

1 **Diversity of germination strategies and seed dormancy in**
2 **herbaceous species of *campo rupestre* grasslands**

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19 **Running title:** Germination strategies in tropical grasslands

20 **Abbreviations:** MGT: Mean Germination Time; \bar{E} : germination synchrony; GLM:
21 Generalized Linear Models.

22

23 **Abstract**

24 The effects of fire on the vegetation vary across continents. However, in Neotropical fire-
25 prone grasslands, the relationship between fire and seed germination is still poorly
26 understood, while their regeneration, especially after strong anthropogenic disturbance, is
27 challenging for their conservation. In the present study, we assessed diversity of germination
28 strategies in 15 dominant herbaceous species from Neotropical altitudinal grasslands (locally
29 known as *campos rupestres*). We exposed seeds to several fire-related treatments. We also
30 compared germination between regularly and post-fire fruiting species. Finally, we
31 investigated the diversity of dormancy classes aiming at better understanding the
32 biogeography and phylogeny of seed dormancy. Germination strategies varied among
33 families. Velloziaceae and Xyridaceae produced non-dormant, fast-germinating seeds.
34 Cyperaceae and Poaceae showed an extremely low or null germination, due to a high
35 proportion of unviable or embryoless seeds. The seeds of *campo rupestre* grasslands are fire-
36 resistant, but there is no evidence that fire triggers germination in this fire-prone ecosystem.
37 Although heat and charred wood did not promote germination, smoke enhanced germination
38 in one grass species, and decreased the mean germination time and improved synchrony in
39 Xyridaceae and Velloziaceae. Fire had a positive effect on post-fire regeneration by
40 stimulating fruit set in some Cyperaceae and Poaceae species. These species produced faster-
41 germinating seeds with higher germination percentage and synchrony compared to regularly
42 fruiting Cyperaceae and Poaceae species. This strategy of dispersion and regeneration seems
43 to be an alternative to the production of seeds with germination triggered by fire.
44 Physiological dormancy is reported for the first time in several clades of Neotropical plants.
45 Our data help advance the knowledge on the role of fire in the regeneration of Neotropical
46 grasslands.

47

48 **Key-words:** Fire-related germination; Neotropical grasslands; physiological dormancy;

49 temperature fluctuation.

50

51 INTRODUCTION

52 In fire-prone environments plants show adaptive strategies that enable them to persist
53 or thrive in the face of recurrent fires (Bond & Keeley 2005). Fire-resistant or fire-persistent
54 species show two different regeneration strategies. “Resprouters” (Clarke *et al.* 2013) recover
55 from fire via vegetative regeneration, whereas “seeders” germinate from the seed bank or
56 from newly dispersed seeds (Whelan 1995, Bond & Van Wilgen 1996, Hoffmann 1998;
57 Keeley & Fotheringham 2000; Pausas *et al.* 2004; Bond & Keeley 2005).

58 In the case of obligate seeders (species which are not able to resprout after fire),
59 regeneration from seeds is the only way to recover from this kind of disturbance (Keeley
60 1986, Whelan 1995). Hence, germination in these species is expected to be triggered by fire-
61 related cues. The persistence of seeders at a site depends on: (1) their ability to produce seeds
62 during the inter-fire period, (2) seed survival probability during fire, and (3) the degree to
63 which recruitment is enhanced by fire (Pausas *et al.* 2004). Several species in fire-prone
64 environments have part of their recruitment process restricted to the first year after fire (Bond
65 & Van Wilgen 1996), because growth and reproduction are stimulated by fire-related cues. In
66 those cases, germination is usually triggered by heat, smoke, or charred wood, as direct
67 consequences of fire include increase in aboveground temperatures and smoke production
68 (Bond & Keeley 2005; Keeley & Fotheringham 2000).

69 However, fire effects on vegetation vary across continents (Lehmann *et al.* 2014). In
70 Neotropical grasslands most species are perennial and resprout after fire (Hoffmann 1998).
71 However, germination in herbaceous species is poorly documented and, hence, the
72 relationship between fire and germination in Neotropical species should be further studied.
73 Fire-stimulated flowering and fruit set is also pointed out as another alternative strategy; it is a
74 mechanism of recruiting seedlings after fire (Bond & Van Wilgen 1996, Bond and Keeley

75 2005). Flowering occurs just after fire on sprouts and allows producing a large amount of
76 seeds able to germinate in the first or second years after fire.

77 The relationship between germination and fire is well documented in fire-prone
78 environments, such as Mediterranean vegetation (Keeley & Fotheringham 1997; Keeley &
79 Bond 1997; Keeley & Fotheringham 1998, Pérez-Fernández & Rodríguez-Echeverría 2003;
80 Moreira *et al.* 2010, Santana *et al.* 2013), Australian vegetation (Williams *et al.* 2003, 2005),
81 African savannas (Dayamba *et al.* 2008, 2010), and African *fynbos* (Keeley & Bond 1997).
82 However, the effects of fire on the germination of herbaceous species in Neotropical
83 grasslands and the effects of fire-related cues remain poorly understood (Ribeiro *et al.* 2013,
84 Ribeiro & Borghetti 2014). In Neotropical grasslands, such as *campos rupestres*, some studies
85 assessed the effect of light and temperature on germination. However, no study has addressed
86 the impact of fire on germination (but see Fichino *et al.* 2012).

87 *Campos rupestres*, a part of the Cerrado biome, are species-rich grasslands that grow
88 on quartzite-derived soils at altitudes between 800 and 2,000 m a.s.l. They constitute a mosaic
89 of grasslands and scattered rocky outcrops dominated by ericoid, sclerophyllous evergreen
90 shrubs and sub-shrubs (Alves & Kolbek 2010; Le Stradic *et al.* 2014; Appendix 1) that grow
91 on shallow, highly acid, nutrient-poor soils (Benites *et al.* 2007; Le Stradic 2012). *Campos*
92 *rupestres* harbour high floristic diversity (4,000 estimated species) with one of the highest
93 levels of endemism found in Brazil (Alves & Kolbek 2010; Echternacht *et al.* 2011; Giuliatti
94 *et al.* 1997). Like all savannas, the Cerrado is subjected to recurrent fires (Simon *et al.* 2009),
95 which is an important factor that regulates vegetation dynamics in these ecosystems (Bond *et*
96 *al.* 2005; Cochrane 2009). Simon *et al.* (2009) have argued that fire acts as an ecological tool
97 in the Cerrado, as it maintains open some types of vegetation such as the *campos rupestres*.

