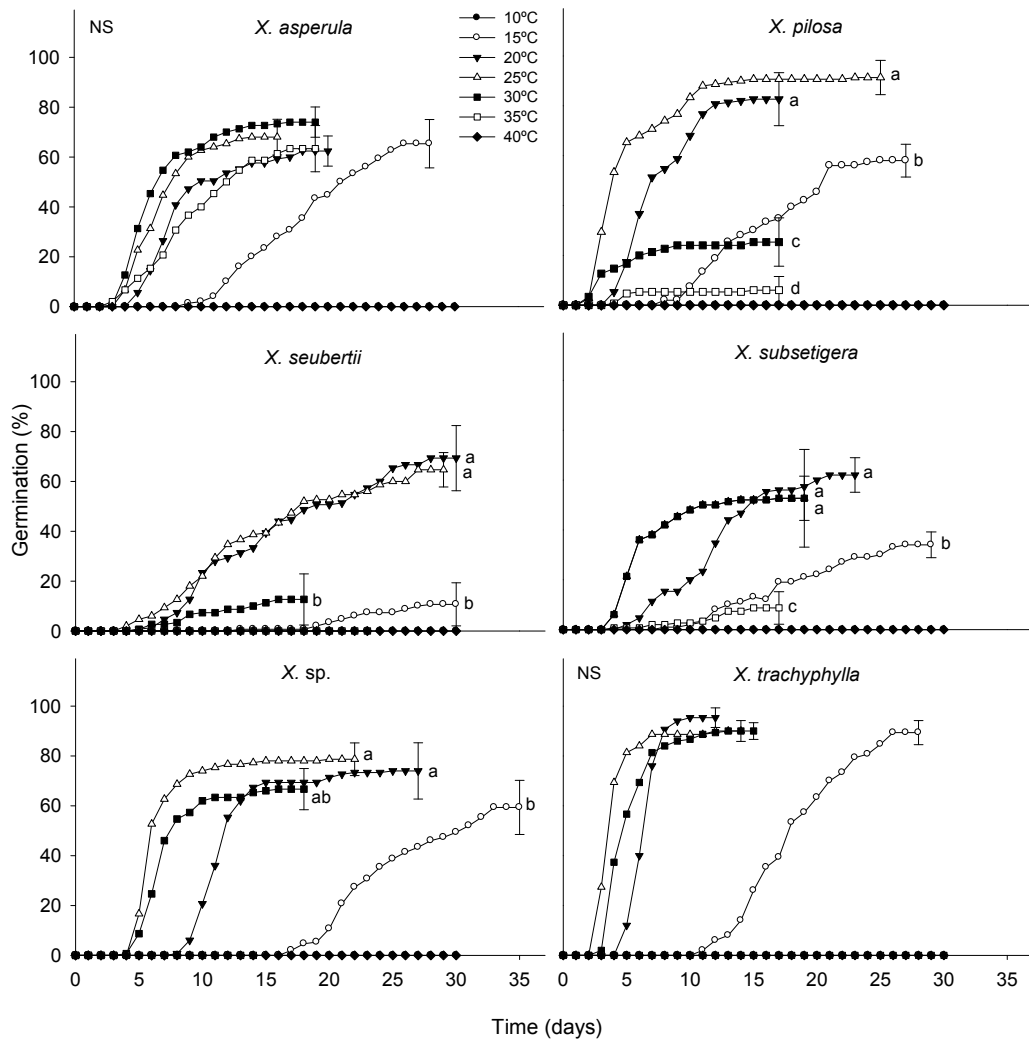
FIG. 1. Germination percentages (%) of *Xyris* seeds in different germination temperatures. Equal letters indicate absence of significant differences by Tukey test at 5% probability. NS = non-significant. Bars represent the mean ± standard deviation.
