

1 **Avoiding the dry season: dispersal time and syndrome mediate seed dormancy in**

2 **Neotropical savanna grasses**

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16 **Abstract**

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

30 **Location:** Open savannas and wet grasslands in Central Brazil.

31 **Methods:** We collected seeds of 29 grass species and tested viability and dormancy  
32 using germination trials of fresh seeds, which was then repeated after dry storage of 3,  
33 6, 9 and 12 months. Generalized Linear Mixed Models were used to test whether the  
34 degree of dormancy was dependent **on habitat type, seed dispersal time and seed**  
35 **dispersal syndrome.**

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

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

47 **Keywords:** *Cerrado*; Dry storage; Germination; Open savanna; Poaceae; Wet  
48 grassland.

## 49 **Introduction**

50 Savannas are biomes composed of a continuous grass layer and scattered trees,  
51 under a seasonal climate consisting of an annual cycle of wet and dry seasons (Scholes  
52 & Archer 1997). The *Cerrado* is a Neotropical biome in Central Brazil dominated by  
53 savannas, but also containing grasslands and forests. Soil moisture is one of the main  
54 determinants of vegetation physiognomies; for example, rainforests are associated with  
55 **shaded valleys** and riparian areas while wet grasslands occur in more open habitats  
56 where the water table reaches close to the surface (Cianciaruso & Batalha 2008). In both  
57 of these examples, soils are moist throughout the year. However, in savanna  
58 **physiognomies** the water table sits deeper below the soil surface (Rossatto et al. 2012),  
59 leading to soils and the herbaceous layer drying out during the annual dry season. Grass  
60 species within the *Cerrado* biome are mostly restricted to **these** open physiognomies of  
61 savannas and grasslands due to their shade intolerance.

62 In savannas, rainfall seasonality is among the major limiting factors determining  
63 seed germination and seedling establishment, due to its direct effects on water  
64 availability in the soil (Jurado & Flores 2005; Baskin & Baskin 2014). Rainfall  
65 seasonality is therefore likely to be a strong selective pressure acting on seed dormancy,  
66 and indeed seed dormancy has been found in a higher number of species in many  
67 seasonal rainfall environments in comparison to less seasonal environments (Jurado &  
68 Flores 2005; Baskin & Baskin 2014). Grasses are one of the most highly represented  
69 families (Poaceae) in the herbaceous layer of the *Cerrado* (around 600 species,  
70 Filgueiras et al. 2014), with species from wet grasslands and open savannas occurring  
71 under the same seasonal macro-climate. However, in wet grasslands the water table is  
72 situated near the surface throughout the year (Cianciaruso & Batalha 2008) and hence  
73 the impact of rainfall seasonality in the soil is buffered by the water table and the soil

74 surface stays moist for longer during the dry season (Cianciaruso & Batalha 2008).  
75 Subsequently, if seed dormancy is a strategy of species from seasonal environments to  
76 deal with water shortage, this selective pressure would be weaker in wet grasslands.  
77 Thus, we would expect a deeper seed dormancy among seeds of species occurring in  
78 open savannas in comparison to seeds of species occurring in wet grasslands.

79 Grass flowering and fruiting follow a seasonal pattern restricted to the wet  
80 season in open savannas and wet grasslands (Munhoz & Felfili 2007; Ramos et al.  
81 2014), presumably due to resources availability (i.e. water) to allow reproduction.  
82 However, there is large variation in the timing of seed dispersal among grass species  
83 (Munhoz & Felfili 2007; Ramos et al. 2014), with some species dispersing early in the  
84 wet season, others dispersing later, and some species dispersing seeds even during the  
85 dry season. Seeds dispersed early in the wet season would have the entire growing  
86 season to germinate and recruit, whereas seeds dispersed late in the wet season, or  
87 during the dry season (when low amounts of rain usually occurs in Brazilian savannas),  
88 would very probably face extreme water shortages and low relative air humidity, which  
89 potentially could be a risky strategy for seedling survival. Avoiding germination prior to  
90 and during the dry season would be a key strategy for reducing the probability of  
91 seedling mortality during the dry season, and seed dormancy would therefore play an  
92 important role in delaying seed germination to the next wet season. Consequently, we  
93 would expect deeper seed dormancy among seeds dispersed late in the wet season, and  
94 during the dry season, in comparison to seeds dispersed early in the wet season.

