Disturbance as a factor in breaking dormancy and enhancing invasiveness of African grasses in a Neotropical Savanna

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ABSTRACT
The Cerrado is threatened by wildfires and invasive species. We aimed to evaluate in laboratory conditions whether temperature fluctuation at the soil surface, resulting from the absence of vegetation due to fire, can affect the germination of *Urochloa decumbens* and *U. brizantha*, two invasive African grasses. Seeds of both species were submitted to simulations: 1) temperature during fire at 1cm belowground (F); 2) temperature fluctuation at 1cm belowground without vegetation cover for a month (TF); 3) (F) + (TF); 4) control at 25ºC. After treatments, seeds were put to germinate at 25ºC for 40 days. We had four replicates per treatment and three temporal replicates. We compared germination percentage and the mean germination time among treatments using ANOVA. The treatments TF and F+TF had the highest germination values for both species. The results showed that fire per se could not stimulate seed germination, however, they suggest that a disturbance that produces a pattern of temperature fluctuation is able to break dormancy and enhance seed germination and, consequently, increase the invasiveness of the study species. Vegetation gaps resulting from disturbance may become new sites of invasion. This information is important for making management decisions regarding the control of these species.

Keywords: Cerrado, fire, germination percentage, invasive species, physiological dormancy, seed dormancy, temperature fluctuation, *Urochloa brizantha*, *Urochloa decumbens*

Introduction

One of the major challenges in invasion ecology is whether it is possible to identify a set of traits that enables a species to be invasive. Reproductive traits are important determinants of the success of invasive species (Rejmánek 1996; Pyšek et al. 2015). The most relevant plant traits related to invasiveness are those associated with seed germination and seed bank longevity. These traits are related to germination capability as well as to the extended germination over time. Together, they enable fast establishment and allow the species to wait for better environmental conditions (Pyšek & Richardson 2007).

Disturbance is well recognized as a triggering factor in a number of cases of biological invasion (Hobbs & Huenneke 1992). Species whose seeds can germinate in both intact and disturbed environments are more likely to spread and occupy new habitats than those with restricted germination requirements. Thus, the ability to germinate in a wide range of environmental conditions should also be mentioned as a trait associated with plant invasiveness (Luo & Cardina 2012).

Events of disturbance create open spaces within the vegetation and lead to alterations in the microclimate (mainly through soil temperature and moisture), and usually increase the availability of soil nutrients or change in the soil microbiota (Davis et al. 2000; Carvalho et al. 2010).
Disturbance may also alter a number of species interactions, and thus favor invasibility in the plant community (Lonsdale 1999; Davis et al. 2000). In addition, the type and intensity of a disturbance are critical in determining the success of exotics and their impact on the invaded community (Lockwood et al. 2007).

Fire is a natural disturbance in many ecosystems and it has been an important selective force in the evolution of seeds of fire-prone plant species (Keeley & Forthofer Ham 2000; Bond & Keeley 2005). High temperatures can break the physical dormancy of seeds resulting in higher germination (Hanley & Lamont 2000; Adkins et al. 2002) and other permeable seeds (Luo & Cardina 2012). On the other hand, some studies have shown decreased germination (Auld & O’Connel 1991; Ribeiro et al. 2013), or no relevant effect (e.g. Le Stradic et al. 2015; Fichino et al. 2016).

Besides fire direct effects on germination through high temperatures and smoke (Santana et al. 2012; Moreira & Pausas 2012), it also promotes indirect effects by changing the environmental conditions: gaps are open in the vegetation, that lead to higher solar radiation and light spectrum, as well as greater temperatures at the soil surface. In consequence, the range of daily soil temperatures is changed, as shown in different ecosystems (Coutinho 1990; Fidelis & Blanco 2014; Santana et al. 2012). The fluctuation of soil temperature can also break seed dormancy (Baeza & Roy 2008; Santana et al. 2012; Musso et al. 2015) and increase the germination of some "gap dependent" species (Ooi et al. 2014). Therefore, the establishment and persistence of invasive species in a community can be directed by fire through its influence on seed germination and seed bank longevity, and according to the burning regime and fire parameters.