98 Seed dormancy evolved several times in species that occur in seasonal environments,
99 and it prevents seedlings from establishing under unfavourable conditions (Baskin & Baskin

100 2014). There has been some progress in understanding the germination of *campo rupestre*
101 plants. However, we still need more studies on the phylogeny of seed dormancy, in order to
102 understand the dynamics of adaptive strategies in its flora and the relationship between
103 dormancy and plant traits (Jurado & Flores 2005, Baskin & Baskin 2014). Seed dormancy in
104 many woody plants of *campos rupestres* appears to be phylogenetically conserved (Gomes *et*
105 *al.* 2001; Silveira & Fernandes 2006). Nevertheless, the causes of dormancy in herbs that
106 occur in *campos rupestres* and the historical forces driving its evolution are poorly known
107 (Garcia *et al.* 2011).

108 The aim of the present study was to assess the diversity of germination strategies in
109 herbs that occur in *campos rupestres*. In fire-prone environments, plant and vegetation
110 responses to fire differ among continents (Lehmann *et al.* 2014). Hence, studying seed
111 germination ecology in *campos rupestres* will allow comparisons with African and Australian
112 grasslands. We described the germination ecology of 15 dominant species under several
113 conditions and tested whether seeds respond positively to fire-related cues. We also assessed
114 seed germination in four post-fire fruiting species and compared their germination behaviour
115 with that of regularly fruiting species. We report some insights on the seed dormancy of the
116 studied species and discuss our results in the context of results obtained in other fire-prone
117 environments in different biogeographic regions.

118

119 **MATERIAL AND METHODS**

120 **Seed collection**

121 The present study was carried out at Serra do Cipó (19°17 S; 43°33 W), in the
122 southern part of the Espinhaço Mountain Range (state of Minas Gerais, south-eastern Brazil),
123 where *campos rupestres* (rocky/rupestrian grasslands) is the dominant vegetation. The climate
124 is classified as Cwb, with a cool dry season (from May to October) and a warm rainy season

125 (from November to April), according to the Köppen system (Köppen 1900). The average
126 annual rainfall is 1,622 mm and the average annual temperature is 21.2 °C (Madeira &
127 Fernandes 1999). Natural fires occur at the end of the dry season, whereas man-made fires are
128 frequent in the mid dry season.

129 Fifteen dominant species from *campos rupestres* were selected from a
130 phytosociological study (Le Stradic 2012) based on seed availability. Our sample was
131 composed of species that produce seeds regularly (i.e. every year), including five Cyperaceae:
132 *Lagenocarpus tenuifolius*, *Lagenocarpus alboniger*, *Rhynchospora ciliolata*, *R.*
133 *consanguinea*, and *R. riedeliana*; two Poaceae: *Aristida torta* and *Echinolaena inflexa*; four
134 Velloziaceae: *Vellozia epidendroides*, *V. caruncularis*, *V. resinosa*, and *V. variabilis*; two
135 Xyridaceae: *Xyris obtusiuscula* and *X. pilosa*; and two Asteraceae: *Richterago arenaria* and
136 *Lessingianthus linearifolius* (Appendix 2). All studied species are perennial hemicryptophytes
137 except for *V. variabilis*, which is nanophanerophyte, and have abiotic seed dispersal. All
138 species resprout after fire (Le Stradic 2012). Seeds were manually collected from randomly
139 selected individuals of different populations ($n > 50$ per species) in unburned areas (for at
140 least the past five years) during the fruit set peak of 2010. For Poaceae, we used the entire
141 diaspore without removing accessory structures, such as lemma and palea (Baskin *et al.*
142 2006). We will henceforth refer to the achenes of Asteraceae as seeds.

143 To compare seed germination between regularly and post-fire fruiting species, we
144 analysed seed germination in four additional resprouter species, which fruit only after fire
145 (Appendix 3). We collected from a recently burned area (fire in August 2011) seeds of two
146 Cyperaceae: *Bulbostylis emmerichiae* and *B. paradoxa*; and two Poaceae: *Homolepis*
147 *longispicula* and *Paspalum pectinatum*. Nearly two weeks after fire those plants had already
148 resprouted and produced flowers (Le Stradic, personal observation). Seeds from Cyperaceae
149 and Poaceae were collected in December 2011 and January 2012, respectively.

150

151 **Germination experiments**

152 We assessed the effects of temperature, dry heat, smoke water, charred wood, and soil
153 on seed germination. In the soil treatment, seeds were sown on a 1-cm deep layer of soil
154 collected from randomly picked locations in the natural grasslands where the species occur.
155 We expected the soil to decrease the imbibition rate by decreasing water availability. In the
156 dry heat treatment, seeds were exposed to 100 °C for 5 min in an oven prior to sowing
157 (González-Rabanal & Casal 1995; Keeley & Bond 1997; Ribeiro *et al.* 2013), in accordance
158 with data obtained for the Cerrado (Miranda *et al.* 1993; Ribeiro *et al.* 2013). In the smoke
159 water treatment, smoke was obtained from burning leaves and wooden stems and funnelled
160 through a hose into a distilled water sample. First-day seeds were watered with a 1:10 diluted
161 smoked water solution. From the second day on, seeds were watered with distilled water
162 (Clarke & French 2005). In the charred wood treatment, seeds were watered with 10 ml of an
163 aqueous suspension of charred wood (González-Rabanal & Casal 1995; Pérez-Fernández &
164 Rodríguez-Echeverría 2003). This suspension was obtained from the combustion of dried
165 plant material (i.e. hay of grasses and forbs) collected in grasslands so that it contained
166 representative species of *campos rupestres*. An initial biomass of 408 g was burned, resulting
167 in 50 g of charred wood, and then sieved (2mm). The charred wood was diluted with distilled
168 water to a concentration of 10 g of charred wood per litre.

169 Seeds were put to germinate in Petri dishes (five replicates of 20 seeds per treatment),
170 covered with filter paper, and moistened with Nistatina® suspension to prevent fungi growth.
171 Seeds were placed in germination chambers kept at a constant temperature of 25 °C under a
172 12-h photoperiod, because these are optimum conditions for the germination of *campo*
173 *rupestre* plant species. As an additional treatment, seeds were also exposed to fluctuating
174 temperatures between 15 °C and 25 °C and between 20 °C and 30 °C, based on a 12-h

175 photoperiod (with the highest temperature corresponding to daytime). Seeds were exposed to
176 $27 \mu\text{mol m}^{-2} \text{s}^{-1}$ light incidence, because small-sized seeds of *campos rupestres* herbs are
177 light-demanding (Abreu & Garcia 2005; Oliveira & Garcia 2012; Mota & Garcia 2013;
178 Silveira *et al.* 2013). All seeds were monitored for 30 consecutive days (Baskin *et al.* 2006) to
179 make our data comparable to other studies (Baskin & Baskin 2014). Germination was
180 checked every 24 h and germination was determined through radicle emergence.

