Interactions between substrate temperature and humidity in signalling cyclical dormancy in seeds of two perennial tropical species

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Abstract

The involvement of environmental factors in dormancy cycling is well known in temperate annual species, but it is not known how interaction between soil temperatures and humidity can modulate dormancy in perennial tropical species. In this study the effects were evaluated of substrate temperature and humidity on the modulation of the acquisition and overcoming of secondary dormancy in the buried seeds of two endemic Eriocaulaceae species from the rocky fields (*campos rupestres*) vegetation in south-eastern Brazil. Fresh seeds of *Comanthera bisulcata* and *Syngonanthus verticillatus* were buried and subsequently maintained at temperatures of 15, 20, 25 and 30°C, under three substrate humidity levels (boggy, humid and humid/dry). The seeds were exhumed every 3 months and tested for germination (20°C, 12 h photoperiod) and viability (tetrazolium test). The seeds of both species acquired dormancy after burial in all of the treatments. During the experimental period they demonstrated cycles of acquisition and overcoming of dormancy that were most evident in the treatments involving alterations of the substrate humidity (humid/dry regime) that coincided with the environmental conditions found naturally in the region of origin of the species. The seeds gradually lost dormancy during the dry period and re-acquired it when exposed again to humidity; dormancy would once again be overcome during the subsequent dry period. Burial promoted the acquisition of dormancy in *C. bisulcata* and *S. verticillatus* seeds; the lowest temperature tested favoured overcoming dormancy; and varying the humidity regime signalled the acquisition and the overcoming of secondary dormancy.

Introduction

Seed dormancy can be defined as the temporary incapacity to germinate under favourable conditions (Bewley, 1997), and it can be regulated by environmental factors such as temperature, soil water content, light and oxygen concentrations, and by internal factors such as plant hormones (Hoang *et al.*, 2013). Seeds can experience changes in their germination capacity under natural environmental conditions, thus synchronizing their dormancy cycles with the seasons of the year (Footitt *et al.*, 2011). Dormancy cycling is an endogenous mechanism that regulates seed germination in environments with marked seasonal variations (Baskin *et al.*, 1993), so that the seasonality of their emergence in the field is the result of the seasonality of their surrounding environmental conditions, and favours species recruitment only at certain times of the year.

Temperature is considered to be one of the principal environmental factors that regulate changes in dormancy states (Baskin and Baskin, 1988; Handley and Davy, 2005) through continuous exposure to either low or elevated temperatures (Baskin and Baskin, 1998; Kruk and Benech-Arnold, 1998). Studies undertaken with annual summer species in temperate climates have shown that primary dormancy is overcome by low winter temperatures shortly after seed dispersal (Kruk and Benech-Arnold, 1998; Baskin *et al.*, 2000; Meulebrouck *et al.*, 2010), and the acquisition of secondary dormancy is triggered by high summer temperatures (Baskin and Baskin, 1977; Bouwmester and Karssen, 1993). In contrast, dormancy in annual winter species is overcome during

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storage at high temperatures (Christensen *et al.*, 1996; Bauer *et al.*, 1998; Benech-Arnold *et al.*, 2000) and secondary dormancy is acquired by exposure to low temperatures (Baskin and Baskin, 1977).

The role of temperature as a crucial environmental factor in modulating the dormancy states of seeds is widely accepted, although there is evidence that temperature effects in overcoming dormancy can be modulated by soil humidity under some circumstances (Adámoli *et al.*, 1973; de Miguel and Soriano, 1974; Baskin and Baskin, 1992). Seeds buried in superficial soil layers experience seasonal alterations during the year, and fluctuations in the soil water content may influence their state of dormancy and regulate their emergence (Baskin and Baskin, 1976; Batlla and Benech-Arnold, 2010). Studies undertaken under controlled conditions have shown that soil humidity (as well as its fluctuations) can alter the depth of dormancy and, consequently, regulate seed germination (Kruk and Benech-Arnold, 1998, 2000; Batlla and Benech-Arnold, 2006; Batlla *et al.*, 2007).