95 **Moreover, to synchronize germination in the next wet season, we would expect dormant**  
96 **seeds to maintain viability and overcome dormancy through dry storage.**

97 While the season of seed release may be an important driver for variation in  
98 dormancy, dispersal also plays a critical role determining the level of competition faced

99 by emerging seedlings. In broad terms, seed dormancy can reduce extinction risk by  
100 spreading germination over time (Bulmer 1984), while seed dispersal can reduce risk by  
101 spreading seeds and, consequently, germination over space (Venable & Brown 1988).  
102 Parent-offspring (Ellner 1986) and sibling competition can significantly reduce plant  
103 fitness (Satterthwaite 2010; Baskin & Baskin 2014; Saatkamp et al. 2014). Both seed  
104 dormancy and dispersal are costly strategies, thus a trade-off between seed dormancy  
105 and dispersal is often reported between these two bet-hedging traits (Venable and  
106 Brown 1988). Theoretical studies in particular have reported that mean dispersal  
107 distance decreases with increasing dormancy (e.g. Venable & Lawlor 1980; Cohen &  
108 Levin 1991), although this relationship is not always supported where positive temporal  
109 correlations in environment exist (Snyder 2006). Nevertheless, few empirical  
110 assessments of the trade-off between dormancy and dispersal have been made (Rees  
111 1993).

112         The diaspore morphology of savanna grass species is highly variable. Among  
113 several dispersal syndromes, seeds can be characterized by structures which facilitate  
114 wind dispersal (anemochory), such as winged bracts, hairy bracts and hairy rachis.  
115 These structures can be completely absent and dispersal is therefore likely to occur  
116 through gravity (barochory) (Ernst et al. 1992). Barochoric seeds fall near the mother-  
117 plant, while anemochoric seeds have the opportunity to disperse far from the mother-  
118 plant. Anemochory could therefore be favoured to deal with spatial unpredictability and  
119 competition. Hence, we would expect to find a deeper seed dormancy among barochoric  
120 seeds in comparison to anemochoric seeds if there was support for a dispersal-dormancy  
121 trade-off.

122         Seeds of grass species can be non-dormant or physiologically dormant (Baskin  
123 & Baskin 1998), but the occurrence and mechanisms of seed dormancy in grass species

124 from Brazilian ecosystems are poorly understood. Furthermore, investigating the effects  
125 of selective pressures resulting from abiotic factors, or how dormancy is related to  
126 dispersal, is important for understanding the ecology and evolution of plant traits. The  
127 seasonal macro-climate of the Brazilian savanna, the micro-climatic differences in open  
128 savannas and wet grasslands habitats as a consequence of variations of the water table,  
129 and the high variability in seed dormancy and dispersal syndromes of savanna grasses  
130 make the *Cerrado* an ideal ecological system to investigate germination strategies.  
131 Thus, our objectives were to investigate the germination, viability (*i.e.* longevity) and  
132 dormancy levels of both freshly collected and dry-stored seeds: (1) from species living  
133 in habitats with contrasting soil moisture during the dry season (open savannas *versus*  
134 wet grasslands); (2) dispersed at different times (early in the wet season, late in the wet  
135 season and in the dry season) and (3) displaying opposing dispersal syndromes  
136 (barochoric *versus* anemochoric).

## 137 **Methods**

### 138 Study area

139 The *Cerrado* is the largest Neotropical savanna and covers around 2 million  
140 square kilometers in Central Brazil. The climate of the region is seasonal with two well  
141 defined seasons: a wet season from October to March (mean annual precipitation from  
142 800 to 2000 mm; Oliveira-Filho & Ratter 2002) and a dry season from April to  
143 September (Fig. 1). The study was conducted in two protected areas of Brasília:  
144 Fazenda Água Limpa at the University of Brasília (FAL - 15°58'43.06"S and  
145 47°56'21.41"W, 1.197 m above sea level) and the National Park of Brasília (PNB -  
146 15°38'46.22"S and 48°00'19.75"W, 1.178 m above sea level). FAL and PNB are  
147 composed of patches of savanna, forest and grassland. This study was conducted in

148 open savannas and wet grasslands physiognomies. We selected two wet grassland sites  
149 in each area, four open savanna sites in PNB and two open savanna sites in FAL.  
150 Vegetation in open savannas and wet grasslands are composed mainly of grasses and  
151 forbs, with a few scattered trees occasionally visible in open savannas. Soils in open  
152 savannas are well drained, poor in nutrients and rich in aluminum. On the other hand,  
153 soils in wet grasslands are rich in organic matter and the water table sits near the surface  
154 (about 20–80 cm) (Cianciaruso & Batalha 2008), making the soil moist for most of the  
155 year (Cianciaruso & Batalha 2008; Fidelis et al. 2013).

