ABSTRACT

Fire plays an important role in several grassland ecosystems in the world. Fire can trigger germination in several species, by breaking the physical dormancy of their seeds. Thus, we hypothesized that exposure to high temperatures during fire would break seed dormancy and enhance germination. We tested the effect of high temperatures on the germination of six species of legumes from Brazilian subtropical grasslands. We used heat shock experiments with the following treatments: 60, 90, 120 and 150°C for one minute. Seeds were then placed to germinate for 60 days in 12/12 hours light/dark and 20/30°C. Germination was generally low for all study species. Most species was not affected by heat shock treatments. However, *Stylosanthes montevidensis* was the only species that had its physical dormancy broken when exposed to 120°C. The seeds of all the other species were neither stimulated nor killed by high temperatures. Although the exposure to high temperatures did not affect the germination of the study species (except for one), it also did not kill seeds, thereby showing that seeds are resistant to fire. Therefore, the rapid passage of fire in these grasslands is not sufficient to break the dormancy of most of the studied species of legumes.

Keywords: Brazilian Campos grasslands, Fabaceae, fire-resistance, germination percentage, heat shock experiments

Germination triggered by fire cues is an important trait in fire-prone environments, enabling plant population to regenerate after disturbance (Keeley & Fotheringham 2000; Keeley *et al.* 2011). Hard-coated seeds, for an example, remain dormant until suitable environmental conditions take place (Baskin 2003; Willis *et al.* 2014). The exposure to high temperatures (*e.g.* Moreira & Pausas 2012), as well as daily fluctuating temperatures (*see* Santana *et al.* 2013), are major factors related to break of physical dormancy in fire-prone ecosystems. Increasing temperature promotes the breaking of dormancy through cracking the seed coat, allowing imbibition to occur, and promoting germination (Keeley 1991; Morrison *et al.* 1998; Baskin & Baskin 2014).

In fire-prone ecosystems, studies focusing on the effects of heat shock on dormancy break and germination are of great importance. Fire-related germination is found in different types of vegetation, but most studies describe this relationship in Mediterranean ecosystems (*see* Reyes & Trabaud 2009; Moreira *et al.* 2010) and in Australia (*see* Auld & O’Connell 1991; Ooi *et al.* 2014). Keeley *et al.* (2011) concluded thus, that breaking the dormancy or stimulating the germination by fire cues should be considered as evidence for selective pressures in plant evolution. In a plant functional approach, researchers have classified physically dormant seeds as having pyrogenic obligate or facultative dormancy-breaking mechanisms (Ooi *et al.* 2014). On the...
other hand, fire may be irrelevant for some species, so they survive high temperatures but do not show a specific dormancy-breaking bounded by higher temperatures (see Hanley & Lamont 2000; Fichino et al. 2016).

Since heat shock may play a major role in breaking physical dormancy in fire-prone ecosystems, it is critical to determine its role as a fire-related cue in hard-coated seeds from Brazilian subtropical grasslands. Fires in these grasslands are very fast, occurring with low temperatures and intensity (Fidelis et al. 2010a). Moreover, vegetation dynamics are affected by fire, maintaining open physiognomies and promoting plant diversity (Overbeck et al. 2007; Fidelis et al. 2012). Earlier studies found little evidence that germination could be enhanced by seed exposure to high temperatures (Overbeck et al. 2006; Fidelis et al. 2010b). However, recent studies (Silveira & Overbeck 2013) have demonstrated that heat exposure at 80°C led to increased germination by a grassland legume, Desmodium barbatum.

Our study addresses the role of fire temperature (dry heat shocks) to influence seed germination across six Fabaceae species from Brazilian subtropical grasslands, in order to test if high temperatures would break seed. We hypothesize that heat shock would be sufficient to break the dormancy of seeds, or at least that they would survive to the exposure to higher temperatures. The species used in our study belonged to three subfamilies in the Fabaceae family: *Chamaecrista repens* (Vogel) H.S.Irwin & Barneby (forb, Caesalpinoioideae); *Desmanthus tatuhyensis* Hoehne (forb, Mimosoideae); *Collaea stenophylla* (Hook. & Arn.) Benth (shrub), *Crotalaria tweediana* Benth. (forb), *Stylosanthes montevidensis* Vog (forb), and *Zornia reticulata* Sm. (forb, all Papilionoideae). Seeds from Fabaceae are known to have impermeable seed coats (Baskin & Baskin 2000; Jayasuriya et al. 2013) and therefore, we chose species from this family to test our hypothesis.

Seeds of each of the six legume species were collected on different individuals and populations found in natural grasslands, located on Morro Santana, a granitic hill located in Porto Alegre (Southern Brazil, 30°03’ S, 51°07’ W; 311 m a.s.l.). The climate of this region is subtropical humid (Köppen classification Cfa), with annual precipitation of 1350 mm and mean temperatures of 22°C (Livio 1999). Seeds were collected during the dispersal period (from October to February) and kept at room temperature in paper bags. We conducted heat shock experiments two months after seed collection.

