UNIVERSIDADE DE BRASÍLIA

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

Ecologia e funções adaptativas da dormência em sementes de gramíneas campestres brasileiras

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BRASÍLIA

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SUMÁRIO

RESUMO

O Cerrado, a savana brasileira, caracteriza-se por um clima com precipitação sazonal e com recorrente passagem de fogo. Em teoria, o número de espécies com sementes dormentes tende a ser maior em ambientes com sazonalidade hídrica do que em ambientes não-sazonais, porque a estação seca constitui um forte limitante ao recrutamento de plântulas. No entanto, apesar da reprodução das gramíneas nativas no Cerrado ser limitada pela sazonalidade, existe ampla variação entre espécies na presença e duração da dormência. As causas desta variação, bem como suas consequências ecológicas, permanecem desconhecidas. Assim, o objetivo deste estudo foi determinar os padrões de longevidade, germinação e dormência (i.e. estratégia de germinação) de sementes de 29 espécies de gramíneas nativas do Cerrado, investigando possíveis causas evolutivas e consequências ecológicas dessas estratégias, além da potencial aplicabilidade das sementes dessas espécies em restauração ecológica. No capítulo 1, investiguei duas hipóteses para a evolução de dormência: (1) restrição hídrica e (2) competição intraespecífica durante o recrutamento de plântulas. Assumindo que a dispersão limitada resulta em adensamento de sementes e, consequentemente, maior competição durante o desenvolvimento das plântulas, comparei as estratégias de germinação de espécies de habitats que diferem em umidade do solo (campo sujo vs. campo úmido) e épocas de dispersão contrastantes em termos de sazonalidade hídrica (início ou fim da estação chuvosa e estação seca), e diferentes síndromes de dispersão (anemocórica vs. barocórica). Sementes de campos úmidos perderam a dormência mais rapidamente e permaneceram vivas por mais tempo do que sementes de campos sujos. Sementes dispersas na seca e no início da estação chuvosa tiveram maior germinação do que sementes dispersas no final da estação chuvosa. Sementes com dispersão barocórica foram mais dormentes do que sementes com dispersão anemocórica. No capítulo 2, verifiquei se a a probabilidade de uma semente passar por um evento de queima e se as diferenças de temperaturas que ocorrem durante a passagem do fogo nos habitats das espécies de gramíneas determinam a tolerância das sementes a altas temperaturas. Assumindo que sementes dormentes passam mais tempo no solo do que sementes não dormentes e, portanto, estão mais susceptíveis a passar por um evento de queima, comparamos a tolerância a choques-térmicos (80 °C e 110 °C) em sementes dormentes e não dormentes. Também testamos a tolerância a choques-térmicos em sementes de espécies coletadas em habitats com menores temperaturas do fogo (campo úmido) e maiores temperaturas do fogo durante a queima (campo sujo). Sementes de ambos os habitats foram negativamente afetadas por altas temperaturas. Entretanto, sementes dormentes foram mais tolerantes a altas temperaturas do que sementes não dormentes. Por fim, no capítulo 3, verificamos as variações temporais nos padrões de longevidade e germinação de 29 espécies de gramíneas, e identificamos 20 espécies com potencial para utilização na recuperação de áreas degradadas. Em conclusão, nossos resultados sugerem que (1) a dormência em sementes de gramíneas do Cerrado evoluiu em resposta à restrição hídrica e à competição intraespecífica; (2) e a tolerância ao fogo é possivelmente uma consequência ecológica da dormência.

Palavras-chave: Cerrado, germinação, Poaceae, longevidade, sazonalidade, síndrome de dispersão, fogo.

ABSTRACT

Cerrado, the Brazilian savanna, is characterized by a climate with seasonal precipitation and frequent fire events. In theory, the number of species with seed dormancy is higher in seasonal environments compared to species in non-seasonal environments, due to the fact that dry seasons may strongly limit seedling recruitment. However, although reproduction of grass species in the *Cerrado* is limited by seasonality, there is a high variation in the presence of seed dormancy and its duration among these species. The origin and the ecological consequences of this variation remain unknown. Therefore, the aim of this study was to determine the longevity, germination, and dormancy patterns (i.e. germination strategies) of seeds from 29 native grass species from the *Cerrado*. This study also aimed to investigate the possible evolutionary causes and ecological consequences of these strategies, as well as the potential applicability of these species in restoration ecology. In chapter 1, we investigated two hypotheses for seed evolution: (1) water restriction and (2) intraspecific competition during seedling recruitment. We assumed that limited dispersion results in seed crowding and, consequently, higher competition during seedling growth. Thus, comparisons were conducted regarding the germination strategies of species from contrasting habitats (open savanna vs. wet grassland), seed dispersal season in terms of water availability, and different dispersal syndromes (anemochoric vs. barochoric). Seeds from wet grassland species lost dormancy faster and were viable for longer periods than seeds from open savanna species. Seeds dispersed during the dry season or early in the rainy season germinated more than those dispersed late in the rainy season. Species with barochoric seeds had more seed dormancy than species with anemochoric seeds. In chapter 2, the aim was to verify if the likelihood of a seed to burn and the differences in temperature that occur during fires in species' habitats may determine seed tolerance to heat. Assuming that dormant seeds might remain in the soil longer than non-dormant seeds, increasing their likelihood to burn, the heat tolerance (80 and 110 °C) of species with dormant and non-dormant seeds was compared. In addition, the heat tolerance of seeds from species from habitats with lower (wet grasslands) and higher (open savannas) fire temperatures was also tested. Seeds from both habitats were negatively affected by high temperatures, but dormant seeds had a higher tolerance than non-dormant seeds. Finally, in chapter 3, the variation in temporal patterns of seed longevity and germination in 29 grass species were verified, and 20 species with ecological restoration potential were identified. In conclusion, the results suggest that (1) seed dormancy in grass seeds from the *Cerrado* evolved in response to water restriction and intraspecific competition; (2) and seed tolerance to fire is probably an ecological consequence of seed dormancy.

Key words: *Cerrado*, seed germination, Poaceae, seed longevity, seasonality, dispersal syndrome, fire.

INTRODUÇÃO GERAL

Sazonalidade da precipitação e dormência

A dormência pode ser caracterizada pela não germinação de uma semente em condições adequadas (i.e. água, temperatura, oxigênio) para que a germinação ocorra. A dormência em sementes pode ser vista como uma resposta adaptativa a condições de estresse, muitas destas associadas à sazonalidade, como por exemplo, o dessecamento do solo durante a estação seca (Garwood 1983; Mathias & Kisdi 2002). Uma das consequências da dormência é a distribuição da germinação no tempo em dada população de sementes (Bewley 1997). Esta característica proporciona maior aptidão a plantas de ambientes sazonais, pois reduz a probabilidade de germinação em época não favorável para o estabelecimento da plântula (Keya 1997). Tendo isso em conta, o número de espécies com sementes dormentes encontrada é maior em ambientes com algum nível de sazonalidade na precipitação ou temperatura (~80%) do que (~40%) em ambientes não sazonais, ou seja, com ausência de uma estação seca e/ou fria (Jurado & Flores 2005).

O Cerrado é composto por um mosaico de fisionomias de savanas, florestas e campos na região central do Brasil. O clima é marcadamente sazonal, com um inverno seco de abril a setembro e um verão chuvoso de outubro a março (precipitação média entre 800 – 2000 mm), com temperatura média de 18°C e 28°C, respectivamente (Oliveira-Filho & Ratter 2002). Com cerca de 600 espécies a família Poaceae tem alta representatividade no Cerrado (Filgueiras et al. 2014). Além disso, é uma família com alta abundância e frequência em áreas campestres do Cerrado. Por possuírem sistema radicular não profundo, a absorção de água em gramíneas está limitada às camadas superficiais do solo (5-50cm; ver Rossatto et al. 2013) e, consequentemente, são afetadas pela escassez de água durante a seca. Assim, a floração e frutificação é restrita, principalmente, à estação chuvosa (Tannus et al. 2006; Munhoz & Felfili 2007; Ramos et al. 2014). Porém, a dispersão das sementes ocorre principalmentes durante a estação chuvosa com algumas espécies dispersando também durante a seca (Silva & Ataroff 1985; Almeida 1995). Em teoria, sementes dispersas no início da chuva, entre outubro e janeiro, tem toda a estação chuvosa para germinar e estabelecimento das plântulas (cerca de sete meses). Por outro lado, a germinação nos últimos meses de chuva, entre fevereiro e abril, pode ser arriscada e o estabelecimento das plântulas prejudicado, devido ao curto período que resta de estação chuvosa (menos de quatro meses). Além disso, o solo pode estar úmido no início da estação seca devido as últimas chuvas da estação chuvosa. Assim, a germinação de sementes dispersas na estação seca, entre maio e setembro, pode ser prejudicial para o estabelecimento das plântulas devido ao dessecamento do solo nos meses seguintes. Diante disto, pode-se esperar a existência de um maior número de espécies com dormência entre as espécies que dispersam no final da estação chuvosa e na seca em comparação as espécies que dispersam no início da estação chuvosa.

Além da sazonalidade climática da região existe uma variação na sazonalidade hídrica relacionada a umidade do solo em fisionomias campestres do Cerrado. Nos campos sujos, que possuem lençol freático profundo, as camadas superficiais do solo secam durante a estação seca. Por outro lado, nos campos úmidos as camadas superficiais do solo permanecem úmidas por um determinado tempo dentro da estação seca, uma vez que o lençol freático se encontra mais próximo a superfície. A sazonalidade hídrica é menos marcante em campos úmidos do que em campos sujos e, por esse motivo, espera-se que as sementes de espécies de gramíneas coletadas em campos úmidos tenham menor dormência do que sementes de espécies coletadas em campos sujos.

Trade-off entre estratégia de dispersão e dormência

Além da função de evitar períodos de seca em ambientes com sazonalidade hídrica, outra explicação alternativa existente é de que a dormência pode ter evoluído em resposta, à competição por recursos entre organismos da mesma espécie, incluindo parentes (*e.g.* planta-mãe ou irmãos; hipótese da competição com parentes – Howe & Smallwood 1982; Kobayashi & Yamamura 2000; Cheplick 1992). A existência de alguma modalidade de dormência que espalhasse germinação das sementes ao longo do tempo a poderia contribuir com a redução da competição direta intraespecífica. Neste contexto, a estratégia de dispersão poderia ser um fator de relevante influência na evolução da dormência em sementes (Rees 1993, 1994; Baskin & Baskin 2014). Por exemplo, espécies que apresentam sistemas de dispersão que possibilitam maior distância de dispersão, como o anemocórico, teriam em consequência menor pressão de competição por recursos entre as plântulas de determinada progênie e entre estas e a planta-mãe (Venable & Brown 1988; Cheplick 1993). Assim, em teoria, espécies que apresentam maior distância de dispersão das sementes teriam menor pressão seletiva no estabelecimento da dormência (Lu et al. 2010), de maneira que os propágulos de espécies que fossem dispersos a grandes distâncias apresentariam menor grau de dormência do que propágulos de espécies dispersos demasiadamente próximos a planta-mãe (Fenner & Thompson 2005). Além disto, espécies com sementes sem dormência evitariam os custos associados à dormência, como o maior tempo de exposição à predação e a patógenos, assim como a evolução de mecanismos de defesa como compostos secundários (Thompson 1987).

A maior parte das espécies de gramíneas possui fruto seco, chamado cariopse, que não se separa da semente (Filgueiras 1986). Este fruto, na maioria das vezes, é disperso com brácteas e apêndices, que tornam estes diásporos complexos (a partir daqui será referido como semente). As sementes das gramíneas apresentam considerável variação quanto à síndrome de dispersão (Peart 1979, 1981, 1984; Silberbauer-Gottsberger 1984; Ernst et al. 1992; Guerrero & Tye 2009), dentre as quais destacam-se a dispersão pelo vento, anemocoria (*Andropogon* L.), por gravidade, barocoria (algumas espécies de *Paspalum* L. e *Panicum* L.) e ingestão por animais, endozoocoria (*Lasiacis* (Griseb.) Hitchc.) (Davidse & Morton 1973). Algumas espécies, como *Echinolaena inflexa* (Zuloaga & Morrone 2003), de dispersão primária barocórica possuem elaiossomos (Davidse 1986), estruturas que atraem as formigas, que podem possibilitar a dispersão secundária (van der Pijl 1982). A endozoocoria e a anemocoria têm sido reconhecidas como eficientes mecanismos de dispersão a longas distâncias (Ernst et al. 1992; Couvreur et al. 2004a; Couvreur et al. 2004b). Estruturas como tricomas longos podem facilitar a dispersão dos diásporos de gramíneas de savanas pelo vento (Chase & Sendulsky 1991). Desta forma, estes diásporos podem atingir maiores distâncias de dispersão. Por outro lado, espécies que possuem estratégia de dispersão barocórica dispersam suas sementes próximas à planta mãe e, a menos que essas espécies possuam dispersão secundária, a germinação de suas sementes teoricamente poderá resultar em competição por recursos entre as plântulas e estas com a planta mãe, em caso de plantas perenes (Howe & Smallwood 1982; Kobayashi & Yamamura 2000; Cheplick 1992).

Efeitos do fogo na sobrevivência e germinação de sementes em savanas

As queimadas recorrentes são características das savanas e o estrato herbáceo é o principal combustível para o fogo (Kauffman et al. 1994; Mistry 1998; Hoffmann et al. 2012). Durante a passagem do fogo as sementes ficam expostas fatores como altas temperaturas e fumaça, que podem ter efeitos na germinação e sobrevivência das mesmas (Gashaw & Michelsen 2002; Clarke & French 2005; Dayamba et al. 2008; Scott et al. 2010). Estudos sugerem que a frequência do fogo e as altas temperaturas geradas durante as queimadas influenciam os padrões de sobrevivência em sementes (Ribeiro et al. 2012; Ribeiro & Borghetti 2013). A sobrevivência das sementes a altas temperaturas é considerada uma adaptação ao fogo em espécies de plantas de ambientes propensos a queimadas (Gashaw & Michelsen 2002; Clarke & French 2005; Overbeck et al. 2006; Dayamba et al. 2008; Scott et al. 2010). Assim, sementes de espécies de habitats que queimam frequentemente (cerrado típico) são mais tolerantes a altas temperaturas do que sementes de espécies de habitats com menor frequência ou ausência de queimadas (matas de galeria) (Ribeiro et al. 2012; Ribeiro & Borghetti 2013).

No Cerrado, o comportamento do fogo difere entre fisionomias de campo sujo e campo úmido (Miranda et al. 2009; Schmidt 2011). A intensidade do fogo é menor em campos úmidos (1083 kJ s^{-1} m^{-1}) do que em campos sujos (20393 kJ s^{-1} m^{-1}), provavelmente devido a maior umidade do combustível nos campos úmidos (Miranda et al. 2009; Schmidt 2011). Outro fator importante é que as temperaturas máximas do ar (1 cm acima do nível do solo) que ocorrem durante a passagem do fogo são maiores em campos sujos do que em campos úmidos. Além disto, o tempo de residência de temperaturas acima de 60 °C é menor em campos úmidos do que em campos sujos (Schmidt 2011). Assim, podemos esperar menor tolerância a choques térmicos com altas temperaturas em sementes de espécies de gramíneas de campos úmidos em relação a sementes de espécies de campos sujos.

Em savanas existe uma grande variação nos níveis de dormência em sementes de gramíneas (Baskin & Baskin 1998; Baskin & Baskin 2014). As sementes podem ser dormentes quando dispersam ou não ter dormência. Sementes dormentes, normalmente perdem a dormência após um certo tempo no solo, sincronizando a germinação com a estação chuvosa seguinte. Por outro lado, as sementes não dormentes podem germinar assim que dispersam, ficando menos tempo no solo. Por ficarem mais tempo no solo, as sementes dormentes aumentam a probabilidade de passar por um evento de queima do que sementes não dormentes. Diante disto, espera-se que sementes de espécies com dormência tenham maior tolerância a altas temperaturas do que sementes de espécies que não tenham dormência.

OBJETIVO GERAL

- Determinar os padrões de longevidade, estratégias de germinação e dormência de sementes de espécies de gramíneas nativas do Cerrado, investigando possíveis causas evolutivas e consequências ecológicas dessas estratégias, além da potencial aplicabilidade das sementes dessas espécies em restauração ecológica.

OBJETIVOS E HIPÓTESES POR CAPÍTULO

Capítulo 1:

Objetivo 1: Testar os efeitos da época de dispersão (estação seca, início e final da estação chuvosa) e do tipo de habitat da coleta das sementes (campos sujos e campos úmidos) na germinação e níveis de dormência de sementes de gramíneas nativas do Cerrado.

