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## Effect of high temperature on germination of four legumes from a forest-grassland mosaic in Southern Brazil

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**Abstract:** Passage of a fire can break dormancy of the seeds of many plant species in fire-prone ecosystems. This response to fire is especially well known for the Fabaceae family, but has been poorly studied in Southern Brazil. We collected seeds of four Fabaceae species present in grasslands-forest mosaics of Southern Brazil: *Mimosa bimucronata*, *Desmodium barbatum*, *Sesbania virgata* and *Collaea stenophylla*. Seeds were exposed to different heat treatments (exposure to 60° and 80 °C for 5 minutes, to 100 °C for 2 minutes, control without heat treatment); not all species were tested in all treatments. After the treatment, the seeds were kept in a germination chamber with a light period of 12/12 hours and temperature of 25 °C. Germinated seeds were counted every 2 days. The results were analyzed by randomization testing. Germination of *D. barbatum* and *S. virgata* was increased after exposure to high temperatures (80° and 60 °C, respectively), while *M. bimucronata* showed reduced germination after temperatures of 80 °C and *C. stenophylla* no response. This study is the first for Southern Brazil to show a positive response of germination for grassland species exposed to high temperatures, simulating fire effects. As the study presented distinct responses of species to the heat treatment, it seems important to conduct more works with other species from the family, in order to be able to detect more general patterns.

**Keywords:** *Campos Sulinos, ecophysiology, Fabaceae, fire, grassland, heat shock.*

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**Resumo:** A passagem do fogo pode quebrar a dormência de sementes de muitas espécies em ecossistemas suscetíveis ao fogo. Essa resposta ao fogo é especialmente bem conhecida para espécies da família Fabaceae, mas tem sido pouco estudado no Sul do Brasil. Coletamos sementes de quatro espécies de Fabaceae presentes em mosaicos de campo-florestas do Sul do Brasil: *Mimosa bimucronata*, *Desmodium barbatum*, *Sesbania virgata* e *Collaea stenophylla*. As sementes foram expostas a diferentes tratamentos de calor (60 °C e 80 °C, ambos por 5 minutos, e 100° C por 2 minutos, mais controle sem tratamento de calor). Nem todas as espécies foram submetidas a todos os tratamentos. Após o tratamento, as sementes foram mantidas em câmara de germinação com fotoperíodo 12/12 horas e temperatura de 25 °C. A porcentagem de sementes germinadas foi contada a cada 2 dias. Os resultados foram analisados por teste de aleatorização. A taxa de germinação de *D. barbatum* e *S. virgata* aumentou quando expostas a altas temperaturas (80 °C e 60 °C, respectivamente), enquanto *M. bimucronata* mostrou redução e *C. stenophylla* nenhuma resposta aos tratamentos. Esse estudo foi o primeiro para o Sul do Brasil a mostrar uma resposta positiva na germinação de espécies campestres expostas a altas temperaturas, simulando os efeitos do fogo. Como o estudo apresentou respostas distintas, é importante conduzir trabalhos com outras espécies da família a fim de detectar padrões gerais.

**Palavras-chave:** *Campos Sulinos, ecofisiologia, Fabaceae, fogo, vegetação campestre, choque térmico.*

## Introduction

Fire is a major determinant of the global distribution of vegetation types (Keeley et al. 2008), as well as an important selective force in plant communities around the world (Van Staden et al. 2000) – in the words of Bond & Keeley (2005), a “global herbivore”. The importance of fire in the history of grasslands in Southern Brazil has been highlighted by Behling et al. (2009). Grasslands in this region had dominated the landscape under cooler and drier glacial and warmer and drier postglacial climates, and have been subject to forest expansion only since about 4000 years BP, when climate became moister and cooler (Behling et al. 2004). In Southern Brazil, like in many other open vegetation types of the world (Bond 2005), fire is considered to be a decisive factor for persistence of grasslands, preventing encroachment of woody species under climatic and edaphic conditions favorable for forest development (Overbeck et al. 2007).