In spite of the fact that the involvement of environmental factors in both the acquisition and overcoming of secondary dormancy is well supported (Baskin and Baskin, 1978, 1980; Karssen, 1980/1981; Kruk and Benech-Arnold, 1998, 2000), there have been no studies demonstrating that interaction between soil temperatures and soil humidity can modulate secondary dormancy in tropical species. Previous studies undertaken with endemic species in areas of *campos rupestres* (rocky, open, altitudinal) vegetation in Brazil have shown that two species of Xyridaceae (Garcia *et al.*, 2012) and three species of Eriocaulaceae (Garcia *et al.*, 2014) demonstrate marked cycles of annual dormancy. Nonetheless, the precise roles of each of these environmental factors (temperature and humidity) in the processes of acquiring and overcoming secondary dormancy have not yet been established.

The present study tested, for the first time, the effects of substrate temperature and humidity on secondary dormancy modulation in the seeds of two perennial tropical species endemic to *campos rupestres* vegetation in Brazil (*Comanthera bisulcata* and *Syngonanthus verticillatus*). These species occur in mesic habitats, with soil humidity very close to saturation during the rainy season (Oliveira and Garcia, 2011). Their seeds are very small, require light for germination (Oliveira and Garcia, 2011), and demonstrate annual cycles of dormancy under natural conditions (Garcia *et al.*, 2014). In light of this information, the present study sought to: (1) evaluate the influences of temperature and soil humidity on the acquisition and overcoming of secondary dormancy in the seeds of *C. bisulcata* and *S. verticillatus*; and (2) analyse the interactions of these environmental factors in regulating the annual cycles of dormancy in those buried seeds.

Materials and methods

Seed collection

Capitula of *C. bisulcata* (Koern.) L.R. Parra & Giul. and *S. verticillatus* (Bong.) L.R. Parra & Giul. were collected in natural populations in *campos rupestres* vegetation in the Serra do Cipó mountains of Minas Gerais State, Brazil (19°20′64″S 43°35′63″W and 19°14′97″S 43°32′59″W, respectively) at the time of dispersal. The regional climate there is characterized by two well-defined seasons: a dry season (autumn/winter, from April to September; mean values ± standard deviation of temperature: maximum 31.9 ± 0.51°C and minimum 8.2 ± 0.8°C; total precipitation 151.9 ± 65.9 mm); and a rainy season (spring/summer, from October to March; maximum 34.2 ± 0.92°C and minimum temperature 14.5 ± 1.2°C; total precipitation 1327.2 ± 278.5 mm). The soils are shallow and poorly drained (Romero and Nakajima, 1999), favouring boggy conditions during the rainy period.

The effects of temperature and soil humidity on dormancy changes

Lots containing approximately 1 mg of seeds were placed in nylon bags and buried 5 cm deep in polyethylene planting pots wrapped in aluminium foil and containing soil from the region where the species occurred mixed with washed and sterilized sand (1:1); hereafter called the substrate. The pots were stored at four different temperatures (15, 20, 25 and 30°C) under three different substrate humidity regimes: (1) boggy, in which the substrate was maintained with excess water for the entire storage/test period; (2) humid, in which the substrate was maintained at field capacity; and (3) humid/dry, in which the substrate was maintained at field capacity (humid) for 4 months (simulating the rainy season; the experiment was initiated in November), followed by the suspension of irrigation for a period of 7 months (gradual drying of the substrate, which was completely dry at the end of the period, simulating the dry season), after which irrigation was re-initiated (5 months) and once again the irrigation was suspended (approximately 2.5 months). The field capacity (treatments 2 and 3) of the substrate had been determined in earlier experiments, and during the current testing the pots were weighed regularly and water added to the substrate as needed. During 18 months, at intervals of 3 months, four seed lots exposed to each treatment were exhumed and submitted to germination tests.

Germination tests

The exhumed seeds (four replicates of 50 seeds each) were washed with distilled water to remove any

adhering substrate and placed immediately in 9-cm Petri dishes lined with a double layer of filter paper that had been humidified with 5 ml of a solution of 1% nystatin, and then maintained at 20°C (the optimum germination temperature for both species) (Oliveira and Garcia, 2011). Germination was evaluated on a daily basis until the germination response became constant (or for 30 d in experiments showing low germinability); the criterion utilized for germination was the emergence of the vegetative axis (Garcia *et al.*, 2014). The seeds that did not germinate were submitted to tetrazolium testing (1% 2,3,5-triphenyl tetrazolium chloride in the dark at 25°C for 24 h); seeds were considered viable if their embryos stained rose-pink.