156 To characterize the soil moisture of the studied areas (FAL and PNB) we  
157 measured the water potential (in Megapascals – MPa/ $\Psi$ ) of six open savanna sites and  
158 four wet grassland sites every 30 days during the dry season, from June to August in  
159 2015. Soil samples were collected during the dry season to verify whether soils of wet  
160 grasslands stayed wet longer than the soils of open savannas after the end of the wet  
161 season. We collected soil samples in three plots previously set in each area equidistant  
162 (50m) from each other in a triangle arrangement, totaling 18 plots in open savannas and  
163 12 plots in wet grasslands. The plots were divided into three subsamples, one for each  
164 month of collection. In each subsample soil samples were collected at two depths:  
165 between 2 to 3 centimeters and between 10 to 11 centimeters. These depths were  
166 selected because 1) most seeds in the soil seed banks in Brazilian savannas were shown  
167 to occur in the first few centimeters below the surface (Andrade & Miranda 2014), and  
168 2) root biomass of grasses was shown to occur mostly between 0 to 20cm depth in  
169 Brazilian grasslands (Fidelis et al. 2013), so a depth between 2 to 11cm is well within  
170 the *perfil-position* that roots of grass seedlings would grow during the initial  
171 development stages of germination. The soil samples were placed in hermetically sealed  
172 sample cups and stored in a coolbox. The water potential was measured in the



173 laboratory with a WP4C water potential meter (Decagon Devices, Pullman-USA, 2015),  
174 within two days of sampling.

#### 175 Seed collection

176 The dispersal units in grasses are generally complex structures composed of a  
177 caryopsis (fruit), a caryopsis with bracts (lemma and palea) or occasionally a caryopsis  
178 with inflorescence structures attached (hereafter called seeds). Seeds of 28 native  
179 perennial grass species and one annual *Digitaria lehmanniana* (Table 1) from open  
180 savannas and wet grasslands habitats were collected in 2012 and 2013, in FAL and  
181 PNB. Plants were monitored regularly to ensure that seeds were mature at dispersal and  
182 then collected by hand. After collection the seeds were stored in paper bags at room  
183 temperature (27 °C -maxima and 17 °C -minima), measured throughout dry storage with  
184 a thermometer. The average relative air humidity in the region (Distrito Federal) was  
185 43-80% during the period of study (data from BDMEP/INMET). For accurate  
186 identification, we collected vouchers of the studied grass species, which were deposited  
187 in the Embrapa Genetic Resources and Biotechnology (CEN) Herbarium, headquartered  
188 in Brasilia.

189 The monitoring of the grass populations selected for this study allowed us to  
190 estimate the period of seed dispersal for each species. Thus, we classified the grass  
191 species into three groups according to their dispersal times: a) species dispersing early  
192 in the wet season (October to January); b) species dispersing late in the wet season  
193 (February to April) and c) species dispersing in the dry season (May to September).  
194 Based on their external morphology, we categorized the seeds into one of two dispersal  
195 syndromes: anemochoric or barochoric (van der Pijl 1982). We considered those seeds  
196 with structures that facilitate dispersal by wind, such as winged bracts and/or presenting

197 hairs in the bracts as anemochoric. Seeds without winged bracts or hairs were  
198 considered barochoric (Table 1).

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

200 To determine the level of dormancy and to test the effect of dry storage on the  
201 **level** of dormancy, viability and germination of seeds for each species, germination  
202 trials were conducted with both freshly collected seeds as well as with seeds **dry**-stored  
203 for three, six, nine and twelve months. The germination experiments were conducted in  
204 germination chambers regulated **set at an alternating temperature cycle of 28/18°C**  
205 day/night, under a photoperiod of 12h of white light. These temperatures were set  
206 according to the average minimum and maximum temperatures recorded during the wet  
207 season (Fig. 1), which represents the growing season for most savanna species in the  
208 *Cerrado*, including grasses. Seeds were placed in petri dishes lined with two sheets of  
209 filter paper and moistened with distilled water. The germination was recorded daily for  
210 up to 30 days, using radicle emergence as a criterion for seed germination. Five  
211 replicates of 20 seeds **each** were used for each species for each treatment, except for *P.*  
212 *maculosum*, *A. goyazense*, *H. longispicula* and *S. sanguineum*, where we used five  
213 replicates of 10 seeds **each** per treatment due to the limited quantity of seeds. After each  
214 germination trial, the viability of the **ungerminated** seeds was tested using 1%  
215 tetrazolium chloride solution. **The ungerminated** seeds were placed in contact with  
216 tetrazolium solution for 24 hours in the dark at 30 °C in a germination chamber. We  
217 considered the seeds **whose** embryos were stained dark pink or red as viable. The total  
218 viability of each seed sample was set as the number of germinated seeds during the  
219 experiments plus the positive results from the tetrazolium test. **The results for the**  
220 **viability were used to measure seed longevity across dry storage times.**