In the Cerrado (Brazilian Tropical Savanna), fire has been acting as a selective force on plant communities since millions of years (Simon et al. 2009) and several species – especially in the herb layer – are believed to be fire-dependent (Coutinho 1982; 1990). Tolerance or enhanced germination at high temperatures have been reported for some Cerrado species (Coutinho 1982; Gottsberger & Silberbauer-Gottsberger 2006; Ribeiro & Borghetti 2014).

Therefore, the aim of this study was to evaluate in laboratory conditions whether the increase of soil temperature (direct effect of fire) and/or the daily fluctuation of soil temperature (indirect effect of fire) would affect the germination of two African grasses, Urochloa brizantha and U. decumbens, which show very aggressive invasive behavior in the Cerrado (Pivello et al 1999 a; b). The results of this study will help us to understand the germination strategies of invasive grasses in disturbed environments, giving support to management actions regarding the adequacy of prescribed fires in Cerrado invaded by these grasses.

Materials and Methods

Climate and fire in Cerrado

Cerrado has a tropical climate with wet summers and dry winters. The mean annual rainfall varies from 1200 to 1800 mm, and mean annual temperatures are 22-23°C (Coutinho 2002). The maximum temperatures do not vary much throughout the year and can reach more than 40°C, however, minimum temperatures may vary greatly and reach values close to zero in the coldest months (May-July), producing high temperature amplitudes in the coldest months (Coutinho 2002). The vegetation of Cerrado is characterized by a mosaic of different formations that include grasslands (Campo limpo), savanna-like vegetation (e.g. Campo sujo; Cerrado sensu stricto), and forest formations (Cerradão) (Coutinho 1982; Furley 1999). In a Campo sujo physiognomy in Central Brazil, Fidelis et al. (unpubl. res.) registered temperatures of 50°C at soil surface during the hottest hours of the day, which decreased to 12 - 17°C at night during the dry season (September).

Fires in the Cerrado are ‘surface fires’, which run fast over the soil and rapidly consume the herbaceous biomass, causing no intense heating belowground. In Cerrado physiognomies the temperatures registered during a fire at 1 cm aboveground ranged from 85°C to 840°C (for more details, see Miranda et al. 1993) but belowground they increased only a few degrees, being the maximum values registered by Miranda et al. (1993; 2002) between 29°C (3 cm) and 55°C (1 cm).

Study species

We used two African species that are considered some of the most common and severe invasive species in the Cerrado: Urochloa decumbens (Stapf) R. D. Webster (signal grass) and Urochloa brizantha (Hochst. ex A. Rich.) R.D.Webster (palisade grass) (Pivello et al. 1999 a; b; Almeida-Neto et al. 2010; Lannes et al. 2012). They are C₄ perennial grasses widely used in tropical pastures due to their high palatability, productivity and resistance to intensive grazing and trampling (Loch 1977; Kissmann 1997). Urochloa species were introduced in Brazil in the mid-1950s as cattle fodder (Kissmann 1997), and readily adapted to the low-fertile and aluminum-rich soils of Cerrado (Rao et al. 1996). The seeds of U. decumbens disperse in December/January and of U. brizantha from April to June. However, some individuals of both species continue to disperse all over the year (personal observation). These invasive species have a strong competitive ability and they can rapidly dominate the Cerrado herbaceous community.
(Pivello et al. 1999 a; Almeida-Neto et al. 2010). In addition, the presence of Urochloa spp. in Cerrado can change fire behavior by affecting fire intensity, maximum temperature and flame height (Gorgone-Barbosa et al. 2015).

In the experiments of this study we used commercialized seeds of Urochloa decumbens and Urochloa brizantha, since they are widely used in planted pastures. The seeds were not submitted to any previous treatment, being stored at room temperatures of 25ºC.