Statistical analyses

The data were analysed using Statistica version 7.0. software (StatSoft Inc., Tulsa, Oklahoma, USA), employing multiple regression tests through the construction of general linear models (LM) that were tested by the premises proposed by Eisenlohr (2013).

Results

The recently collected seeds of *C. bisulcata* and *S. verticillatus* showed germination percentages of 50 and 86% respectively, and viability levels greater than 90%. After 3 months of burial the germinability of *C. bisulcata* and *S. verticillatus* seeds fell to below 10%, although their viability remained above 75%, except at 30°C (Figs 1 and 2). Over the course of the experimental period, the germinability and viability of the seeds of both species demonstrated variations depending on the temperature and humidity regimes to which the substrate was exposed.

The buried seeds of *C. bisulcata* that had been maintained at 15°C demonstrated gradual alleviation of secondary dormancy after the sixth month of burial,
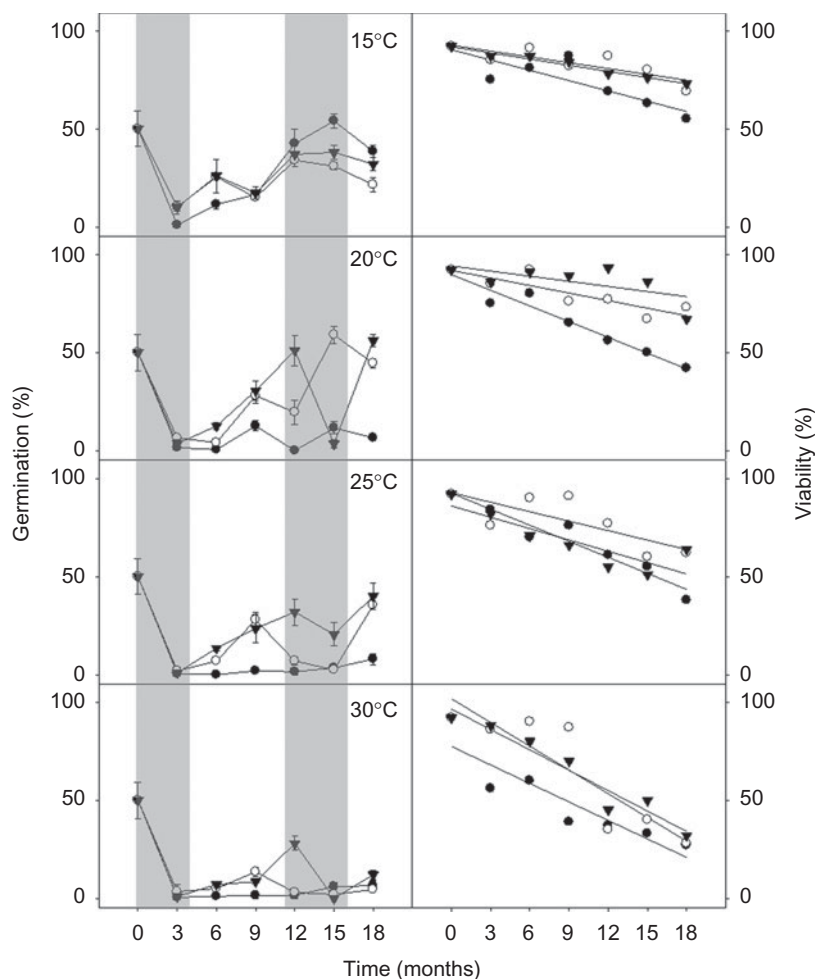

Figure 1. Percentages of germination and viability of *Comanthera bisulcata* seeds at 15, 20, 25 and 30°C in boggy (filled circles), humid (empty circles) or humid/dry substrates (inverted triangles). Germination tests were performed at 20°C (four replicates of 50 seeds). Vertical bars indicate the standard errors; the shaded areas indicate the duration time of the humid portion of the humid/dry treatment.