Subtropical grasslands are very rich in plant species (450-500 species; see Aguiar 1986) and they are composed of a mixture of C₃ and C₄ grasses, which are characteristic in Southern Brazil (Fidelis 2010). Fire has been present in the study area since 1200 BP (Behling et al. 2007) with fire frequency estimated between 2-5 years. Further, fires are mostly set by local residents to clean fields and to stimulate resprouting of medicinal plants. Fire is fast and temperatures at soil surface range from 48 to 537°C and temperatures >150°C never lasted more than 60s (Fidelis et al. 2010a). Although temperatures below soil surface (where seeds from the seed bank are) have not been measured, we believe that they are much lower than at soil surface, as observed by Miranda et al. (1993) for Cerrado areas. In fact, Bradstock & Auld (1995) registered temperatures from 0-3cm soil depth ranging from 60°C to 120°C. Moreover, we used temperatures similar to the ones used by Overbeck et al. (2006) and Silveira & Overbeck (2013) in studies on seed germination of Brazilian subtropical grasslands. Therefore, we chose the range from 60°C (which is usually measured in the upper layers of soils during fire) to 150°C (extreme temperatures) to test our hypothesis.

Seeds were exposed to different heat shock temperatures lasting one minute: 60°, 90°, 120°, 150°C and control (no heat shock treatment). Each species and treatment had five replicates (except for *Chamaecrista repens* and *Collaea stenophylla*, with three replicates each). We used 20 seeds/replicate, except for *Chamaecrista repens* (15 seeds/replicate). Such differences in seed numbers and replicates across species were related to constraints on seed availability.

We applied heat shock treatments using a preheated oven, with insertion and removal of replicates in aluminum dishes. Each replicate was heated separately to avoid pseudoreplication (Morrison & Morris 2000). Both treated and non-treated seeds were placed in Petri dishes, on three layers of sterilized filter papers. All dishes were moistened with distilled water during 60 days in germination chamber. Since this study aimed to reproduce real conditions from the field, 20°/30°C temperature, 12/12 hours dark/light conditions were chosen. Seeds were kept moistened while we tracked germination rates weekly. When radicle and/or cotyledons could be observed, they were counted and removed from Petri dishes.

By the end of the germination tests, non-germinated seeds (except for *Desmanthus tatuhyensis* and *Collaea stenophylla*) were submitted to a tetrazolium viability test, in order to test if seeds were still viable after treatments. Some species showed several seeds damaged by fungi, and thus, we also counted the damaged seeds and used only the non-damaged seeds for the tetrazolium test. Since the seeds did not show any sign of imbibition, we punctured them and left to imbibe before they were cut and soaked in a 2,3,5-triphenyl-tetrazolium chloride solution (0.075%; Brasil 2009), at 30°C for four hours. When embryos stained red, seeds were considered to be viable, and viable seeds (%) were considered the germinated + stained seeds at the end of the experiment.

We used a one-way analysis of variance (ANOVA) to test whether there were statistical differences of germination percentages across the different temperatures (main fixed factor) for each study species. We used randomization tests (Euclidean distances between replicates, 10000 iterations), since there is no restriction about normal distribution of...
data (for more details, see Manly 2007). All statistical analyses were performed using MULTIV (Pillar 2008).

Most of the species germinated less than 20% across all treatments, remaining visibly impermeable by the end of the tests (e.g., no changes in seed size). In *Crotalaria tweediana*, we observed ca. of 20% of germinable seeds in the control treatment; and although the species almost doubled germination with the heat shocks, differences between treatments were not found (Fig. 1A, $P > 0.05$). Also an increase in germination was observed with the high temperatures in *Collaea stenophylla* (Fig. 1B), but differences were not statistically significant ($P > 0.05$).

Seeds of *Desmanthus tatuyensis* showed low germination percentages in all treatments (<10%) and the exposure to high temperatures did not affect germination ($P > 0.05$, Fig. 1C). *Chamaecrista repens* seeds tended to germinate more after being exposed to 60º (Fig. 1D, $P > 0.05$). Seeds of *Zornia reticulata* (Fig. 1E) also did not show any response to the heat shocks, with germination percentages <10% in all treatments ($P > 0.05$). The only species affected by the heat shock treatments was *Stylosanthes montevidensis*: a higher germination percentage was observed after seeds were exposed to 120ºC (20%) in relation to the control (2.4%, $P = 0.01$, Fig. 1F).

Seed total viability (germinated + stained seeds) was higher in *Zornia reticulata* and *Crotalaria tweediana* (Tab. 1), presenting a low percentage of damaged seeds. On the other hand, 88% and 86.6% of non-germinated seeds of *S. montevidensis* and *C. repens* were damaged by fungi and thus, they also had low viability (Tab. 1).

