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

Desirée M. Ramos iD, Pedro Diniz, Mark K.J. Ooi, Fabian Borghetti & José F.M. Valls

Ramos, D.M. (corresponding author, desibio@gmail.com)[1,2],
Diniz, P. (pdadiniz@gmail.com)[3],
Ooi, M.K. (mark.ooi@unsw.edu.au)[4,5],
Borghetti, F. (fborghet@unb.br)[1],
Valls, J.F. (jose.valls@embrapa.br)[1,2]

[1]Department of Botany, University of Brasília, Brasília 70910-970, Brazil;
[2]Brazilian Corporation of Agricultural Research, Embrapa, Brasília 70770-917, Brazil;
[3]Department of Zoology, University of Brasília, Brasília 70910-900, Brazil;
[4]Centre for Sustainable Ecosystem Solutions, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia;
[5]Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia

Abstract

Question: In seasonal rainfall systems, seed dormancy is a strategy to avoid germination and seedling emergence in the dry season. Grass species in Brazilian savannas (*Cerrado*) show variation in seed dispersal timing and mechanisms, and occur in different habitat types (distinguished by soil moisture) within a seasonal rainfall environment. However, it is unknown whether dormancy has evolved in these systems as a dominant way in which germination is deferred, or how it correlates with other key traits such as dispersal, where known trade-offs exist for avoiding competition. We asked whether seed germination and dormancy vary with dispersal and abiotic factors in savanna systems. Specifically, we assessed dormancy by comparing seeds: (1) from species living in habitats with contrasting soil moisture during the dry season (open savannas vs wet grasslands); (2) dispersed at different times (early in the wet season, late in the wet season and in the dry season); and (3) showing alternate dispersal syndromes (barochoric vs anemochoric).

Location: Open savannas and wet grasslands in central Brazil.

Methods: We collected seeds of 29 grass species and tested viability and dormancy using germination trials with fresh seeds, which was then repeated after dry storage for 3, 6, 9 and 12 mo. GLMM were used to test whether the degree of dormancy was dependent on habitat type, seed dispersal time and seed dispersal syndrome.

Results: Seeds from wet grasslands lived longer and had consistently higher germination rates than seeds from open savannas. Additionally, fresh seeds dispersed late in the wet season had higher levels of seed dormancy compared to seeds dispersed early in the wet season. Finally, we found that anemochoric seeds had lower levels of dormancy than barochoric seeds.

Conclusions: Seed dormancy among Neotropical grasses was higher for seeds of species from dry habitats, dispersed late in the wet season, and with short distance dispersal (barochory). These results suggest that seed dormancy is a key mechanism by which seeds avoid seedling emergence in the dry season, an effect offset by habitat-specific soil moisture availability. The trade-off between dormancy and seed dispersal suggests that both strategies are costly and had non-additive benefits.

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 & Archer 1997). The *Cerrado* is a Neotropical biome in central Brazil dominated by savannas, but also containing grasslands and forests. Soil moisture is one of the main determinants of vegetation physiognomies; for example, rain forests are associated with shaded valleys and riparian areas, while wet grasslands occur in more open habitats where the water table reaches close to the surface (Cianciaruso & Batalha 2008). In both of these examples, soils are moist throughout the year. However, in savanna physiognomies the water table is further below the soil surface (Rossatto et al. 2012), leading to soils and the herbaceous layer

drying out during the annual dry season. Grass species within the *Cerrado* biome are mostly restricted to these 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 & Flores 2005; Baskin & Baskin 2014). Rainfall seasonality is therefore likely to be a strong selective pressure acting on seed dormancy. Indeed, seed dormancy has been found in a higher number of species in many seasonal rainfall environments in comparison to less seasonal environments (Jurado & Flores 2005; Baskin & 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. 2015, http://floradobrasil.jbrj.gov.br), with species from wet grasslands and open savannas occurring under the same seasonal macroclimate. However, in wet grasslands the water table is situated near the surface throughout the year (Cianciaruso & Batalha 2008). 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 & Batalha 2008). Subsequently, if seed dormancy is a strategy in species from seasonal environments used to deal with water shortage, this selective pressure would be weaker in wet grasslands. Thus, we would expect higher seed dormancy among seeds of species occurring in open savannas in comparison to seeds of species occurring in wet grasslands.

Grass flowering and fruiting follow a seasonal pattern restricted to the wet season in open savannas and wet grasslands (Munhoz & Felfili 2007; Ramos et al. 2014), presumably due to resource availability (i.e. water) to allow reproduction. However, there is considerable variation in the timing of seed dispersal among grass species (Munhoz & 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. 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 (when low amounts of rain usually occurs in Brazilian savannas), would very probably face extreme water shortages and low relative air humidity, 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 higher seed dormancy among seeds dispersed late in the wet season, and during the dry season, in comparison to seeds dispersed early in the wet season. Moreover, to synchronize germination in the next wet season, we would expect dormant seeds to maintain viability and overcome dormancy through dry storage.