Hipóteses:

H1a) Sementes de espécies com dispersão no início da estação chuvosa são menos dormentes do que sementes de espécies com dispersão no final da estação chuvosa e na estação seca;

H1b) Sementes de espécies com dormência aumentam a germinação, ou seja, perdem a dormência, com o armazenamento;

H2a) Sementes de espécies coletadas em campos sujos (com maior restrição hídrica) são mais dormentes do que as sementes de espécies coletadas em campos úmidos (com menor restrição hídrica);

H2b) Sementes de espécies coletadas em campos úmidos tem menor duração de dormência do que sementes de espécies coletadas em campos sujos.

Objetivo 2: Investigar a existência de um trade-off entre síndrome de dispersão (anemocórica ou barocóricas) e a presença de dormência em sementes de espécies de gramíneas nativas do Cerrado.

Hipóteses:

H1) Sementes de espécies com síndrome de dispersão barocórica tem maior dormência do que sementes de espécies com síndrome de dispersão anemocórica;

H2) Sementes de espécies com síndrome de dispersão barocórica perdem a dormência com o armazenamento.

Capítulo 2:

Objetivo 1: Verificar a tolerância de sementes de gramíneas a choques-térmicos com altas temperaturas, de espécies coletadas em habitats com diferentes temperaturas de fogo (campos sujos e campos úmidos);

Hipótese:

H) Sementes de espécies coletadas em campos sujos são mais tolerantes a altas temperaturas do que sementes de espécies coletadas em campos úmidos;

Objetivo 2: Verificar a tolerância de sementes de espécies de gramíneas com dormência e sem dormência a choques-térmicos com altas temperaturas.

Hipótese:

H) Sementes de espécies com dormência são mais tolerantes a altas temperaturas do que sementes de espécies sem dormência.

Capítulo 3:

Objetivo: Verificar os padrões temporais de longevidade e germinação de sementes de 29 espécies de gramíneas nativas do Cerrado com aplicabilidade na recuperação de áreas degradadas;

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CAPÍTULO 1

Avoiding the dry season: dispersal time and syndrome mediate seed dormancy in Neotropical savanna grasses

Avoiding the dry season: dispersal time and syndrome mediate seed dormancy in Neotropical savanna grasses

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Abstract

• Background and Aims: Seed dormancy is often viewed as a strategy to avoid seed germination in dry periods in plants from seasonal rainfall environments and also trades-off with seed dispersal syndrome, because both can be costly strategies to avoid crowding and sibling competition. Grass species in Brazilian savannas (*Cerrado*) show large variation in habitat type, seed dispersal times, syndromes and live in a rainfall seasonal environment. Our aims are to test dormancy levels and seed germination comparing seeds: (1) dispersed under different times (early rainy season, late rainy season and dry season); (2) living in habitats with contrasting soil moisture during the dry season (open savannas *versus* wet grasslands), and (3) showing opposite dispersal syndromes (barochoric *versus* anemochoric). We expected to find the highest dormancy levels in late rainy season dispersed seeds, wet grasslands and barochory (short dispersal) seeds.

• Methods We investigated viability, dormancy and germination of fresh seeds and after dry storage (3, 6, 9 and 12 months) of 29 grass species from open savannas and wet grasslands of Central Brazil.

• Key Results Grass seed dormancy varied with seed dispersal timing and syndrome, and habitat seasonality. First, fresh seeds dispersed late in the rainy season had higher levels of seed dormancy compared to the early dispersed seeds. Second, seeds from wet grasslands lived longer and had constant higher germination rates than seeds from open savannas. Finally, we found that anemochoric seeds had less dormancy than barochoric seeds.

• Conclusions We found that indeed seed dormancy is higher prior to the dry season, in dry habitats and in short dispersed (barochory) seeds for grasses in Brazilian savannas. These results suggest seed dormancy probably avoid costly seedling recruitment in the dry season and this effect seems to be attenuated by habitat specific soil moisture. Seed dormancy traded-off with seed dispersal, which suggests both strategies are costly and had non-additive benefits.

Key words: *Cerrado*, dry storage, germination, open savanna, Poaceae, wet grassland.

INTRODUCTION

Savannas are biomes composed of a continuous grass layer and scattered trees, under a seasonal climate consisting of an annual cycle of wet and dry seasons (Scholes and Archer, 1997). The *Cerrado* is a Neotropical biome in Central Brazil composed mostly of savannas but also includes grasslands and forests. Soil moisture is one of the main determinants of vegetation physiognomies; for example, rain forests are associated with waterfalls and riparian areas while wet grasslands occur where the water table reaches close to the surface. In both of these examples, soils are moist throughout the year. However, in savannas the water table sits deeper below the soil surface, leading to soils and the herbaceous layer drying out during the annual dry season. Grass species within the *Cerrado* biome are mostly restricted to the open physiognomies of savannas and grasslands due to their shade intolerance.

In savannas, rainfall seasonality is among the major limiting factors determining seed germination and seedling establishment, due to its direct effects on water availability in the soil (Jurado and Flores, 2005; Baskin and Baskin, 2014). Rainfall seasonality is therefore likely to be a strong selective pressure acting on seed dormancy, and indeed seed dormancy has been found in a higher number of species in seasonal rainfall environments in comparison to less seasonal environments (Jurado and Flores, 2005; Baskin and Baskin, 2014).

Grasses are one of the most highly represented families (Poaceae) in the herbaceous layer of the *Cerrado* (around 600 species, Filgueiras *et al.*, 2014). Grasses occurring in savanna physiognomies of the *Cerrado* are subjected to seasonal variation in soil water availability throughout the year. For most species the flowering and fruiting periods are restricted to the rainy season (Tannus *et al.*, 2006; Munhoz and Felfili, 2007; Ramos *et al.*, 2014), presumably as a strategy to avoid water shortage during the dry season. However, there is large variation in the timing of seed dispersal among grass species (Almeida, 1995; Munhoz and Felfili, 2007; Ramos *et al.*, 2014), with some species dispersing early in the wet season, others dispersing later, and some species dispersing seeds even during the dry season (Almeida, 1995). Seeds dispersed early in the wet season would have the entire growing season to germinate and recruit, whereas seeds dispersed late in the wet season, or during the dry season, would very probably face extreme water shortages, which potentially could be a risky strategy for seedling survival. Avoiding germination prior to and during the dry season would be a key strategy for reducing the probability of seedling mortality during the dry season, and seed dormancy would therefore play an important role in delaying seed germination to the next wet season. Consequently, we would expect deeper seed dormancy among seeds dispersed late in the rainy season, and during the dry season, in comparison to seeds dispersed early in the rainy season for species from savanna habitats. In contrast, in wet grasslands the water table is situated near the surface throughout the year (Cianciaruso and Batalha, 2008; Meirelles *et al.*, 2010) and hence the impact of rainfall seasonality in the soil is buffered by the water table and the soil surface stays moist for longer during the dry season (Cianciaruso and Batalha, 2008; Meirelles *et al.*, 2010). Subsequently, if seed dormancy is a strategy of species from seasonal environments to deal with water shortage, this selective pressure would be weaker in wet grasslands. Thus, we would expect a deeper seed dormancy among seeds of species occurring in open savannas in comparison to seeds of species occurring in wet grasslands.

While the season of seed release may be an important driver for variation in dormancy, dispersal also plays a critical role determining the level of competition faced by emerging seedlings. In broad terms, seed dormancy can reduce extinction risk by spreading germination over time (Bulmer, 1984), while seed dispersal can reduce risk by spreading seeds and, consequently, germination over space (Venable and Brown, 1988). Parent-offspring (Ellner, 1986) and sibling competition can significantly reduce plant fitness (Satterthwaite, 2010; Baskin and Baskin, 2014; Saatkamp *et al.*, 2014). Both seed dormancy and dispersal are costly strategies, thus a trade-off between seed dormancy and dispersal is often reported between these two bet-hedging traits (Venable and Brown, 1988). Theoretical studies in particular have reported that mean dispersal distance decreases with increasing dormancy (e.g. Venable & Lawler, 1980; Cohen & Levin, 1991), although this relationship is not always supported where positive temporal correlations in environment exist (Snyder, 2006). Nevertheless, few empirical assessments of the trade-off between dormancy and dispersal have been made (Rees, 1993).

The diaspore morphology of savanna grass species is highly variable, and among several dispersal syndromes, seeds can be characterized by structures which facilitate wind dispersal (anemochory) such as winged bracts, hairy bracts and hairy rachis, or these structures can be completely absent and dispersal probably occurs through gravity (barochory) (Silberbauer-Gottsberger, 1984; Ernst *et al.*, 1992). Barochoric seeds fall near the mother-plant, while anemochoric seeds have the opportunity to disperse far from the mother-plant. Thereby, anemochory could be favoured to deal with spatial unpredictability and competition. Hence, we would expect to find a deeper seed dormancy among barochoric seeds in comparison to anemochoric seeds if there was support for a dispersal-dormancy trade-off.

The seasonal climate of the Brazilian savanna, the micro-climatic differences in their physiognomies as a consequence of variations of the water table, and the high variability in seed dormancy and dispersal syndromes of savanna grasses make the *Cerrado* an ecological system to investigate germination strategies. Thus, our aims were to test dormancy levels and seed germination comparing seeds: (1) dispersed under different times (early rainy season, late rainy season and dry season); (2) living in habitats with contrasting soil moisture during the dry season (open savannas *versus* wet grasslands), and (3) displaying opposing dispersal syndromes (barochoric *versus* anemochoric). We expected to find the highest dormancy levels in late rainy season dispersed seeds, wet grasslands and barochoric (short dispersal) seeds.

MATERIALS AND METHODS

Study area and seed collection

The *Cerrado* is the largest Neotropical savanna and covers around 2 million square kilometers in Central Brazil. The dispersal units in grasses are generally complex structures composed of a caryopsis (fruit), a caryopsis with bracts (lemma and palea) or occasionally a caryopsis with inflorescence structures attached (hereafter called seeds). Seeds of 28 native perennial grass species and one annual *Digitaria lehmanniana* (Table 1) from Brazilian open savannas and wet grasslands were collected in 2012 and 2013, in two protected areas of Brasília: Fazenda Água Limpa at the University of Brasília (FAL - 15º58'43.06"S and 47º56'21.41"W, 1.197 m) and the National Park of Brasília (PNB - 15º38'46.22"S and 48º00'19.75"W, 1.178 m). For an accurate identification we collected vouchers of the studied grass species, which were deposited in the Embrapa Genetic Resources and Biotechnology (CEN) Herbarium, headquartered in Brasilia. The climate of the region is seasonal with two well defined seasons: a wet season from October to March (mean annual precipitation from 800 to 2000 mm; Oliveira-Filho and Ratter, 2002) and a dry season from April to September (Fig. 1). The study sites are composed of patches of savanna, forest and grassland. Vegetation in open savannas and wet grasslands are composed mainly of grasses and forbs, with a few scattered trees occasionally visible in the first case. Soils in open savannas are well drained, poor in nutrients and rich in aluminum. On the other hand, soils in wet grasslands are rich in organic matter and the water table sits near the surface (about 20–80 cm) (Cianciaruso and Batalha, 2008; Meirelles *et al.*, 2010), making the soil moist for most of the year (Cianciaruso and Batalha, 2008; Fidelis *et al.*, 2013).

Plants were monitored regularly to ensure that seeds were mature at dispersal and then collected by hand. After collection the seeds were stored in paper bags at room temperature (27 ºC -maxima and 17 ºC -minima), measured throughout dry storage with a thermometer. The average relative air humidity in the region (Distrito Federal) was 43-80% during the period of study (data from BDMEP/INMET).

To characterize the soil moisture of the studied areas (FAL and PNB) we measured the water potential (in Megapascals – MPa/Ψ) of six sites of open savannas and four sites of wet grasslands every 30 days during the dry season, from June to August in 2015. Soil samples were collected during the dry season to verify whether soils of wet grasslands stay wet for longer than soils of open savannas after the end of the rainy season. We collected soil samples in three plots previously set in each area equidistant 50m from each other in a triangle arrangement, totally 18 plots in open savannas and 12 plots in wet grasslands. The plots were divided into three subsamples, one for each month of collection. In each subsample soil samples were collected at two depths: between 2 to 3 centimeters and between 10 to 11 centimeters. These depths were selected because 1) most seeds in the soil seed banks in Brazilian savannas were shown to occur in first centimeters underground (Andrade *et al.*, 2002; Zago de Andrade and Miranda, 2014) and 2) root biomass of grasses were shown to occur mostly between 0 to 20cm depth in Brazilian grasslands (Castro and Kauffman, 1998; Fidelis *et al.*, 2013), so a depth between 2 to 11cm is well within the *perfil-position* roots of grass seedlings would be found in the case of germination. The soil samples were placed in hermetically sealed sample cups, and as soon as possible the water potential of each sample was measured in the laboratory with WP4C water potential meter (Decagon Devices, Pullman-USA, 2015).

Dispersal syndrome and seed dispersal time

Based on external morphology of dispersal structure, the seeds were categorized as either anemochoric and barochoric (van der Pijl, 1982). We considered as anemochoric those seeds presenting structures that facilitate dispersal by wind, like winged bracts and/or presenting hairs in the bracts. Seeds without winged bracts or hairs were considered barochoric (Table 1). The monitoring of the grass populations selected for this study allowed us to estimate the periods of seed dispersal for each species. Thus, we divided the grass species in three groups: a) species dispersing early in the rainy season (October to January); b) species dispersing late in the rainy season (February to April) and c) species dispersing in the dry season (May to September).

Dormancy level of freshly harvested and stored seeds

To determine the level of dormancy and to test the effect of dry storage on the degree of dormancy, viability and germination of the seeds for each species' germination trial were conducted with both freshly collected seeds as well as with seeds stored for three, six, nine and twelve months. The germination experiments were conducted in germination chambers regulated for 28/18°C cycle at day/night temperatures, respectively, under a photoperiod of 12h of white light. These temperatures were set according to the average minimum and maximum temperatures recorded during the wet season (Fig. 1), which represents the growing season for most savanna species in the *Cerrado*, including grasses. Seeds were placed in petri dishes lined with two sheets of filter paper and moistened with distilled water. The germination was recorded daily up to 30 days, using the radicle emergence as a criterion for seed germination. Five replicates of 20 seeds were used for each species for each treatment, except for *P. maculosum*, *A. goyazense*, *H. longispicula* and *S. sanguineum*, which were used five replicates of 10 seeds per treatment due to the limited quantity of seeds. After each germination trial, the viability of the non-germinated seeds was tested using 1% tetrazolium chloride solution. For that, the remaining seeds were placed in contact with tetrazolium solution for 24 hours in dark at 30 ºC in a germination chamber. We considered as viable the seeds which embryos were dark pink or red. The total viability of each seed sample was set as the number of germinated seeds during the experiments plus the positive results of tetrazolium test.

Statistical analysis

All analysis were done using the R 3.1.2 statistical software (R Core Team 2014). We used Generalized Linear Mixed Models (GLMM, binomial distribution, see Zuur *et al.*, 2009) (R package: lme4; see Bates *et al.*, 2014) to test whether the effect of storage treatments on seed viability and germination is dependent on seed dispersal time (early or late in the rainy season and in the dry season), seed dispersal strategy (anemochoric or barochoric) and habitat of seed collection (open savannas or wet grasslands). We analysed seed viability and germination (presence/absence) as the response variables, separately. In this analysis, we used each seed as an experimental unit. We included a two-way interaction term between habitat, seed dispersal time and seed dispersal strategy and dry storage treatment (zero, three, six, nine and twelve months), as fixed independent variables. We included genus, species and replication as nested random factors in all models. As the seeds placed inside the petri dishes are under the same environment, which can result in autocorrelation of errors (Sileshi, 2012), we included the replication (N=5 *per* species) at the random component of the model in order to control for autocorrelation of errors. As all interactions were significant, it was not necessary to perform a model selection, so we made only a single step with the full model. We made post-hoc pairwise comparisons between levels of the significant fixed factors: dry storage and habitat, dry storage and seed dispersal time and dry storage and seed dispersal strategy. We used the single step method to P-values adjustment and the glht command of multcomp package for the pairwise comparisons (Hothorn *et al.*, 2008).

