Seed germination after fire: a study with a plant inhabiting non-fire-prone areas

Germinación de semillas después del fuego: un estudio con una planta que habita áreas no propensas al fuego

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Abstract. After a fire event, germination of seeds with hard coats can be enhanced. In the present study, we investigated whether germination of Mimosa bimucronata (DC.) Kuntze (Fabaceae) seeds, which present hard coats, is increased after fire, even though these trees usually occur in damp areas, where fire is a transient event. Fire events were manipulated in trays filled with soil covered with different amounts of dry grasses, characterizing three treatments: a) 0g (control group); b) 300g, and c) 450g of dry grass. Burning treatments were applied to seeds positioned either on the soil surface or buried in the soil. Germination experiments were carried out at 0-h and 24-h after fire to obtain the germination percentage and the Germination Speed Index (GSI). For buried seeds, the control and the 300g treatments presented significantly higher germination percentages than the 450g treatment. On the other hand, the GSI was significantly higher in the 300g treatment. Significant differences were not found between 0-h and 24-h. Seeds on the soil surface did not survive after burning. Our study showed that temperatures around 100 °C significantly decrease the germinability of M. bimucronata seeds. However, when seeds were buried, the germination percentage did not differ between the control group and the 300g treatment. Results also suggest that temperatures around 60 °C may enhance the velocity of germination of M. bimucronata seeds.

Keywords: Germination; Hard seed coat; High temperatures; Fabaceae.

Resumen. Después del fuego, la germinación de semillas que poseen tegumento duro puede aumentar. En el presente estudio investigamos si la germinación de semillas de Mimosa bimucronata (DC.) Kuntze (Fabaceae), cuyos tegumentos son duros, aumenta después del fuego, a pesar de que estos árboles por lo general son de áreas húmedas, donde el fuego es un evento raro. Los incendios fueron provocados en bandejas llenas de tierra cubierta con cantidades diferentes de pasto seco, caracterizando tres tratamientos: a) 0g (grupo control); b) 300g, y c) 450g de pasto seco. Las semillas fueron localizadas en la superficie o enterradas en el suelo que iba a ser quemado. Los experimentos de germinación fueron realizados en los tiempos 0-h y 24-h después del fuego para obtener los porcentajes y el Índice de Velocidad de Germinación (IVG). Los resultados mostraron que para el grupo control y para el grupo con 300g de pasto seco la germinación de las semillas enterradas fue significativamente mayor que en el grupo con 450g de pasto. Por otra parte, el IVG fue significativamente más alto con el tratamiento de 300g. No se observaron diferencias significativas entre los tiempos 0-h y 24-h. Las semillas colocadas en la superficie del suelo no sobrevivieron a la quema. Nuestro estudio mostró que las temperaturas alrededor de 100 °C disminuyen significativamente la capacidad germinativa de las semillas de M. bimucronata, cuando fueron enterradas el porcentaje de germinación no fue diferente entre el grupo control y el tratamiento con 300g. Los resultados también sugieren que temperaturas alrededor de 60 °C pueden aumentar la velocidad de germinación de las semillas de M. bimucronata.

Palabras clave: Germinación; Semillas con tegumentos duros; Temperaturas elevadas; Fabaceae.

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INTRODUCTION

Seed germination can be triggered by a number of environmental factors and, as such, may constitute an adaptive response (Keeley & Fortheringham, 1997; Vandelook et al., 2008). After environmental perturbations, seed bank germination is crucial for the re-establishment of plant communities due to changes in the aboveground plant biomass. Temperature is widely known as one of these perturbations and plays a crucial role in regulating germination, thus affecting significantly seed dormancy conditions (Silvertown & Charlesworth, 2001; Gurevitch et al., 2002).

Seed dormancy allows plants to germinate under favorable conditions, which is of great selective value. Seed dormancy is also an important strategy against environmental variability because it can promote heterogeneity in germination over time (Bewley & Black, 1994; Li & Foley, 1997; Silvertown & Charlesworth, 2001; Lacerda et al., 2004). Seeds with water-impermeable seed coats usually present an important form of innate primary dormancy (physical dormancy), which is found in many legume species (Baskin & Baskin, 1998; Silvertown & Charlesworth, 2001). This type of physical dormancy can frequently be broken by the passage through the gut of vertebrate dispersal agents (Traveset, 1998; Robertson et al., 2006) or by environmental factors such as precipitation and fire (Garwood, 1983; Baskin & Baskin, 1998; Keeley & Fortheringham, 2000; Auld & Denham, 2006; Morbidoni et al., 2008). When the tegument is water-impermeable, germination of seeds buried in the soil can be facilitated by fire because this barrier is usually broken in response to high temperatures (Brits et al., 1993; Pickup et al., 2003; Bossuyt & Honnay, 2008; Morbidoni et al., 2008).

