DOES FIRE ENHANCE GERMINATION IN LEGUMES IN BRAZILIAN CAMPOS GRASSLANDS?

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INTRODUCTION

Fire is an important factor influencing vegetation dynamics in several ecosystems in the world. Subtropical grasslands from Brazil, also known as Brazilian Campos grasslands, are unique ecosystems, with a very rich flora. Disturbance affects vegetation dynamics and physiognomy, as well as maintains plant diversity. The major disturbances in Brazilian Campos grasslands are grazing and fire (Overbeck *et al.*, 007).

Plant populations are affected by fire in different ways. It can promote population growth, by enhancing both resprouting and germination (Bond & van Wilgen, 1996, Whelan, 1995). In some "flammable ecosystems", species flower and/or even release their seeds only after fire events (Bond & van Wilgen, 1996). Seed establishment is enhanced, since seed dormancy can be broken by the exposure to high temperatures (Hanley & Fenner, 1998; Hanley & Lamont, 2000) or even to smoke (Keith, 1997). Such effect was already described for some species that show physical dormancy due to their hard seeds, e.g. species of Leguminosae (Keith, 1997).

In Brazilian Campos grasslands, the principal regeneration strategy is resprouting. Recruitment of seedlings is rare, but even in fewer amounts, it guarantees genetic variability of plant community (Fidelis 2008). Overbeck *et al.*, (2007) and Fidelis *et al.*, (2007) did not find any evidence that germination was enhanced by seed exposure to high temperatures. However, most of the study species showed no hard seeds, except for Desmanthus tatuhyensis and Collaea stenophylla (both Leguminosae, Fidelis *et al.*, 007).

OBJECTIVES

Therefore, we aim to evaluate the effects of seed exposure to high temperatures (heat shock experiments) of four species of Leguminosae, in order to test if fire would enhance germination in Campos grasslands. We hypothesize that germination rates will be higher at the exposure of higher temperatures, since seed dormancy will be broken.

MATERIAL AND METHODS

Seeds were collected on plant canopy from different populations of four legume species during

Summer on Morro Santana, a granitic hill located in Porto Alegre (southern Brazil, $30^0$03' S, $51^0$07' W; 311 m a.s.l.). Seeds were stored in paper bags (room temperature) during 8 months. Afterwards, they were sorted, weighted and measured before germination experiments. The study species were: Stylosanthes montevidensis Vog (forb), Crotalaria tweediana Benth. (forb), Zornia reticulta Sm. (forb) and Chamaecrista repens (Vogel) H.S.Irwin & Barneby.

For the experiments, seeds were subjected to different heat shock temperatures during 1 minute: 60º, 90º, 120º, and 150ºC. Each species and treatment had 5 replicates (except for Chamaecrista repens, 3 replicates). We used 20 seeds/replicate, except for Stylosanthes montevidensis (25 seeds/replicate) and Chamaecrista repens (15 seeds/replicate). Such differences were related to constraints on available number of seeds. Treatment tried to simulate some range of temperature and time found in Brazilian Campos grasslands by Fidelis *et al.*, (submitted). In these areas, fire spreads very fast, intensities are low and temperature ranges from 61ºC to 319ºC (Fidelis *et al.*, submitted). The chosen temperatures were measured in the field at least for one minute. Heat treatments used a preheated oven, with insertion and removal of replicates in aluminium dishes. Untreated seeds were used as controls. Both treated and untreated seeds were placed in Petri dishes, with three layers of sterilized filter papers. All dishes were moistened with distilled water and put to germinate during 60 days in germination chamber. Since this study aimed to reproduce real conditions from the field, 20º/30ºC temperature, 12/12 hours dark/light conditions were chosen. Seed were kept moist and observations were performed every week. When radicle and/or cotyledons could be observed, they were counted and removed from Petri dishes. Seeds that at the end of the experiment did not germinate were submitted to the tetrazolium test to check if they were still viable. For this, moist seeds were cut, imbibed by 2,3,5 - tiphenyl - tetrazolium chloride (0.075%, BRASIL, 1992), kept at 30ºC during four hours and evaluated later. If embryos were pink, it confirmed that seed was still viable.

Analysis of variance was performed to verify statistical differences between treatments (temperatures) for each study species. Randomization tests were used applied to Euclidean distances (10000 iterations), since there is no restriction about normal distribution of data (for more details, see Manly 2007).