181 After germination tests, seed viability was assessed in all species through seed
182 dissection procedures followed by a tetrazolium test applied to fresh seeds. Seeds that did not
183 germinate were cut and placed in a 1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride
184 (TTC) for 48 h under dark conditions in a chamber at 25 °C (Peters & Lanham 2005).

185

186 **Germination of regularly and post-fire fruiting species**

187 Four species did not produce fruits regularly but had massive fruit production right
188 after fire: *Bulbostylis emmerichiae*, *B. paradoxa*, *Homolepis longispicula*, and *Paspalum*
189 *pectinatum*. Seeds of these post-fire fruiting species were placed in germination chambers
190 (five replicates of 20 seeds/species) kept at a constant temperature of 25°C, in a 12-h
191 photoperiod. Germination behaviour of these Poaceae and Cyperaceae seeds was then
192 compared to the germination behaviour of Poaceae and Cyperaceae seeds of regularly fruiting
193 species exposed to the same conditions (i.e. constant temperature of 25 °C and a 12-h
194 photoperiod).

195

196 **Determination of seed dormancy classes**

197 In the present study, seed dormancy was defined as the absence of germination in
198 viable seeds subjected to favourable germination conditions (Hilhorst 2011). Seed dormancy
199 classes followed Baskin and Baskin (2004). Physical dormancy implies that the seed or fruit

200 coat is impermeable to water. To determine whether diaspores were water impermeable, four
201 replicates of 25 seeds (100 seeds for Xyridaceae species) were weighed on a digital balance,
202 soaked in distilled water for 72h at room temperature, and then reweighed. Seed permeability
203 was determined by the increase in seed mass between dry and soaked seeds. We dissected dry
204 seeds under a stereomicroscope to determine embryo developmental patterns and infer on
205 morphological and morphophysiological dormancy (Baskin & Baskin 2004).

206

207 **Statistical analyses**

208 For each replicate, we calculated final germination percentage, mean germination time
209 (MGT), and germination synchrony (\bar{E}) (Ranal & Santana 2006), using the formula:

$$210 \quad MGT = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

211 where t_i is the time from the start of the experiment to the i^{th} observation, n_i is the number of
212 germinated seeds at time i (not the accumulated number), and k is the last time of
213 germination. And the formula:

$$214 \quad \bar{E} = -\sum_{i=1}^k f_i \log_2 f_i \quad \text{with} \quad f_i = n_i / \sum_{i=1}^k n_i$$

215 where f_i is the relative frequency of germination, n_i is the number of germinated seeds on
216 day i , and k is the final day of observation. Low \bar{E} values indicate more synchronized
217 germination and high \bar{E} values indicate less synchronized germination.

218 We used generalized linear models (GLM), with quasi-binomial distribution and a
219 logit link function to compare the final germination percentage among treatments (25°C,
220 15°C/25°C, 20°C/30°C, soil, heat, smoke water, and charred wood) for the species: *A. torta*,
221 *L. linearifolius*, *V. caruncularis*, *V. epidendroides*, *V. resinosa*, *V. variabilis*, *X. obtusiuscula*,
222 and *X. pilosa* (other species showed low or no germination). We used a GLM with a gamma

223 distribution and an inverse link function to compare mean germination time among treatments
224 for the same species. To compare germination synchrony among treatments per each species,
225 we used simple analyses of variance (ANOVA), followed by *post hoc* tests (Tukey test), in
226 which germination synchrony was treated as the dependent variable and treatment as the
227 factor. Normality and homoscedasticity assumptions were checked and a square root
228 transformation was applied whenever necessary (Sokal & Rohlf 1998). In addition, we used a
229 GLM with a quasi-binomial distribution and a logit link function to test for differences in the
230 percentage of viable and embryoless seeds among species.

231 We compared the final germination percentage between regularly and post-fire fruiting
232 species with a GLM (quasi-binomial distribution and logit link function). We tested for
233 differences in mean germination time among species using a GLM (gamma distribution and
234 inverse link function) and used a t-test to compare germination synchrony between the two
235 treatments. At the species level, we compared the final germination percentage of all species
236 (regularly fruiting species vs. post-fire fruiting species) with a GLM, using a quasi-binomial
237 distribution and logit link function. We also compared mean germination time among species
238 with a GLM (gamma distribution and inverse link function). We tested for differences in
239 germination synchrony among species with an ANOVA, followed by *post hoc* Tukey tests, in
240 which germination synchrony was treated as the dependent variable and species as the factor.
241 Normality and homoscedasticity assumptions were previously checked and a square root
242 transformation was applied whenever necessary.

243 We analysed seed coat permeability with paired t-tests, in order to test for differences
244 in weight gain between dried and soaked seeds. All analyses were carried out in R 2.9.1 (R
245 Development Core Team 2010).

246

247 **RESULTS**

248 **Seed germination requirements and effects of fire-related cues**

249 Seeds of *E. inflexa*, *L. alboniger*, *L. tenuifolius*, *R. ciliolata*, *R. consanguinea*, *R.*
250 *riedeliana*, and *R. arenaria* showed low or no germination regardless of the treatment (Table
251 1). *Vellozia caruncularis* and *V. variabilis* had high germination percentage (> 75% and >
252 88%, respectively) regardless of the treatment (Table 1). *Vellozia epidendroides*, *V. resinosa*,
253 and *X. pilosa* had high germination percentages at 25 °C, but experienced a germination
254 decrease at temperatures fluctuating between 15 °C and 25 °C. *Xyris obtusiuscula* and *A. torta*
255 were not affected by fluctuating temperatures (Table 1). The soil treatment had a negative
256 effect on the germination of *L. linearifolius*, *V. epidendroides*, and *V. resinosa* (Table 1).

257 The responses of seeds to fire-related cues were idiosyncratic. Dry heat had no effect
258 on germination percentage in any species, except for *A. torta* (Table 1). *Aristida torta* showed
259 its highest germination percentage (21%) in the smoke water treatment, whereas heat
260 decreased its germination to 1% (Table 1). No fire-related cues affected germination
261 percentage in *L. linearifolius* (Table 1). Charred wood affected germination percentage
262 negatively in *V. epidendroides*, *X. obtusiuscula*, and *X. pilosa*.