While the season of seed release may be an important driver for variation in dormancy, dispersal also plays a critical role in 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 & Brown 1988). Parent–offspring (Ellner 1986) and –sibling competition can significantly reduce plant fitness (Satterthwaite 2010; Baskin & 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 & Brown 1988). Theoretical studies in particular have reported that mean dispersal distance decreases with increasing dormancy (e.g. Venable & Lawlor 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. Among several dispersal syndromes, seeds can be characterized by structures that facilitate wind dispersal (anemochory), such as winged bracts, hairy bracts and hairy rachis. These structures can be completely absent and dispersal is therefore likely to occur through gravity (barochory; Ernst et al. 1992). Barochoric seeds fall near the mother plant, while anemochoric seeds have the opportunity to disperse far from the mother plant. Anemochory could therefore be favoured to deal with spatial unpredictability and competition. Hence, we would expect to find higher seed dormancy among barochoric seeds in comparison to anemochoric seeds if there was evidence of a dispersal–dormancy trade-off.

Seeds of grass species can be non-dormant or physiologically dormant (Baskin & Baskin 1998), but the occurrence and mechanisms of seed dormancy in grass species from Brazilian ecosystems are poorly understood. Furthermore, investigating the effects of selective pressures resulting from abiotic factors, or how dormancy is related to dispersal, is important for understanding the ecology and evolution of plant traits. The seasonal macro-climate of the Brazilian savanna, the micro-climatic differences in open savannas and wet grasslands habitats as a consequence of variations in the water table, and the high variability in seed dormancy and dispersal syndromes of savanna grasses make the *Cerrado* an ideal ecological system to investigate germination strategies. Thus, our objectives were to

investigate the germination, viability (i.e. longevity) and dormancy levels of both freshly collected and dry-stored seeds: (1) from species living in habitats with contrasting soil moisture during the dry season (open savannas vs wet grasslands); (2) dispersed at different times (early in the wet season, late in the wet season and in the dry season); and (3) displaying alternate dispersal syndromes (barochoric vs anemochoric).

Methods

Study area

The *Cerrado* is the largest Neotropical savanna and covers around 2 million square kilometers in central Brazil. The climate of the region is seasonal with two well-defined seasons: a wet season from Oct to Mar (mean annual precipitation from 800 to 2000 mm; Oliveira-Filho & Ratter 2002) and a dry season from Apr to Sept (Fig. 1). The study was conducted in two protected areas of Brasília: Fazenda Água Limpa at the University of Brasília (FAL – 15°58′43.06″ S, 47°56′21.41″ W, 1.197 m a.s.l.) and the National Park of Brasília (PNB – 15°38′46.22″ S, 48°00′19.75″ W, 1.178 m a.s.l.l). FAL and PNB are composed of patches of savanna, forest and grassland. This study was conducted in open savannas and wet grasslands. We selected two wet grassland sites in each area, four open savanna sites in PNB and two open savanna sites in FAL. Vegetation in open savannas and wet grasslands are composed mainly of grasses and forbs, with a few scattered trees occasionally visible in open savannas. Soils in open savannas are well drained, poor in nutrients and rich in aluminium. On the other hand, soils in wet grasslands are rich in organic matter and the water table is near the surface (about 20–80 cm; Cianciaruso & Batalha 2008), making the soil moist for most of the year (Cianciaruso & Batalha 2008; Fidelis et al. 2013).

To characterize the soil moisture of the studied areas (FAL and PNB) we measured the water potential (MPa/Ψ) of six open savanna sites and four wet grassland sites every 30 d during the dry season, from Jun to Aug 2015. Soil samples were collected during the dry season to verify whether soils of wet grasslands stayed wet longer than the soils of open savannas after the end of the wet season. We collected soil samples in three plots previously set in each area equidistant (50 m) from each other in a triangular arrangement, totaling 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 cm and between 10 to 11 cm. These depths were selected because (1) most seeds in the soil seed banks in Brazilian savannas occur in the first few centimeters below the surface (Andrade & Miranda 2014), and (2) root biomass of grasses occurs mostly between 0 to 20 cm depth in
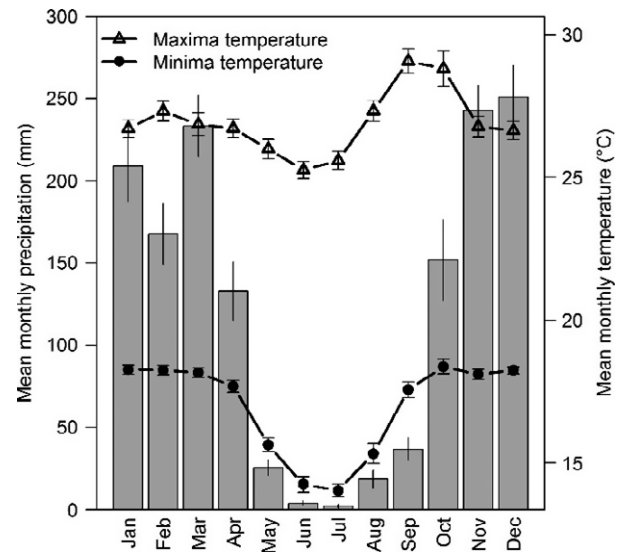