Figure 2. Percentages of germination and viability of *Syngonanthus verticillatus* seeds at 15, 20, 25 and 30°C in boggy (filled circles), humid (empty circles) or humid/dry substrates (inverted triangles). Germination tests were performed at 20°C (four replicates of 50 seeds). Vertical bars indicate the standard errors; the shaded areas indicate the duration time of the humid portion of the humid/dry treatment.
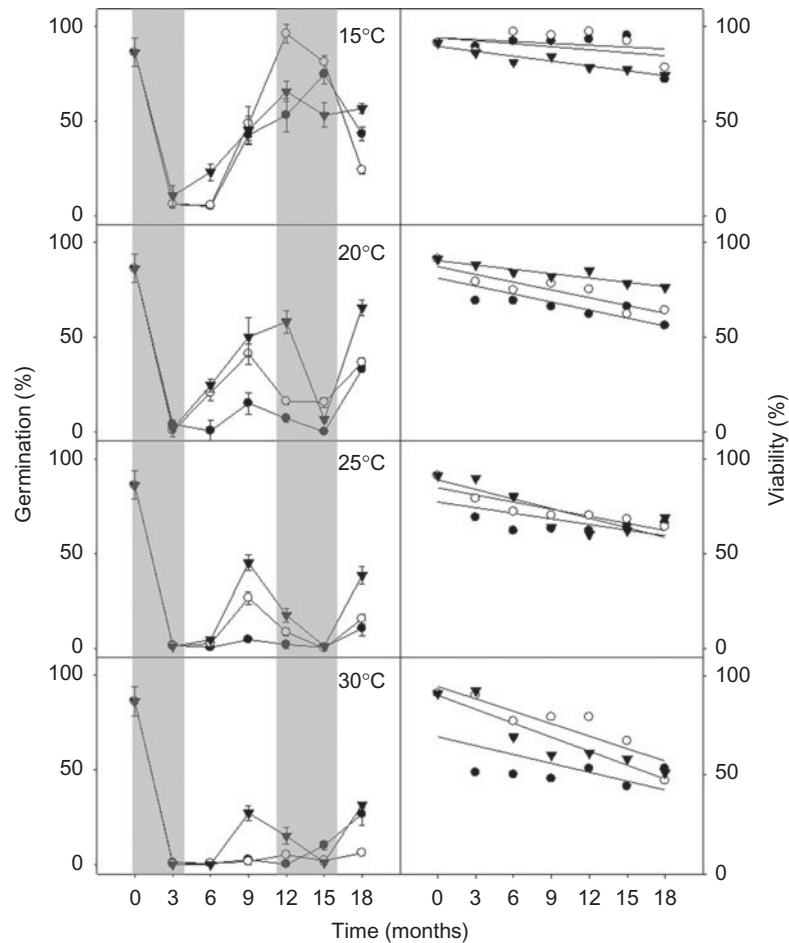
independent of the substrate water regime, attaining maximum germinability in the 12th month in the humid and humid/dry treatments, and in the 15th month in the boggy treatment (Fig. 1). Seed viability gradually diminished over the 18-month period of burial, with a higher rate in the boggy treatment. The seeds exposed to the humid treatment at 20°C demonstrated diminishing dormancy until the 15th month of burial, while seeds maintained under boggy conditions remained dormant during the entire evaluation period (Fig. 1). Seeds of *C. bisulcata* maintained under an alternating humidity regime (humid/dry) demonstrated gradually diminishing dormancy during the dry period but reacquired dormancy when exposed to substrate humidity conditions, which was overcome again during the subsequent dry period. Decreases in seed viability were slightly more accentuated at this temperature (20°C), especially for seeds exposed to the boggy regime. Seeds submitted to the humid and humid/dry treatments at 25°C demonstrated partial recuperation

of their germination capacity, while those that had been exposed to the boggy treatment remained completely dormant during the entire period (Fig. 1). Seeds remained dormant in all of the substrate humidity treatment regimes at 30°C, although the dormancy level may have been overestimated since the seed viability decreased significantly after 9 months' burial (Fig. 1).