263 Overall, temperatures fluctuating between 15 °C and 25 °C and between 20 °C and 30
264 °C increased the mean germination time of all species (Table 2). They also increased the
265 germination synchrony in *V. epidendroides* and *X. pilosa* (Table 3). Soil treatment reduced the
266 mean germination time of *V. variabilis* (Table 2). In most species, dry heat had no effect on
267 mean germination time, but it reduced moderately mean germination time in *V. epidendroides*
268 and *V. resinosa* (Table 2). Charred wood increased mean germination time and synchrony in
269 *V. resinosa* (Table 1, Table 2). In addition, smoked water improved mean germination time in
270 *X. pilosa* (Table 2) and increased germination synchrony in *V. epidendroides*, *V. resinosa*, and
271 *X. pilosa* (Table 3).

272

273 **Viability**

274 *Aristida torta*, *L. alboniger*, *R. ciliolata*, *L. linearifolius*, and *R. arenaria* had over
275 50% of embryoless seeds (the percentage of embryoless seeds differed significantly among
276 species; $F = 88.76$, $P < 0.001$) (Table 4). In Xyridaceae and Velloziaceae, the percentage of
277 embryoless seeds was often below 10%. *Echinolaena inflexa*, *L. alboniger*, *R. ciliolata*, and
278 *R. arenaria* exhibited seed viability below 10%. Seed viability in *A. torta*, *R. riedeliana*, *L.*
279 *tenuifolius*, *R. consanguinea*, and *L. linearifolius* ranged from 10% to 42%, whereas in
280 Xyridaceae and Velloziaceae seed viability was above 85% (the percentage of viable seeds
281 differed significantly among species; $F = 75.81$, $P < 0.001$) (Table 4).

282

283 **Germination of regularly and post-fire fruiting species**

284 Among post-fire fruiting species, the germination percentage of *H. longispicula*, *B.*
285 *emmerichiae*, and *B. paradoxa* was above 75%, whereas *P. pectinatum* showed lower
286 germination percentage ($F = 28.08$, $P < 0.001$, Figure 1). Both post-fire fruiting Poaceae (*H.*
287 *longispicula* and *P. pectinatum*) showed higher germination percentage than regularly fruiting
288 Poaceae ($F = 30.37$, $P < 0.001$). The same pattern was observed in Cyperaceae, in which
289 germination percentages were higher in the post-fire fruiting species (*B. emmerichiae*, and *B.*
290 *paradoxa*) than in regularly fruiting species ($F = 600.85$, $P < 0.001$).

291 The two Poaceae species (*H. longispicula* and *P. pectinatum*) had shorter mean
292 germination time than *B. emmerichiae*, *B. paradoxa*, and *A. torta* ($F = 33.46$, $P < 0.001$,
293 Figure 1). *Paspalum pectinatum* showed higher synchrony than the three other post-fire
294 fruiting species ($F = 16.87$, $P < 0.001$), but this could be an artefact caused by the lower
295 germination rate of this species (Figure 1).

296

297 **Determination of seed dormancy classes**

298 In eight out of 15 species, seed germination percentages were below 10% under
299 suitable germination conditions (Table 1). *Echinolaena inflexa*, *L. alboniger*, *R. ciliolata*, and
300 *R. arenaria* produced high percentages of unviable seeds and embryoless seeds (Table 4).
301 Thus, in these four species, lack of germination is attributed to low seed quality, and no safe
302 conclusions on seed dormancy can be drawn from these species. Seeds of *A. torta*, *L.*
303 *tenuifolius*, *R. consanguinea*, and *R. riedeliana* were considered to have at least some degree
304 of dormancy, with percentages ranging from 68 to 100% (Table 4). Although 29% of the *X.*
305 *obtusiuscula* seeds germinated, nearly 67% of its seeds were viable (Table 4). Hence, the
306 seeds of these five species were considered primarily dormant.

307 The increase in weight after soaking seeds in tap water ranged from 6.2 to 217%. This
308 increase in seed weight was significant for all studied species (and nearly significant for *V.*
309 *epidendroides*, Appendix 4). Hence, all species produced water-permeable seed coats and did
310 not present physical dormancy. Mature seeds of the five dormant species (*A. torta*, *L.*
311 *tenuifolius*, *R. consanguinea*, *R. riedeliana*, and *X. obtusiuscula*) produced differentiated
312 embryos that occupied most of the seed cavity. These seeds are, therefore, physiologically
313 dormant (PD).

314

315 **DISCUSSION**

316 Our results show that herbaceous plants of the *campos rupestres* have a large diversity
317 of seed germination strategies. There were clear interspecific and interfamily differences in
318 germination. Seven out of 15 species of Poaceae and Cyperaceae had only sporadic
319 germination and produced high percentages of unviable or dormant seeds. We could not
320 expect responses to fire-related cues from those species (*E. inflexa*, *L. alboniger*, *L.*
321 *tenuifolius*, *R. ciliolata*, *R. consanguinea*, *R. riedeliana*, and *R. arenaria*), due to their low
322 viability, our discussion is thus focused on the other group of species that produced non-

323 dormant, fast-germinating seeds.

324 Our results on fluctuating temperatures corroborate other studies carried out in Cerrado
325 and *campo rupestre* species, which showed that alternating temperatures either decrease or
326 have no effect on germination (Velten & Garcia 2005; Silveira *et al.* 2013). Seeds of *L.*
327 *linearifolius*, *Vellozia*, and *Xyris* are non-dormant and germinated without undergoing pre-
328 germination treatments. Our germination results using *campo rupestre* soil suggest that some
329 species, such as *L. linearifolius*, *V. epidendroides*, and *V. resinosa*, might be particularly
330 sensitive to the imbibition phase. Water imbibition is crucial to seed germination: when a dry
331 seed enters the soil, imbibition occurs prior to germination and the seed must absorb enough
332 water to germinate. However, the critical hydration level is species-specific (Hadas & Russo
333 1974).

334 Seed germination of shrubs and trees of Mediterranean-like ecosystems or savannas can
335 be stimulated by fire-related cues, such as charred wood or dry heat exposure (Keeley & Bond
336 1997; Pérez-Fernández & Rodríguez-Echeverría 2003; Dayamba *et al.* 2008; Ribeiro *et al.*
337 2013). However, our results do not support the hypothesis that fire-related cues trigger
338 germination in Neotropical herbs. As already observed in herbaceous species of savannas
339 (Dayamba *et al.* 2008, 2010; Fichino *et al.* 2012) no fire-related cue had a marked effect on
340 seed germination. Unlike Mediterranean species, germination in *X. pilosa*, *X. obtusiuscula*, *V.*
341 *epidendroides*, and *V. resinosa* was inhibited or decreased by charred wood. However, smoke
342 stimulated germination in *A. torta*, decreased the mean germination time, and increased
343 germination synchrony in most of the studied species.