Fig. 1. Mean (95% CI) monthly minimum and maximum (95% CI) temperatures and precipitation in Brasília-DF from 1994 to 2014. Data from BDMEP/INMET.

Brazilian grasslands (Fidelis et al. 2013), so a depth between 2 to 11 cm is well within the *perfil-position* that roots of grass seedlings would grow during the initial development stages of germination. The soil samples were placed in hermetically sealed sample cups and stored in a cool-box. The water potential was measured in the laboratory with a WP4C water potential meter (Decagon Devices, Pullman, NY, USA), within 2 d of sampling.

Seed collection

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 open savanna and wet grassland habitats were collected in 2012 and 2013 in FAL and PNB. 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 °C maximum, 17 °C minimum), measured throughout dry storage using a thermometer. The average relative air humidity in the region (Distrito Federal) was 43–80% during the period of study (data from BDMEP/INMET). For accurate identification, we collected voucher specimens of the studied grass species, which were deposited in the Embrapa Genetic Resources and Biotechnology (CEN) Herbarium, headquartered in Brasilia.

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 (Nees) Kuhlm.	Barochory	Late	Open savanna	FAL
Ctenium cirrosum (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 ex Trin.	Anemochory	Dry	Wet grassland	FAL
Saccharum villosum Steud.	Anemochory	Early	Wet grassland	PNB
Sacciolepis myuros (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 (Oct–Jan), late in the wet season (Feb–Apr) and during the dry season (May–Sept). Site of occurrence: PNB (National Park of Brasília) and FAL (Água Limpa farm).

The monitoring of the grass populations selected for this study allowed us to estimate the period of seed dispersal for each species. Thus, we classified the grass species into three groups according to their dispersal times: (1) species dispersing early in the wet season (Oct to Jan); (2) species dispersing late in the wet season (Feb to Apr); and (3) species dispersing in the dry season (May to Sept). Based on their external morphology, we categorized the seeds into one of two dispersal syndromes: anemochoric or barochoric (van der Pijl 1982). We considered those seeds with structures that facilitate dispersal by wind, such as winged bracts and/or presenting hairs in the bracts as anemochoric. Seeds without winged bracts or hairs were considered barochoric (Table 1).

Germination, viability and dormancy levels of freshly harvested and dry-stored seeds

To determine the level of dormancy and to test the effect of dry storage on the level of dormancy, viability and germination of seeds for each species, germination trials were conducted with both freshly collected seeds as well as with seeds dry-stored for 3, 6, 9 and 12 mo. The germination experiments were conducted in germination chambers set at an alternating temperature cycle of 28/18 °C day/night, under a photoperiod of 12 h 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 for up to 30 d, using radicle emergence as a criterion for seed germination. Five replicates of 20 seeds each were used for each species for each treatment, except *Paspalum maculosum*, *Agenium goyazense*, *Homolepis longispicula* and *Schizachyrium sanguineum*, where we used five replicates of ten seeds each 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. The non-germinated seeds

were placed in contact with tetrazolium solution for 24 h in the dark at 30 °C in a germination chamber. We considered the seeds whose embryos were stained dark pink or red as viable. The total viability of each seed sample was set as the number of germinated seeds during the experiments plus the positive results from the tetrazolium test. The results for viability were used to measure seed longevity across dry storage times.

The level of dormancy was interpreted as the difference between the estimated viability and the number of germinated seeds for each seed sample (see statistical analysis). As low germination can be the result of low seed viability of the seed sample and not dormancy *per se*, we verified whether seed viability varied between treatments (i.e. dispersal syndromes, habitat of seed collection and dry storage times; see Results and Tables S1–S5).