S. verticillatus seeds maintained at 15°C demonstrated a gradual alleviation of dormancy until the 12th month in the constant and fluctuating substrate humidity regimes; seeds maintained in the boggy substrate regime demonstrated a gradual alleviation of dormancy until the 15th month. Germination was observed to diminish after 18 months of storage at this temperature (15°C), although viability remained high during the entire period (Fig. 2). Although dormancy was alleviated in the humid and in the boggy treatments at 20°C, dormancy was more dramatically overcome in the humid/dry regime while accompanying the variations in substrate humidity (the

acquisition of dormancy during the humid period and overcoming dormancy during the dry period). The seeds remained dormant at 25 and 30°C, although in the humid/dry treatments they demonstrated the same pattern observed at lower temperatures, overcoming dormancy during the dry period (Fig. 2). The viability of *S. verticillatus* seeds gradually diminished with increasing storage temperatures, being especially

affected by the 30°C regime regardless of the substrate humidity treatments (Fig. 2).

The overcoming of dormancy in *C. bisulcata* and *S. verticillatus* was significantly greater in seeds buried at 15°C (Figs 3A and 4A) and in the humid/dry treatment (Figs 3B and 4B) ($P < 0.001$). The analyses of the effects of storage duration indicated that *C. bisulcata* and *S. verticillatus* seeds demonstrated
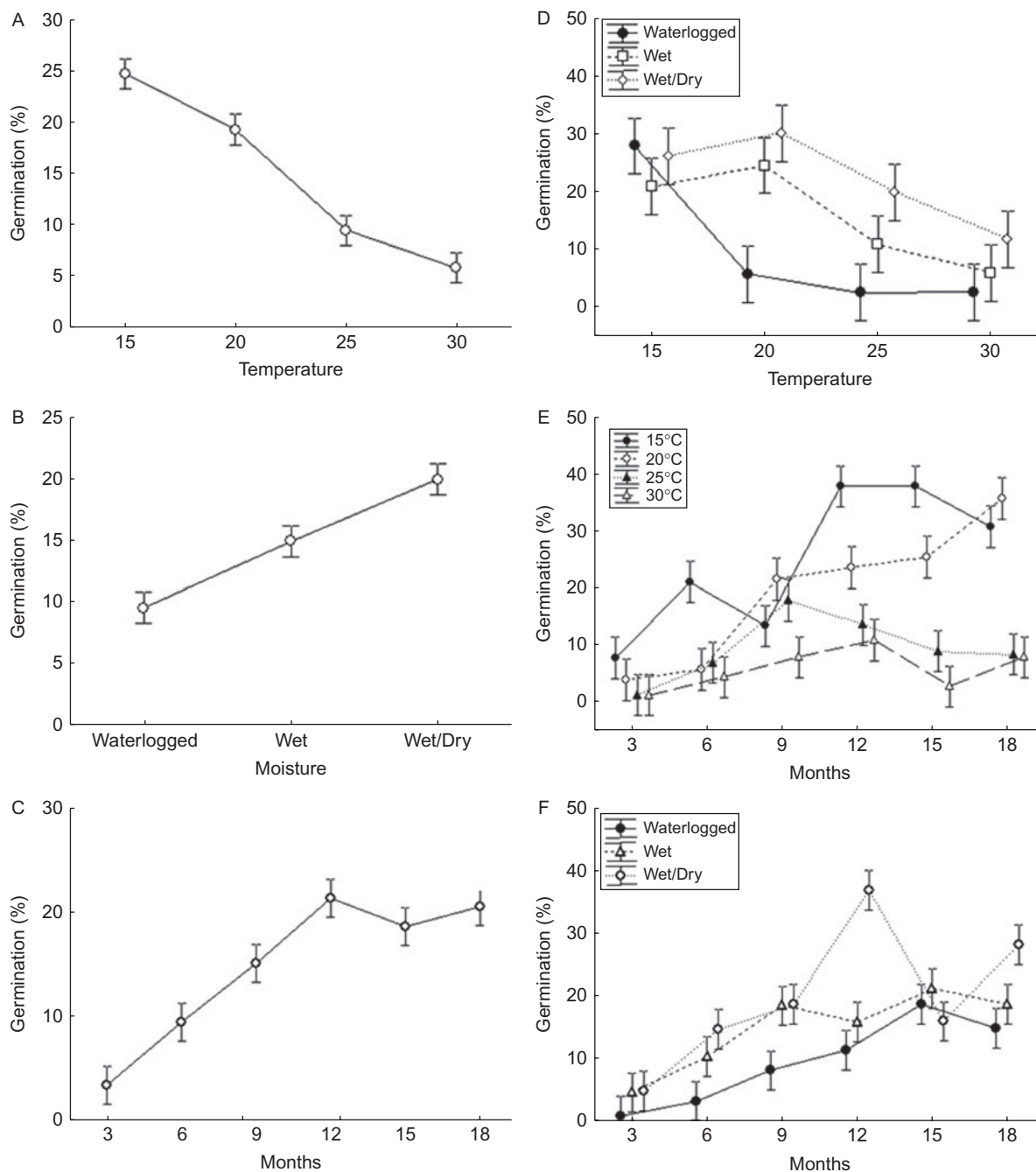