The degree of seed germination responses to high temperatures will depend on a combination of intensity and duration of exposure to this adverse condition (Keeley & Fortheringham, 2000). Therefore, some studies have been carried out to test the effect of fire on germination in a range of temperatures and exposure times (Herranz et al., 1998; Banda et al., 2006; Vivar-Evans et al., 2006; Herrero et al., 2007). Little is known about seed tolerance to fire for many tree species inhabiting transient fire-prone areas (where fire occasionally occurs). This is because the post-fire germination hypothesis has been mostly investigated in semi-arid areas with a high frequency of fires (Pugnaire & Lozano, 1997). Moreover, many pioneering plants inhabiting transient fire-prone areas are potentially important for habitat restoration. Consequently, understanding the germination responses of their seeds after exposure to high temperatures is relevant.

*Mimosa bimucronata* (DC.) Kuntze (Fabaceae: Mimosoideae) is a perennial tree native to Brazil, Paraguay, Argentina, and Uruguay (Burkart, 1959) that can reach a height of 10 m and presents intense branch ramification. *Mimosa bimucronata* is an important agent for restoration of degraded areas, even though this plant is also a weed growing in dense populations in pasture areas (Lorenzi, 2000). This species occurs predominantly in damp lowlands, floodplains and on the margins of rivers and lakes (Lorenzi, 2000). Although *M. bimucronata* populations commonly occur in damp areas, these plants usually live associated with grasses, which become desiccated during the dry season, when most *M. bimucronata* fruits have already matured (indehiscent, craspedium fruits) and seeds are then dispersed. Therefore, a fire-prone scenario is created during the dry season. It is known that approximately 56% of *M. bimucronata* seeds germinate without any scarification (Kestring et al., 2009). However, scarification methods such as hot water at 80 °C (Ribas et al., 1996) and sulfuric acid application for 10 minutes (Fowler & Carpanezzi, 1998; Tomaz et al., 2007) significantly enhanced the percentage and the velocity of *M. bimucronata* seed germination, indicating that these seeds present hard coats (Kestring et al., 2009). In this study we investigated whether the germinability of *M. bimucronata* seeds (percentage and velocity) is significantly enhanced after exposure to high temperatures created by fire-simulated experiments, even though this species normally occurs in non-fire-prone areas.

MATERIALS AND METHODS

Seed collection. *Mimosa bimucronata* fruits were collected from two populations located within Botucatu municipality in the State of São Paulo, Brazil; the first at the Faculdade de Ciências Agrárias, Unesp/Botucatu Campus (22° 50’ 52” S; 48° 29’ 23” W; =10,000 m²). At both sites, plants are surrounded mainly by grasses. Mature fruits were randomly collected in May 2007, transported to the laboratory, and then kept within white paper bags for one week in an incubator at light/dark (18/6 h) at 25 °C. After this period, fruits from both populations were mixed and organized into a single sample and then manually dissected for the experiments. A total of 2080 mature, not injured seeds were randomly extracted from fruits.

Fire-simulated experiment 1: buried seeds. To assess the first fire-simulated experiment, three metallic trays (aluminum) (35 cm x 25 cm x 5 cm) were used. Each tray was filled with 5.5 kg of oven-sterilized soil (120 °C for 1-h), and seeds were buried at the depth of 2.0 cm. One of the trays had 120 seeds (control group) and the other two had 200 seeds each. Seeds were positioned in the center of each tray, isolated 0.5 cm from each other, so that they were exposed to similar temperatures while grass was burned. After that, grasses were randomly collected from the surroundings of each *M. bimucronata* population to simulate natural conditions. Two areas of 1 m² each, with marked differences in grass densities, were selected in both areas for collection, which determined the different
amounts of plant material used in the experiment. All plant material was oven-dried (120 °C for 1-h) in the laboratory to facilitate grass burning. Three treatments were established, determined by the burning of different quantities of dry plant material previously weighed on a semi-analytical scale: a) 0g (control group); b) 300g, and c) 450g of dry grass. Therefore, one tray had 0g, and the other two had 300g and 450g of dry grass each. Grasses were carefully placed over the trays, covering the entire soil surface.