To analyse the differences in soil water potential of wet grasslands and open savannas during the dry season months we also used Generalized Linear Mixed Models (GLMM, normal distribution, see Zuur *et al.*, 2009). As we intended to test the differences in soil water potential between vegetation in each month, we made a model for each month separately. We used the water potential as response variable and as fixed independent variables a two-way interaction between vegetation (wet grassland or open savanna) and depth (2-3 or 10-11cm). We included the studied areas (FAL or PNB) and plots (3 *per* site) nested as random factors in models. As the water potential did not follow a normal distribution and range from negative to zero values, we made a transformation lognormal +1 to normalize it and we add +1 due to zeros present in the data. As is not possible to transform negative values in lognormal we reflected the data, multiplying by -1, to change it to positive before perform the lognormal transformation. We made post-hoc pairwise comparisons between levels of the significant fixed factors: vegetation and depth. We used the single step method for P-values adjustment and the glht command of multcomp package for the pairwise comparisons (Hothorn *et al.*, 2008).

RESULTS

Habitat seasonality and germination

The soil water potential at each depth differed between vegetation types with exception of 10 - 11cm in June and July (Table 2). In wet grasslands the soil water potential was higher than in open savannas for all dry season months (Fig. 2). The mean soil water potential at shallower depths (2 - 3cm) was higher in wet grasslands than in open savannas, ranging from -0.5 MPa (June) to -1.2 MPa (August) and from -3.2 MPa (June) to -5 MPa (August), respectively (Fig. 2).

The germination of grass seeds during dry storage was influenced by habitat of seed collection ($LRT_4 = 27.583$, $P<0.001$; Fig. 3). The germination among species from open savannas increased after six months of dry storage and stayed constant until twelve months of dry storage (Table 3; Fig. 3). On the other hand, the germination increased faster among species from wet grasslands, after three months of dry storage, and also stayed constant until twelve months of dry storage (Table 3; Fig. 3). Similar to seed germination, seed viability response along the storage time was also influenced by species habitat ($LRT_4 = 10.323$, $P<0.05$). In open savannas the seed viability was lower after nine ($\beta = -0.57$, $P<0.05$), and twelve ($\beta = -0.75$, $P<0.05$) months of dry storage in comparison to freshly collected seeds, while in wet grasslands the seed viability did not differ during the storage time (Table 3; Table S1 - supplementary data).

Dormancy level comparison against dispersal time and dispersal syndrome

The germination of grass seeds during dry storage was influenced by the seed dispersal time ($LRT_8 = 110.738$, $P<0.001$; Fig. 4). Freshly collected seeds dispersed early in the rainy season and in the dry season had higher probability of germination than seeds dispersed late in the rainy season (Table 3; Fig. 4). After three months of dry storage the probability of germination was not influenced by the dispersal time of the seeds (Fig. 4). The viability between seed dispersal times differ in six months of dry storage, in which early-dispersed seeds had lower viability than late dispersed seeds in the rainy season ($\beta = -0.9635$, $P<0.05$; Table S4 - supplementary data). In addition, after twelve months of dry storage the viability of early-dispersed seeds was lower than late-dispersed seeds ($\beta = -0.9258$, $P<0.05$; Table S4 - supplementary data) and of seeds dispersed in the dry season ($\beta = -2.2161$, $P<0.001$; Table S4 - supplementary data).

We found a significant effect of seed dispersal syndrome ($LRT_4 = 28.785$, $P<0.001$; Fig. 5) on germination probability during dry storage. The germination in barochoric seeds was lower than in anemochoric ones for freshly collected seeds and also after three months of dry storage (Table 3; Fig. 5). After six months of dry storage the probability of germination was no longer influenced by the seed dispersal syndrome (Fig. 5). The viability of barochoric and anemochoric seeds did not differ (Table S2 - supplementary data), except at six months of dry storage, when barochoric seeds showed a lower viability than anemochoric seeds (Table 3; $\beta = -0.7955$, $P<0.05$).

DISCUSSION

There were contrasting effects of dry storage on the germination and viability of species from open savannas and wet grasslands. In open savannas the germination of fresh seeds was low, increasing after six months, followed by seed mortality after nine months of storage. These results indicate that germination in seeds of species from open savannas may be delayed, at most, until the onset of the next rainy season. On the other hand, seeds of species from wet grasslands were longer-lived than seeds from open savannas, remaining viable after one year of dry storage. Additionally, germination levels increased and stayed constant high after three months of dry storage.

In wet grasslands, the soil remained wet during the dry season in comparison to open savannas, presenting values around -1 MPa at 2 to 3 cm depth (Fig. 2). Water potential of around -1 MPa is not limiting for the germination of grass species (Qui and Redmann 1993), thus suggesting that the water potential measured in the wet grasslands studied here would not limit the germination of seeds present in the soil. Moreover, the soil moisture and the high percentages of germination of grass species from wet grasslands suggest that germination might occur even during the dry season.

Although water seasonality does not seem to be a strong selective pressure on seed germination of species from wet grasslands, due to the high soil moisture even during the dry season, another factor, such as the pressure of stablished vegetation, has been demonstrated to negatively influence seedling establishment in savannas (Zimmermann *et al.*, 2008). Wet grasslands can be strongly competitive environments for seedlings, as they have dense and closed aboveground plant biomass of around 765 g m^{-2} (Fidelis *et al.*, 2013). Fire frequently occurs in wet grasslands and can reduce competition by removing the established vegetation (Zimmermann *et al.*, 2008). The high longevity of seeds, combined with high germination rates, of grass species from wet grasslands can result in an opportunistic strategy for taking advantage of gaps, for example after a fire event, to allow growth and establishment in a less competitive environment.

Seed dormancy at the end of the rainy season may avoid risky germination

We show that seed dormancy is related to the dispersal time of Neotropical grass species (Fig. 4). Seeds dispersed late in the rainy season showed lower probability of germination than seeds dispersed either early in the rainy season, or dispersed during the dry season. The high levels of dormancy among late dispersed seeds may represent a drought-avoidance syndrome, a strategy already observed among grass species of savanna environments (Mott, 1978; Veenendaal *et al.*, 1996; O'Connor and Everson, 1998; McIvor and Howden, 2000; Scott *et al.*, 2010). Seeds dispersed in the beginning of the rainy season are expected to have about seven months to germinate and establish. On the other hand, seed germination at the end of the rainy season would be very risky, since the seedlings would not have time enough to grow and acquire a minimal size to enable them to tolerate the harsh conditions expected during the dry season. Thus, the presence of seed dormancy here might prevent germination during times of low chances of recruitment, and hold back emergence to the onset of the next rainy season.

Contrary to our expectations, the germination of seeds dispersed in the dry season was not different from seeds dispersed early in the rainy season. Seeds dispersed during the dry season showed low levels of seed dormancy. Since sporadic rains are expected to occur in the dry season (Fig. 1), we did not expected to find high germination in seeds dispersed in the dry season. However, the amount of rain is extremely low (Fig. 1) and potentially not sufficient to induce the start of germination. Additionally, the next rainy season is close and, by not having seed dormancy, these dry season dispersed seeds may rapidly germinate and take advantage of the entire growing season to establish.

Trade-off between seed dispersal and level of dormancy

We found a negative relationship between seed dispersal and seed dormancy. Anemochoric seeds germinated to significantly greater levels and (consequently) had lower levels of dormancy than barochoric seeds. As far as we know, this is the first study to demonstrate the trade-off between seed dispersal and dormancy across species from the same family (Poaceae). Moreover, most of both the theoretical and empirical studies investigating the evolution of seed dispersal and seed dormancy have been made for plants from unpredictable environments, mostly in deserts (Volis and Bohrer, 2013).

Our between-species comparison in a seasonally wet ecological system provide empirical results showing that barochoric grass seeds are significantly more dormant than anemochoric ones.

Seed dispersal and seed dormancy may be viewed as bet-hedging strategies to enable species to cope with environmental patch heterogeneity and climatic unpredictability (Bulmer, 1984; Venable and Brown, 1988), the former by spreading risk over time (Bulmer, 1984), and the latter by spreading risk over space (Venable and Brown, 1988). However, even in the absence of patch heterogeneity, seed dispersal can be favoured by kin selection (Venable and Brown, 1988). Additionally, in environments without inter-annual variation in precipitation, seed dormancy can be advantageous where sibling competition is high (Volis and Bohrer, 2013). Although water availability affects seedling establishment in savannas (Medina and Silva, 1990; Davis *et al.*, 1999; Sharam *et al.*, 2006; Higgins *et al.*, 2000) and might explain seed dormancy, it does not explain our results that anemochoric seeds had lower seed dormancy than barochoric seeds. In open savannas and wet grasslands of the *Cerrado* there is no clear patch heterogeneity, as exists in deserts, so it is less likely that this has driven a seed dispersal-dormancy trade-off in our studied species. Alternatively, we suggest that competition between siblings, conspecifics or heterospecifics, might drive the dispersal-dormancy trade-off that we found in our study species.

Conspecific competitor densities during seedling growth negatively influences plant fitness (Orrock and Christopher, 2010). By dispersing far and not being dormant, grass seeds with an anemochoric dispersal strategy might enhance the chances of a seed landing in a more open patch, enabling it to take advantage of sites with lower competition for resources. Moreover, fire is a frequent disturbance in Brazilian open savannas and wet grasslands, and fire occurrence can open up gaps in the herbaceous vegetation. As such, these anemochoric species may be favored by germinating earlier and growing in gaps opened by fire events. In contrast, the outcome for barochoric seeds is that they fall near to the mother plant and siblings. Because they disperse throughout the rainy season, there is sufficient moisture to enable seed germination, and seedlings could grow in clumps if no other mechanism were available. Thus, seed dormancy can be a way to reduce immediate germination and distribute seedling emergence over time. Alternatively, deeper dormancy among barochoric seeds could give seeds more time to enable a secondary dispersal event. Indeed, some of the barochoric grass species studied have structures recognized to favor secondary dispersal, including *Echinolaena inflexa* and *Icnanthus camporum*, which both have elaiosomes that are known to attract ants. Furthermore, *Aristida* species have awns, which can attach to rodents providing potential longer-distance dispersal.

In conclusion, our results show that seed dormancy is a strategy to synchronize the seed germination of grasses at the beginning of rainy season in seasonal environments. The coevolution of both strategies – seed dormancy and the timing of seed dispersal –contribute to enabling seeds to avoid germination during periods with low chances of successful recruitment, such as at the end of the rainy season. We show, with a large representative number of grass species from Neotropical savannas that seed dormancy is a critical life history trait that allows persistence in seasonal environments with dry periods. Moreover, our results also provide empirical evidence for a trade-off between seed dispersal and seed dormancy. These results indicate that rainfall seasonality is not the only selective pressure driving evolution of seed dormancy in grass species and we suggest that competition can have an important influence selecting for the germination timing of seeds from seasonal and predictable environments.

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Table 1. Time of seed dispersal, seed dispersal syndrome and habitat of occurrence of grass species from open savannas and wet grasslands of Central Brazil.

Species	Seed dispersal syndrome	Seed dispersal (Season)	Habitat	Site
Agenium goyazense (Hack.) Clayton	Barochory	Dry	Open savanna	PNB
Andropogon leucostachyus Kunth	Anemochory	Early	Wet grassland	FAL
Anthaenantia lanata (Kunth) Benth.	Anemochory	Early	Open savanna	PNB
Aristida gibbosa (Nees) Kunth	Barochory	Dry	Open savanna	FAL
Aristida recurvata Kunth	Barochory	Dry	Open savanna	PNB
Aristida riparia Trin.	Barochory	Dry	Open savanna	FAL
Aristida setifolia Kunth	Barochory	Late	Open savanna	FAL
Arthropogon villosus Nees	Barochory	Early	Wet grassland	PNB
Axonopus siccus var. siccus (Nees) Kuhlm.	Barochory	Late	Open savanna	FAL
Ctenium cirrhosum (Nees) Kunth	Anemochory	Late	Open savanna	PNB
Digitaria lehmanniana Henrard	Barochory	Late	Wet grassland	PNB
Echinolaena inflexa (Poir.) Chase	Barochory	Late	Open savanna	FAL
Elionurus muticus (Spreng.) Kuntze	Anemochory	Early	Open savanna	PNB
Eragrostis polytricha Nees	Barochory	Early	Wet grassland	PNB
Homolepis longispicula (Döll) Chase	Anemochory	Early	Wet grassland	PNB
Ichnanthus camporum Swallen	Barochory	Late	Open savanna	FAL

Mesosetum ferrugineum (Trin.) Chase	Anemochory	Early	Wet grassland	PNB
Panicum olyroides Kunth	Barochory	Early	Open savanna	PNB
Paspalum carinatum Humb. & Bonpl. ex Flüggé	Anemochory	Late	Open savanna	FAL
Paspalum erianthum Nees ex. Trin.	Anemochory	Early	Open savanna	PNB
Paspalum glaucescens Hack.	Barochory	Late	Open savanna	PNB
Paspalum guttatum Trin.	Anemochory	Early	Open savanna	PNB
Paspalum maculosum Trin.	Barochory	Late	Wet grassland	PNB
Paspalum pectinatum Nees ex Trin.	Anemochory	Early	Open savanna	PNB
Paspalum polyphyllum Nees	Anemochory	Dry	Wet grassland	FAL
Saccharum villosum Steud.	Anemochory	Early	Wet grassland	PNB
Sacciolepis myurus (Lam.) Chase	Barochory	Dry	Wet grassland	FAL
Schizachyrium sanguineum (Retz.) Alston	Barochory	Dry	Open savanna	PNB
Setaria parviflora (Poir.) Kerguélen	Barochory	Late	Open savanna	PNB

Time period of seed dispersal: Early (October - January), late in the rainy season (February - April) and during the dry season (May - September). Site of occurrence: PNB (National Park of Brasília) and FAL (Água Limpa farm). G = Germination; V = Viability; GV = Germination proportion of viable seeds.

Table 2. Multiple comparisons of differences in soil water potential between open savannas and wet grasslands from Central Brazil during the dry season months.

Month	LRT_1	P	Habitat	Depth (cm)	β ± SE	P
June	17.562	$P<0.001$	OS – WG = 0	2-3	0.6346 ± 0.2192	$P<0.05$
			OS – WG = 0	10-11	0.4418 ± 0.2189	$P=0.05$
July	21.405	$P<0.001$	OS – WG = 0	2-3	0.6161 ± 0.2334	$P<0.05$
			OS – WG = 0	10-11	0.4293 ± 0.2328	$P=0.08$
August	39.049	$P<0.001$	OS – WG = 0	2-3	0.9121 ± 0.2199	$P<0.05$
			OS – WG = 0	10-11	0.6127 ± 0.2194	$P<0.05$

OS = Open Savannas, WG = Wet Grasslands. β ± Standard Error.

Table 3. Seed germination and viability of grass species from *Cerrado* according to dispersal time, seed dispersal syndrome and habitat of seed collection along twelve months of dry storage.

	Dry storage (Months)				
	0	3	6	9	12
SEED VIABILITY (%)					
Dispersal time					
Early	73.8 ± 2.8	69.9 ± 3.4	65.0 ± 4.0	76.3 ± 3.4	50.0 ± 4.7
Late	70.3 ± 3.7	68.7 ± 4.2	63.9 ± 3.7	57.6 ± 4.3	52.1 ± 4.6
Dry	72.7 ± 3.9	81.4 ± 2.0	77.4 ± 3.0	66.8 ± 4.8	81.5 ± 5.7
Habitat					
Open savanna	72.4 ± 2.4	69.8 ± 2.7	63.1 ± 2.93	62.6 ± 3.33	46.7 ± 4.21
Wet grassland	72.1 ± 3.5	78.2 ± 2.7	76.3 ± 2.98	76.1 ± 3.05	63.7 ± 4.56
Seed dispersal					
Anemochoric seeds	71.1 ± 2.8	67.2 ± 3.5	67.7 ± 3.8	66.7 ± 4.2	44.5 ± 4.7
Barochoric seeds	73.1 ± 2.6	77.4 ± 2.1	67.6 ± 2.8	68.2 ± 2.9	61.3 ± 4.1
SEED GERMINATION (%)					
Dispersal time					
Early	59.8 ± 4.3	59.7 ± 4.5	60.0 ± 4.5	68.3 ± 4.2	43.7 ± 4.8
Late	7.6 ± 2.0	18.5 ± 4.0	23.9 ± 4.8	29.5 ± 4.9	26 ± 4.4
Dry	33.6 ± 6.1	43.6 ± 6.0	54.3 ± 4.9	38.0 ± 4.7	59.5 ± 5.2
Habitat					
Open savanna	30.3 ± 3.6	40.1 ± 3.8	38.6 ± 3.6	40.2 ± 3.8	27.5 ± 3.8
Wet grassland	45.2 ± 5.4	48.0 ± 5.8	60.6 ± 5.1	62.1 ± 5.0	54.9 ± 5.0
Dispersal syndrome					
Anemochoric seeds	59.8 ± 3.9	63.3 ± 3.7	61.4 ± 4.5	65.6 ± 4.4	43.8 ± 4.7
Barochoric seeds	20.6 ± 3.5	29.5 ± 4.1	36.9 ± 3.8	36.4 ± 3.9	34.4 ± 4.5
SAMPLE SIZE (N)					
Dispersal time					
Early	60; 12	45; 9	60; 12	60; 12	60; 12
Late	50; 10	30; 6	50; 10	50; 10	40; 8
Dry	35; 7	35; 7	35; 7	25; 5	10; 2
Habitat					
Open savanna	95; 19	65; 13	95; 19	85; 17	65; 13
Wet grassland	50; 10	45; 9	50; 10	50; 10	45; 9
Seed dispersal					
Anemochoric seeds	55; 11	45; 9	55; 11	55; 11	50; 10
Barochoric seeds	90; 18	65; 13	90; 18	80; 16	60; 12

Mean ± Standard Error. Sample size = Number of petri dishes; Number of species.