RESULTS AND DISCUSSION

Germination rates of all study species were low. Crotalaria tweediana showed the highest germination rates (ca. 41%), whilst Zornia reticulata had the lowest rates (ca. 5%). In general, the legume seeds did not show significant differences between treatments ($p > 0.05$). The maximum germination rate found was for Crotalaria tweediana at 90º and 120ºC (50%). However, Stylosanthes montevidensis was influenced by high temperatures. Higher germination rates were found at 120ºC ($p \leq 0.05$). All other study species were not affected by higher temperatures: germination rates did not increase. However, high temperatures did not kill seeds.

Several studies showed the effects of exposure to high temperatures on the germination of different plant species. Temperature is known to regulate both dormancy and germination of many species (Finch - Savage& Leubner - Metzger, 2006). Especially hard - seeded species, such as Citus had their germination rates increased after the exposure to temperatures exceeding 100ºC (Hanley & Fenner, 1998). Luna *et al.*, (2007) found maximum germination rates at 80ºC, whilst Hanley & Lamont (2000) demonstrated that at 100ºC, Acacia pulchella showed its maximum germination rates.

Other studies have already showed the effects of heat shock treatments on germination of legume species. Auld & O'Connell (1991) evaluated 35 legume species in Australia and observed that nearly all species had their germination rates increased by heat shock treatments. The highest germination rates were obtained at 80ºC. However, at 120ºC, germination was reduced (Auld & O'Connell, 1991). Gashaw & Michelsen (2002) also observed this trend: increase of germination of legume species exposed to heat shock treatments, however at higher temperatures (120º, 150º and 200ºC) there was a decrease in germination of study species. Such effects can also be related to the time of exposure. Herranz *et al.*, (1998) affirmed that if exposure to higher temperatures is not so long, a positive effect can still be observed.

This effect was not observed in our study, since there was no significant difference between control and temperatures at 120º and 150ºC (except for Stylosanthes montevidensis), which can be related to the time of exposure. Fidelis *et al.*, submitted) observed that residence time of fire (time that fire reached temperatures at least 60ºC) ranged among 5 to 330 s. Therefore, a longer time of exposure can provide different results, even with the increase of germination rates of plant species. Thus, further studies using different times of exposures should be carried out.

Few seeds showed to be not viable. Seeds of Zornia reticulata showed the highest numbers of not viable seeds (22%), whilst all seeds of Stylosanthes montevidensis were viable. 10% of all seeds of Crotalaria tweediana were not viable and only 0.4% of seeds of Chamaecrista repens showed not live embryo. After fire, few species showed seedlings (ca. 10%, Fidelis 2008). However, legume seedlings could be found (pers. obs), emphasizing the importance of seedling recruitment for legume species in Campos grasslands.

Fungi attacked several seeds, since seeds were not treated to prevent fungi before experiments were carried out, in order to simulate natural conditions. 88.7% of all seeds of Stylosanthes montevidensis and 87% of seeds of Chamaecrista repens were destroyed by fungi. Seeds of Crotalaria tweediana showed only 14.6% of damaged seeds, whilst seeds of Zornia reticulata had only 8.25% affected by fungi. Climate in Campos grasslands is subtropical humid, with high precipitation levels distributed all over the year. An excluded area is characterized by a tall grass matrix with few species growing underneath. Fungi can be a factor affecting germination in these ecosystems, since they find the propitious conditions to grow.

Overbeck *et al.*, (2006) and Fidelis *et al.*, (2007) did not find any effect of heat shock treatments on germination rates in Brazilian Campos grasslands. Both authors studied mostly Asteraceae and Poaceae species and had the same results: neither positive nor detrimental effects on germination. They also used short exposure times, emphasizing thus, the importance of further studies with longer periods of exposure to high temperatures.

Low germination rates can be related to several factors. The high death rates of seeds by fungi can be an important factor, since most of seeds were still viable. In addition, other fire related cues can affect directly and indirectly germination, such as charred wood, smoke (Keith, 1997), ash (González - Rabanal & Casal, 1995), and changes in light regime (Bell, 1999). Additionally, according to Luna *et al.*, (2007), resprouters showed reduced germination rates than seeders when exposed to heat shock treatments. All study species are resprouters, showing developed belowground organs. Therefore, the most important regeneration strategy of this species might be resprouting after biomass loss after fire, as observed for most of plant species in Brazilian Campos grasslands by Fidelis (2008). However, the few seedlings that successfully establish and survive can guarantee genetic diversity for these ecosystems (Fidelis, 2008).

CONCLUSION

Although only one species showed to be affected by the exposure to high temperatures, our results show that seeds were not killed by high temperatures. Germination viability was low, showing the necessity of further studies, including other factors such as smoke and addition of ash to evaluate the effects of fire related cues on germination of legume species in Brazilian Campos grasslands. Resprouting is the most important regeneration strategy in these ecosystems. However, germination guarantees genetic diversity and population survival of plant species in Campos grasslands.

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