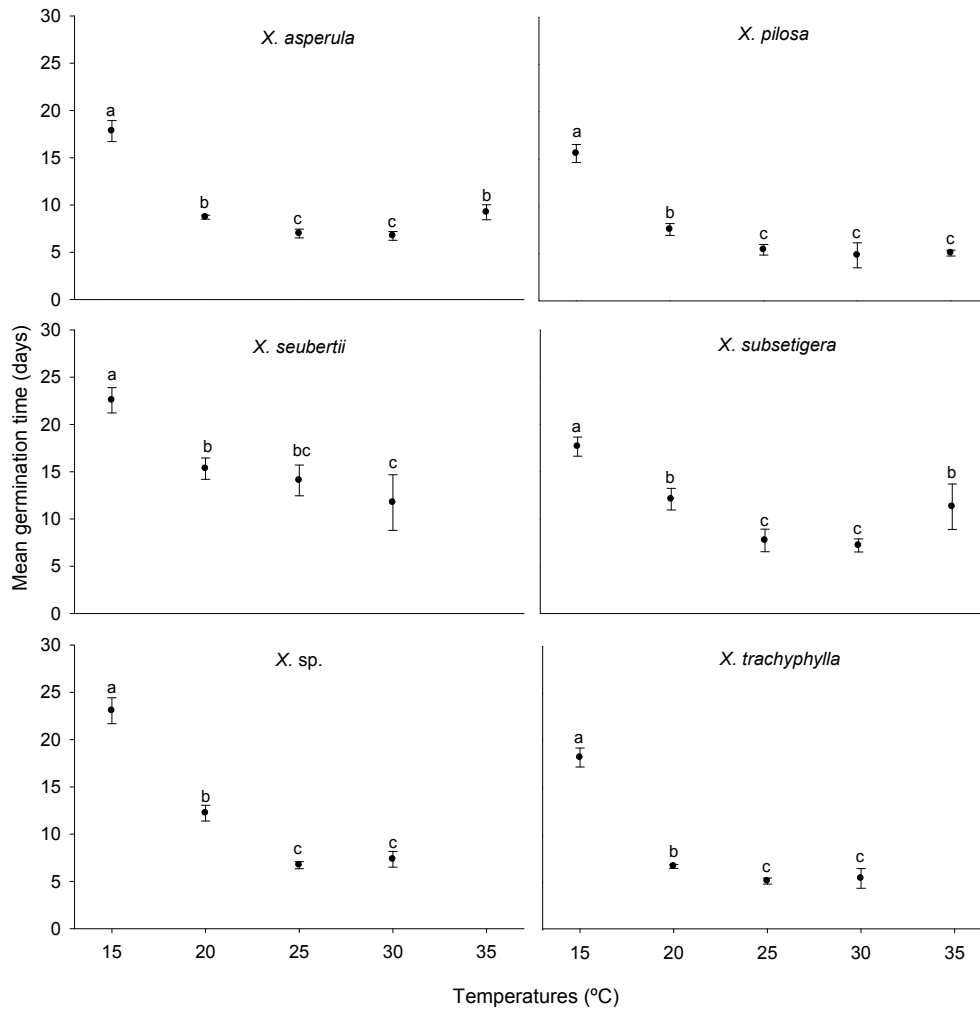
FIG. 2. Mean germination time (days) of *Xyris* seeds in different germination temperatures. Equal letters indicate absence of significant differences by Tukey test at 5% probability. Bars represent the mean ± standard deviation.
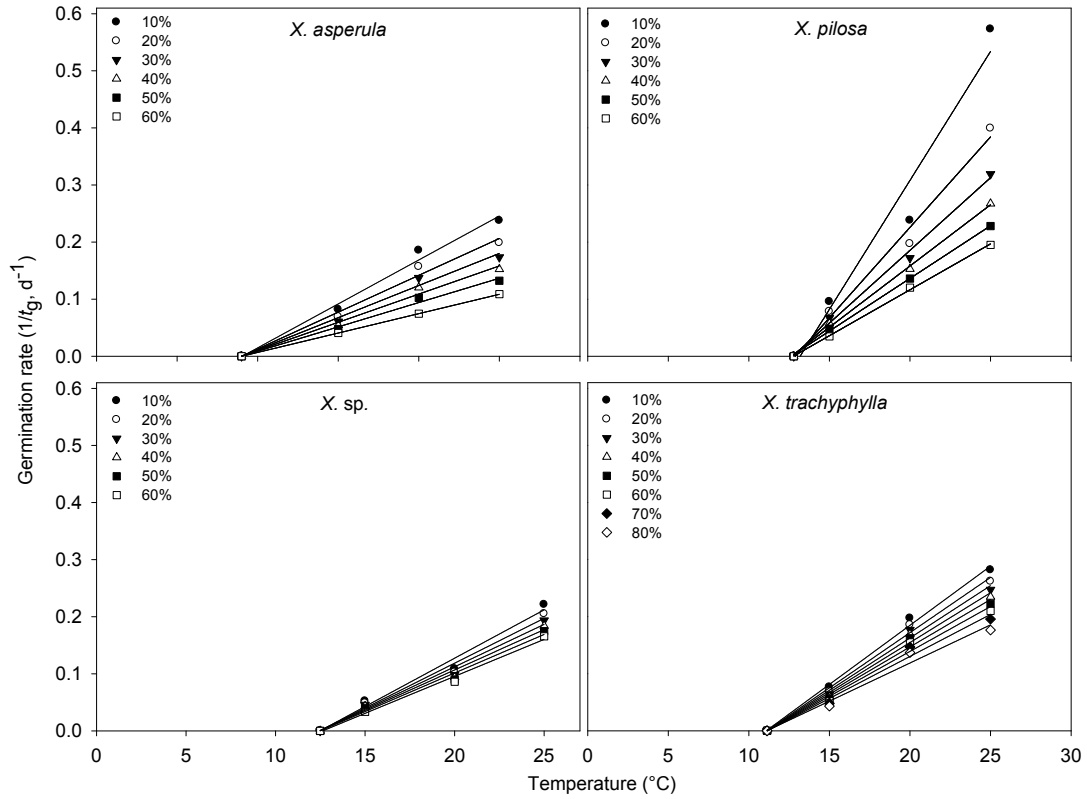
FIG. 3. Base temperatures ($T_b$) calculated for different germination percentiles of four *Xyris* species. For each species, the linear regressions for the different percentiles were constrained to pass to the common value of $T_b$.