Statistical analysis

All analyses were done using the R 3.1.2 statistical platform (R Foundation for Statistical Computing, Vienna, AT). To analyse differences in soil water potential of wet grasslands and open savannas during the dry season months, we used GLMM, normal distribution (Zuur et al. 2009). As we intended to test for these differences at each month (Jun, Jul and Aug), we made a model for each month separately. We used the water potential as response variable, and included a two-way interaction with habitat (wet grassland or open savanna) and depth (2–3 or 10–11 cm) as fixed independent variables. We included the studied areas (FAL or PNB) and plots (three per site) nested as random factors in the models. As the water potential did not follow a normal distribution and ranged from negative to zero values, we log-transformed (+1) data. As it is not possible to obtain the log of negative values, we multiplied the data by −1 to change it to a positive value before performing the log transformation. We made post-hoc pair-wise comparisons between levels of the significant fixed factors: habitat and depth. We used the single step method for *P*-value adjustment and the *glht* command of multcomp package for the pair-wise comparisons (Hothorn et al. 2008).

We used GLMM binomial distribution (Zuur et al. 2009) to test whether the effect of dry storage (0, 3, 6, 9 and 12 mo) on seed viability and germination is dependent on seed dispersal time (early in the wet season, late in the wet season and in the dry season), seed dispersal syndrome (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 syndrome and dry storage treatment (0, 3, 6, 9 and 12 mo) 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 in the same environment, which can result in autocorrelation of errors (Sileshi 2012), we included the number of replicates (five per species) as 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 and used likelihood ratio tests (LRT) to test the significance of the fixed independent variables. We made post-hoc pair-wise comparisons between levels of the significant fixed factors: dry storage and habitat, dry storage and seed dispersal time and dry storage and seed dispersal syndrome. We used the single step method for *P*-value adjustment and the *glht* command of multcomp package for the pair-wise comparisons (Hothorn et al. 2008).

Results

Habitat seasonality and germination

The soil water potential at each depth differed between habitat types during the dry season months of Jun, Jul and Aug, with the exception of 10–11 cm in Jun and Jul (Fig. 2, 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 (2–3 cm) was higher in wet grasslands than in open savannas, ranging from −0.5 MPa (Jun) to −1.2 MPa (Aug) and from −3.2 MPa (Jun) to −5 MPa (Aug), respectively (Fig. 2, Table 2).
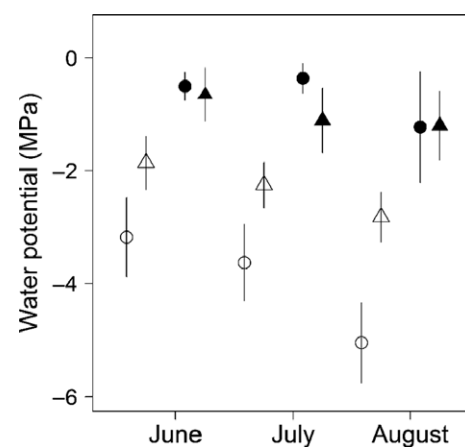

Fig. 2. Soil water potential (MPa) of wet grasslands and open savannas during the dry season. Open symbols = open savannas; Closed symbols = wet grasslands; Circles = 2–3-cm depth; Triangles = 10–11-cm depth. Mean + 95% CI.

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

Month	LRT_1	P	Habitat	Depth (cm)	$\beta \pm SE$	P
Jun	17.562	<0.001	OS − WG = 0	2–3	0.6346 ± 0.2192	<0.05
			OS − WG = 0	10–11	0.4418 ± 0.2189	0.05
Jul	21.405	<0.001	OS − WG = 0	2–3	0.6161 ± 0.2334	<0.05
			OS − WG = 0	10–11	0.4293 ± 0.2328	0.08
Aug	39.049	<0.001	OS − WG = 0	2–3	0.9121 ± 0.2199	<0.05
			OS − WG = 0	10–11	0.6127 ± 0.2194	<0.05

OS, Open Savannas; WG, Wet Grasslands.

The germination of grass seeds during dry storage was influenced by habitat of seed collection (likelihood ratio test, $LRT_4 = 27.583$, $P < 0.001$; Fig. 3). The germination among species from open savannas increased after 6 mo of dry storage relative to freshly collected seeds and stayed constant until 12 mo of dry storage (Table 3, Fig. 3). On the other hand, germination increased at a higher rate for species from wet grasslands after 3 mo of dry storage relative to freshly collected seeds, and also stayed constant until 12 mo of dry storage (Table 3, Fig. 3). Similar to seed germination, seed viability response over storage time was also influenced by species habitat ($LRT_4 = 10.323$, $P < 0.05$). In open savannas the seed viability was lower after 9 ($\beta = -0.57$, $P < 0.05$) and 12 ($\beta = -0.75$, $P < 0.05$) months of dry storage in comparison to freshly collected seeds, while in wet grasslands the seed viability did not differ during storage time (Tables 3 and S1).