344 In our study, heat did not improve seed germination in herbaceous species. Heat-
345 stimulated seeds exhibit physical dormancy imposed by a dense palisade tissue (Keeley &
346 Fotheringham 2000; Ribeiro *et al.* 2013). Heat disrupts this tissue, which increases water
347 permeability. All studied species have water-permeable seed coats and lack physical

348 dormancy, which may be restricted to some clades, such as Fabaceae (Gomes *et al.* 2001;
349 Silveira & Fernandes 2006). Heat is a direct effect of fire (Keeley & Fotheringham 2000).
350 Other combinations of temperatures and exposure times should be tested to further assess the
351 role of heat on the germination of *campo rupestre* plants.

352 In our study, the effects of charred wood and smoke were contradictory, whereas in the
353 Mediterranean region, smoke and charred wood increased germination (Keeley &
354 Fotheringham 1998; Pérez-Fernández & Rodríguez-Echeverría 2003). The first hypothesis to
355 explain those differences is that the chemical compounds produced by smoke and charred
356 wood are different and, thus, have different effect on germination. The second hypothesis is
357 that the studied seeds need time to become sensitive to fire-related cues (e.g., following wet–
358 dry cycles and dry periods after ripening (Long *et al.* 2011a; Long *et al.* 2011b). The third
359 hypothesis is that seed germination triggered by smoke carrying karrikinolide compounds
360 (Bradshaw, Dixon, Hopper, Lambers, & Turner, 2011; Flematti, Ghisalberti, & Dixon, 2004)
361 is not a fire-adapted plant trait (Bradshaw *et al.* 2011). Indeed, organic matter decay was
362 already pointed out as the first driver in the development of smoke-mediated germination
363 (Bradshaw *et al.* 2011).

364 Despite the lack of evidence of fire-induced germination, fire might still play an
365 important role in the regeneration of plant communities in *campos rupestres*. Firstly, all but
366 one species showed high tolerance to high temperatures, which suggests that fire-tolerance is
367 common in *campo rupestre* species similar to other fire-prone grasslands in southern and
368 central Brazil (Overbeck *et al.* 2005; Overbeck & Pfadenhauer 2007; Fichino *et al.* 2012).
369 Secondly, post-fire fruiting species had high, fast, and synchronous germination, which
370 indicates an adaptive advantage of regeneration after fire. Indeed, regularly fruiting Poaceae
371 and Cyperaceae had low or null germination rate, whereas post-fire fruiting Poaceae and
372 Cyperaceae species had high germination rate and short mean germination time. The massive

373 flower production just after fire favours cross-breeding, which promotes vigorous seedlings
374 with a wide habitat tolerance (Lamont & Wiens 2003). Moreover, fire decreases competition
375 and increases resource availability (i.e. nutrients, light, and space). Species that produce seeds
376 only after fire may have been selected, because relaxed interspecific competition after fire is
377 expected to increase their likelihood of establishment (Santana *et al.* 2013). Like seeders, our
378 four post-fire fruiting species (resprouters) produced several viable seeds after fire. This fact
379 may indicate that the ultimate role of seeds in resprouter species is dispersal instead of
380 survival. However, our results should be interpreted with caution, as we did not test for a
381 phylogenetical signal in the comparison between regularly and post-fire fruiting species.

382 On the other hand, all studied species are resprouters, which suggests that resprouting is
383 the dominant mechanism for regeneration after fire in *campos rupestres* (Simon *et al.* 2009).
384 This may lead to lower investment in sexual reproduction and explains why many of the
385 studied species, especially the Poaceae and Cyperaceae, had almost no germination (Paula &
386 Pausas 2008). Resprouters represent a large group of Neotropical fire-prone communities
387 (Hoffmann 1998; Overbeck & Pfadenhauer 2007) Resprouting provides an alternative to the
388 establishment from seeds after fire, and allows the plant to persist in the environment
389 (Hoffmann 1998; Fidelis *et al.* 2010). Our results indicate that some resprouters from *campos*
390 *rupestres* produce many embryoless or unviable seeds, especially wind-pollinated species
391 (Cyperaceae and Poaceae). This pattern seems to be widespread in Cyperaceae, Asteraceae,
392 and Poaceae (Leck & Schutz 2005; Overbeck & Pfadenhauer 2007; Velten & Garcia 2005).
393 Low fecundity among resprouters in comparison to non-sprouters can be explained by
394 resource limitation, breeding system limitation, and genetic load (Lamont *et al.* 2011; Lamont
395 & Wiens 2003). Conversely, the Xyridaceae and Velloziaceae, which are also resprouter
396 species, showed a high germination rate, regardless of the treatment. Hence, this pattern is not
397 applicable to all families.

398 Our data suggest that populations of *A. torta*, *X. obtusiuscula*, *L. tenuifolius*, *R.*
399 *consanguinea*, and *R. riedeliana* produce physiologically dormant seeds (*sensu* Baskin &
400 Baskin 2004). Physiological dormancy occurs in most Cyperaceae (Leck & Schutz 2005) and
401 Poaceae species (Baskin & Baskin 2014). However, despite recent reports on secondary
402 dormancy (Garcia *et al.* 2011), there were no reports of primary dormancy in Xyridaceae.
403 Seed dormancy in Poaceae is a consequence of chemical inhibition or mechanical resistance
404 of glumes, lemmas, and palea (Gasque & García-fayos 2003; Ma *et al.* 2010). To the best of
405 our knowledge, this is the first record of primary dormancy in Cyperaceae, Poaceae, and
406 Xyridaceae species of *campos rupestres*. Further efforts to study other species are especially
407 important to shed light on the relationships between seed dormancy and species life history
408 (Jurado & Flores 2005).