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

#### 227 Statistical analysis

228           All analyses were done using the R 3.1.2 statistical platform (R Core Team  
229 2014). To analyse the differences in soil water potential of wet grasslands and open  
230 savannas during the dry season months we used Generalized Linear Mixed Models  
231 (GLMM, normal distribution, see Zuur et al. 2009). **As we intended to test for these**  
232 **differences at each month (June, July and August), we made a model for each month**  
233 **separately.** We used the water potential as response variable, and included a two-way  
234 interaction with **habitat** (wet grassland or open savanna) and depth (2-3 or 10-11cm) **as**  
235 **fixed independent variables.** We included the studied areas (FAL or PNB) and plots (3  
236 *per site*) nested as random factors in the models. As the water potential did not follow a  
237 normal distribution and ranged from negative to zero values, **we log transformed (+1)**  
238 **data.** As it is not possible to obtain the log of negative values, we multiplied the data **by**  
239 **-1** to change it to a positive value before performing the log transformation. We made  
240 post-hoc pairwise comparisons between levels of the significant fixed factors: **habitat**  
241 and depth. We used the single step method for P-values adjustment and the `glht`  
242 command of `multcomp` package for the pairwise comparisons (Hothorn et al. 2008).

243           We used Generalized Linear Mixed Models (GLMM, binomial distribution, see  
244 Zuur et al. 2009) (R package: `lme4`; see Bates et al. 2014) to test whether the effect of  
245 **dry storage (zero, three, six, nine and twelve months)** on seed viability and germination

246 is dependent on seed dispersal time (early in the wet season, late in the wet season and  
247 in the dry season), seed dispersal syndrome (anemochoric or barochoric) and habitat of  
248 seed collection (open savannas or wet grasslands). We analysed seed viability and  
249 germination (presence/absence) as the response variables, separately. In this analysis,  
250 we used each seed as an experimental unit. We included a two-way interaction term  
251 between habitat, seed dispersal time and seed dispersal syndrome and dry storage  
252 treatment (zero, three, six, nine and twelve months), as fixed independent variables. We  
253 included genus, species and replication as nested random factors in all models. As the  
254 seeds placed inside the petri dishes are under the same environment, which can result in  
255 autocorrelation of errors (Sileshi 2012), we included the number of replicates (5 per  
256 species) at the random component of the model in order to control for autocorrelation of  
257 errors. As all interactions were significant, it was not necessary to perform a model  
258 selection, so we made only a single step with the full model and we used likelihood ratio  
259 tests (LRT) to test the significance of the fixed independent variables. We made post-  
260 hoc pairwise comparisons between levels of the significant fixed factors: dry storage  
261 and habitat, dry storage and seed dispersal time and dry storage and seed dispersal  
262 syndrome. We used the single step method to P-values adjustment and the glht  
263 command of multcomp package for the pairwise comparisons (Hothorn et al. 2008).

## 264 **Results**

### 265 Habitat seasonality and germination

266 The soil water potential at each depth differed between habitat types during the  
267 dry season months of June, July and August, with the exception of 10 - 11cm in June  
268 and July (Fig. 2; Table 2). In wet grasslands, the soil water potential was higher than in  
269 open savannas for all dry season months (Fig. 2). The mean soil water potential at

270 shallower depths (2 - 3cm) was higher in wet grasslands than in open savannas, ranging  
271 from -0.5 MPa (June) to -1.2 MPa (August) and from -3.2 MPa (June) to -5 MPa  
272 (August), respectively (Fig. 2; Table 2).

273 The germination of grass seeds during dry storage was influenced by habitat of  
274 seed collection (Likelihood-Ratio Test,  $LRT_4 = 27.583$ ,  $P < 0.001$ ; Fig. 3). The  
275 germination among species from open savannas increased after six months of dry  
276 storage relative to freshly collected seeds and stayed constant until twelve months of dry  
277 storage (Table 3; Fig. 3). On the other hand, the germination increased at a greater rate  
278 for species from wet grasslands after three months of dry storage relative to freshly  
279 collected seeds, and also stayed constant until twelve months of dry storage (Table 3;  
280 Fig. 3). Similar to seed germination, seed viability response over storage time was also  
281 influenced by species habitat ( $LRT_4 = 10.323$ ,  $P < 0.05$ ). In open savannas the seed  
282 viability was lower after nine ( $\beta = -0.57$ ,  $P < 0.05$ ), and twelve ( $\beta = -0.75$ ,  $P < 0.05$ )  
283 months of dry storage in comparison to freshly collected seeds, while in wet grasslands  
284 the seed viability did not differ during storage time (Table 3; Table S1 - supplementary  
285 data).