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 wet season, and in the dry season had a higher probability of germination than seeds dispersed late in the wet season (Table 3, Fig. 4). After 6 mo of dry storage the probability of germination was no longer influenced by the dispersal time of the seeds (Fig. 4). After 6 mo of dry storage, early-dispersed seeds had lower viability than late-dispersed seeds in the wet season ($\beta = -0.9635$, $P < 0.05$; Table S4). After 12 mo of dry storage the viability of early-dispersed seeds was lower than late-dispersed seeds ($\beta = -0.9258$, $P < 0.05$; Table S4) and of seeds dispersed in the dry season ($\beta = -2.2161$, $P < 0.001$; Table S4).

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 seeds for freshly collected seeds and also after 3 mo of dry storage (Table 3, Fig. 5). After 6 mo of dry storage the probability of germination was no longer influenced by the seed dispersal
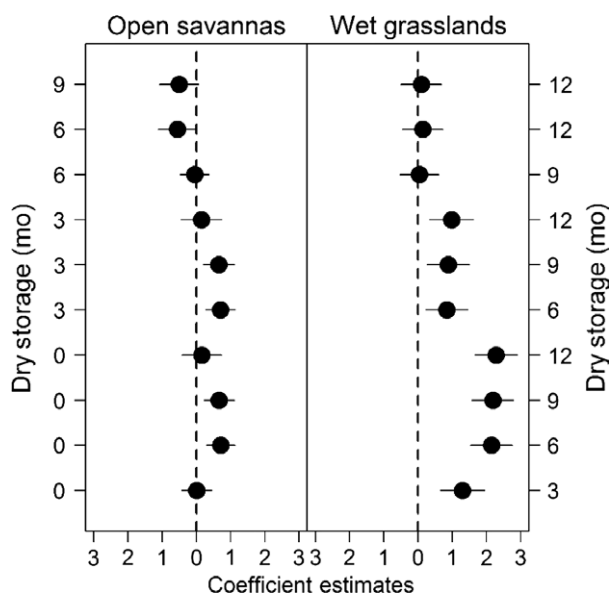

Fig. 3. Coefficient estimates from pair-wise multiple comparisons of germination probability between months of dry storage in grass species from open savannas and wet grasslands. Closed circles denote mean values, error bars denote 95% of lower and upper CI. The central dotted line (zero value) means no statistical difference.

syndrome (Fig. 5). The viability of barochoric and anemochoric seeds did not differ (Table S2), except at 6 mo of dry storage, when barochoric seeds showed lower viability than anemochoric seeds (Table S2).

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 initially low, increasing after 6 mo of storage, but then followed by seed mortality after 9 mo of storage. These results indicate that germination in seeds of species from open savannas may be delayed due to dormancy, at most until the onset of the next wet season. On the other hand, seeds of species from wet grasslands were longer-lived than seeds from open savannas, remaining viable

Table 3. Mean percentage level of dormancy (D), seed germination (G) and viability (V) of grass species from *Cerrado* according to dispersal time, seed dispersal syndrome and habitat of seed collection over 12 mo of dry storage.

	Dry Storage (Months)														
	0			3			6			9			12		
	D	G	V	D	G	V	D	G	V	D	G	V	D	G	V
Dispersal Time															
Early in Wet Season	17	60	74	11	58	70	10	60	65	9	68	76	27	44	50
Late in Wet Season	90	8	70	71	18	69	71	24	64	52	29	58	53	26	52
Dry Season	55	34	73	32	44	81	49	54	77	36	38	67	24	59	81
Habitat															
Open Savanna	58	30	72	39	40	70	43	39	63	34	40	63	48	27	47
Wet Grassland	39	45	72	40	48	78	23	61	76	23	62	76	19	55	64
Seed Dispersal															
Anemochoric Seeds	15	60	71	5	63	67	11	61	68	4	66	67	22	44	44
Barochoric Seeds	74	21	73	64	29	77	52	37	68	47	36	68	47	34	61

Level of dormancy (D) = number of non-germinated viable seeds over total viable seeds.
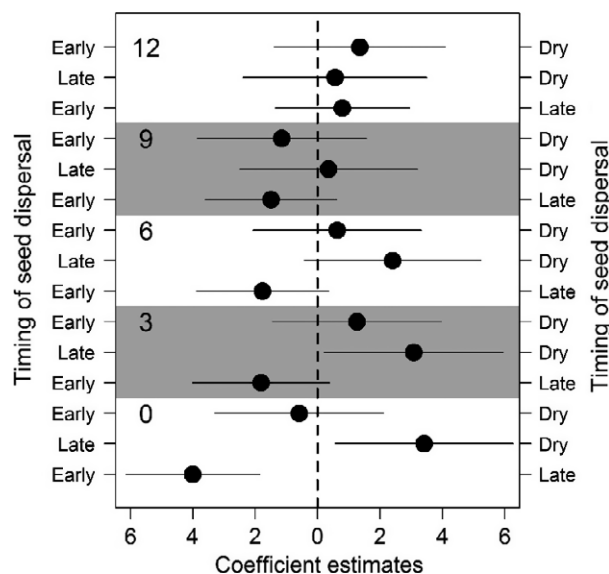

Fig. 4. Coefficient estimates from pair-wise multiple comparisons of germination probability between grass seeds dispersed early, late in the wet season and in the dry season during dry storage (0, 3, 6, 9 and 12 mo). Closed circles denote mean values, error bars denote 95% of lower and upper CI. Central dotted line (zero value) is no statistical difference.

after 1 yr of dry storage. Additionally, germination levels increased and remained consistently high after 3 mo of dry storage.