409 In summary, in contrast to other fire-prone vegetation types, our study shows that
410 seed germination of herbaceous species of *campos rupestres* is unresponsive to fire-related
411 cues. These contrasting results may be related to the evolutionary history of plants in fire-
412 prone environments in different continents (Lehmann *et al.* 2014). Our study also shows
413 marked differences in germination among families. However, fire may play an important role
414 in regeneration in *campos rupestres*. Most studied species are fire-tolerant and, at least in
415 Cyperaceae and Poaceae, post-fire fruiting species germinate quicker and more synchronously
416 than regularly fruiting species. Finally, we report new records of physiological dormancy in
417 many clades, which contributes to the understanding of phylogenetic and biogeographic
418 distribution of seed dormancy (Baskin & Baskin 2014). The relationship between germination
419 and fire seems quite different in Neotropical grasslands compared to African or
420 Mediterranean fire-prone environments. We hope our data improve cross-continental
421 comparisons of fire-related cues on seed germination and provide important information for
422 fire management of this threatened ecosystem.

423

424 **ACKNOWLEDGEMENTS**

425 We thank William Bond and Gregory Mahy for their suggestions on the text, Kolo D Wamba
426 who revised the English and anonymous reviewers for their comments to improve the
427 manuscript. This work was supported by the Conselho Nacional de Pesquisa [CNPq
428 561883/2010-6]; Fundação do Amparo a Pesquisa de Minas Gerais [Fapemig APQ-04105-
429 10], Natural Reserva Vellozia and the Ministère Français des affaires étrangères et
430 européennes [EGIDE 2009/657176K].

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600

Table 1. Germination percentage (mean \pm standard error) for graminoid and forb species of *campos rupestres* grasslands in the various treatments. GLM procedures (with quasibinomial distribution) were performed for *A.torta*, *L. linearifolius*, *V. caruncularis*, *V. epidendroides*, *V. resinosa*, *V. variabilis*, *X. obtusiuscula* and *X. pilosa*. Lack of data means no germination under this specific condition. Within a species, treatment values with different lower case letters were significantly different according to the GLM results.

Species	Control	15°C/25°C	20°C/30°C	Charred wood	Water smoke	Dry heat	Soil	GLM
<i>Aristida torta</i>	9.00 \pm 2.10 ^a	5.00 \pm 3.06 ^a	10.00 \pm 3.95 ^a	7.00 \pm 2.24 ^a	21.00 \pm 6.94 ^b	1.00 \pm 1.12 ^c	9.00 \pm 4.81 ^a	F=3.45 **
<i>Echinolaena inflexa</i>	-	-	1.00 \pm 1.12	-	-	-	-	-
<i>Lagenocarpus alboniger</i>	-	-	1.00 \pm 1.12	-	-	-	-	-
<i>Lagenocarpus tenuifolius</i>	-	4.00 \pm 2.10	1.00 \pm 1.12	-	-	-	-	-
<i>Rhynchospora ciliolata</i>	-	-	1.00 \pm 1.12	-	1.00 \pm 1.12	-	-	-
<i>Rhynchospora consanguinea</i>	-	-	-	-	-	-	1.00 \pm 1.12	-
<i>Rhynchospora riedeliana</i>	-	3.00 \pm 2.24	1.00 \pm 1.12	-	-	-	-	-
<i>Lessingianthus linearifolius</i>	41.00 \pm 1.12 ^a	52.00 \pm 5.18 ^a	33.00 \pm 10.69 ^a	24.00 \pm 4.11 ^a	25.00 \pm 6.12 ^a	37.00 \pm 10.69 ^a	7.00 \pm 3.79 ^b	F=5.71 ***
<i>Richterago arenaria</i>	-	-	-	-	1.00 \pm 1.12	-	-	-
<i>Vellozia caruncularis</i>	84.00 \pm 4.11	86.00 \pm 6.22	80.00 \pm 6.85	90.00 \pm 3.95	82.00 \pm 3.79	75.00 \pm 6.37	86.00 \pm 7.37	F=0.87 ^{NS}
<i>Vellozia epidendroides</i>	83.00 \pm 3.79 ^a	32.00 \pm 1.37 ^b	78.00 \pm 7.20 ^a	9.00 \pm 2.44 ^c	85.00 \pm 3.06 ^a	85.5 \pm 1.63 ^a	27.5 \pm 6.79 ^b	F=48.80 ***
<i>Vellozia resinosa</i>	91.00 \pm 3.01 ^a	37.00 \pm 4.63 ^b	93.50 \pm 1.12 ^a	90.00 \pm 3.95 ^a	94.00 \pm 4.47 ^a	86.00 \pm 3.14 ^a	30.00 \pm 5.86 ^b	F=43.28 ***
<i>Vellozia variabilis</i>	88.00 \pm 3.35	90.00 \pm 1.77	92.00 \pm 2.85	97.00 \pm 3.35	97.00 \pm 2.24	96.00 \pm 2.09	95.00 \pm 2.50	F=1.75 ^{NS}
<i>Xyris obtusiuscula</i>	29.00 \pm 8.95 ^a	28.5 \pm 6.94 ^a	10.00 \pm 2.34 ^b	2.00 \pm 1.37 ^c	41.00 \pm 8.91 ^a	27.00 \pm 6.02 ^a	-	F=9.79 ***
<i>Xyris pilosa</i>	79.50 \pm 5.03 ^a	28.5 \pm 4.29 ^b	74.50 \pm 5.55 ^a	15.00 \pm 3.54 ^c	72.00 \pm 7.20 ^a	71.00 \pm 6.03 ^a	-	F=38.72 ***

Table 2. Mean germination time [MGT in days (mean \pm standard error)] for graminoid and forb species of *campo rupestre* grasslands in the various treatments. GLM procedures (with Gamma distribution) were performed for *A. torta*, *L. linearifolius*, *V. caruncularis*, *V. epidendroides*, *V. resinosa*, *V. variabilis*, *X. obtusiuscula* and *X. pilosa*. Lack of data means no germination under this specific condition. Within a species, treatment values with different lower case letters were significantly different according to the GLM results.