286 Dormancy level comparison against dispersal time and dispersal syndrome

287 The germination of grass seeds during dry storage was influenced by the seed  
288 dispersal time ( $LRT_8 = 110.738$ ,  $P < 0.001$ ; Fig. 4). Freshly collected seeds dispersed  
289 early in the wet season and in the dry season had a higher probability of germination  
290 than seeds dispersed late in the wet season (Table 3; Fig. 4). After six months of dry  
291 storage the probability of germination was no longer influenced by the dispersal time of  
292 the seeds (Fig. 4). After six months of dry storage, early-dispersed seeds had lower  
293 viability than late dispersed seeds in the wet season ( $\beta = -0.9635$ ,  $P < 0.05$ ; Table S4 -  
294 supplementary data). After twelve months of dry storage the viability of early-dispersed

295 seeds was lower than late-dispersed seeds ( $\beta = -0.9258$ ,  $P < 0.05$ ; Table S4 -  
296 supplementary data) and of seeds dispersed in the dry season ( $\beta = -2.2161$ ,  $P < 0.001$ ;  
297 Table S4 - supplementary data).

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

## 306 Discussion

307 There were contrasting effects of dry storage on the germination and viability of  
308 species from open savannas and wet grasslands. In open savannas the germination of  
309 fresh seeds was initially low, increasing after six months of storage, but then followed  
310 by seed mortality after nine months of storage. These results indicate that germination  
311 in seeds of species from open savannas may be delayed due to dormancy, at most, until  
312 the onset of the next wet season. On the other hand, seeds of species from wet  
313 grasslands were longer-lived than seeds from open savannas, remaining viable after one  
314 year of dry storage. Additionally, germination levels increased and stayed consistently  
315 high after three months of dry storage.

316 In wet grasslands, the soil remained wet during the dry season in comparison to  
317 open savannas, presenting values around -1 MPa at 2 to 3 cm depth (Fig. 2). Water  
318 potential of around -1 MPa is not limiting for the germination of grass species (Qi &

319 Redmann 1993), thus suggesting that the water potential measured in the wet grasslands  
320 studied here would not limit the germination of seeds present in the soil. Moreover, the  
321 soil moisture and the high percentages of germination of grass species from wet  
322 grasslands suggest that germination might occur even during the dry season.

323         Although **rainfall** seasonality does not seem to be a strong selective pressure on  
324 seed germination of species from wet grasslands, due to the high soil moisture even  
325 during the dry season, other factors, such as the pressure of established vegetation, has  
326 been demonstrated to negatively influence seedling establishment in savannas  
327 (Zimmermann et al. 2008). Wet grasslands can be strongly competitive environments  
328 for seedlings, as they have dense and closed aboveground plant biomass of around 765  
329 g m<sup>-2</sup> (Fidelis et al. 2013). Fire frequently occurs in wet grasslands and can reduce  
330 competition by removing the established vegetation (Zimmermann et al. 2008). The  
331 high longevity of seeds, combined with high germination rates, can result in an  
332 opportunistic strategy for taking advantage of gaps by grass species from wet  
333 grasslands, for example after a fire event, to allow growth and establishment in a less  
334 competitive environment.

335 Seed dormancy at the end of the wet season may avoid risky germination

336         We show that seed dormancy is related to the dispersal time of Neotropical grass  
337 species (Fig. 4). Seeds dispersed late in the wet season showed lower probability of  
338 germination than seeds dispersed either early in the wet season, or dispersed during the  
339 dry season. The high levels of dormancy among late dispersed seeds may represent a  
340 drought-avoidance syndrome, a strategy already observed among grass species of  
341 savanna environments (Mott 1978; Veenendaal et al. 1996; McIvor & Howden 2000;  
342 Scott et al. 2010; Salazar et al. 2011), and **Melastomataceae species from high**  
343 **grasslands in other parts of Brazil (Silveira et al. 2012)**. Seeds dispersed in the

344 beginning of the wet season are expected to have about seven months of relatively  
345 stable water availability to enable germination and establishment. On the other hand,  
346 seed germination at the end of the wet season would be very risky, since seedlings  
347 would not have time enough to grow and acquire a minimal size that enable them to  
348 tolerate the harsh conditions expected during the dry season. Thus, the presence of seed  
349 dormancy here might prevent germination during times of low chances of recruitment,  
350 and hold back emergence until the onset of the next wet season.

351         Contrary to our expectations, the germination of seeds dispersed in the dry  
352 season was not different from seeds dispersed early in the wet season. Seeds dispersed  
353 during the dry season showed low levels of seed dormancy. Since sporadic rains are  
354 expected to occur in the dry season (Fig. 1), we did not expected to find high  
355 germination in seeds dispersed during this time. However, the amount of rain is  
356 extremely low (Fig. 1) and may potentially not be sufficient to induce germination.  
357 Additionally, the following wet season occurs relatively soon after dispersal and, by not  
358 having seed dormancy, these dry season dispersed seeds may rapidly germinate and take  
359 advantage of the entire growing season to establish.

360 Trade-off between seed dispersal and level of dormancy

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



369 empirical results showing that barochoric grass seeds are significantly more dormant  
370 than anemochoric ones.