In wet grasslands, the soil remained wet during the dry season in comparison to open savannas, with values around −1 MPa at 2–3-cm depth (Fig. 2). Water potential of around −1 MPa is not limiting for the germination of grass species (Qi & 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 germination of grass species from wet grasslands suggest that germination might occur even during the dry season.

Although rainfall 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, other factors, such as the pressure of established vegetation, have 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 above-ground 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, can result in an opportunistic strategy that takes advantage of gaps in grass species from wet grasslands, for example after a fire event, to allow growth and establishment in a less competitive environment.

Seed dormancy at the end of the wet 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 wet season had lower probability of germination than seeds dispersed either early in the wet 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; McIvor & Howden 2000; Scott et al. 2010; Salazar et al. 2011), and
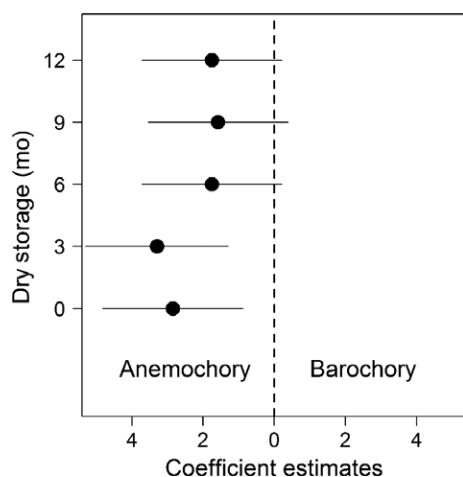
Fig. 5. Coefficient estimates from pair-wise multiple comparisons of germination probability between grass species with anemochory and barochory dispersal syndromes during dry storage (0, 3, 6, 9 and 12 mo). Closed circles denote mean values, error bars denote 95% of lower and upper CI. Central dotted line (zero value) means no statistical difference.

Melastomataceae species from high grasslands in other parts of Brazil (Silveira et al. 2012). Seeds dispersed at the beginning of the wet season are expected to have about 7 mo of relatively stable water availability to enable germination and establishment. On the other hand, seed germination at the end of the wet season would be very risky, since seedlings would not have time enough to grow and acquire a minimum size that enables them to tolerate the harsh conditions expected during the dry season. Thus, the presence of seed dormancy here might prevent germination during periods when there is a low probability of recruitment, and hold back emergence until the onset of the next wet season.

Contrary to our expectations, the germination of seeds dispersed in the dry season was not different from seeds dispersed early in the wet season. Seeds dispersed during the dry season had low levels of seed dormancy. Since sporadic rains are expected to occur in the dry season (Fig. 1), we did not expect to find high germination in seeds dispersed during this time. However, the amount of rain is extremely low (Fig. 1) and may potentially be insufficient to induce germination. Additionally, the following wet season occurs relatively soon after dispersal 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 a significantly higher degree and consequently had lower levels of dormancy than barochoric seeds. As far as we know, this is the first study to demonstrate a trade-off between seed dispersal and dormancy across species from the same family (*Poaceae*). Moreover, most theoretical and empirical studies investigating the evolution of seed dispersal and seed dormancy have been made for plants from unpredictable environments, mostly in deserts (Ellner & Shmida 1981; Volis & Bohrer 2013). Our between-species comparison in a seasonal rainfall ecological system provides empirical results showing that barochoric grass seeds are significantly more dormant than anemochoric seeds.

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 & Brown 1988); the former by spreading risk over space (Bulmer 1984), and the latter by spreading risk over time (Venable & Brown 1988). However, even in the absence of patch heterogeneity, seed dispersal can be favoured by kin selection (Venable & Brown 1988). Additionally, in environments without inter-annual variation in precipitation, seed dormancy can be advantageous where sibling competition is high (Volis & Bohrer 2013). Although water availability affects seedling establishment in savannas (Medina & Silva 1990; 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 found in our study species.