Species	Control	15°C/25°C	20°C/30°C	Charred wood	Water smoke	Dry heat	Soil	GLM
<i>Aristida torta</i>	12.16 \pm 1.34 ^a	22.66 \pm 5.06 ^b	19.62 \pm 3.25 ^a	16.12 \pm 3.45 ^a	9.44 \pm 1.09 ^a	6.00 \pm 0.00 ^c	15.12 \pm 3.84 ^a	F=3.45 ***
<i>Echinolaena inflexa</i>	-	-	25.00 \pm 0.00	-	-	-	-	
<i>Lagenocarpus alboniger</i>	-	-	26.00 \pm 0.00	-	-	-	-	
<i>Lagenocarpus tenuifolius</i>	-	29.66 \pm 0.28	30.00 \pm 0.00	-	-	-	-	
<i>Rhynchospora ciliolata</i>	-	-	29.00 \pm 0.00	-	20.00 \pm 0.00	-	-	
<i>Rhynchospora consanguinea</i>	-	-	-	-	-	-	23.00 \pm 0.00	
<i>Rhynchospora riedeliana</i>	-	23.75 \pm 0.17	26.00 \pm 0.00	-	-	-	-	
<i>Lessingianthus linearifolius</i>	5.99 \pm 0.31 ^a	8.90 \pm 0.64 ^b	8.15 \pm 0.43 ^c	6.05 \pm 0.16 ^a	6.59 \pm 0.14 ^a	6.38 \pm 0.43 ^a	5.20 \pm 1.52 ^a	F=9.84 ***
<i>Richterago arenaria</i>	-	-	-	-	7.00 \pm 0.00	-	-	
<i>Vellozia caruncularis</i>	5.28 \pm 0.38 ^a	9.75 \pm 1.26 ^b	7.24 \pm 0.43 ^c	5.11 \pm 0.59 ^a	5.02 \pm 0.69 ^a	5.45 \pm 0.52 ^a	4.05 \pm 0.20 ^d	F=11.21 ***
<i>Vellozia epidendroides</i>	13.93 \pm 0.68 ^a	23.87 \pm 1.18 ^b	10.29 \pm 0.71 ^c	13.26 \pm 1.90 ^a	13.65 \pm 0.38 ^a	10.33 \pm 0.58 ^c	12.32 \pm 0.79 ^a	F=20.30 ***
<i>Vellozia resinosa</i>	7.04 \pm 0.40 ^a	21.02 \pm 0.31 ^b	8.53 \pm 0.55 ^c	7.96 \pm 0.46 ^c	7.21 \pm 0.30 ^a	7.91 \pm 0.58 ^c	6.46 \pm 0.12 ^a	F=93.86 ***
<i>Vellozia variabilis</i>	5.86 \pm 0.38 ^a	7.94 \pm 0.29 ^b	6.86 \pm 0.54 ^a	6.04 \pm 0.59 ^a	5.27 \pm 0.39 ^a	5.77 \pm 0.29 ^a	3.75 \pm 0.26 ^c	F=13.30 ***
<i>Xyris obtusiuscula</i>	15.51 \pm 0.29 ^a	15.17 \pm 0.68 ^a	23.80 \pm 1.45 ^b	13.00 \pm 0.00 ^a	16.51 \pm 1.91 ^a	15.10 \pm 1.50 ^a	-	F=8.40 ***
<i>Xyris pilosa</i>	10.24 \pm 0.44 ^a	17.77 \pm 1.06 ^b	14.89 \pm 0.87 ^c	10.76 \pm 0.69 ^a	8.10 \pm 0.80 ^d	10.55 \pm 0.77 ^a	-	F=22.24 ***

Table 3. Germination synchrony (mean \pm standard error) of graminoid and forb species of *campos rupestres* grasslands. Low values indicate more synchronized germination and high values indicate asynchronous germination. Within a species, treatment values with different lower case letters were significantly different according to the GLM results.

Species	Control	15°C/25°C	20°C/30°C	Charred wood	Water smoke	Dry heat	Soil	ANOVA
<i>Aristida torta</i>	0.40 \pm 0.18	0.21 \pm 0.18	0.31 \pm 0.18	0.34 \pm 0.20	0.75 \pm 0.28	0.00 \pm 0.00	0.50 \pm 0.32	F=0.71 ^{NS}
<i>Lessingianthus linearifolius</i>	0.87 \pm 0.16 ^{ab}	1.53 \pm 0.14 ^a	1.23 \pm 0.37 ^a	0.96 \pm 0.17 ^{ab}	0.82 \pm 0.13 ^{ab}	1.11 \pm 0.21 ^{ab}	0.26 \pm 0.26 ^b	F=3.54 *
<i>Vellozia caruncularis</i>	1.56 \pm 0.08	1.87 \pm 0.07	1.90 \pm 0.21	1.53 \pm 0.07	1.50 \pm 0.20	1.60 \pm 0.11	1.58 \pm 0.06	F=1.96 ^{NS}
<i>Vellozia epidendroides</i>	2.57 \pm 0.03 ^a	1.64 \pm 0.06 ^b	2.16 \pm 0.06 ^{ab}	1.10 \pm 0.15 ^d	1.63 \pm 0.06 ^b	2.24 \pm 0.08 ^{ac}	1.90 \pm 0.26 ^{bc}	F=18.50 ***
<i>Vellozia resinosa</i>	1.92 \pm 0.07 ^a	1.98 \pm 0.10 ^a	1.78 \pm 0.10 ^a	0.96 \pm 0.12 ^b	0.79 \pm 0.10 ^b	2.21 \pm 0.12 ^a	1.45 \pm 0.09 ^b	F=32.50 ***
<i>Vellozia variabilis</i>	1.55 \pm 0.04	1.69 \pm 0.07	1.88 \pm 0.09	1.52 \pm 0.17	1.42 \pm 0.16	1.57 \pm 0.07	1.54 \pm 0.08	F=2.29 ^{NS}
<i>Xyris obtusiuscula</i>	1.53 \pm 0.14 ^a	1.62 \pm 0.22 ^a	0.82 \pm 0.29 ^a	0.00 \pm 0.00 ^b	1.37 \pm 0.17 ^a	0.99 \pm 0.33 ^a	-	F=4.82 **
<i>Xyris pilosa</i>	2.22 \pm 0.13 ^a	1.88 \pm 0.19 ^a	1.14 \pm 0.16 ^b	0.72 \pm 0.10 ^b	1.13 \pm 0.23 ^b	2.46 \pm 0.06 ^a	-	F=24.60 ***

Table 4. Viable, embryoless and dormant seeds (mean percentage \pm standard error) for graminoid and forb species of *campos rupestres* grasslands. Dormant seeds were calculated as the final germination percentage over the total number of viable seeds. ND: non-dormant seeds. NC: non-conclusive data. Embryoless seeds and viable seeds are in respect to total number of seeds.