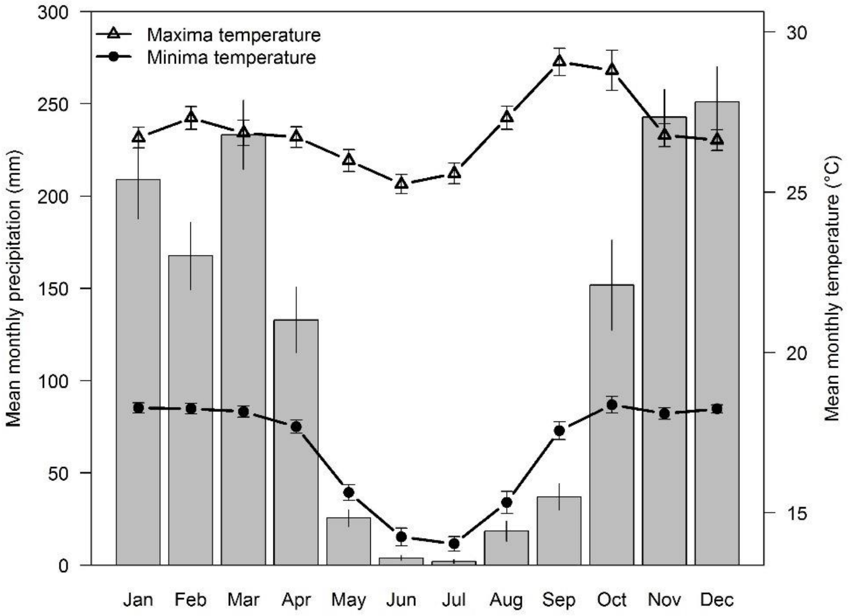

Figure 1. Mean monthly minimum and maximum temperatures and precipitation in Brasília-DF from 1994 to 2014. Data from BDMEP/INMET.

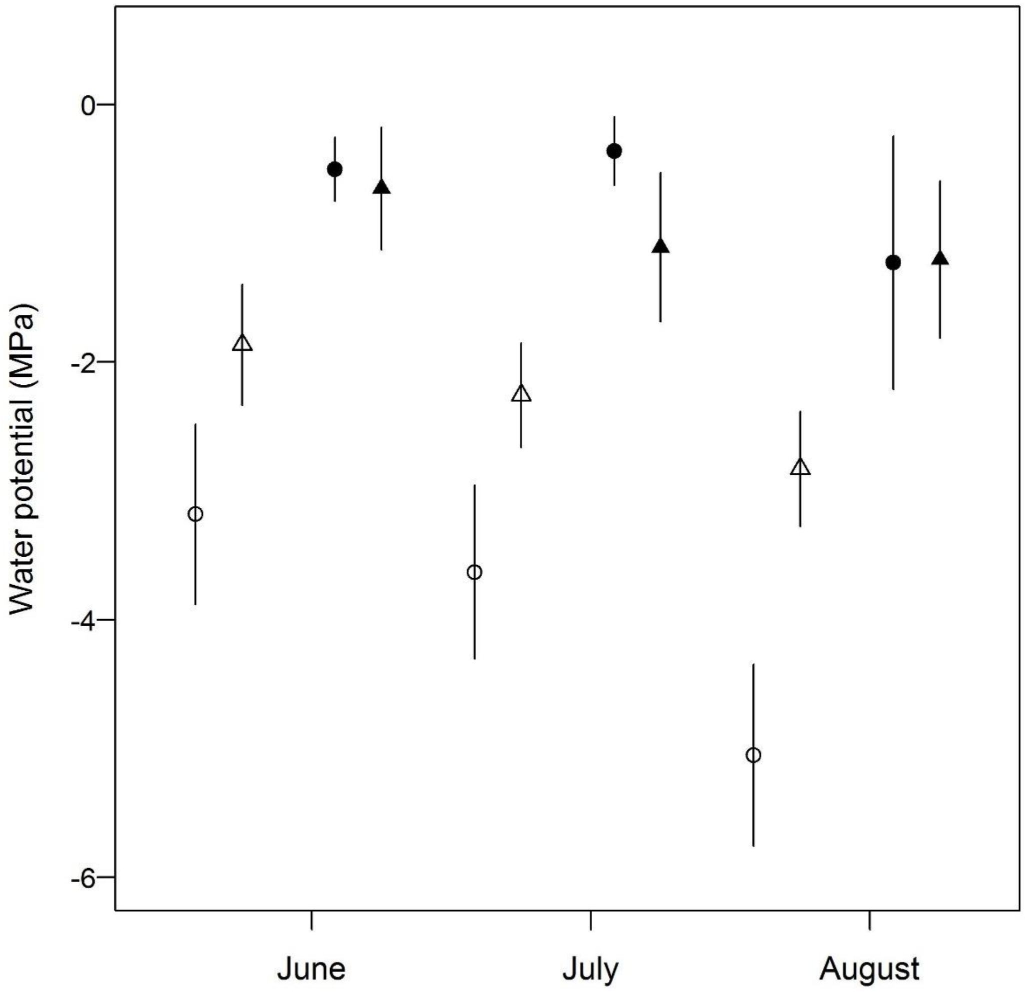

Figure 2. Soil water potential (MPa) of wet grasslands and open savannas during the dry season. Open symbols = open savannas; Closed symbols = wet grasslands; Circles = 2 - 3 cm depth; Triangles = 10 - 11 cm depth. Mean + 95% Confidence Interval.

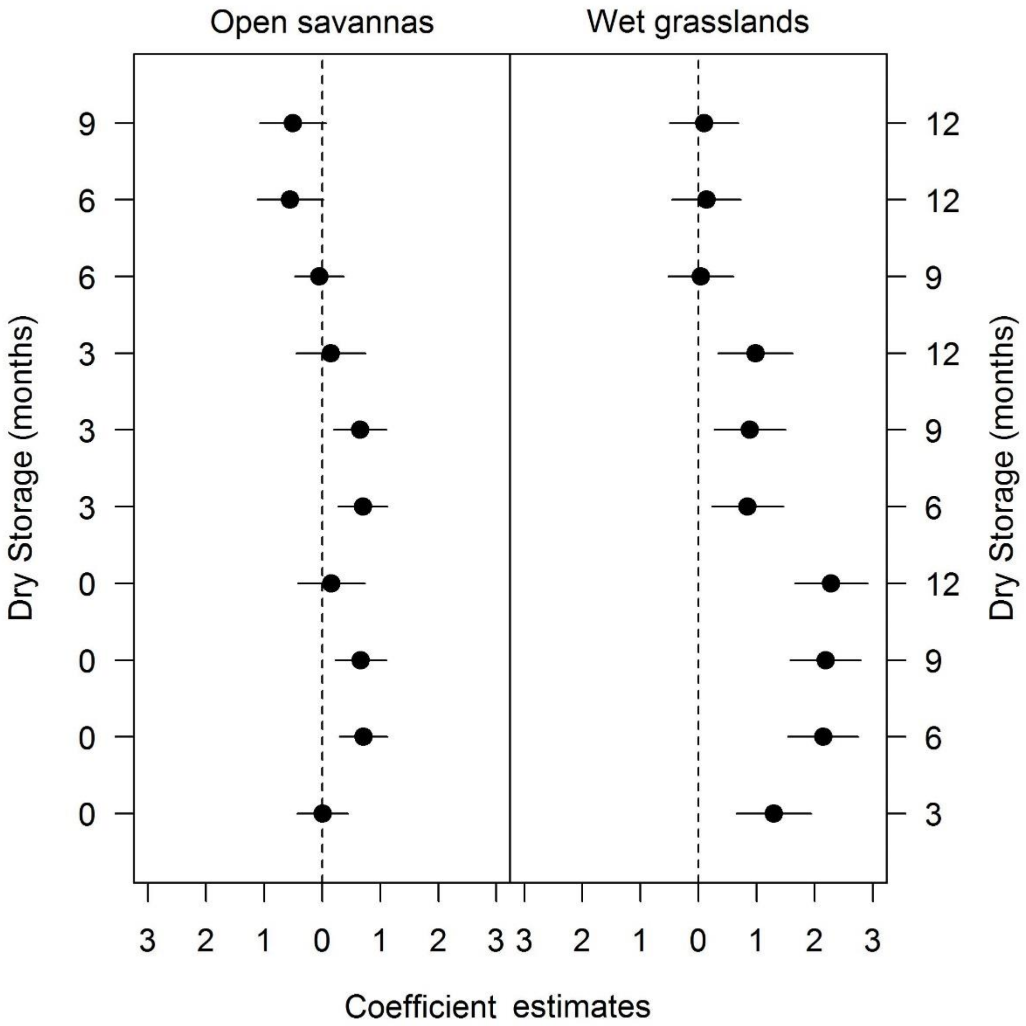

Figure 3. Coefficient estimates from pairwise multiple comparisons of germination probability between months of dry storage in grass species from open savannas and wet grasslands. The closed circles denote the mean values, the error bars denote 95% of lower and upper confidence intervals. The central dotted line (zero value) means no statistical difference.

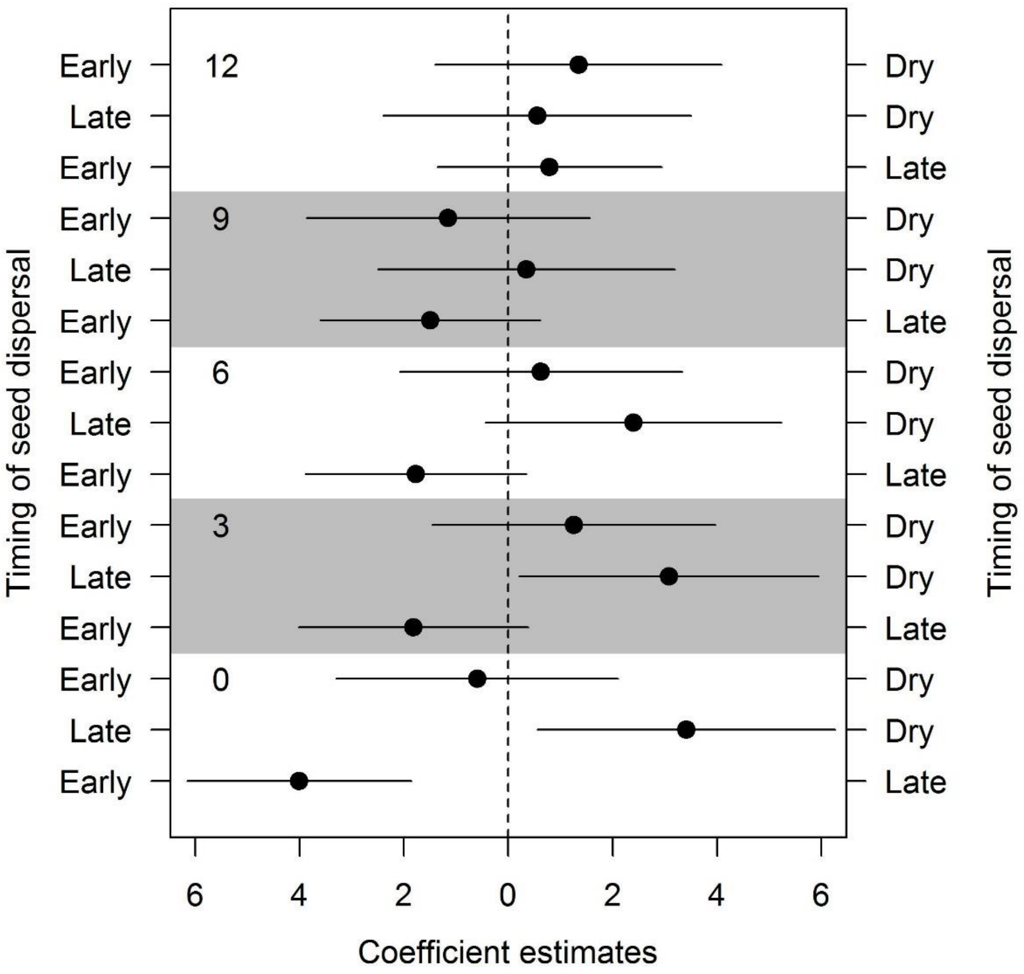

Figure 4. Coefficient estimates from pairwise multiple comparisons of germination probability between grass seeds dispersed early, late in the rainy season and in the dry season along dry storage (0, 3, 6, 9 and 12 months). The closed circles denote the mean values, the error bars denote 95% of lower and upper confidence intervals. The central dotted line (zero value) means no statistical difference.

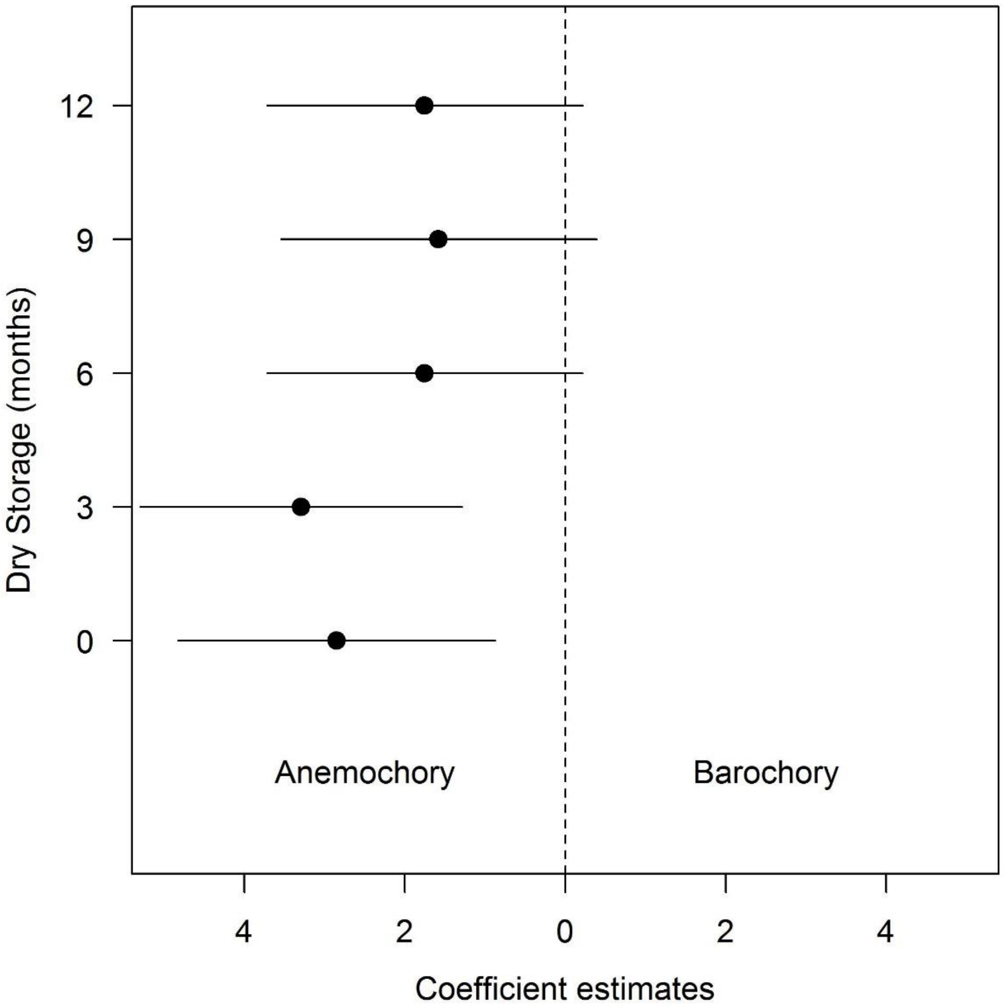

Figure 5. Coefficient estimates from pairwise multiple comparisons of germination probability between grass species with anemochory and barochory dispersal syndromes along dry storage (0, 3, 6, 9 and 12 months). The closed circles denote the mean values, the error bars denote 95% of lower and upper confidence intervals. The central dotted line (zero value) means no statistical difference.