Conspecific competitor densities during seedling growth negatively influence plant fitness (Orrock & Christopher 2010). By dispersing long distances and not being dormant, grass seeds with an anemochoric dispersal syndrome 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 (Ellner 1986; Venable & Brown 1988; Cohen & Levin 1991). Moreover, fire is a frequent disturbance in Brazilian open savannas and wet grasslands, and fire occurrence can open up gaps in the herbaceous vegetation. Thus, these anemochoric species may be favoured 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 their own siblings. Because they disperse throughout the wet 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, higher 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 favour secondary dispersal, including *Echinolaena inflexa* and *Icnanthus camporum*, which both have elaiosomes that are known to attract ants (Giladi 2006). 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 germination of grasses at the beginning of the wet season in seasonal environments. The co-evolution of both strategies – seed dormancy and the timing of seed dispersal –contributes to enabling seeds to avoid germination during periods with low chances of successful recruitment, such as at the end of the wet season. We show, using 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 the evolution of seed dormancy in grass species. Furthermore, we suggest that competition can have an important influence in selecting for the timing of germination of seeds from seasonal and predictable environments.

Acknowledgements

References

Andrade, L.A.Z. & Miranda, H.H.S. 2014. The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. *Plant Ecology* 215: 1199–1209.

Baskin, C.C. & Baskin, J.M. 1998. Ecology of seed dormancy and germination in grasses. In: Cheplick, G.P. (ed.) *Population biology of grasses*. Cambridge University Press, Cambridge, UK.

Baskin, C.C. & Baskin, J.M. 2014. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. Academic Press, New York, NY, US.

Bulmer, M.G. 1984. Delayed germination of seeds: Cohen's model revisited. *Theoretical Population Biology* 26: 367–377.

Cianciaruso, M.V. & Batalha, M.A. 2008. A year in a Cerrado wet grassland: a non-seasonal island in a seasonal savanna environment. *Brazilian Journal of Biology* 68: 495–501.

Cohen, D. & Levin, S.A. 1991. Dispersal in patchy environments: the effects of temporal and spatial structure. *Theoretical Population Biology* 39: 63–99.

Ellner, S. 1986. Germination dimorphisms and parent–offspring conflict in seed germination. *Journal of Theoretical Biology* 123: 173–185.

Ellner, S. & Shmida, A. 1981. Why are adaptations for long-range seed dispersal rare in desert plants? *Oecologia* 51: 133–144.

Ernst, W.H.O., Veenendaal, E.M. & Kebakile, M.M. 1992. Possibilities for dispersal in annual and perennial grasses in a Savanna in Botswana. *Vegetatio* 102: 1–11.

Fidelis, A., Lyra, M.F.S. & Pivello, V.R. 2013. Above- and below-ground biomass and carbon dynamics in Brazilian Cerrado wet grasslands. *Journal of Vegetation Science* 24: 356–364.

Filgueiras, T.S., Longhi-Wagner, H.M., Viana, P.L., Zanin, A., Oliveira, R.C., Canto-Dorow, T.S., Shirasuna, R.T., Oliveira, R.P., Rodrigues, R.S., (...) & Valls, J.F.M. 2015. *Poaceae in Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro, BR.

Giladi, I. 2006. Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. *Oikos* 112: 481–492.

Higgins, S.I., Bond, W.J. & Trollope, W.S. 2000. Fire, resprouting and variability: a recipe for grass–tree coexistence in savanna. *Journal of Ecology* 88: 213–229.

Hothorn, T., Bretz, F. & Westfall, P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50: 346–363.

Jurado, E. & Flores, J. 2005. Is seed dormancy under environmental control or bound to plant traits? *Journal of Vegetation Science* 16: 559–564.

McIvor, J.G. & Howden, S.M. 2000. Dormancy and germination characteristics of herbaceous species in the seasonally dry tropics of northern Australia. *Austral Ecology* 25: 213–222.

Medina, E. & Silva, J.F. 1990. Savannas of northern South America: a steady state regulated by water–fire interactions on a background of low nutrient availability. *Journal of Biogeography* 17: 403–413.

Mott, J.J. 1978. Dormancy and germination in five native grass species from savannah woodland communities of the northern territory. *Australian Journal of Botany* 26: 621–631.

Munhoz, C.B.R. & Felfili, J.M. 2007. Reproductive phenology of an herbaceous–subshrub layer of a savannah (Campo Sujo)

in the Cerrado Biosphere Reserve I, Brazil. *Brazilian Journal of Biology* 67: 299–308.

Oliveira-Filho, A.T. & Ratter, J.A. 2002. Vegetation physiognomies and woody flora of the Cerrado Biome. In: Oliveira, P.S. & Marquis, R.J. (eds.) *The cerrados of Brazil: ecology and natural history of a Neotropical savanna*, pp. 121–140. Columbia University Press, New York, NY, US.

Orrock, J.L. & Christopher, C.C. 2010. Density of intraspecific competitors determines the occurrence and benefits of accelerated germination. *American Journal of Botany* 97: 694–699.