371         Seed dispersal and seed dormancy may be viewed as bet-hedging strategies to  
372 enable species to cope with environmental patch heterogeneity and climatic  
373 unpredictability (Bulmer 1984; Venable & Brown 1988), the former by spreading risk  
374 over space (Bulmer 1984), and the latter by spreading risk over time (Venable & Brown  
375 1988). However, even in the absence of patch heterogeneity, seed dispersal can be  
376 favored by kin selection (Venable & Brown 1988). Additionally, in environments  
377 without inter-annual variation in precipitation, seed dormancy can be advantageous  
378 where sibling competition is high (Volis & Bohrer 2013). Although water availability  
379 affects seedling establishment in savannas (Medina & Silva 1990; Higgins et al. 2000)  
380 and might explain seed dormancy, it does not explain our results that anemochoric seeds  
381 had lower seed dormancy than barochoric seeds. In open savannas and wet grasslands of  
382 the *Cerrado* there is no clear patch heterogeneity, as exists in deserts, so it is less likely  
383 that this has driven a seed dispersal-dormancy trade-off in our studied species.  
384 Alternatively, we suggest that competition between siblings, conspecifics or  
385 heterospecifics, might drive the dispersal-dormancy trade-off found in our study  
386 species.

387         Conspecific competitor densities during seedling growth negatively influences  
388 plant fitness (Orrock & Christopher 2010). By dispersing far and not being dormant,  
389 grass seeds with an anemochoric dispersal syndrome might enhance the chances of a  
390 seed landing in a more open patch, enabling it to take advantage of sites with lower  
391 competition for resources (Ellner 1988; Venable & Brown 1988; Cohen & Levin 1991).  
392 Moreover, fire is a frequent disturbance in Brazilian open savannas and wet grasslands,  
393 and fire occurrence can open up gaps in the herbaceous vegetation. As such, these

394 anemochoric species may be favored by germinating earlier and growing in gaps opened  
395 by fire events. In contrast, the outcome for barochoric seeds is that they fall near to the  
396 mother plant and their own siblings. Because they disperse throughout the wet season,  
397 there is sufficient moisture to enable seed germination, and seedlings could grow in  
398 clumps if no other mechanism were available. Thus, seed dormancy can be a way to  
399 reduce immediate germination and distribute seedling emergence over time.

400 Alternatively, deeper dormancy among barochoric seeds could give seeds more time to  
401 enable a secondary dispersal event. Indeed, some of the barochoric grass species studied  
402 have structures recognized to favor secondary dispersal, including *Echinolaena inflexa*  
403 and *Icnanthus camporum*, which both have elaiosomes that are known to attract ants.  
404 Furthermore, *Aristida* species have awns, which can attach to rodents providing  
405 potential longer-distance dispersal.

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

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536 **Supplementary material**

537 Table S1. Post-hoc comparisons of probability of seed viability between dry storage  
538 months according to habitat types.

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

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

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



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
<i>Agenium goyazense</i> (Hack.) Clayton	Barochory	Dry	Open savanna	PNB
<i>Andropogon leucostachyus</i> Kunth	Anemochory	Early	Wet grassland	FAL
<i>Anthaenantia lanata</i> (Kunth) Benth.	Anemochory	Early	Open savanna	PNB
<i>Aristida gibbosa</i> (Nees) Kunth	Barochory	Dry	Open savanna	FAL
<i>Aristida recurvata</i> Kunth	Barochory	Dry	Open savanna	PNB
<i>Aristida riparia</i> Trin.	Barochory	Dry	Open savanna	FAL
<i>Aristida setifolia</i> Kunth	Barochory	Late	Open savanna	FAL
<i>Arthropogon villosus</i> Nees	Barochory	Early	Wet grassland	PNB
<i>Axonopus siccus</i> var. <i>siccus</i> (Nees) Kuhlm.	Barochory	Late	Open savanna	FAL
<i>Ctenium cirrhosum</i> (Nees) Kunth	Anemochory	Late	Open savanna	PNB
<i>Digitaria lehmanniana</i> Henrard	Barochory	Late	Wet grassland	PNB
<i>Echinolaena inflexa</i> (Poir.) Chase	Barochory	Late	Open savanna	FAL
<i>Elionurus muticus</i> (Spreng.) Kuntze	Anemochory	Early	Open savanna	PNB
<i>Eragrostis polytricha</i> Nees	Barochory	Early	Wet grassland	PNB
<i>Homolepis longispicula</i> (Döll) Chase	Anemochory	Early	Wet grassland	PNB
<i>Ichnanthus camporum</i> Swallen	Barochory	Late	Open savanna	FAL
<i>Mesosetum ferrugineum</i> (Trin.) Chase	Anemochory	Early	Wet grassland	PNB
<i>Panicum olyroides</i> Kunth	Barochory	Early	Open savanna	PNB
<i>Paspalum carinatum</i> Humb. & Bonpl. ex Flügge	Anemochory	Late	Open savanna	FAL
<i>Paspalum erianthum</i> Nees ex. Trin.	Anemochory	Early	Open savanna	PNB
<i>Paspalum glaucescens</i> Hack.	Barochory	Late	Open savanna	PNB
<i>Paspalum guttatum</i> Trin.	Anemochory	Early	Open savanna	PNB
<i>Paspalum maculosum</i> Trin.	Barochory	Late	Wet grassland	PNB
<i>Paspalum pectinatum</i> Nees ex Trin.	Anemochory	Early	Open savanna	PNB
<i>Paspalum polyphyllum</i> Nees	Anemochory	Dry	Wet grassland	FAL
<i>Saccharum villosum</i> Steud.	Anemochory	Early	Wet grassland	PNB