SUPPLEMENTARY DATA

Table S1. Post-hoc comparisons of probability of seed viability between dry storage months according to habitat types. Significant differences in bold.

Open savanna

Dry storage (months)	β	SE	Z	P
12 - 9	-0.17	0.25	-0.69	0.96
12 - 6	-0.32	0.25	-1.29	0.69
12 - 3	-0.24	0.26	-0.93	0.88
12 - 0	-0.75	0.25	-3.05	**0.02**
9 - 6	-0.14	0.18	-0.78	0.93
9 - 3	-0.07	0.20	-0.34	1.00
9 - 0	-0.58	0.19	-3.13	**0.01**
6 - 3	0.08	0.19	0.40	0.99
6 - 0	-0.44	0.18	-2.48	0.09
3 - 0	-0.51	0.19	-2.69	0.05

Wet grassland

Dry storage (months)	β	SE	Z	P
12 - 9	-0.26	0.25	-1.03	0.84
12 - 6	-0.49	0.25	-1.97	0.28
12 - 3	-0.63	0.26	-2.39	0.12
12 - 0	-0.22	0.25	-0.87	0.91
9 - 6	-0.23	0.24	-0.96	0.87
9 - 3	-0.37	0.25	-1.45	0.59
9 - 0	0.04	0.24	0.18	1.00
6 - 3	-0.14	0.25	-0.54	0.98
6 - 0	0.27	0.24	1.15	0.78
3 - 0	0.41	0.25	1.64	0.47

Table S2. Post-hoc comparisons of probability of seed viability between seeds with contrasting dispersal syndromes across dry storage months. Significant differences in bold.

	Barochory – Anemochory = 0			
Dry storage (months)	β	SE	Z	P
0	-0.31	0.33	-0.93	0.35
3	-0.18	0.41	-0.47	0.65
6	-0.80	0.33	-2.38	**0.02**
9	0.22	0.34	0.66	0.51
12	0.43	0.36	1.22	0.22

Table S3. Post-hoc comparisons of probability of seed viability between dry storage months according to the timing of seed dispersal. Significant differences in bold.

	Dry season			
Dry storage (months)	β	SE	Z	P
12 - 9	1.31	0.47	2.78	**0.04**
12 - 6	0.54	0.46	1.16	0.77
12 - 3	0.44	0.47	0.94	0.88
12 - 0	1.05	0.46	2.26	0.15
9 - 6	-0.78	0.32	-2.45	0.10
9 - 3	-0.87	0.33	-2.64	0.06
9 - 0	-0.27	0.32	-0.84	0.92
6 - 3	-0.09	0.31	-0.31	1.00
6 - 0	0.51	0.29	1.75	0.40
3 - 0	0.60	0.31	1.97	0.27
	Early rainy season			
Dry storage (months)	β	SE	Z	P
12 - 9	-1.75	0.22	-7.90	**<0.001**
12 - 6	-0.85	0.21	-4.00	**<0.001**
12 - 3	-1.15	0.25	-4.57	**<0.001**
12 - 0	-1.42	0.22	-6.58	**<0.001**
9 - 6	0.89	0.22	4.07	**<0.001**
9 - 3	0.60	0.25	2.37	0.12
9 - 0	0.33	0.22	1.51	0.55
6 - 3	-0.29	0.25	-1.17	0.77
6 - 0	-0.56	0.21	-2.64	0.06
3 - 0	-0.27	0.25	-1.09	0.81
	Late rainy season			
Dry storage (months)	β	SE	Z	P
12 - 9	-0.21	0.29	-0.74	0.95
12 - 6	-0.89	0.28	-3.14	**0.01**
12 - 3	-0.60	0.33	-1.79	0.38
12 - 0	-1.09	0.29	-3.77	**<0.01**
9 - 6	-0.68	0.26	-2.67	0.06
9 - 3	-0.38	0.30	-1.29	0.70
9 - 0	-0.87	0.26	-3.40	**0.01**
6 - 3	0.30	0.30	0.99	0.86
6 - 0	-0.19	0.26	-0.75	0.94
3 - 0	-0.49	0.30	-1.63	0.48

Table S4. Post-hoc comparisons of probability of seed viability between seeds dispersed in different periods across dry storage months. Significant differences in bold.

Timing of seed dispersal	β	SE	Z	P
Dry storage = 0 month				
Early x Late	-0.59	0.36	-1.65	0.22
Early x Dry	0.25	0.42	0.59	0.83
Late x Dry	0.84	0.38	2.20	0.07
Dry storage = 3 months				
Early x Late	-0.38	0.45	-0.83	0.68
Early x Dry	-0.63	0.46	-1.35	0.37
Late x Dry	-0.25	0.40	-0.63	0.81
Dry storage = 6 months				
Early x Late	-0.96	0.36	-2.68	**0.02**
Early x Dry	-0.83	0.42	-1.97	0.12
Late x Dry	0.14	0.38	0.36	0.93
Dry storage = 9 months				
Early x Late	0.61	0.36	1.68	0.21
Early x Dry	0.85	0.44	1.92	0.13
Late x Dry	0.23	0.41	0.57	0.84
Dry storage = 12 months				
Early x Late	-0.93	0.39	-2.39	**0.04**
Early x Dry	-2.22	0.54	-4.11	**<0.001**
Late x Dry	-1.29	0.58	-2.23	0.06

CAPÍTULO 2

Seed tolerance to heating is better predicted by seed dormancy than by habitat type in Neotropical savanna grasses

Seed tolerance to heating is better predicted by seed dormancy than by habitat type in Neotropical savanna grasses

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Running head: Seed tolerance to heating in savanna grasses

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Abstract

Open savannas and wet grasslands occur under the same seasonal macro-climate in central Brazil. However, in open savannas temperatures during fires are higher than in wet grasslands. Grasses dominate both ecosystems and exhibit large variation in seed dormancy. We hypothesize that seeds of species from open savannas are more tolerant to heating than those of wet grasslands. Assuming that dormant seeds might remain longer in the soil than non-dormant seeds, thus being more likely to burn, we expect that dormant seeds are more tolerant to heating than non-dormant seeds. We tested the effects of heating at 80 and 110°C for 2.5 and 5.0 min on the survival of seeds of 14 species, seven from each community, containing dormant and non-dormant species. Seeds of most species survived at 80°C, but seeds from open savannas maintained greater survival for 5 min than seeds from wet grasslands. Seeds of most species died at 110°C, but dormant seeds survived more than non-dormant seeds. We conclude that species with seed dormancy experience selection for covarying characteristics that allow tolerance to heating in hotter fires. Our findings suggest that both latency to germinate and habitat-specific fire temperatures may drive the evolution of seed fire tolerance in Neotropical savannas.

Additional Keywords: Heat shock, dormancy, fire, habitat seasonality, open savanna, wet grassland.

Summary: We investigated whether seed tolerance of high temperatures was related to dormancy or habitat type for grass species from Brazilian fire-prone savannas. Seeds from wetter habitats had lower tolerance while dormant seeds had high tolerance, suggesting both dormancy and habitat moisture drive the evolution of seed tolerance to heat.

Introduction

Savannas are ecosystems characterized by a continuous herbaceous-grass layer, with a large representation of grasses, and a discontinuous distribution of shrubs and trees (Eiten 1972; Eiten 1978; Bourlière and Hadley 1983). Savannas are environments with a strongly seasonal climate, with two well defined seasons during the year, alternating between a rainy summer and a dry winter (Medina 1982; Sarmiento 1984). Consequently, the grass layer dries out during the dry season, becoming a highly flammable fuel for subsequent fires (Kauffman *et al*. 1994; Mistry 1998). Brazilian savannas and grasslands are known for their high propensity of fire occurrence (Kauffman *et al*. 1994; Mistry 1998; Miranda *et al*. 2010; Hoffmann *et al*. 2012), which is likely to have contributed to the evolution of seed tolerance to heating in several plant species (Ribeiro *et al*. 2012; Ribeiro and Borghetti 2013).

Seed survival to heating is considered an adaptation to fire in several taxa of plants from fire-prone environments (Overbeck *et al*. 2006). Nevertheless, there is considerable variability in seed survival after heat shock between species inhabiting fire-prone environments (Gashaw and Michelsen 2002; Overbeck *et al*. 2006; Paula and Pausas 2008; Ribeiro and Borghetti 2013; Ooi *et al*. 2014). The probability of seeds being burnt and the associated life-history cost to adult plants may be relevant mechanisms driving the evolution of seed tolerance to fire. For instance, previous studies have shown that seed tolerance to heat shock is negatively related to adult plant survival after fire in Mediterranean and Australian fire-prone environments (Paula and Pausas 2008; Ooi *et al*. 2014), and positively related to the probability of being exposure to burnt (*e.g*. when comparing habitats with different fire regimes in Australia and central Brazil) (Thomas *et al*. 2003; Ribeiro *et al*. 2012; Ribeiro and Borghetti 2013).

Seed dormancy is a mechanism to prevent germination in an unfavorable time for seedling growth and recruitment (Baskin and Baskin 2014). In general, seeds of grass species from savannas can either be non-dormant or physiologically dormant (Baskin and Baskin 2004; Aires *et al*. 2013; Baskin and Baskin 2014). As a consequence of seasonal climate, the reproductive period of herbaceous species in wet grasslands and open savannas in Brazil, including the production and dispersal of seeds, occurs mainly in the rainy season (Tannus *et al*. 2006; Munhoz and Felfili 2007b; Ramos *et al*. 2014). Therefore, seed germination and seedling recruitment of dormant grass seeds are expected to occur only at the beginning of the following rainy season, while the non-dormant seeds can germinate in the same dispersal season (Mott 1978; Veenendaal *et al*. 1996). Considering that dormant seeds might remain longer in soil than non-dormant seeds, thus being more likely to burn, we could expect that dormant seeds are more tolerant to high temperatures than non-dormant seeds.

In general, grass seeds from fire-prone environments are thought to survive heating between 70 and 100°C, but seeds from most species do not survive at higher temperatures, especially at prolonged exposure times (*e.g.* 5 minutes) (Gashaw and Michelsen 2002; Overbeck *et al*. 2006; Dayamba *et al*. 2008; Reyes and Trabaud 2009; Gonzalez and Ghermandi 2012). However, in these studies it is clear that seeds of some species survive at the high temperatures and exposure times tested. But, it remains unclear which plant traits drive the variation between grass species from the same environment in tolerance to survive at high temperatures (> 100°C).

The maximum temperatures reached during fires in the environment in which species occur is among the potential factors influencing the tolerance of their seeds to heat shock. Fire temperatures can be highly variable and is dependent on the fuel amount and humidity across all fire-prone ecosystems, including within savannas and grasslands (Miranda *et al*. 1993; Williams *et al*. 2003). Wet grasslands (known as *campo úmido*) and open savannas (known as *campo sujo*) are common vegetation types within Brazilian savannas and are susceptible to fire (Munhoz and Felfili 2007a,b; Cianciaruso and Batalha 2008; Schmidt 2011). The top of the water table is deep below the soil surface in open savannas (about 1.1–4 m) (Rossatto *et al*. 2012), leading soil and the herbaceous layer to dry out during the dry winter season. In contrast, the water table sits near the surface in wet grasslands (about 20–80 cm) (Cianciaruso and Batalha 2008; Meirelles *et al*. 2010), making the soil moist for most of the year (Cianciaruso and Batalha 2008; Fidelis *et al*. 2013).

In both vegetation types, dried leaves of living herbaceous plants or dead leaf litter from these plants are the main fuel source (Kauffman *et al*. 1994; Hoffmann *et al*. 2012). Wet grasslands generally produce lower aboveground (1cm high) maximum temperatures during fire occurrence than open savannas, ranging from 57 to 330°C (Schmidt 2011) compared to 288 to 350°C (Miranda *et al*. 1993), respectively. We therefore, could expect that seeds of species from open savannas are more tolerant to heating at high temperatures than those of wet grasslands.

In this study, we use 14 perennial grass species (Poaceae) from either wet grasslands or open savannas of Central Brazil, to examine whether seed tolerance to fire can be explained by habitat type (opens savannas *versus* wet grasslands) and/or by seed dormancy status (non-dormant *versus* dormant). We predict that grass species occurring in wet grasslands, which burns less intensely than open savannas, produce seeds less tolerant to heat shock (simulating fire-generated temperatures) than seeds of open savannas grass species. We also predict that seeds of dormant species will be more tolerant to heating than seeds of non-dormant species.

Materials and Methods

Study area and seed collection

Mature seeds of 14 native perennial grass species from Brazilian wet grasslands and open savannas were harvested manually from January/2012 to June/2013, in two areas of

Brasília, Federal District: Fazenda Água Limpa - FAL (Água Limpa farm, University of Brasília- 15º58'43.06"S and 47º56'21.41"W, 1.197 m) and the Parque Nacional de Brasília - PNB (National Park of Brasília - 15º38'46.22"S and 48º00'19.75"W, 1.178 m). All study species are closely related, from within the same clade (Grass Phylogeny Working Group II 2012), with 11 representatives of the tribe Paniceae. We monitored plants regularly, about weekly, to ensure that seeds were mature at the time of collection. We selected sites protected from cattle and with two different soil moisture conditions within these areas. The sites were either open savannas, an environment with strong seasonality, where the soils dry out during the dry season, or wet grasslands, a less seasonal environment, where the soils are moist for most of the year (Table 1). To ensure seeds were representative of a number of genotypes across the population, and since grasses have vegetative reproduction by rhizomes, we collected seeds from at least 30 individuals randomly selected across a large area of the habitat.

To ensure accurate identification we also collected voucher specimens of these species which were deposited at National Center of Genetic Resources - Cenargen/Embrapa herbarium (CEN). After collection caryopsis were stored in paper bags at laboratory at 20.6 and 26.6°C minimum and maximum temperatures, respectively, with storage times varying slightly dependent on ripening phenology (Table 1).

Initial seed dormancy

Based on germination trials conducted with fresh collected seeds (about one week after collection), we categorized species as either dormant or non-dormant (Table 1). For germination experiments we used five replicates of 20 seeds for each species, in an incubation chamber, with 12h photoperiod with 28/18°C cycle at day/night, respectively. Temperatures vary little over the year in this climatic region (~3°C), and the experimental trials represents the mean maxima and minima temperatures during the wet summer, which is the main period of seed dispersal and growth of grasses in these environments. We recorded the germination daily for 30 days and used radicle emergence as the criterion for seed germination. After the germination experiments, we categorized species as dormant if more than 50% of the viable seed lot did not germinate (Table 1).

Heat shock treatments

Aiming to simulate the effects of a fire component (high temperatures) on seed survival, the following treatments were applied on the seed samples: 80°C for 2.5 min (t1); 80°C for 5 min (t2); 110°C for 2.5 min (t3); 110°C for 5 min (t4). Unheated seeds were the controls. The temperatures and exposure times were chosen to simulate the conditions experienced at 1 cm aboveground in the soil in Brazilian wet grasslands and open savannas during prescribed fires (Miranda *et al.* 1993; Schmidt 2011). Although these studies registered their highest maximum temperatures above 80°C and 110°C (*e.g.* 330°C, Schmidt 2011; 350°C, Miranda *et al*. 1993), lower maximum temperatures of 56°C (Schmidt 2011) and 106°C (Marinho and Miranda 2013) were also commonly recorded. The exposure times of 2.5 min and 5 min were chosen because the residence times of higher temperatures (> 60°C) range from 1 min to 4 min in wet grasslands (Schmidt 2011) and from 1.5 min to 4.5 min in open savannas (Miranda *et al*. 1993). The heat shock treatments were simulated in an oven with air circulation (Marconi - MA035/1). Four replications of 25 seeds were used per species, in each treatment, except for *Homolepis longispicula*, where only four replications of 10 seeds were possible due to limited seed numbers.

Germination and viability

The germination and viability of seeds were tested after the heat shock treatments (t1, t2, t3, t4 and control). Seeds were placed on two sheets of 9-cm-diameter filter paper in petri dishes and moistened with distilled water. The germination trials were conducted in a germination chamber (Marconi/MA402), at a constant single temperature of 30 ºC and 12 hours of dark/light photoperiod for comparative purposes between species and treatments. The germination was checked every 2 days for 15 days. We used the radicle emergence followed by its gravitropic curvature as a criterion of seed germination. After each germination trial the viability of non-germinated seeds were tested using 1% tetrazolium chloride solution. The seeds were placed in contact with tetrazolium solution for 24 hours in dark at 30 ºC in a germination chamber. We considered as viable the seeds that the embryos were dark pink or red. Germination and viability data were combined to calculate the total viable seeds remaining after heat treatments.