Figure 3. Results of the general linear model (LM) for the germination of *Comanthera bisulcata* seeds as functions of the variables: temperature (A), humidity (B), storage time (C), and their interactions, temperature × humidity (D), temperature × time (E) and time × humidity (F).

a gradual alleviation of dormancy over time (Figs 3C and 4C). Temperature × humidity interactions were significant for both species ($P < 0.001$). There were no differences between the different substrate humidity regimes (boggy, humid or humid/dry) at 15°C (Figs 3D and 4D), although the fluctuating humidity regime (humid/dry) favoured overcoming dormancy in *S. verticillatus* seeds stored at 20, 25 and 30°C (Fig. 4D).

Temperature × time interactions were significant for *C. bisulcata* and *S. verticillatus* ($P < 0.001$), with temperatures of 15°C favouring overcoming dormancy in *C. bisulcata* after 12 and 15 months, and after 9, 12 and 15 months of burial with *S. verticillatus* (Figs 3E and 4E). The time × humidity interactions demonstrated that overcoming dormancy was significantly greater in the humid/dry regime in *C. bisulcata* after 12 months, and
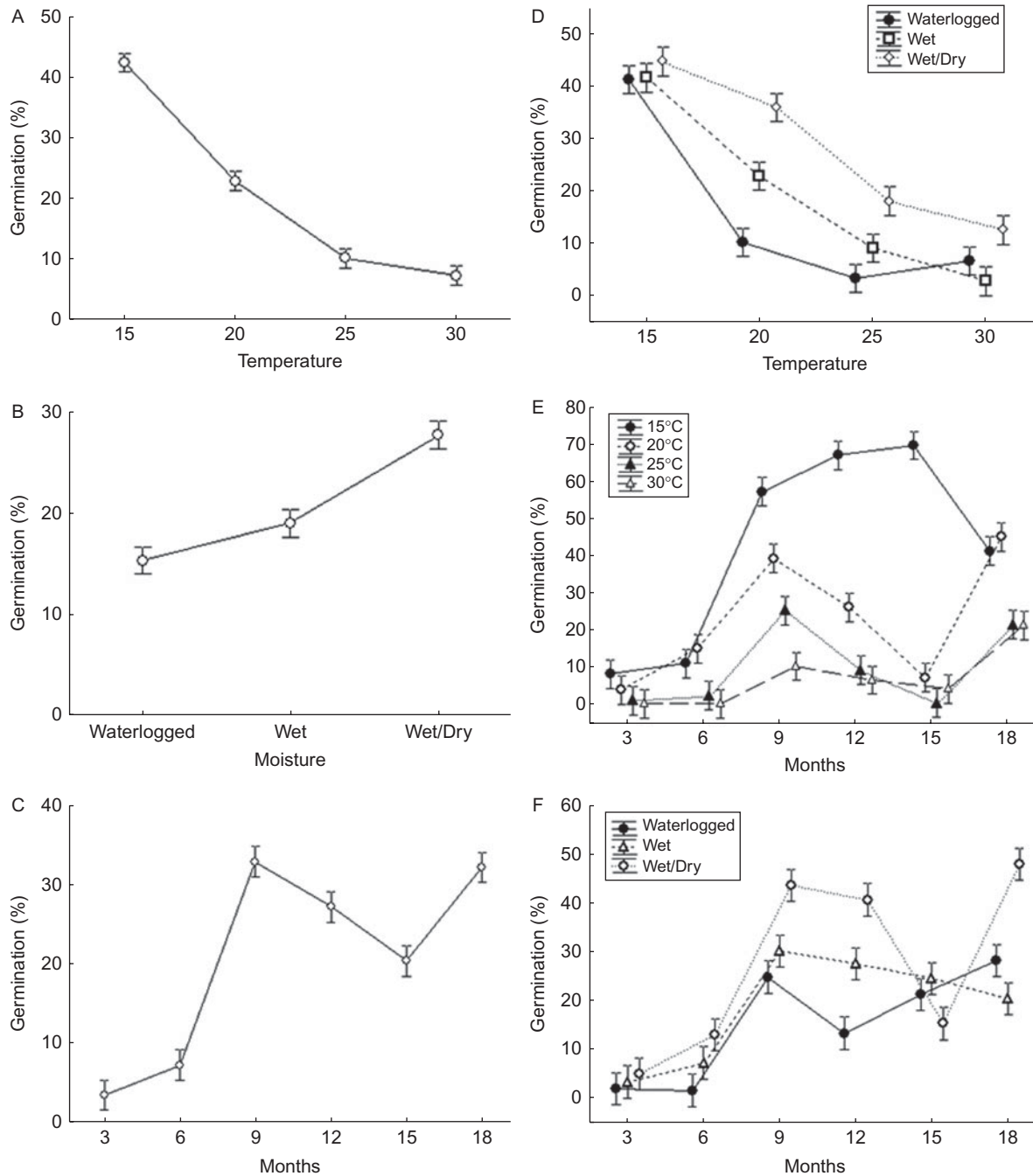