A key and pervading concept accepted by most environmental managers is that combustible ecosystems have traditionally been burned because plants are adapted to fire (Bradshaw et al. 2011). Seeds from plants of fire-prone ecosystems can exhibit a high tolerance to fire (Keeley 1994, Williams et al. 2005, Scott et al. 2010) when compared to forest species (Ribeiro et al. 2013) and can benefit from it (Whelan et al. 2002, Newton et al. 2006) or even require fire as a cue to the onset of germination (González-Rabanal & Casal 1995, Pugnaire & Lozano 1997). Breaking of seed dormancy is one important plant strategy in relation to fire (Bond & Van Wilgen 1996). Seed dormancy is a key factor in the dynamics of many natural populations and ensures that seedling emergence occurs at the most advantageous time and place (Baskin & Baskin 1985). Several direct and indirect means to break dormancy due to effects of fire exist, such as high temperatures (González-Rabanal & Casal 1995), presence of smoke (Keeley & Fotheringham 1997), presence of ashes and releases of nitrogenous compounds due to combustion of plant matter (González-Rabanal & Casal 1995). Dry heat is effective in breaking dormancy in seeds of a number of species (Baskin & Baskin 1998) through its physical effect on seed coat structure (Bell et al. 1993) and/or physiological effect on the seed embryo (Bell & Williams 1998). This response to fire is especially well known for the Fabaceae family, as seeds from many species of this family exhibit physical dormancy (Baskin et al. 2000).

Exposure to temperatures between 80° and 100 °C is sufficient to break the seed dormancy of several legumes of eucalypt savannas in north-eastern Australia, but temperatures greater than 100-120 °C have been shown to be lethal for seeds of these species (Williams et al. 2003). Williams et al. (2004) demonstrated that breaking seed dormancy is possible with temperatures below 80 °C and 100 °C for *Indigofera hirsuta* and *Galactia tenuiflora*, respectively. Suñé & Franke (2006) have tested different treatments to break dormancy of native plants of Rio Grande do Sul (Brazil) with potential for

agricultural use and have found that thermic scarification with immersion in water a 60 °C for 5 minutes was one of the best to surpass or overcome dormancy of *Desmanthus depressus* and *Trifolium riograndense*. Franke & Baseggio (1998) have suggested water immersion at temperatures as high as 70 °C to efficiently break dormancy of *Lathyrus nervosus* e *Desmodium incanum*.

Regarding grasslands and forest-grassland mosaics in Rio Grande do Sul, the only legume species for which possible germination responses after increased temperatures, simulating passage of a fire (i.e., without water immersion), have been analyzed up to date were *Collaea stenophylla* e *Desmanthus tathiensis*. For those two species, neither a temperature of 60 °C nor of 120 °C for a period of 1 minute influenced germination (Fidelis et al. 2007). The aim of this work is to assess the impact of high temperatures stimuli on germination of four legumes species of grassland-forest mosaics in Southern Brazil.

## Material and Methods

Seeds of four legume species (Tab. 1) were collected in grassland formations and at forest-grassland edges on and around Santana Hill in Porto Alegre city (30° 03' S and 51° 07' W, max. altitude 311m, subtropical humid climate), Southern Brazil, between May and June 2011. The species rich grasslands on Morro Santana are dominated by C4 tussock grasses and are under a regime of frequent burns (average fire return intervals: ca. 3 years, in some parts more often; Overbeck et al. 2005, 2006a), which prevents expansion of forest over grassland (e.g. Müller et al. 2012). According to Silveira & Miotto (2013), 62 species of Fabaceae occur on this granitic hill.

Seeds were stored at dry conditions and ambient temperature until the start of the experiments in October 2011. Each treatment consisted of five replicate batches with 25 seeds each for *Mimosa bimucronata* (DC.) Kuntze, *Desmodium barbatum* (L.) Benth. and *Sesbania virgata* (Cav.) Pers., while for *Collaea stenophylla* (Hook. et Arn.) Benth. only 20 seeds were used, due to lower availability of seeds. All are rather frequent species on the hill (Silveira & Miotto 2013).

Temperature treatments were 60°, 80° and 100° C, plus control treatment without exposition to elevated temperature. Due to differences in availability of seeds, the experiment could be concluded with all treatments only for *M. bimucronata*, while for the other species, only one or two treatments could be conducted (Table 1). All treatments consisted of exposure to dry heat in an oven. Duration of treatments was 5 minutes for exposure to 60 °C and 80 °C and 2 minutes for exposure to 100 °C, thus simulating exposure to different temperatures and time spans. It can be expected that faster exposure to high temperatures is enough to break dormancy, but continued exposure to high temperatures could kill the seeds. Longer exposure periods at lower temperatures reflect the slow decrease in temperature in the uppermost soil layer (Bradstock & Auld 1995, Overbeck et al. 2006b).