Statistical analysis

We analyzed all data with R 3.1.2 software (R Core Team 2014). First, we analyzed the effect of heat shock treatments on seed viability (presence/absence) with Generalized Linear Models (GLM, binomial error distribution), separately for each species. We included the heat shock treatments (t1, t2, t3, t4 and control) as fixed independent variable. Seeds inside the petri dishes are under the same environment which can affect the germination pattern of neighbors'seeds (Tielbörger and Prasse 2009), resulting in autocorrelation of errors (Sileshi 2012). So, we used each seed as an experimental unity and the replication (N=4) as a fixed independent variable, in order to control this autocorrelation of errors. We used likelihood ratio tests coupled with stepwise backward procedure to run the model selection. We made post-hoc multiple comparisons between control and heating treatments, using the single step method to P-values adjustment and the glht command of multcomp package (Hothorn *et al*. 2008).

We used Generalized Linear Mixed Models (GLMM, binomial distribution, see Zuur *et al*. 2009) (package: lme4; see Bates *et al*. 2014) to test whether the effect of heating treatments on seed viability is dependent of seed dormancy and habitat of seed collection. We included species and replication as nested random factors in all models. We first included seed viability (presence/absence) as the response variable and a three-way interaction between heat treatment (t1, t2, t3, t4 and control), habitat (open savannas or wet grasslands) and seed dormancy (Dormant or Non-dormant), as fixed independent variables. Then we checked for collinearity effects using the variance inflation factor (VIF), assuming accepted values with VIF<10 (Dormann *et al*. 2013). Because we found high values of VIF (>10) in this first model, we built a second model without the three-way interaction. In this new model, we included two-way interactions between heating treatment (t1, t2, t3, t4 and control) and habitat (Open savannas or Wet grasslands), and between heating treatment and seed dormancy (Dormant or Non-dormant). Finally, we carried out the same procedures of model selection and multiple comparisons described for the prior analysis.

Results

Heat shock effects on seed viability

With the exception of *Anthaenanthia lanata*, *Saccharum villosum*, *Digitaria lehmanniana* and *Homolepis longispicula*, seeds of all species maintained high seed viability after being exposed to 80°C, irrespective of the exposure time (Fig. 1). However, there was a clear negative effect on their viability when the seeds were exposed to 110°C, for 2.5 and 5 minutes (Fig.1). With the exception of *Aristida riparia*, *Aristida setifolia* and *Sacciolepis myurus*, whose seeds kept high viability after being subjected to 110°C, seeds of all species significantly lost viability after this heat shock, at both exposure times (Fig.1).

Effects of dormancy status and habitat on seed viability after heat shock

There was no difference in viability between controls of all multiple comparisons groups: non-dormant and dormant (Fig. 2), wet grasslands and open savannas (Fig. 3). So, there was no influence of initial viability (Fig. 2 and 3) and the following comparisons between all treatments (t1, t2, t3 and t4) were possible. The effect of heat shock treatments on seed viability was dependent on seed dormancy (LRT_4 = 246.14, P<0.0001). Although no differences were found between dormant and non-dormant seeds in respect to their response to heat shock of 80°C (Fig. 2), dormant seeds survived more than non-dormant seeds after exposure to 110°C (Fig. 2).

The effect of heat shock treatments on seed viability was dependent on habitat (LRT_4 = 44.99, P<0.0001). With exception of the treatment of 80°C for 5 min, in which seeds from open savannas survived more than seeds from wet grasslands, no statistical differences were found in the viability of seeds collected in open savannas and wet grasslands (Fig. 3).

Discussion

Seeds of most grass species (10 out of 14) survived heat shock of 80°C for 2.5 and 5.0 min. This result was similar to that recorded for grass seeds in both African (Gashaw and Michelsen 2002) and Australian savannas (Clarke and French 2005; Dayamba *et al*. 2008; Scott *et al*. 2010), in Mediterranean Shrubland (Reyes and Trabaud 2009) and in South American grasslands (Overbeck *et al*. 2006; Gonzalez and Ghermandi 2012). Soil temperatures at 1 cm depth have been recorded reaching 29-55°C during a fire in Brazilian savannas (Miranda *et al*. 1993; 2009), a depth where the majority of the grass seed bank is maintained (Musso *et al*. 2014). In Brazilian wet grasslands, temperatures at 1 cm aboveground may stay under 100°C most part of the time, with temperatures above 60°C having less than 2 min of residence time during a fire event (Schmidt 2011). Taken together, the high survival rates displayed by these seeds after the 80°C treatment suggests that grass seeds in the soil seed bank and over the soil surface would be able to survive low temperatures generated during a fire in both open savanna and wet grassland.

In contrast to the effects of heat shock of 80 ºC, we found that in 11 out of 14 species there was a negative effect of heat shock after 110°C treatments for 2.5 and 5 min on seed survival. Studies conducted with species from fire-prone ecosystems show contrasting results:

Seeds of many grass species of an Australian savanna survived after treatment of 120°C for 2 min (Clarke and French 2005), seeds of five native grass species of a Sudanese savanna tolerated temperatures of 120°C for 2.5 min (Dayamba *et al*. 2008), seeds of two grass species of the Mediterranean Shrubland survived after being exposed to 110°C for 5 and 10 min (Reyes and Trabaud 2009), while in Ethiopia, seeds in two out of three species survived well at 120°C for 1 min, but did not survive 120 ºC for 5 min treatment (Gashaw and Michelsen 2002). In a Brazilian temperate grassland on the other hand, seeds of three grass species survived after a heat shock of 110°C for 2 min but another three did not (Overbeck *et al*. 2006). Unlike our study sites, the majority of the habitats listed above have a shrub component to their community composition, and are likely to produce longer duration burns that could increase soil heating and residence times. This would result in more species having adapted to tolerate heating up to 110°C or higher. So, with exception of *Aristida riparia*, *Aristida setifolia* and *Sacciolepis myurus*, which survived well to 110°C, our study species might not tolerate hotter burns, with soil temperatures above this level rarely occurring.

Dormancy and habitat effects on seed tolerance to heating

Dormant seeds were shown to be more tolerant to the heat shock than non-dormant seeds, irrespective of whether the seeds were from open savanna or wet grassland. Seed dormancy in grass species from seasonal savannas has been suggested to constitute a drought-avoidance syndrome, synchronizing germination to the beginning of next rainy season, thus reducing the probability of seedling death during the dry season (Mott 1978; Veenendaal *et al*. 1996; Scott *et al*. 2010). Brazilian grass species disperse their seeds through the rainy season (Tannus *et al*. 2006; Munhoz and Felfili 2007b; Ramos *et al*. 2014) and the natural time of fire occurrence is at the beginning of the rainy season (Ramos-Neto and Pivello 2000). So, when the fire occurs seeds of grasses from previous reproduction are already dispersed and presumably are in the soil.

Seed dormancy and tolerance to heat shock in these environments seems to provide an advantage that could increase fitness of grass species in two ways, firstly by reducing the probability of germination before the next rainy season, and secondly by maintaining seed viability while they remain in the soil seed bank during fires. Germinating in a less competitive post-fire environment could be a further strategy to increase plant fitness (Zimmermann *et al*. 2008). Seeds of *Aristida setifolia*, *Axonopus barbigerus*, *E. inflexa* and *Gymnopogon spicatus* (Musso *et al*. 2014), grass species from Brazilian savannas, have shown increased germination at temperatures mimicking post-fire soil conditions (45°C/10°C), providing further evidence that these species have adapted to taking advantage of the post-fire environment.

Lower temperatures occur in wet grasslands during fires than in open savannas and species survived more in open savannas at 80°C (5 min) than in wet grasslands. However, there were no differences in survivorship at 80°C (2.5 min) or 110°C heating treatments. Thus, we partially corroborate our hypothesis that seeds of species from open savannas are more tolerant to heating than those of wet grasslands. The temperature of 80°C can occur in low temperature fires and our results suggest that seeds of species from open savannas might survive more than seeds from wet grasslands in prolonged fires. Natural fires are caused by lightning and are concentrated at the transitions between dry to rainy season and at the beginning of the rainy season (Ramos-Neto and Pivello 2000). On the other hand, human induced fires are concentrated in the dry season (Ramos-Neto and Pivello 2000).

The impacts of fire can be related to fire season (Williams *et al*. 2004); high temperatures (> 100°C) can penetrate deeper into the soil in late dry season fires compared to early dry season fires in Australian savannas (Williams *et al*. 2004). Our results suggest that seeds of open savanna grass species are therefore better adapted to low temperature fires that occur during the rainy season, suggesting that a change in fire season might negatively impact seed survival of these species via an increase in soil temperatures. Additionally, the residence times of high temperatures are influenced by fire season; temperatures above 60°C had shorter residence times in early dry season fires (around 1 min) than in late dry season fires (more than 5 min) in African savannas (Dayamba *et al*. 2010). Seeds of species from wet grasslands do not tolerate prolonged times of exposure (5 min), even to low temperatures, and a change in fire season may therefore negatively affect seed survival of species from this habitat. Subsequently, changes in fire season might negatively affect seed survival of grass species in open savannas and wet grasslands due to an increase in soil temperatures and high temperatures residence times, respectively.

Environment features such as fuel moisture and air temperature strongly influence fire temperature and behavior during burning (Kauffman *et al*. 1994; Mistry 1998). Mean air temperature increases at both global and local scales, as well as changes in precipitation distribution, are projected to occur over the next century due to climate change (IPCC 2013). A prolonged dry season might make the fuel dryer, resulting in more severe and prolonged fires. Our findings suggest that small increases in fire temperatures (from 80°C to 110°C) can kill grass seeds and thus negatively affect seedling recruitment in most grass species from open savannas and wet grasslands. In general, climate change may result in higher survival for fire tolerant grass species (only three species *Aristida riparia*, *Aristida setifolia* and *Sacciolepis myurus* in our study) in comparison with fire non-tolerant grass species, which in turn may deeply affect population dynamics and community structure in Brazilian open savannas and wet grasslands.

In conclusion, our results suggest that species with dormant seeds experience selection for covarying characteristics that allow tolerance to heating in high temperature fires. Additionally, grass seeds from Brazilian open savannas and wet grasslands are tolerant to low temperature fire events. Habitat moisture and seed dormancy display either a negative or positive relationship with fire tolerance, respectively, suggesting that (i) dry habitats may suffer higher temperatures or frequent fire events than wet habitats, and (ii) the duration of time that seeds stay exposed to burn may be a key driver of the evolution of seed fire tolerance.

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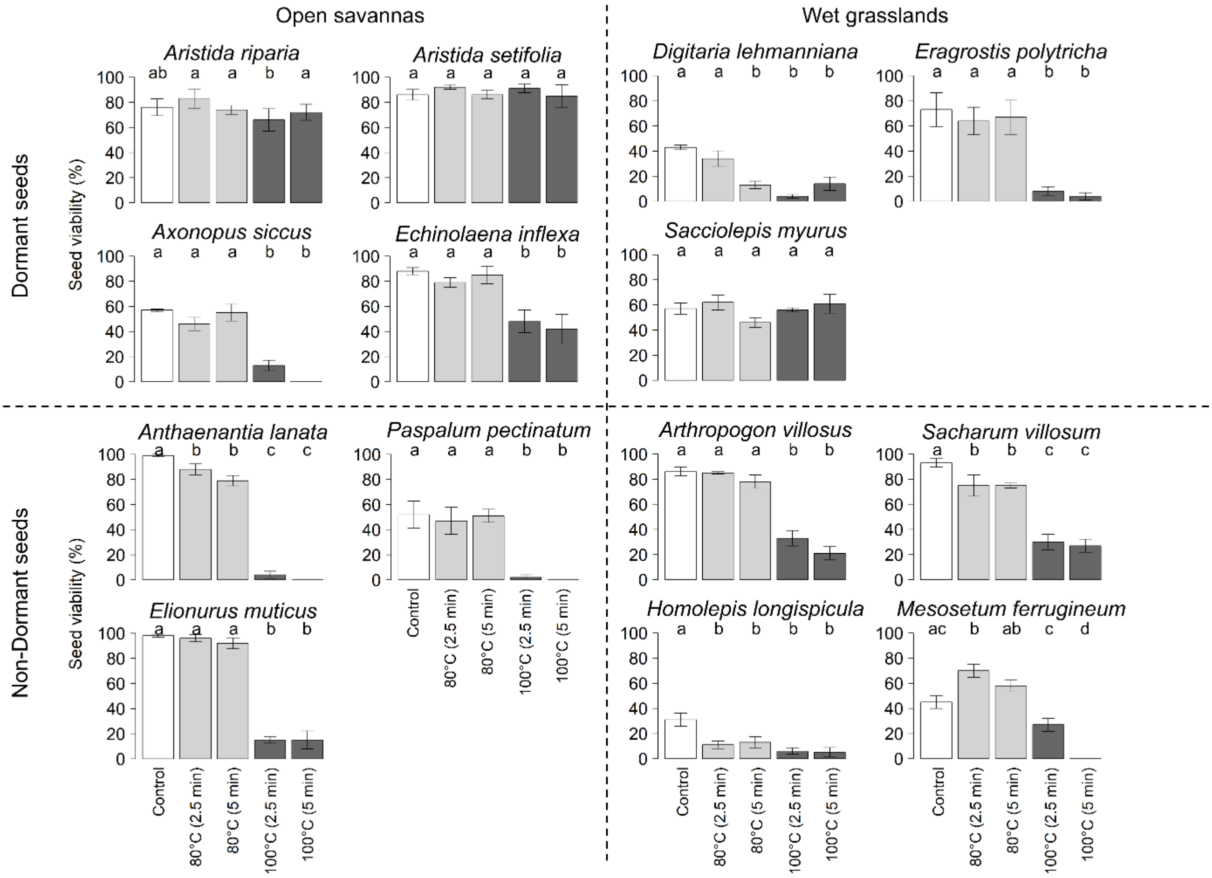

Fig.1 Effects of heat shock treatments on the viability of seeds of Brazilian grass species from open savannas and wet grasslands. Mean viability (%) ± Confidence Interval. Means followed by the same letter do not differ significantly according to multiple comparisons made with glht command.

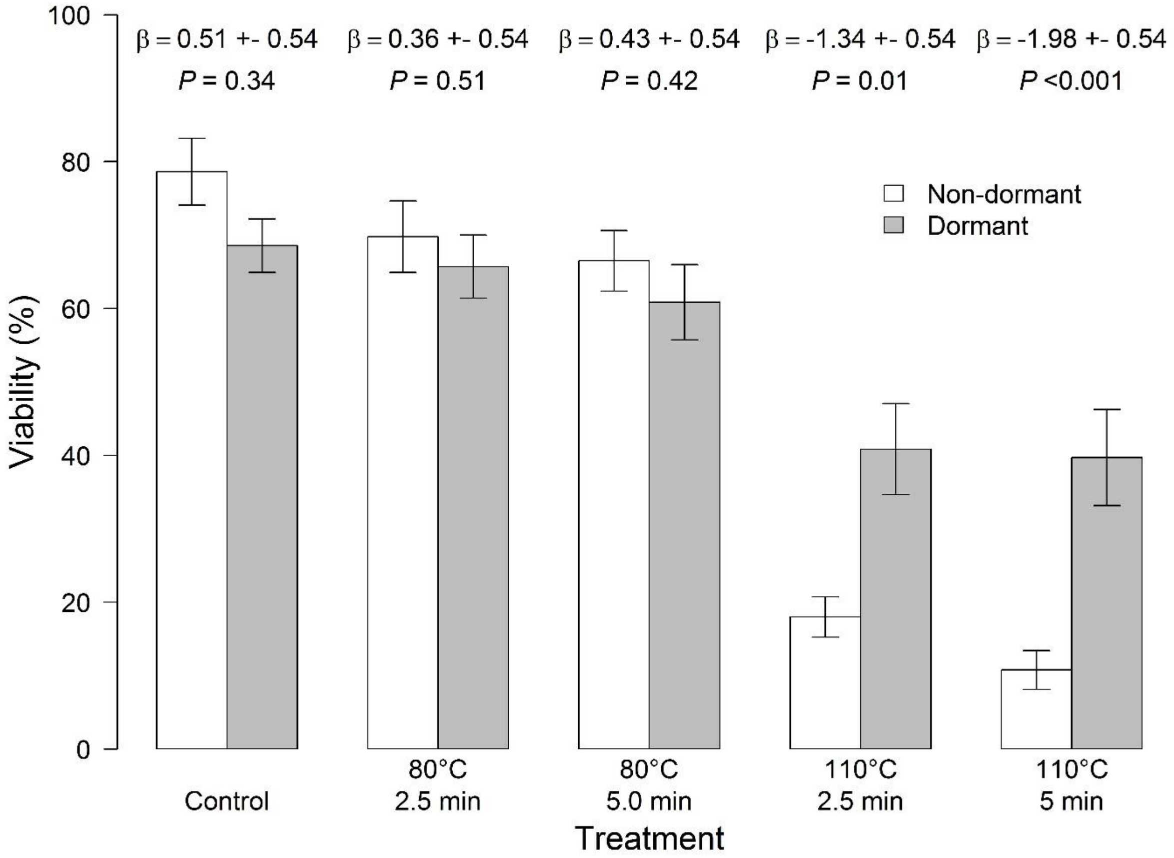

Fig.2 Effects of heat shock treatments on the viability of dormant and non-dormant seeds of Brazilian grass species from open savannas and wet grasslands. White bars = Non-dormant seeds; Grey bars = Dormant seeds. Mean viability (%) ± Confidence Interval.