Figure 4. Results of the general linear model (LM) for the germination of *Syngonanthus verticillatus* seeds as functions of the variables: temperature (A), humidity (B), storage time (C), and their interactions, temperature × humidity (D), temperature × time (E) and time × humidity (F).

in *S. verticillatus* after 9 and 12 months (Fig. 3F and 4F). There were significant decreases in seed germinability after the 12th month of the humid/dry treatments in both *C. bisulcata* and *S. verticillatus* that coincided with the humid period, followed by an increase in the 18th month (dry period) (Figs 3F and 4F).

Discussion

The results reported here using recently collected *C. bisulcata* seeds suggest that some of them showed dormancy at the moment of dispersal, as their viability was significantly greater than their germinability (92% and 50%, respectively). The temperature and humidity treatments applied to those seeds during their burial were not sufficient to alleviate primary dormancy, a theme that will need to be more closely investigated in subsequent studies.

The non-dormant seeds of *C. bisulcata* and *S. verticillatus* acquired dormancy after burial in all of the treatments tested, as was observed with storage under natural conditions (Garcia *et al.*, 2014). It is known that temperature, humidity, light and oxygen concentrations are the principal environmental factors involved in seed-bank dormancy (Benech-Arnold *et al.*, 2000) and that they can signal secondary dormancy soon after burial. From an ecophysiological point of view, the acquisition of dormancy soon after burial appears to be related to ecological adaptations designed to avoid seed losses resulting from germination under conditions unfavourable to seedling establishment (Sester *et al.*, 2006).

The dynamics of the germination responses of *C. bisulcata* and *S. verticillatus* varied among the different treatments as well as with storage time. Changes in dormancy states can be induced by continuous exposure to low or high temperatures (Baskin and Baskin, 1998). Our results suggest that both species behave as annual summer plants in relation to temperature, acquiring secondary dormancy after exposure to higher summer temperatures, which is then alleviated by exposure to lower winter temperatures (Bouwmeester and Karssen, 1993; Baskin and Baskin, 1997). It should be emphasized, however, that the species investigated here were perennials growing in a tropical climate with two well-defined seasons: a dry period that occurs in the Austral autumn/winter; and a rainy period during the Austral spring/summer. *In situ* studies have shown that annual dormancy cycles are aligned with environmental seasonality, indicating that dormancy is highly responsive to local environmental conditions (Handley and Davy, 2005). Additionally, while dormancy diminishes over time, it can be re-induced if environmental conditions again become unfavourable (Graeber *et al.*, 2012).