Temperature treatments and simulated curves

We submitted the seeds of both species to four different treatments to simulate both the effect of belowground temperatures during the passage of fire and the daily temperature fluctuations after the removal of vegetation by fire. The seeds were placed in Petri dishes in an incubator (Binder KB E5.1) where all temperature treatments occurred. We applied the following treatments: Fire (F) = simulation of fire temperatures at 1 cm belowground: the initial temperature was 25ºC, rose up to 50ºC in 20 minutes (based on previous data from Miranda et al. 1993) and then decreased continuously to 25ºC in the following 30 minutes; Temperature fluctuations (TF) = simulation of the daily temperature fluctuation at 1 cm belowground without vegetation cover: seeds were subjected to alternating temperature (10ºC to 40ºC, that represent the mean range of soil temperatures after fire during the dry season, measured in the field by A. Fidelis et al., (unpubl. res.) during thirty days; Fire + Temperature fluctuations (F+TF) = combination of the two treatments, to simulate both the passage of fire and daily temperature fluctuations resulting from vegetation removal; and Control (C): seeds were stored at a constant temperature of 25ºC until put to germinate. According to the manual for the analyses of seeds of the Brazilian Ministry of Agriculture (Brasil 2009), temperatures recommended for germinating U. decumbens and U. brizantha must be between 15 and 35ºC. As the mean temperature in open cerrados is around 25ºC (Coutinho 2002) we chose it as the control temperature in our experiments, although it has been reported in the literature that germination and dormancy breaking of Urochloa spp. was not influenced by light or temperature (Adkins et al. 2002).

Seed germination experiments

Before starting the experiments we tested the seed viability through the Tetrazolium test 1%. The mean viability was 81% for U. decumbens and 87% for U. brizantha.

Urochloa decumbens and U. brizantha seeds were divided into four samples for each treatment (30 seeds/replication/treatment) and temporal replication. We replicated the experiment three times (temporal replication) to avoid pseudoreplication, since we had only one incubator (30 seeds x 4 samples x 4 treatments x 3 temporal replications = 1440 seeds/species). The entire duration of the experiment was 90 days (30 days for each replicate). Although a single species viability analysis was performed, the time elapsed between replicates was not considered to have influenced the results of the experiment, as it was depicted form previous tests. Seeds were placed in Petri dishes, covered with one layer of filter paper, moistened with distilled water, and put in germination chambers at 25ºC and dark conditions to simulate the buried seeds. The germination was recorded every other day, for 40 days. We considered germinated those seeds with 1 mm long radicle, and removed them from the Petri dishes after being counted. Seeds that did not germinate in 40 days were submitted to Tetrazolium test to check their viability.

Analyses

We calculated the percentage of germination and the mean germination time (MGT) in days, according to Ranal & Santana (2006).

To compare percentage of germination, MGT and viability between treatments, we used one-way analysis of variance (ANOVA, factor = treatment) applied to randomization tests (Euclidean distance between sampling units, 10000 interactions). All statistical analyses were performed using the software MULTIV (Pillar 2005).

Results

Seeds of both U. decumbens and U. brizantha showed percentage of germination which varied from 45% to 75%, independently of the treatment. Moreover, both species showed a similar pattern of response to the different treatments. A significant increase in the percentage of germination of both species was observed after TF and F+TF treatments when compared to the control (Fig.1).

The percentages of germination of U. decumbens were 75±10% and 66±12% respectively for TF and F+TF, and not statistically different (P= 0.09), whereas the control (C, 48±10%) and F treatment (45±12%) values were lower (P< 0.0001, for all comparisons) and statistically similar to each other (P= 0.64) (Fig. 1A). The seeds of U. brizantha also showed the highest percentages of germination after TF and F+TF treatments (62±10% and 59±9%, respectively) and not statistically different between each other (P= 0.10), but significantly differed from the control (46±13%, P= 0.004 and P= 0.02, respectively, Fig. 1B) while F treatment resulted in percentage of germination (54±10%) not statistically different from those of the other treatments (P> 0.05, Fig. 1B). Considering all the viable seeds (germinated + not germinated, but still viable), the TF and F+TF treatments proportionally increased the percentage of germination for both species, since viability between the
treatments did not differ (Tab. 1, *U. decumbens*, *P* = 0.31 and *U. brizantha*, *P* = 0.80).

The mean germination time (MGT) did not differ between the treatments for both species (Fig. 2 - *U. decumbens*, *P* = 0.83 and *U. brizantha*, *P* = 0.63). The MGT of *U. decumbens* varied from 4.8±1.9 days in C to 3.7±1.1 days in F+TF.

**Discussion**

The ability to successfully germinate in a wide range of environments and to withstand disturbances are key reproductive traits that increase invasiveness of alien species (Pyšek & Richardson 2007; Luo & Cardina 2012). The two study species germinate more than 40%, which could be already advantageous to colonize and spread in invaded sites. Nevertheless, our results support the idea that part of both species’ seeds (14-37%, considering the viable seeds of the treatments) are dormant, and such dormancy could be broken by daily fluctuation of temperature, generated for example, after disturbance events that remove the aboveground vegetation. Such temperature fluctuation enhanced germination, and consequently their invasiveness by increasing the probability of establishment of the species and therefore increasing the chance of a successful invasion.