Peterson, P.M., Soreng, R.J., Davidse, G., Filgueiras, T.S., Zuloaga, F.O. & Judziewicz, E.J. 2001. Catalogue of New World Grasses (*Poaceae*): II. Subfamily Chloridoideae. *Contributions from the United States National Herbarium* 41: 1–255.

Qi, M.Q. & Redmann, R.E. 1993. Seed germination and seedling survival of C_3 and C_4 grasses under water stress. *Journal of Arid Environments* 24: 277–285.

Ramos, D.M., Diniz, P. & Valls, J.F.M. 2014. Habitat filtering and interspecific competition influence phenological diversity in an assemblage of Neotropical savanna grasses. *Brazilian Journal of Botany* 37: 29–36.

Rees, M. 1993. Trade-offs among dispersal strategies in British plants. *Nature* 366: 150–152.

Rossatto, D.R., Silva, L.C.R., Villalobos-Veja, R., Stenberg, L.S.L. & Franco, A.C. 2012. Depth of water uptake in woody plants relates to groundwater level and vegetation structure along a topographic gradient in a Neotropical savanna. *Environmental and Experimental Botany* 77: 259–266.

Saatkamp, A., Poschlod, P. & Venable, D.L. 2014. The functional role of soil seed banks in natural communities. In: Gallagher, R.S. (ed.) *Seeds: the ecology of regeneration in plant communities*, pp. 263–295. CABI, Wallingford, UK.

Salazar, A., Goldstein, G., Franco, A.C. & Miralles-Wilhem, F. 2011. Timing of seed dispersal and dormancy, rather than persistent soil seed-bank, control seedling recruitment of woody plants in Neotropical savannas. *Seed Science Research* 21: 103–116.

Satterthwaite, W.H. 2010. Competition for space can drive the evolution of dormancy in a temporally invariant environment. *Plant Ecology* 208: 167–185.

Scholes, R.J. & Archer, S.R. 1997. Tree–grass interactions in savannas. *Annual Review of Ecology and Systematics* 28: 517–544.

Scott, K., Setterfield, S., Douglas, M. & Andersen, A. 2010. Soil seed banks confer resilience to savanna grass-layer plants during seasonal disturbance. *Acta Oecologica* 36: 202–210.

Sileshi, G.W. 2012. A critique of current trends in the statistical analysis of seed germination and viability data. *Seed Science Research* 22: 145–159.

Silveira, F.A.O., Ribeiro, R.C., Oliveira, D.M.T., Fernandes, G.W. & Lemos-Filho, J.P. 2012. Evolution of physiological dormancy multiple times in Melastomataceae from Neotropical montane vegetation. *Seed Science Research* 22: 37–44.

Snyder, R.E. 2006. Multiple risk reduction mechanisms: can dormancy substitute for dispersal? *Ecology Letters* 9: 1106–1114.

van der Pijl, L. 1982. *Principles of dispersal in higher plants*, 3rd edn. Springer, New York, NY, US.

Veenendaal, E.M., Ernst, W.H.O. & Modise, G.S. 1996. Effect of seasonal rainfall pattern on seedling emergence and establishment of grasses in a savanna in south-eastern Botswana. *Journal of Arid Environments* 32: 305–317.

Venable, D.L. & Brown, J.S. 1988. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *The American Society of Naturalists* 131: 360–384.

Venable, D.L. & Lawlor, L. 1980. Delayed germination and dispersal in desert annuals: escape in space and time. *Oecologia* 46: 272–282.

Volis, S. & Bohrer, G. 2013. Joint evolution of seed traits along an aridity gradient: seed size and dormancy are not two substitutable evolutionary traits in temporally heterogeneous environment. *New Phytologist* 197: 655–667.

Zimmermann, J., Higgins, S.I., Grimm, V., Hoffmann, J., Münkemüller, T. & Linstädter, A. 2008. Recruitment filters in a perennial grassland: the interactive roles of fire, competitors, moisture and seed availability. *Journal of Ecology* 96: 1033–1044.

Zuloaga, F.O., Morrone, O., Davidse, G., Filgueiras, T.S., Peterson, P.M., Soreng, R.J. & Judziewicz, E.J. 2003. Catalogue of New World Grasses (*Poaceae*): III. Subfamilies Panicoideae, Aristidoideae, Arundinoideae, and Danthonioideae. *Contributions from the United States National Herbarium* 46: 1–662.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. 2009. *Mixed effects models and extensions in ecology with R*. Springer, New York, NY, US.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. *Post-hoc* comparisons of probability of seed viability between dry storage months according to habitat type.

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

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

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

Table S5. Percentage dormancy (D), seed germination (G) and viability (V) of grass species from *Cerrado* according to dispersal time, seed dispersal syndrome and habitat of seed collection over 12 mo of dry storage.