This study demonstrated the close relationship of substrate temperature and humidity with the acquisition and overcoming of secondary dormancy in the buried seeds of both species. The seeds of *C. bisulcata* and *S. verticillatus* gradually overcame dormancy when exposed to the lowest temperature tested (15°C) but remained dormant at the highest temperature (30°C), independent of the substrate humidity regime; however, the viability decreased after 9 months' burial. At intermediate temperatures, overcoming dormancy was dependent on substrate humidity, as was demonstrated by the maintenance of dormancy in the boggy treatment during the entire observation period. A study undertaken with *Polygonum aviculare* L. seeds demonstrated that their dormancy levels depended on soil humidity (Batlla and Benech-Arnold, 2006), confirming that humidity can affect seed dormancy levels. The results of our study also demonstrated that high temperatures, in addition to maintaining secondary dormancy, increased seed degradation, with consequent viability losses. Some evidence indicates that, in the current context of global climatic changes, the increase in the soil temperature can reduce seed viability of the soil seed bank by reaching temperatures that are lethal to the seeds (Ooi, 2012). The impact of higher temperatures may confer negative perspectives for seed viability of *C. bisulcata* and *S. verticillatus*, since the highest temperatures occur during the rainy season in *campos rupestres* sites, conditions that favour the degradation of their seeds.

Overcoming dormancy at low temperatures has been observed in annual species growing in temperate regions (Baskin and Baskin, 1998) and may reflect increased gibberellic acid (GA) biosynthesis in the embryo (Hu *et al.*, 2012). Excess soil water may lead to low O_2 concentrations that, associated with increasing temperature, can induce secondary dormancy through the expression of genes involved in the catabolism and synthesis of abscisic acid (ABA) and GA (Hoang *et al.*, 2013). Benech-Arnold *et al.* (2006), Leymarie *et al.* (2007) and Hoang *et al.* (2012) all reported that low O_2 concentrations induced secondary dormancy. The results of the present study are in agreement with those obtained under natural conditions (Garcia *et al.*, 2014), demonstrating that *C. bisulcata* and *S. verticillatus* acquire secondary dormancy during the summer – the period during which soil saturation generally occurs where these species grow naturally.

The results obtained under the humid/dry regime demonstrated that periodic changes in substrate water contents can affect the dormancy status of the buried seeds of *C. bisulcata* and *S. verticillatus*. Seeds submitted to humid/dry treatments demonstrated a gradual alleviation of dormancy after suspending irrigation (dry substrate) but subsequent increases in dormancy levels once the soil became humid again. A number of workers have shown that seed desiccation followed by rehydration can induce

secondary dormancy (Staniforth and Cavers, 1979; Downs and Cavers, 2000), and that periodic changes in soil humidity can affect the dormancy levels of buried seeds (Batlla and Benech-Arnold, 2010) – thus acting as an additional signalling factor for secondary dormancy (Stoller and Wax, 1993; Vleeshouwers, 1997).

The present study therefore demonstrated the existence of interactions between temperature and soil humidity conditions in signalling cyclical dormancy in the buried seeds of *C. bisulcata* and *S. verticillatus*. The lowest temperature favoured overcoming dormancy, with changes in soil humidity modulating both the acquisition and overcoming of secondary dormancy. The lowest temperatures and driest soils in the areas where the species studied here are found, occur during the autumn/winter seasons, in contrast to the higher humidity and temperatures experienced in the spring/summer. According to Bewley *et al.* (2006), dormancy prevents germination when environmental conditions are appropriate for germination but there is only low probability of their continuing to be favourable enough to guarantee successful seedling establishment and growth. As such, the strategies of the species investigated involve having their seeds in a state of maximum capacity for germination at the end of the dry season (an unfavourable season in itself for germination and establishment)/beginning of the rainy season, while avoiding germination in the middle of the rainy season, so as to better guarantee seedling establishment before the arrival of the subsequent dry season. Additionally, the incapacity of buried seeds to germinate will contribute to a persistent soil seed bank of both species (as verified by Garcia *et al.*, 2014), since high temperatures (>25°C) were not observed below the soil surface, especially in wetland environments where high humidity dampens the amplitudes of thermal fluctuations and helps maintain lower soil temperatures (Oliveira and Garcia, 2011).

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Conflicts of interest

None.

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