**Table 1.** Fabaceae species used in this study and their respective subfamily, habit, principal habitat and 1000 seed weight (extrapolated from mean value of the batches of 20/25 seeds used in experiment, see methods).

| Subfamily      | Species   | Habit      | Habitat                     | Seed weight (1000 seeds) |
|----------------|---|------------|-----------------------------|--------------------------|
| Mimosoideae    | <i>Mimosa bimucronata</i> (DC.) Kuntze            | small tree | forest edge, wet conditions | 9.4 g                    |
| Papilionoideae | <i>Desmodium barbatum</i> (L.) Benth.             | herbaceous | grassland                   | 1.16 g                   |
| Papilionoideae | <i>Sesbania virgata</i> (Cav.) Pers.              | small tree | forest edge                 | 74.84 g                  |
| Papilionoideae | <i>Collaea stenophylla</i> (Hook. et Arn.) Benth. | shrub      | grassland                   | 24.7 g                   |

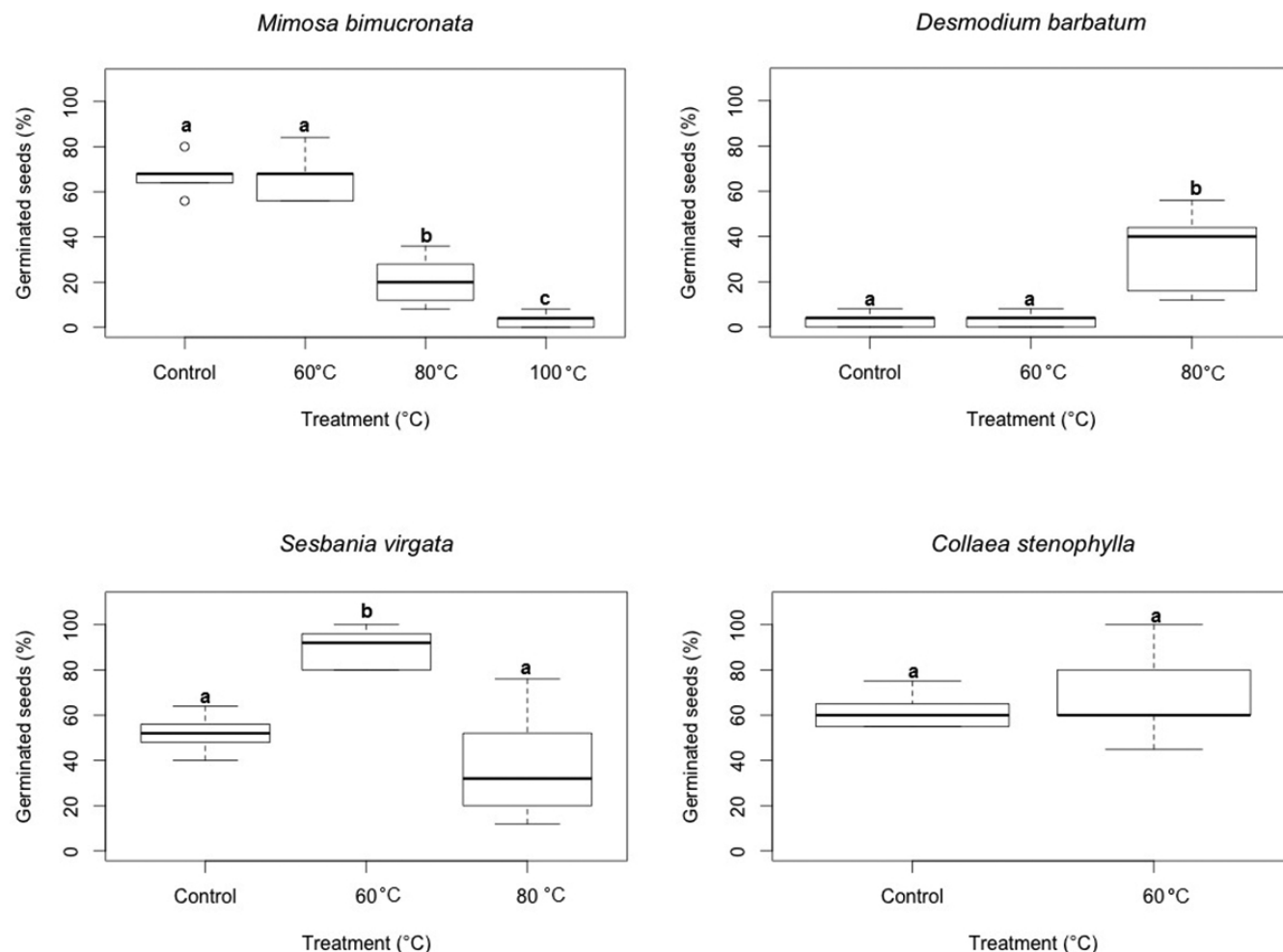
After exposure to heat, seeds were placed into a germination chamber, using Petri dishes with a double layer of filter paper, and kept moist. We followed a light/darkness regime of 12/12 hours. Temperature was kept constant at 25 °C. The Petri dishes were checked for germination every two days, for a period of two months. The experiment was ended when no germination had occurred anymore for a period of two weeks, considering each species separately. Expansion of the radicle was considered as germination and germinated plants were removed from Petri dishes. The percentage of seeds germinated was analyzed by randomization testing (based on Euclidean distance, using 1000 interactions). We used the software MULTIV (Pillar 1997; program and manual available at <http://ecoqua.ecologia.ufrgs.br/ecoqua/MULTIV.html>).