<i>Sacciolepis myuros</i> (Lam.) Chase	Barochory	Dry	Wet grassland	FAL
<i>Schizachyrium sanguineum</i> (Retz.) Alston	Barochory	Dry	Open savanna	PNB
<i>Setaria parviflora</i> (Poir.) Kerguelen	Barochory	Late	Open savanna	PNB

Time period of seed dispersal: Early (October - January), late in the wet season (February - April) and during the dry season (May - September). Site of occurrence: PNB (National Park of Brasília) and FAL (Água Limpa farm).

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 <sub>1</sub>	P	Habitat	Depth (cm)	$\beta \pm SE$	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

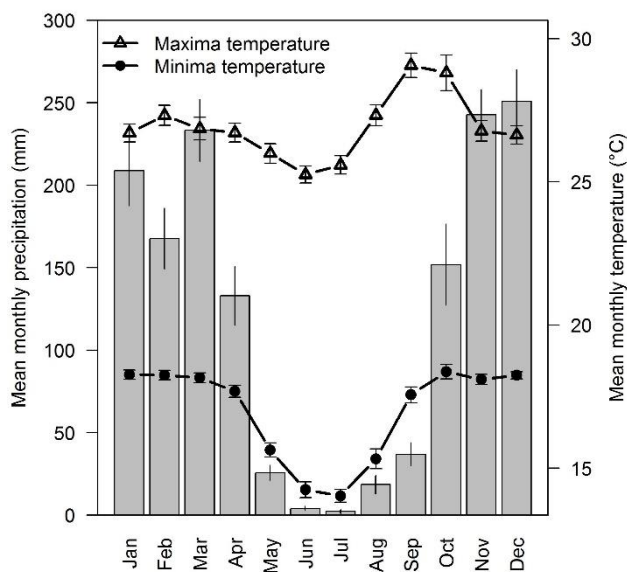
OS = Open Savannas, WG = Wet Grasslands.  $\beta \pm$  Standard Error.

Table 3. Mean of percentage of 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 along twelve months 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
<b>Dispersal time</b>															
Early in the wet season	17	60	74	11	58	70	10	60	65	9	68	76	27	44	50
Late in the 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
<b>Habitat</b>															
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
<b>Seed dispersal</b>															
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

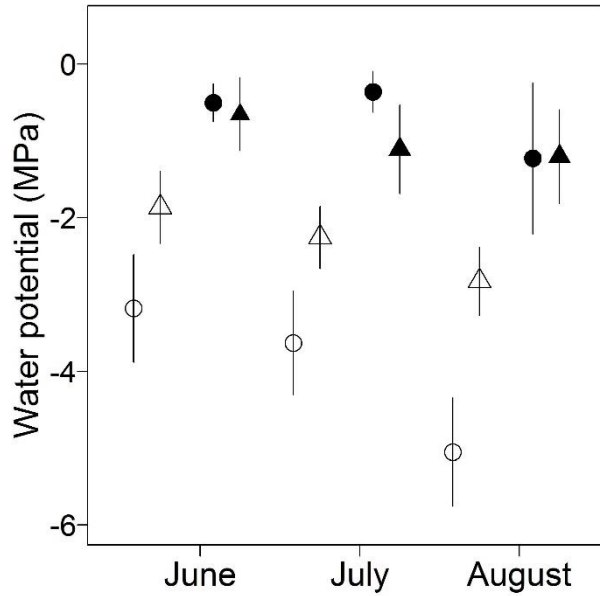
Level of dormancy (D) = number of ungerminated viable seeds over total viable seeds.