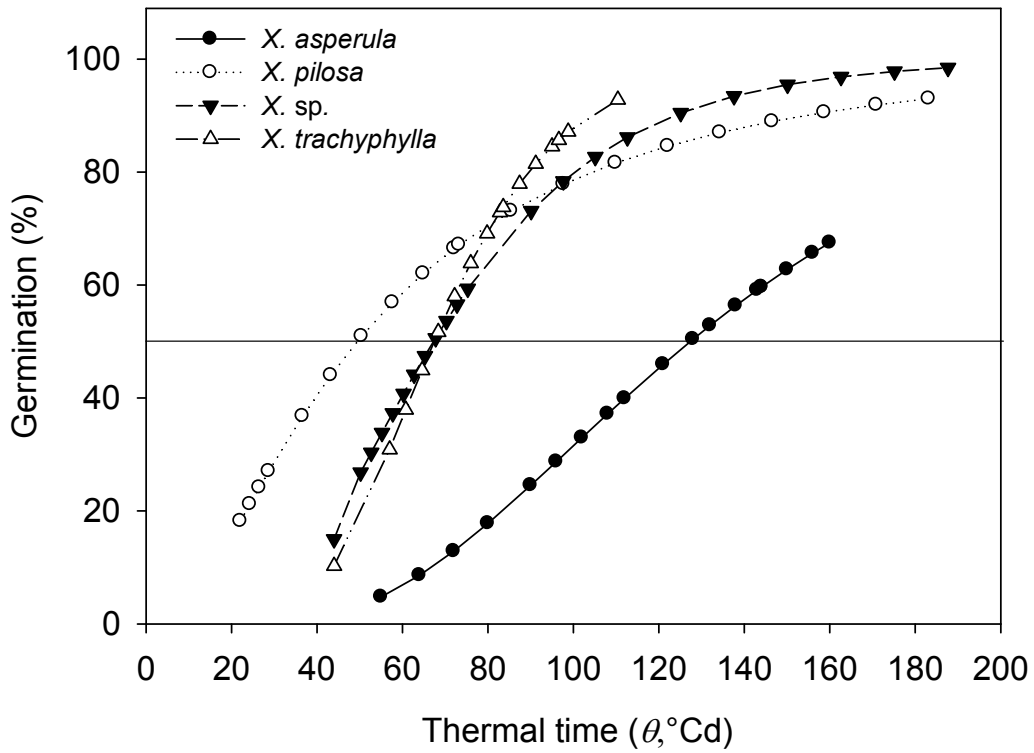
FIG. 4. Seed germination (%) as a function of thermal time requirements ($\theta$, ºCd) for four *Xyris* species. Thermal times were calculated assuming the base temperatures listed in Table 1. Horizontal line indicates thermal times required to reach 50% of germination ($\theta_{50}$).