## Results and Discussion

*D. barbatum* showed higher germination rates after exposure to temperatures of 80 °C, and *S. virgata* after 60 °C, but not after 80 °C. Germination of *M. bimucronata* after exposure to temperatures higher than 60 °C was strongly reduced and *C. stenophylla* apparently was not influenced by the treatment. Our results thus indicate, for the first time, a positive impact of heat on germination of legume species from South Brazilian forest-grasslands mosaics (Figure 1). Previous studies by Overbeck et al. (2006b) and Fidelis et al. (2007) had shown that seeds of some species remain their viability and germination capacity after heat shock, but no stimulation of germination had been detected.

Ribeiro et al. (2013), working with five Brazilian savanna species, had observed distinct responses of seeds to heat shock: species from savanna, i.e. a fire-prone vegetation type, were more tolerant to heat than forest species. In our study, different responses of species also may be linked to different environments where species can be found. *M. bimucronata* seems to be intolerant of heat shocks at higher temperature, while one typical grassland species and one forest edge species were stimulated and the other grassland species not affected in germination. This difference between species groups could be explained by evolutionary history in different types of habitats in the grassland-forest mosaic, which likely differ in fire history.

It has been shown before that *Desmodium* species have different levels of dormancy (Dias Filho & Serrão 1984) which could be broken with temperature treatments up to a maximum at 40 °C (Veasey & Martins 1991). However, our study shows that higher temperatures can even increase germination rates of *D. barbatum*. *D. barbatum* as well *C. stenophylla* are typical species of dry and rocky grasslands (Setubal 2010), where fires are frequent (Overbeck et al. 2005). Temperatures as those used in our experience can be reached in the upper soil layers during a grassland burn (Bradstock & Auld 1995), breaking dormancy and allowing for seedling recruitment. Moreno & Oechel (1991) have suggested that resistance to high temperatures should be an important property primarily in obligate seeders, rather than in resprouters that do not generally rely on regeneration from seeds (Herranz et al. 1998). This make sense especially for



**Figure 1.** Total percentage of seeds germinated by treatment. Figures indicate median, 1<sup>st</sup> and 3<sup>rd</sup> quartile, minimum/maximum values and outliers. Different superscript letters indicate significant differences (randomization testing, 1000 interactions).



*D. barbatum*, a species that invests a lot in production of seeds and does not have special belowground organs that would allow for resprouting after fire (field observations).

The reduced germination rates after exposure to temperatures between 80° and 100 °C in *Mimosa bimucronata* probably are a consequence of the ecological history of this species, which is more common on wet sites and along water courses (Carvalho 1994), i.e. sites usually without direct influence of fire – in contrast to the other species. The germination rate after exposure to 60 °C was the same as in control treatment. Because of this we suppose that this species is tolerant to mild high temperature. However, Ribas et al. (1996) working with different methods to break dormancy of this species, found that immersion in water heated up to 80 °C was an efficient treatment. Thus, relations between life history, evolutionary history and germination cues thus need to be investigated in more detail for this species. For the other tree species studied, *S. virgata*, the heat treatment at 60 °C allowed a higher germination rate, but 80 °C did not show an effect in comparison to control. This response may be explained by its insertion into a grassland environment controlled by fire dynamics, in contrast to *M. bimucronata*. As shown by Fidelis et al. (2007), *C. stenophylla* was not stimulated in germination by a temperature of 60 °C, even though germination rates in our study were higher, indicating that seeds of the species are tolerant at least to mild heat.

In summary, the studied Fabaceae species differed in relation to germination rates after heat treatment. Distinct effects of heat between species – from heat-induced germination to no or negative effects – suggest that the ecological history of different species plays a decisive role, which seems an interesting starting point for future studies. As already suggested by Overbeck et al. (2006b), more studies on families with hard-seeded species seem important in order to better understand the role of fire in evolution and dynamics in South Brazilian grasslands. In this, it would be interesting to include studies on life history and recruitment success in the field.

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