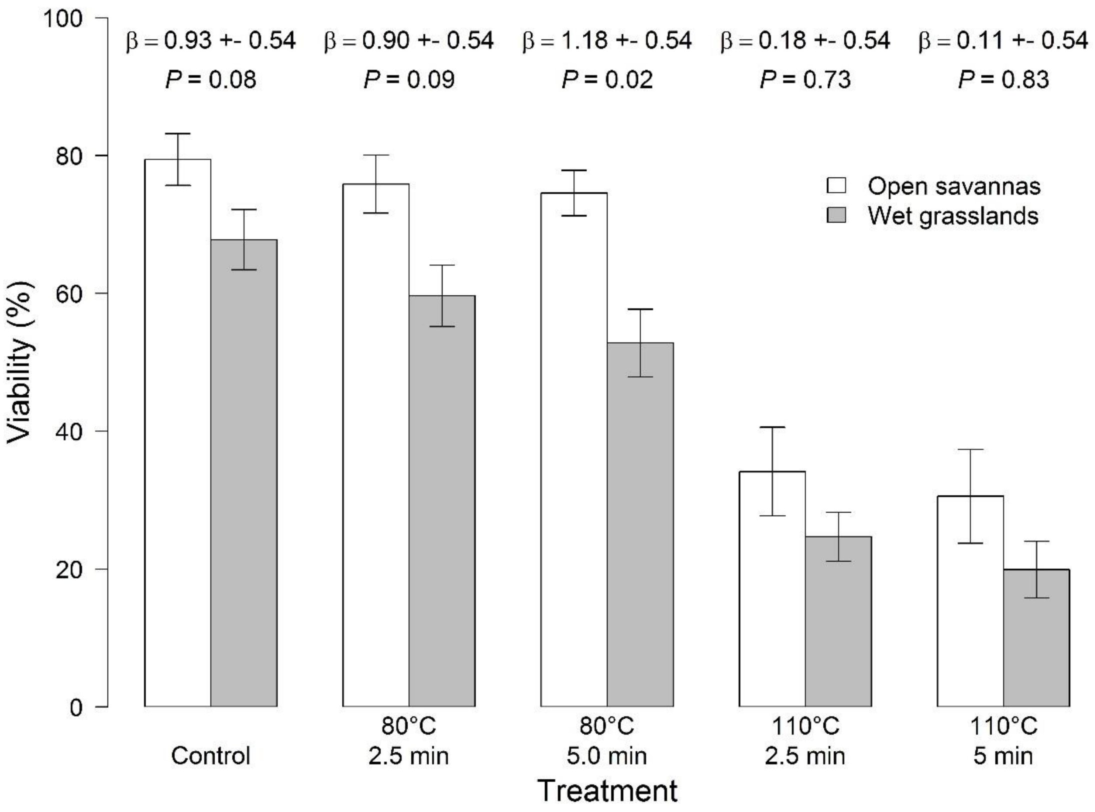

Fig.3 Effects of heat shock treatments on the viability of seeds of Brazilian grass species from open savannas and wet grasslands. White bars = Open savannas; Grey bars = Wet grasslands. Mean viability (%) ± Confidence Interval.

Table 1. Seed germination (G), viability (V) and dormancy of native grass species from open savannas and wet grasslands used in the heat shock treatments. Sites and habitat of collection and months of storage at the heating treatments time.

Species	Habitat	Site	G (%)	V (%)	Dormancy	Storage (Months)
Anthaenantia lanata (Kunth) Benth.	Open savanna	PNB	81	81	ND	8
Aristida riparia Trin.	Open savanna	FAL	10	65	D	5
Aristida setifolia Kunth	Open savanna	FAL	13	82	D	8
Arthropogon villosus Nees	Wet grassland	PNB	97	97	ND	1
Axonopus siccus var. siccus (Nees) Kuhlm.	Open savanna	FAL	1	76	D	7
Digitaria lehmanniana Henrard	Wet grassland	PNB	0	92	D	7
Echinolaena inflexa (Poir.) Chase	Open savanna	FAL	0	97	D	10
Elionurus muticus (Spreng.) Kuntze	Open savanna	PNB	99	99	ND	8
Eragrostis polytricha Nees	Wet grassland	PNB	0	67	D	10
Saccharum villosum Steud.	Wet grassland	PNB	89	92	ND	2
Homolepis longispicula (Döll) Chase	Wet grassland	PNB	57	57	ND	11
Mesosetum ferrugineum (Trin.) Chase	Wet grassland	PNB	68	68	ND	2
Paspalum pectinatum Nees ex Trin.	Open savanna	PNB	54	54	ND	8
Sacciolepis myurus (Lam.) Chase	Wet grassland	FAL	8	94	D	11

PNB = National Park of Brasília; FAL = Fazenda Água Limpa from University of Brasília. D = Dormant; ND = Non-dormant.

CAPÍTULO 3

Temporal changes in seed dormancy, germination and viability of grasses from Neotropical savannas

Temporal changes in seed dormancy, germination and viability of grasses from Neotropical savannas

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Abstract

Savannas are ecosystems with scattered trees and high grass cover. The *Cerrado*, the Brazilian savanna, has been strongly degraded during the past three decades. The use of native grass species to recover degraded areas is strongly advised. However, seed germination and longevity patterns of native species are poorly understood. This study aimed to investigate the viability, dormancy and germination of fresh and dry-stored seeds of 28 native grass species from the *Cerrado*. After seed collection, germination trials were performed every three months for up to one year of dry storage. Dry storage had a negative effect on seed viability for twelve species. Among these species, germination of fresh seeds was high for eight species and four were dormant. Thus, results suggest the use of seeds from these eight species up to one year after collection. On the other hand, viability of seeds from sixteen species was not affected by dry storage. Fresh seeds from five species were non-dormant and able to be sowed at any time for up to one year. Eleven species were dormant and dry storage progressively alleviated the dormancy levels for seven of them. Dry storage is suggested for these seven species to break seed dormancy before sowing.

Keywords: seed dormancy; degraded areas; recovering; germination; open savanna; wet grassland.

Introduction

The *Cerrado*, a tropical savanna covering about 22% of the Brazilian territory (Jepson 2005), has been strongly degraded and replaced by crop cultivation and pasture for cattle during the past three decades (Jepson 2005). Estimates indicate that over 50% of original *Cerrado* area has already disappeared (Klink and Machado, 2005). Open savannas (*campos sujos*) and wet grasslands (*campos úmidos*) are ecosystems mainly characterized by grasses and scattered trees inside the *Cerrado* domain, and are vulnerable ecosystems because they are easily replaced by exotic grasses for cattle forage or crop cultivation. During recent years, the Brazilian government has strongly advised the use of native grass species for recovering degraded areas. However, the use of exotic grass species has been used instead of native species due to the unavailability of their seeds in the market and the lack of information about their viability and germination behavior.

Grasses belong to one of the most representative families (Poaceae) in the herbaceous layer of the *Cerrado* (~ 600 species, Filgueiras et al., 2014) and, since they are affected by water shortage during the dry season, their flowering and fruiting is mostly restricted to the rainy season (Tannus et al., 2006; Munhoz and Felfili, 2007; Ramos et al., 2014). The availability of seeds from these grass species for collection occurs mainly during the rainy season (Silva and Ataroff, 1985; Almeida, 1995), with some species dispersing seeds during the dry season as well (Almeida, 1995). However, seed germination and viability of several grass species from savannas show temporal changes during dry storage, according to seed dormancy and longevity patterns (Baskin and Baskin, 2014). Thus, the knowledge of germination behavior and viability of fresh seeds along a gradient of storage periods may be useful to predict the seed longevity of species from tropical savannas, with strong implications regarding the choice of species for recovering degraded areas.

In savannas, there is a high variation in the level of dormancy among dispersed grass seeds (Baskin and Baskin, 1998; Baskin and Baskin, 2014). The most common type of dormancy in grasses is non-deep physiological dormancy (Baskin and Baskin, 1998; Baskin and Baskin, 2014). The main characteristics of this type of dormancy are that seeds absorb moisture easily when watered, and the level of dormancy is progressively alleviated when the seeds are subjected to dry storage (Baskin and Baskin, 2014; Baskin and Baskin, 2004), increasing germination rates with storage time. Thus, dry storage may be used to improve germination of grass species with the purpose of recovering degraded areas. However, it is extremely necessary to understand whether these seeds remain viable during a period of dry storage.

Despite their importance in savanna dynamics and their large coverage in savannas worldwide, grass seeds' levels of dormancy and patterns of germination, as well as their responses to dry storage, are poorly understood in the Brazilian savannas. Thus, the aim of this study was to verify the temporal patterns of savanna grass species' seed germination, dormancy and longevity, testing germination with freshly collected seeds and after dry storage for up to one year. The results are discussed in relation to the specific potential of native grass species to be used to recover degraded areas based on germination and viability behavior after dry storage.

Material and Methods

Study area and seed collection

The dispersal units in grasses are generally complex structures composed by a caryopsis (fruit), a caryopsis with bracts (lemma and palea) and occasionally inflorescences (commonly called seeds). Seeds from 28 native perennial grass species (Table 1) from Brazilian open savannas and wet grasslands were collected in 2012 and 2013, in two protected areas of Brasília: Fazenda Água Limpa from the University of Brasília (FAL - 15º58'43.06"S and 47º56'21.41"W, 1.197 m) and the National Park of Brasília (PNB - 15º38'46.22"S and 48º00'19.75"W, 1.178 m). The climate of the region is seasonal with two well-defined seasons: a wet summer and a dry winter (Fig. 1). Patches of savannas, forests and grasslands compose the study sites. Plants were monitored regularly to ensure that seeds were mature at dispersal and then manually collected. Monitoring of the grass populations selected for this study allowed the estimate of the seed dispersal periods for each species. After collection, the seeds were stored in paper bags at room temperature (27-maxima and 17 ºC -minima), measured during the storage period with a thermometer of maxima and minima temperatures. The average relative humidity in the region was 43-80% during the study period (data from BDMEP/INMET).

Viability and germination by storage time

To verify the degree of viability and the dormancy level of the seeds, germination trials were conducted with freshly collected seeds. To test the effect of dry storage on the degree of viability and the germination percentages, trials were conducted after 3, 6, 9 and 12 months of dry storage. Five replicates of 20 seeds were used for each species for each treatment, except for *P. maculosum*, *A. goyazense*, *H. longispicula* and *S. sanguineum*, where five replicates of 10 seeds per treatment were used due to the limited quantity of seeds. The germination experiments were conducted in chambers regulated for a 28/18°C cycle for day/night, respectively, under a photoperiod of 12h of white light. The temperatures were set according to the average minimum and maximum temperatures recorded during the wet summer (Fig. 1), which represents the growing season for most savanna species in the *Cerrado*, including grasses. Germination was recorded daily for up to 30 days and the criterion for seed germination was emergence of the radicle. After each germination trial, the viability of the non-germinated seeds was tested using 1% tetrazolium chloride solution. The seeds were placed in contact with tetrazolium solution for 24 hours in the dark at 30 ºC in a germination chamber. Seeds were considered viable when embryos were dark pink or red. We considered as dormant the species whose viable seeds did not reach at least 50% of germination.

Statistical Analysis

We analyzed the effect of dry storage treatments on seed viability and germination for each species separately, because the aim was to verify the species' specific potential for recovering degraded areas. First, viability was analyzed (presence/absence) with Generalized Linear Models (GLM, binomial error distribution, see Zuur et al., 2009). Dry storage treatment (0, 3, 6, 9 and 12 months) was included as a fixed independent variable. Seeds inside the petri dishes were placed under the same environment that could affect the germination pattern of neighbors' seeds (Tielbörger and Prasse 2009), resulting in autocorrelation of errors (Sileshi 2012). Therefore, each seed was used as an experimental unity and replication (N=5) as a fixed independent variable, in order to control the autocorrelation of errors. Post-hoc multiple comparisons were conducted between levels of the significant fixed factors, using the single step method with P-values adjustment and the glht command of multcomp package (Hothorn et al., 2008). Second, germination was analyzed with the same model procedure and steps using viability analysis. We included in the model only the germination of viable seeds, since there is no need to verify germination of a dead seed. In the germination and viability analysis, it is not possible to estimate parameters in GLM in cases of total absence of value, so in these situations one absent value (0) was replaced with one present value (1) in each replication. All data was analyzed with R 3.1.2 software (R Core Team 2014).

Results

Dry storage had a negative effect on seed viability for twelve grass species (Fig. 1). Seeds from these species generally died after six to twelve months of dry storage. Germination of freshly collected seeds was low or absent for four of these species: *Panicum olyroides*, *Paspalum carinatum*, *P. glaucencens* and *I. camporum* (Fig. 1; Table 1). Considering that these species showed moderate to high levels of viability, we conclude that their seeds are dispersed with high levels of dormancy (Table 1). On the other hand, germination of fresh seeds was above 50% for eight species: *Elionurus muticus*, *Arthropogon villosus*, *Mesosetum ferrugineum*, *Paspalum erianthum*, *P. guttatum*, *P. pectinatum*, *Homolepis longispicula* and *Anthaenantia lanata* (Fig. 1; Table 1), so these species disperse seeds with no dormancy.

Dry storage did not affect seed viability for sixteen grass species (Fig. 2). Seeds from these species were viable even after twelve months of dry storage. The germination of these species was variable: Fresh seeds of eleven species did not germinate, and were deemed dormant, while seeds of five species had high levels of germination, and were deemed non-dormant (Fig. 2; Table 1). Germination increased after dry storage for seven of the eleven dormant species (Fig. 2). On the other hand, germination remained low for four of the eleven dormant species: *Paspalum maculosum*, *Setaria parviflora*, *Aristida recurvata* and *Axonopus siccus* (Fig. 2). Seed germination remained high and constant for five of the non-dormant species across all storage periods: *Andropogon leucostachyus*, *Aristida gibbosa*, *Saccharum villosum*, *Schizachyrium sanguineum* and *Paspalum polyphyllum* (Fig. 2).

Discussion

Results show that nearly half of the species had seeds which died with dry storage (12 sp.) and the remaining species had seeds with high longevity across all storage periods (16 sp.). This pattern was also found in grass species from the African savannas (Tessema et al., 2011), where seeds with high longevity patterns can become part of a seed bank (Tessema et al., 2011), increasing resilience after disturbance in savannas (Scott et al., 2010). Among the species negatively affected by dry storage, four are not recommended for restoration purposes due to low levels of germination: *P. olyroides*, *P. carinatum*, *P. glaucencens* and *I. camporum*. Another negative aspect is that *P. carinatum* has a low percentage of fertile seeds (7.8%; Aires et al., 2013) which could become an obstacle for seed collection.

Although seeds of another eight species died after dry storage, they had high levels of germination: *E. muticus*, *A. villosus*, *M. ferrugineum*, *P. erianthum*, *P. guttatum*, *P. pectinatum*, *H. longispicula* and *A. lanata*. These species could be used for restoration during the first months after seed dispersal, which occurs between December and January. Additionally, *E. muticus*, *M. ferrugineum*, *P. erianthum*, and *P. pectinatum* are tall and robust plants (~30-70cm height) and these traits may confer advantage for colonizing areas.