Species	Embryoless seeds (%)	Viable seeds (%)	Dormant seeds (%)
<i>Aristida torta</i>	55.00 \pm 5.00	28.00 \pm 3.79	67.9
<i>Echinolaena inflexa</i>	13.52 \pm 2.45	0.00 \pm 0.00	NC
<i>Lagenocarpus alboniger</i>	54.00 \pm 4.11	7.00 \pm 1.37	NC
<i>Lagenocarpus tenuifolius</i>	14.50 \pm 3.11	38.00 \pm 3.47	100
<i>Rhynchospora ciliolata</i>	80.00 \pm 2.50	5.00 \pm 1.77	NC
<i>Rhynchospora consanguinea</i>	0.50 \pm 0.56	39.50 \pm 5.75	100
<i>Rhynchospora riedeliana</i>	37.00 \pm 2.24	30.00 \pm 4.68	100
<i>Lessingianthus linearifolius</i>	57.00 \pm 1.37	41.00 \pm 1.12	ND
<i>Richterago arenaria</i>	84.00 \pm 4.47	1.00 \pm 1.11	NC
<i>Vellozia caruncularis</i>	11.00 \pm 5.70	86.00 \pm 4.47	ND
<i>Vellozia epidendroides</i>	6.50 \pm 2.27	89.50 \pm 2.98	ND
<i>Vellozia resinosa</i>	3.00 \pm 1.37	93.50 \pm 2.44	ND
<i>Vellozia variabilis</i>	2.00 \pm 2.23	89.00 \pm 3.26	ND
<i>Xyris obtusiuscula</i>	3.00 \pm 1.37	67.00 \pm 2.85	56.7
<i>Xyris pilosa</i>	1.50 \pm 0.68	89.50 \pm 4.79	ND

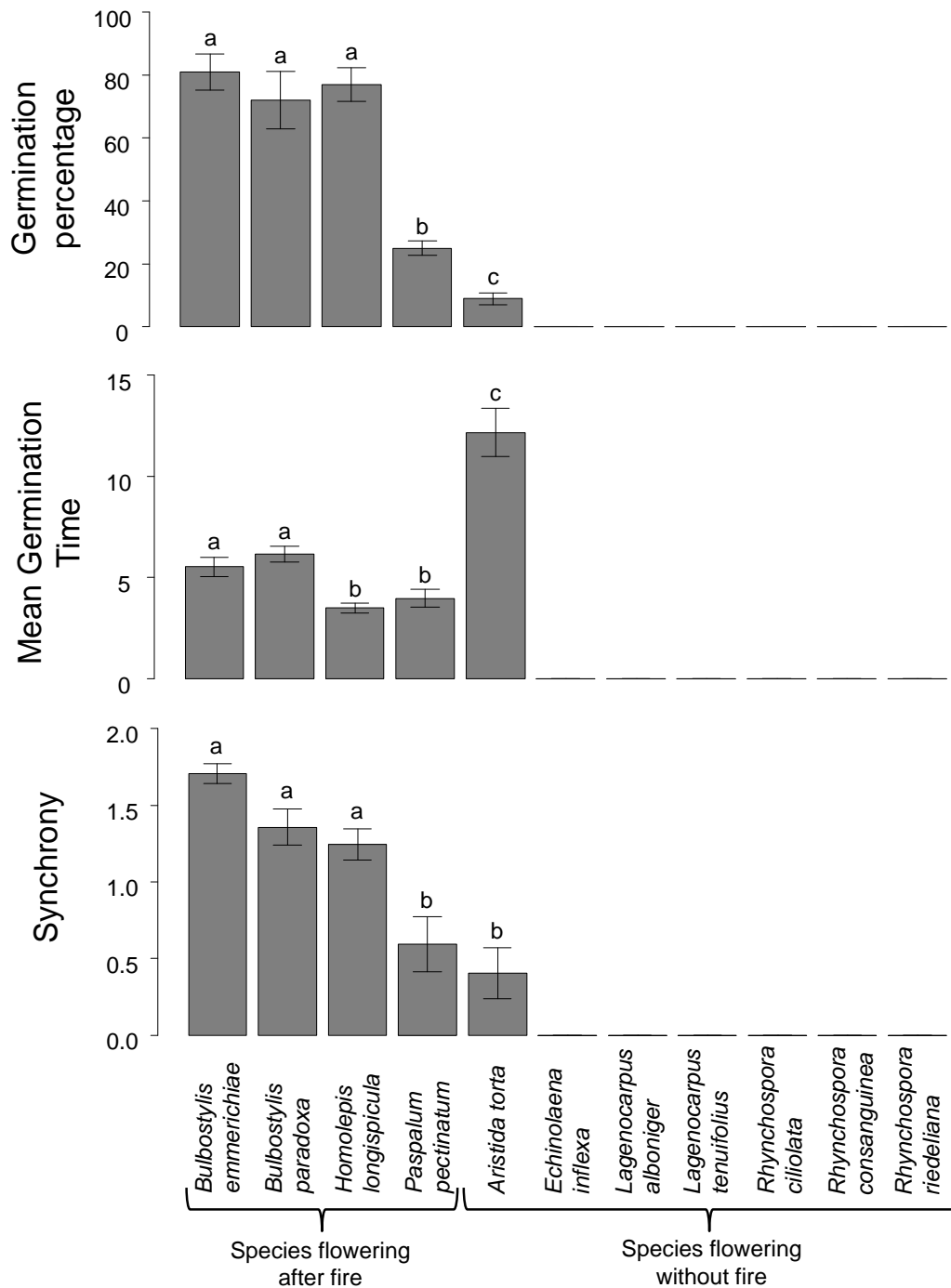


Fig. 1. Germination percentage, mean germination time and synchrony of the fifteen *campo rupestre* species, southeastern Brazil. (a) Germination percentage (%), (b) Mean germination time (days) and (c) synchrony at 25°C, for post-fire fruiting and regularly fruiting Cyperaceae and Poaceae species. Different letters indicate significant differences according (a) GLM procedure (quasibinomial error distribution and logit

link function) with $F=28.08$, $P<0.001$, (b) GLM procedure (Gamma error distribution and inverse link function) with $F=33.46$, $P<0.001$, (c) simple ANOVAs, followed by post-hoc tests (Tukey's "Honest Significant Difference") $F=16.87$, $P<0.001$).

Appendix:

Appendix 1: Pictures of the *campos rupestres* of Serra do Cipó: a) the mosaic of grasslands and rocky outcrops during the dry season, b) the mosaic of grasslands and rocky outcrops during the wet season.

Appendix 2: Plant list with family, plant form, distribution range and seed dispersal period.

Appendix 3: Life-history traits of the four species producing and dispersing seeds after a wildfire in August 2011.

Appendix 4: Average seed mass before and after soaking in tap water for 72h, with the increased seed mass percentage (%) for 15 herbaceous species from *campos rupestres* in Southeastern Brazil. Statistics refer to Wilcoxon tests.