Several grass species are known to have physiological dormancy (Baskin & Baskin 2004), as already reported for the genus *Urochloa* (Whiteman & Mendra 1982; Adkins et al. 2002). This strategy allows for long-term survival of populations by maintaining a persistent soil seed bank and delaying germination until the microclimatic conditions are optimal for seedling establishment (Baskin & Baskin 2004), being advantageous for the invasive species.

Temperature is the main environmental variable involved in the development, control, and the breaking of seed dormancy of species from different ecosystems (Moreira & Pausas 2012; Santana et al. 2012; Presotto et al. 2014). In our study we simulated the heating effect caused by Cerrado fires on seeds stored in the soil seed bank at 1 cm depth, and that mild temperature of 50ºC (F treatment) affected neither *U. decumbens* nor *U. brizantha* germination. A similar result was found by Martins & Silva (2001), where temperatures of 40ºC and 55ºC were not effective on stimulating the germination of *U. brizantha*. Recent studies showed that fire did not enhance the germination of grasses and forbs of open physiognomies of Cerrado but seeds were resistant to high temperatures (Le Stradic et al. 2015; Fichino et al. 2016). Also, heat-shock of 100 °C did not affect germination percentages of invasive species in the Chilean matorral (Gómez-González et al. 2011).

In this study, we found an indirect effect of fire, since both TF and F+TF were able to break seed dormancy and enhance germination. Thus, fire did not affect directly the germination of *U. decumbens* and *U. brizantha* seeds from the soil seed bank, but the temperature fluctuation during one month was the effective triggering element. Musso et al. (2015) also found that *Aristida setifolia* and *Echinolaena inflexa*, two native Cerrado grasses, had their germination increased by temperature fluctuations (10-45°C). However, *Melinis minutiflora*, another Cerrado invasive grass, was not affected by temperature fluctuations (Musso et al. 2015).

Our results agree with the idea that disturbance events could facilitate the invasion process of *U. decumbens* and *U. brizantha* in Cerrado, not only by enhancing invasibility due to the increased resources and changes in competition, but also by enhancing the invasiveness of the species by

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**Figure 1.** Percentage of germination (%) (mean, standard error and mean + 2* standard deviation) after the treatments: C = Control; F = fire; TF = temperature fluctuation; F+TF = fire followed by temperature fluctuation for (A) *Urochloa decumbens* and (B) *Urochloa brizantha*. Different letters represent significant differences, *P* ≤ 0.05.
breaking seed dormancy and facilitating germination. High percentage of germination directly affects the process of invasion by increasing recruitment and reproductive success of the invasive species, and as a consequence, its abundance, subsequent spread and colonization of new areas. Moreover, the pool of seeds in the soil may promote a considerable seed bank to maintain the invasive species in the community (as shown by Carmona 1995; Silva & Dias-Filho 2001). Therefore, the post-disturbance behavior of both *Urochloa* species can be pointed out as an advantage over natives and probably other Cerrado invasive species, such as *Melinis minutiflora*.

It is important to highlight that we used commercial seeds with a high viability, and it is expected that seeds collected in the field would have lower viability. But even so, a percentage of these seeds would be stimulated by post-fire conditions. In fact, a great number of *U. decumbens* seedling was observed after fire in an invaded campo sujo in São Paulo state (E. Gorgone-Barbosa, unpubl. res.).

Our study revealed an important mechanism of the invasion process: increased germination and seedling recruitment after disturbance, which explains in part the invasion success of these species in the burned Cerrado. Local managers should thus consider the gaps created in the vegetation by any kind of disturbance as a potential new site of invasion, if seeds of invasive species are available in the area.

**Conclusion**

Mild temperatures (around 50°C) caused by Cerrado fires in the soil seed bank at 1 cm depth do not affected the germination percentage of both *U. decumbens* and *U. brizantha*, aggressive invasives in that ecosystem. An important ecological mechanism that relates fire to the invasion of *Urochloa* species in disturbed cerrados is the post-fire fluctuation of daily temperatures at the soil level, which may facilitate their initial germination, enhance the percentage of germinated seeds, and probably increase the establishment success in recent burned areas.

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