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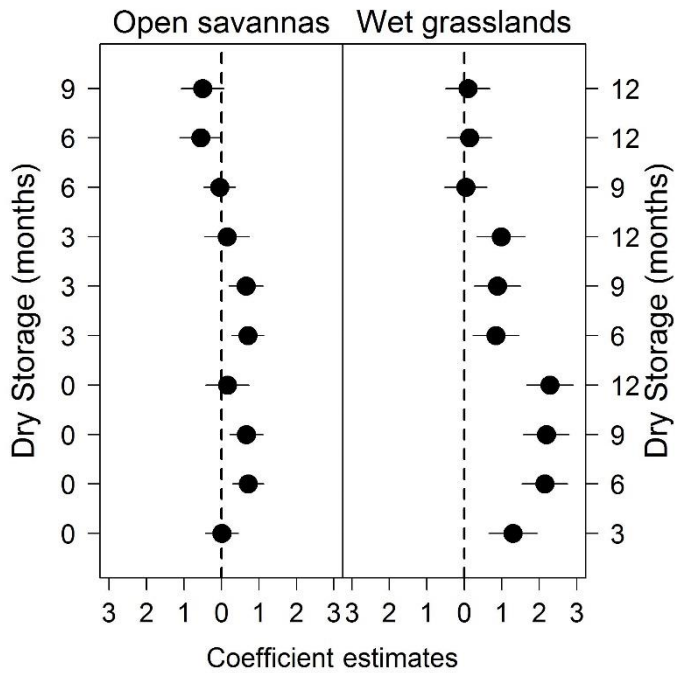
547 Figure 1. Mean monthly minimum and maximum temperatures and precipitation in  
548 Brasília-DF from 1994 to 2014. Data from BDMEP/INMET.



1

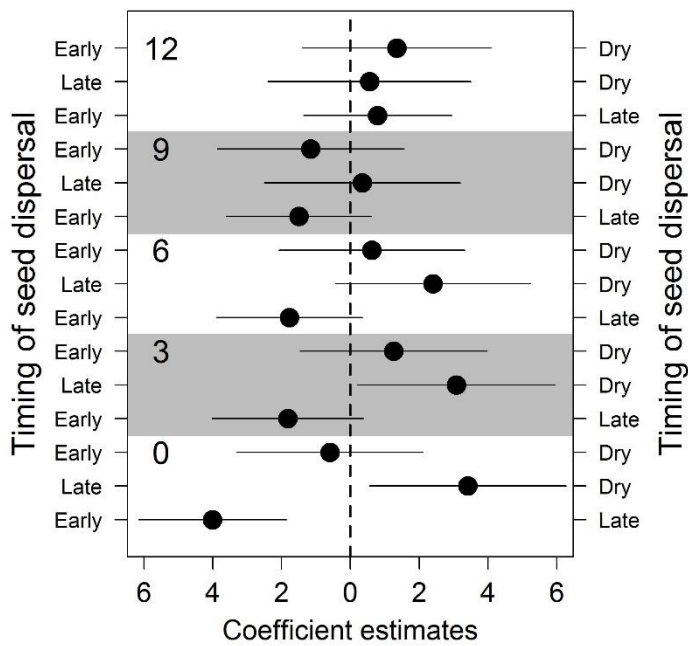
2 Figure 2. Soil water potential (MPa) of wet grasslands and open savannas during the dry  
 3 season. Open symbols = open savannas; Closed symbols = wet grasslands; Circles = 2 -  
 4 3 cm depth; Triangles = 10 - 11 cm depth. Mean + 95% Confidence Interval.

5



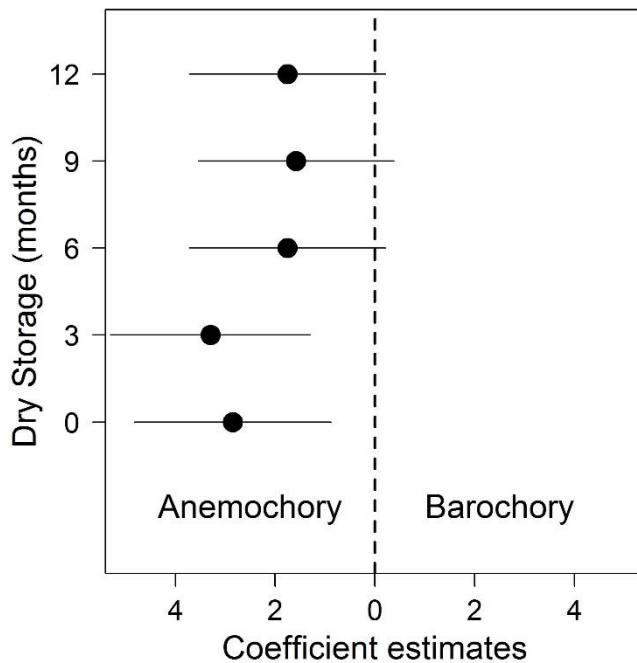
6

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



1

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



7

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