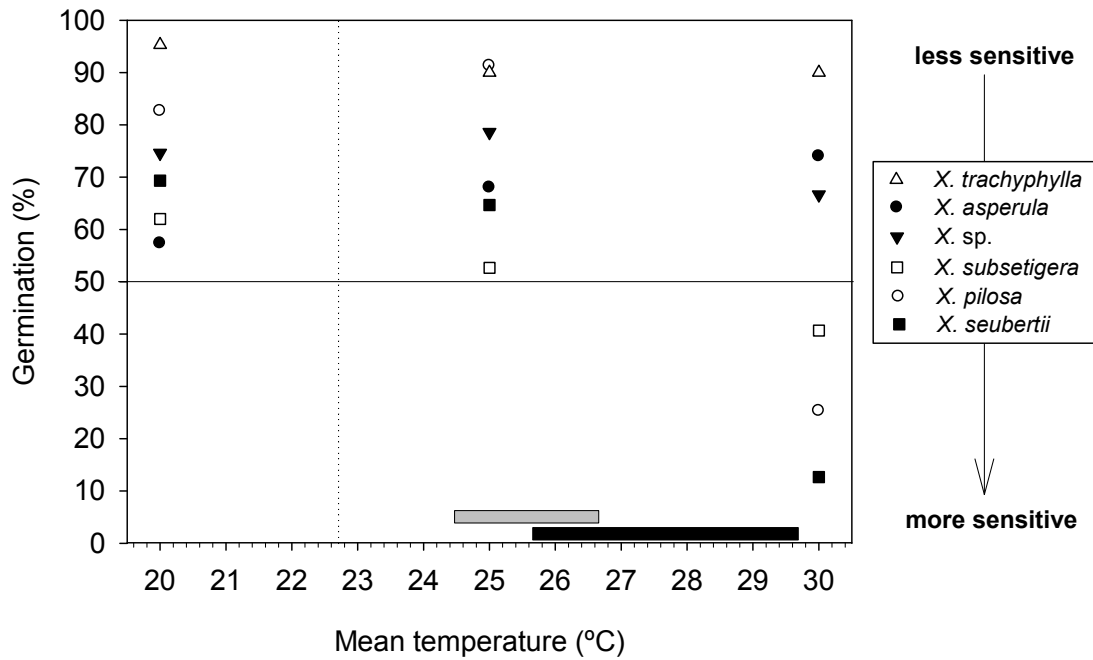
FIG. 5. Seed germination of six *Xyris* species as a function of temperature. Dotted line represents the mean temperature in the 2014 rainy season in Serra do Cipó. Grey and black bars represent respectively an increase between 2 and 4ºC (B2) and between 3 and 7ºC (A2) in the mean temperature. Projections of temperature increase for south-eastern Brazil obtained from Nuñez et al. (2009).

**Considerações Finais**

Foi demonstrado que as espécies de *Xyris* investigadas são capazes de formar bancos de sementes persistentes no solo e que as sementes enterradas exibem ciclos sazonais de germinação e dormência. A longevidade superior a um ano e a ocorrência de ciclos sazonais para todas as espécies de *Xyris* dos campos rupestres estudadas, até o momento, sugerem um padrão para o gênero. O padrão temporal de aquisição e superação da dormência secundária é compartilhado entre todas as espécies. A dormência é adquirida a partir da metade da estação chuvosa e é superada durante a estação seca. A avaliação da germinabilidade em uma ampla faixa de temperatura permitiu pela primeira vez a identificação da dormência condicional em espécies tropicais que apresentam ciclos sazonais de dormência. O próximo passo é definir qual é o papel de cada fator ambiental (umidade e temperatura) na modulação da dormência cíclica nessas espécies.

Os resultados de germinação de coletas de anos 4 consecutivos obtidos sugerem que o ambiente maternal pode afetar o grau de dormência das sementes de *X. longiscapa*. Assim, recomenda-se que estudos de determinação da dormência em espécies de *campo rupestre* utilizem pelo menos dois anos de coleta. Essa medida pode auxiliar na identificação dos componentes genético e ambiental da dormência das sementes.

A amplitude térmica de germinação das sementes, bem como o requerimento térmico (calculado pelo modelo de tempo térmico), podem ser utilizados como preditores da sensibilidade das sementes durante o processo de germinação ao aquecimento global. Em relação à fase da germinação das sementes, foi possível identificar espécies mais vulneráveis aos aumentos de temperatura previstos no atual contexto das mudanças climáticas globais. Por outro lado, sementes de algumas

espécies demonstraram ser mais tolerantes. Ademais do avanço do conhecimento da ecofisiologia das espécies investigadas, os resultados obtidos aqui são importantes para direcionar esforços de conservação, priorizando as espécies mais vulneráveis. Estudos que avaliem a sensibilidade da plântula se fazem necessários para um entendimento mais amplo do efeito das mudanças climáticas no nicho de regeneração das espécies de campos rupestres.