Seed longevity of sixteen grass species was not affected by dry storage and five of them were non-dormant. *Andropogon leucostachyus* produces non-dormant seeds and has a high percentage of fertile seeds (60%; Aires et al., 2013). In addition, seed germination of *A. leucostachyus* was high in fresh seeds and after three, nine and twelve months of dry storage, resulting in broad opportunities for sowing throughout the year. Grass species' seeds commonly have non-deep physiological dormancy and undergo after-ripening during dry storage, increasing germination (Mott, 1978; Baskin and

Baskin, 2014). Indeed, germination increased in seven of the eleven dormant species after dry storage: *S. myurus*, *A. setifolia*, *E. inflexa*, *C. cirrhosum, E. polytricha*, *A. riparia* and *A. goyazense*. These species can be stored without losing viability but their sowing is possible only after about 6 months of dry storage, which is the time necessary to increase germination. *A. riparia* and *A. setifolia* have a high percentage of fertile seeds (90 and 73%, respectively; Aires et al., 2013). Moreover, these species are robust plants (130cm height) and have high longevity patterns associated with high germination rates after dry storage. Thus, *A. riparia* and *A. setifolia* have high potential for the purpose of recovering degraded areas.

This study focused mainly on the use of native grass species from the *Cerrado* for recovering degraded areas. However, there are other important uses of these species that must be considered. First, grasses are commonly used as ornamental plants in gardens around the world. Unfortunately, local researches and producers have not given enough consideration to native species from the *Cerrado*. This may occur because there is not enough information about native species in the literature. Indeed, some species such as *A. riparia* and *C. cirrhosum*, have high ornamental potential to be explored. Second, several native grass species have high forage value for cattle. For example, *E. inflexa* is selected for cattle grazing (Almeida et al., 1987; Leite et al., 1997). Exotic grasses frequently used as forage for cattle are spreading fast, threating local biodiversity (Pivello et al., 1999). Replacement by farmers of exotic grasses for native grass species in pastures could alleviate the pressure on the native biodiversity.

In conclusion, our data shows that seeds from twelve native grass species die after dry storage and can only be used during a short period after collection. On the other hand, viability of seeds from another sixteen species was not affected by dry storage and it is possible to store them for up to one year. We suggest that the species *A.*

leucostachyus, *A. riparia*, *A. setifolia* and *E. inflexa* have good potential to be explored for ornamental or forage usage as well as for recovering degraded areas.

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Table 1. Habitat of occurrence, time of seed dispersal and categorization of seed dormancy based on the results of germination and viability of fresh collected grass seeds from native species from open savannas and wet grasslands of Central Brazil.

Species	G (%)	V (%)	GV (%)	Dormancy	Seed dispersal (Month)	Habitat	Site
Panicum olyroides Kunth	0	98	0	D	Jan	Open savanna	PNB
Paspalum carinatum Humb. & Bonpl. ex Flüggé	0	65	0	D	Feb	Open savanna	FAL
Echinolaena inflexa (Poir.) Chase	0	97	0	D	Mar	Open savanna	FAL
Ichnanthus camporum Swallen	0	93	0	D	Feb	Open savanna	FAL
Eragrostis polytricha Nees	0	67	0	D	Dec	Wet grassland	PNB
Aristida recurvata Kunth	0	61	0	D	Jun	Open savanna	PNB
Axonopus siccus var. siccus (Nees) Kuhlm.	1	76	1.31	D	Feb	Open savanna	FAL
Paspalum glaucescens Hack.	2	68	2.94	D	Feb	Open savanna	PNB
Paspalum maculosum Trin.	2	46	4.35	D	Apr	Wet grassland	PNB
Agenium goyazense (Hack.) Clayton	4	64	6.25	D	Jun	Open savanna	PNB
Sacciolepis myurus (Lam.) Chase	8	94	8.51	D	Sep	Wet grassland	FAL
Aristida riparia Trin.	10	65	15.38	D	Jun	Open savanna	FAL
Aristida setifolia Kunth	13	82	15.85	D	Mar	Open savanna	FAL
Setaria parviflora (Poir.) Kerguélen	20	51	39.21	D	Feb	Open savanna	PNB
Ctenium cirrhosum (Nees) Kunth	38	94	40.42	D	Apr	Open savanna	PNB

Aristida gibbosa (Nees) Kunth	69	81	85.18	ND	Jun	Open savanna	FAL
Saccharum villosum Steud.	89	92	96.74	ND	Nov	Wet grassland	PNB
Paspalum guttatum Trin.	41	41	100	ND	Dec	Open savanna	PNB
Paspalum polyphyllum Nees	52	52	100	ND	Aug	Wet grassland	FAL
Paspalum erianthum Nees ex. Trin.	52	52	100	ND	Dec	Open savanna	PNB
Paspalum pectinatum Nees ex Trin.	54	54	100	ND	Jan	Open savanna	PNB
Homolepis longispicula (Döll) Chase	57	57	100	ND	Dec	Wet grassland	PNB
Mesosetum ferrugineum (Trin.) Chase	68	68	100	ND	Jan	Wet grassland	PNB
Andropogon leucostachyus Kunth	79	79	100	ND	Oct	Wet grassland	FAL
Anthaenantia lanata (Kunth) Benth.	81	81	100	ND	Jan	Open savanna	PNB
Schizachyrium sanguineum (Retz.) Alston	92	92	100	ND	Jun	Open savanna	PNB
Arthropogon villosus Nees	97	97	100	ND	Dec	Wet grassland	PNB
Elionurus muticus (Spreng.) Kuntze	99	99	100	ND	Dec	Open savanna	PNB

Site of occurrence: PNB (National Park of Brasília) and FAL (Água Limpa farm). G = Germination; V = Viability; GV = Germination proportion of viable seeds. D = Dormant; ND = Non-dormant.

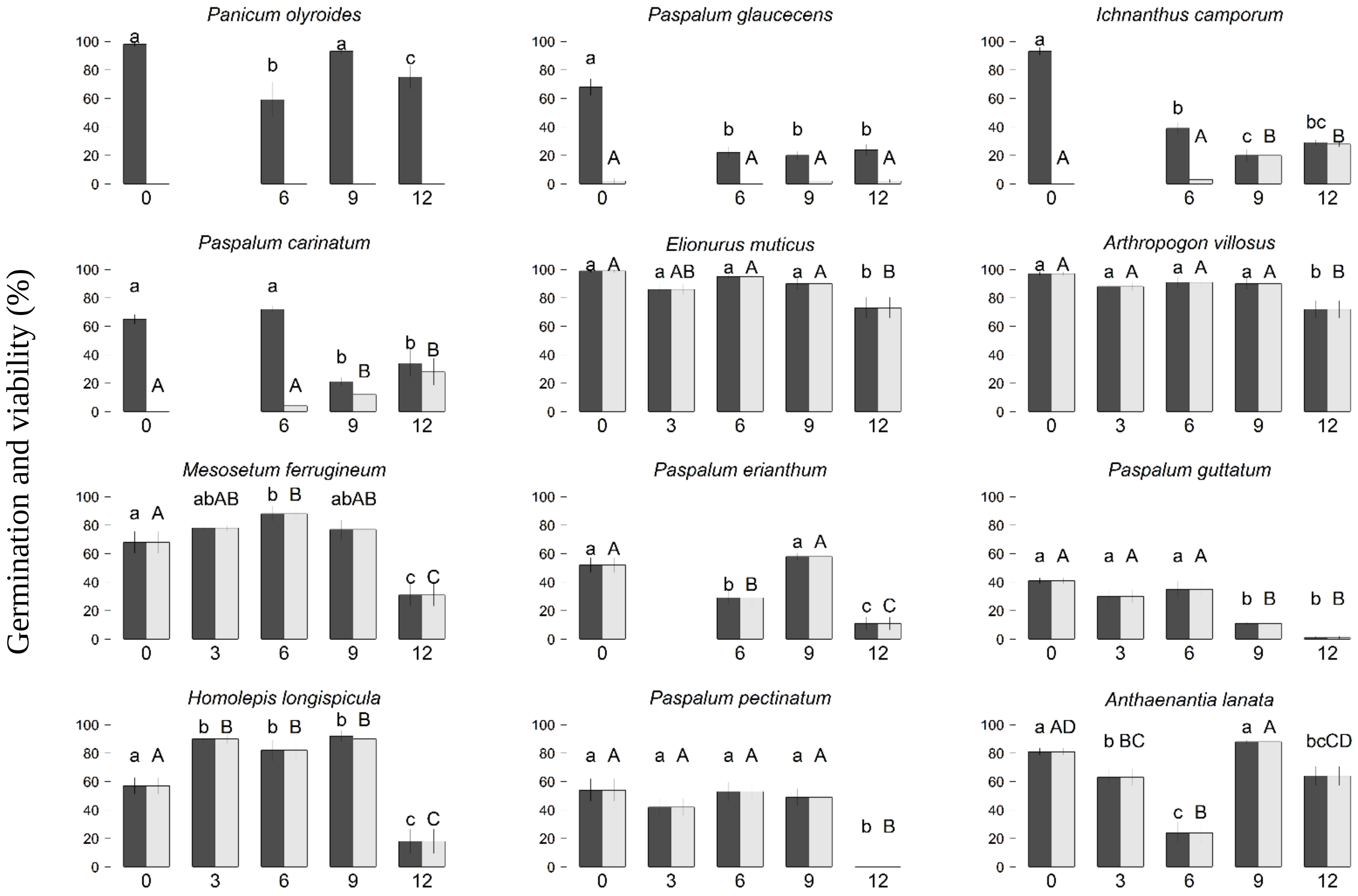

Figure 1. Germination and viability of grass seeds negatively affected by dry storage in species from open savannas and wet grasslands of Central Brazil. Capital letters refers to post-hock test on germination; Lower case letter refers to post-hock test on viability. Dark grey bars = seed viability; Light grey bars = seed germination. Average ± 95% CI.

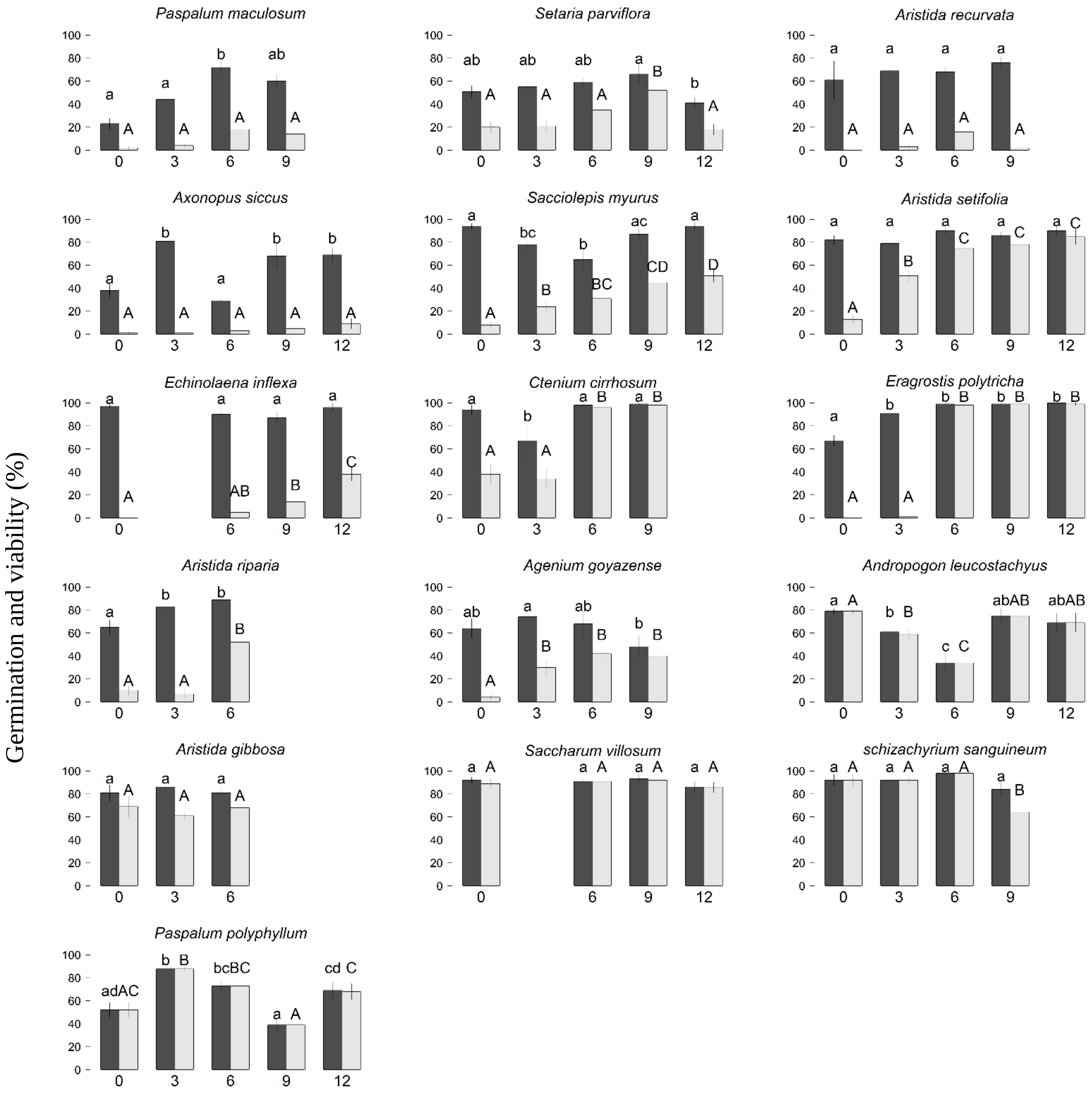

Figure 2. Germination and viability of grass seeds not affected by dry storage in species from open savannas and wet grasslands of Central Brazil. Capital letters refers to post-hock test on germination; Lower case letter refers to post-hock test on viability. Dark grey bars = seed viability; Light grey bars = seed germination. Average ± 95%CI.

CONSIDERAÇÕES FINAIS

O comportamento da germinação ao longo do armazenamento diferiu entre os campos sujos e os campos úmidos. Em ambos os campos a germinação aumentou com o armazenamento, porém nos campos úmidos esse aumento aconteceu mais rápido, após três meses, do que no campo sujo, após seis meses. Em relação à viabilidade, no campo sujo as sementes morreram após doze meses, enquanto que no campo úmido permaneceram vivas e com alta germinação. Estes resultados indicam que a dormência faz com que a germinação nos campos sujos ocorra no máximo na estação chuvosa seguinte após a dispersão das sementes. Por outro lado, nos campos úmidos, aonde a sazonalidade é menos marcante, a germinação pode ocorrer até mesmo nos meses de seca.

A germinação das sementes de gramíneas foi influenciada pela época de dispersão. Sementes dispersas no final da estação chuvosa tiveram maior dormência do que sementes dispersas no início da estação chuvosa e na estação seca. Após três meses de armazenamento, a germinação de sementes dispersas no final da estação chuvosa aumentou, não havendo mais diferenças entre os períodos de dispersão. Sementes dispersas no início da estação chuvosa tem toda a estação chuvosa para que ocorra a germinação e o estabelecimento das plântulas (~7 meses). Por outro lado, a germinação no final da chuva é arriscada pois, estas sementes teriam apenas 3 meses de estação chuvosa pela frente. O alto nível de dormência nestas sementes consiste em uma síndrome de evitar a seca, previamente registrada para espécies de ambientes sazonais (Mott 1978; Veenendaal et al. 1996; O'Connor & Everson 1998; McIvor & Howden 2000; Scott et al. 2010). Já a perda da dormência indica que ocorre uma sincronização da germinação com a estação chuvosa seguinte. A ausência de dormência nas sementes de espécies dispersas na seca pode ser uma estratégia vantajosa uma vez que a estação chuvosa se aproxima.

A estratégia de dispersão teve influência nos padrões de germinação. Sementes frescas de espécies com dispersão barocórica germinaram menos, ouseja foram mais dormentes, do que sementes de espécies com dispersão anemocóricas. A densidade de competidores coespecíficos durante o crescimento das plântulas afeta negativamente o sucesso reprodutivo (Orrock & Christopher 2010). Por dispersar longe e não terem dormência as sementes anemocóricas podem cair em sítios mais abertos e se beneficiar de uma estação de crescimento com menos competidores por recursos. Por outro lado, a dispersão em sementes barocóricas é limitada, causando um adensamento de sementes próximo à planta-mãe. Como estas espécies dispersam durante a estação chuvosa, há umidade suficiente para que a germinação ocorra, aumentando a competição entre plântulas coespecíficas e com a planta-mãe. Entretanto, a dormência diminui esta pressão competitiva, aumentando a chance de ocorrer uma dispersão secundária e, consequentemente, espalhando a germinação no tempo e no espaço.