The increase in germination after fire, both due to heat shock or smoke, is considered an adaptation to fire by some authors (e.g. Keeley et al. 2011). Several species from Mediterranean ecosystems show fire-related cues to germi-

![Figure 1](image-url)

**Figure 1.** Germination percentages (means±1SE) of legumes submitted to heat shock treatments (60ºC, 90ºC, 120º, and 150º) and control (no exposure to high temperatures) for 1 minute. **A**) *Crotalaria tweediana*, **B***) *Collaea stenophylla*, **C***) *Desmanthus tatuyensis*, **D***) *Chamaecrista repens*, **E***) *Zornia reticulata* and **F**) *Stylosanthes montevidensis*. Different letters mean significant differences between temperature treatments ($P \leq 0.05$).
nation (see Moreira et al. 2010), being fire-dependent, while other species can germinate after temperature fluctuations (gap-dependent, Ooi et al. 2014). Our study showed that only seeds of Stylosanthes montevidensis had a fire-related increase in germination after the exposure to 120°C, showing breakage of physical dormancy of at least 20% of the seeds. For most of the species, there was no significant effect in germination after the exposure to high temperatures.

In general, all species showed very low germination percentages and high percentage of hard seeds after experiments, confirming their impermeable coats. Although Moreira & Pausas (2012) showed that dormancy break is fire-related in physically dormant seeds in the Mediterranean Basin, in the present study, the exposure to high temperatures was not sufficient to break the dormancy for most of the studied legume species. However, all of them showed to be resistant to fire, showing no significant decrease in germination when exposed to 120°C and 150°C. Since seeds can survive fire, other mechanisms should be involved in the dormancy-breaking process. Jaganathan (2015) argued that, since seeds have a specific mechanism of losing physical dormancy related to ‘water gaps’, that open as a response to an environmental sign of fluctuating temperatures. Seed responses to fire might be actually expectations, instead of adaptations sensu stricto. In other words, heat-induced traits did not properly evolve related to fire as a selective pressure, being result of a ‘shift’ in its function through the evolutionary process in seasonal ecosystems (Bradshaw et al. 2011). Santana et al. (2013) showed that seeds of Ulex parviflorus had their dormancy broken when exposed to daily fluctuating temperatures, arguing that fire was not the main factor affecting plant germination.

Auld & O’Connel (1991) analysed 35 species of Fabaceae in Australia, and they concluded that the time of exposure was less important than the temperature to germination of these species. Although Stylosanthes montevidensis had the highest germination percentages after the exposure to 120°C, showing that this temperature broke the dormancy of part of the seeds (20%), that means that 80% fraction of the population remained dormant or did not germinate for another reason. In the case of our study, 78% of the remaining seeds were damaged by fungi and thus, it is not possible to affirm if they would have germinated in the absence of the fungi. Silveira & Overbeck (2013) showed that a grassland species, Desmodium barbatum, increased the germination when exposed to 80°C, but it is not clear if the remaining seeds were dormant or dead, since the authors did not mention a viability test by the end of experiments. Most studies addressing the effects of high temperatures on the germination of Fabaceae species found peak germination between 70 – 100°C (Martin et al. 1975; Auld & O’Connel 1991; Williams et al. 2003). Luna et al. (2007) reported that maximum germination for Mediterranean plants was after the exposure to 80°C, and that these temperatures can be reached by the soil during summer, being these species classified as having facultative pyrogenic dormancy, or as gap dependent (Ooi et al. 2014).

Some authors found that long exposure to high temperatures decrease germination or even kill seeds (e.g. Keeley 1991; Tarrega et al. 1992; Herranz et al. 1998). However, our study showed that after the exposure to high temperatures, seeds still germinated. Those results are very consistently with what has been reported to some grass species from the Brazilian subtropical grasslands (Overbeck et al. 2006) and herbaceous species in the Cerrado ecosystem (Fichino et al. 2012; 2016; Le Stradic et al. 2015). When species from Cerrado were compared to forest species, the exposure to high temperatures did not affect negatively the Cerrado species, while forest species showed lower germination percentages (Ribeiro et al. 2013; Ribeiro & Borghetti 2014). Therefore, even with no evidence of fire-related cues to germination, seeds can resist the passage of fire and this trait (fire resistance) should also be considered as an important trait in flammable ecosystems (Fichino et al. 2016).

We showed that the exposure to high temperatures did not break dormancy of legume species in Brazilian subtropical grasslands, except for one species: Stylosanthes montevidensis (20% of seeds). Therefore, other factors related to breaking of dormancy (e.g. temperature fluctuations) might play a more important role than the direct effect of heat shocks. Additionally, we highlight the importance of resistance of seed propagules as a plant functional trait in fire-prone ecosystems.
To resist or to germinate? The effect of fire on legume seeds in Brazilian subtropical grasslands

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References