Trays were taken outside the laboratory (ambient condition) where grasses were burned. Temperatures during fire were recorded at one-second intervals by a thermocouple thermometer (H12-001 Type J) and then transferred to software (Box Car 3.7). In order to record the temperature amplitude to which seeds were in fact exposed during fire, the thermocouple tip (temperature sensitive part) was positioned 2.0 cm below the soil surface, in the central part of each tray. Temperatures were recorded every second for 20 minutes (average time of each fire event). After burning, in the laboratory all seeds were removed from the soil, and germination tests were carried out at 0-h and 24-h after burning. The two distinct moments chosen to start the germination experiments to simulate seed imbibition by rain were then 0-h and 24-h after grass burning. All seeds were placed in transparent Petri dishes (14.5 cm x 1.5 cm) lined with two soaked filter papers (12 mL of distilled water), and kept for 21 days in an incubator at light/dark (18/6 h), 30/20 °C regime (Tomaz et al., 2007). Five replicates of 20 seeds (each Petri dish corresponded to one replicate) were used for each treatment combination, except for the control group, which had three replicates of 20 seeds. The germination percentage of each replicate was evaluated at three-day intervals and germination was defined as growth of 2 mm from the primary root. The Germination Speed Index (GSI), which is the sum of germinated seeds at each evaluation divided by the respective number of days from the start of the experiment (Maguire, 1962), was also calculated for each replicate.

In order to avoid limiting our evaluation to a single fire event per treatment, the entire procedure described above was repeated. Therefore, 240 and 800 seeds were necessary for the control group and for those treated with the burning events, respectively. Two ANOVAs following a split-plot design were run to compare mean germination percentages and the GSI among the different quantities of dry grass burned (0, 300g and 450g), and between the different moments of seed imbibition (0-h and 24-h) (Gotelli & Ellison, 2004). Each analysis was computed considering trays as blocks (two blocks for each quantity of dry grass); blocks had two levels each (two moments of seed imbibition) and the error term was structured as the different moments of seed imbibition nested within blocks (Crawley, 2007). New split-plot ANOVAs were run to compare paired burning treatments for both, the germination percentages and the GSI; in these cases, however, the error structure was defined only with blocks. In order to estimate the variability of the germination percentage and the GSI (raw data), the coefficient of variation (CV) was calculated for each burning treatment by dividing the standard deviation by the mean (Zar, 1999).

All analyses were carried out in the R System for Windows, version 2.7.1 (Crawley, 2007; R Development Core Team, 2008), and the percentage and GSI values were, respectively, arcsin (\(\sqrt{\text{proportion}}\)) and square root transformed; however, mean values computed from the raw data are presented in figures.

**Fire-simulated experiment 2: seeds on the soil surface.** To assess whether *M. bimucronata* seeds survive after a burning event when positioned on the soil surface, all methods described in the section above were repeated. In this experiment, however, seeds were positioned on the soil surface, in the center of each tray. The thermocouple tip was then positioned on the soil surface, in the central part of each tray.

In order to compare temperature fluctuations between both experiments (seeds on the soil and buried), mean temperatures recorded at every minute throughout the burning process were plotted for each quantity of dry grass.

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**RESULTS**

**Temperatures.** Mean temperatures every minute throughout 20 minutes of burning are shown in Figs 1 and 2. When recorded on the soil surface, temperatures were similar between both dry grass treatments during the first minutes of burning (Fig. 1). However, after approximately six minutes, temperatures were much higher in the 450g than in the 300g treatment (Fig. 1). After comparing temperatures taken on the soil surface with those recorded at a depth of 2.0 cm, we verified a great decrease in temperatures when seeds were buried (Figs. 1 and 2); hence, *M. bimucronata* seeds gained considerable protection from the soil. As soil thermal insulation is intimately related to its air concentration, which means that compacted soils are expected to gain more heat per unit of time than loose soils (Baver et al., 1972), the degree of such protection may vary considerably under field conditions.

Considering burning events occurring over buried seeds, the highest recorded temperatures were generally near 60 °C, with a short peak around 70 °C, for the 300g treatment (Fig. 2). However, for the 450g treatment, the highest temperatures recorded were approximately 100 °C (Fig. 2). Therefore, a substantial difference in temperature was observed between the two burning treatments.

**Germination experiments.** As temperatures recorded were extremely high (Fig. 1), seeds on the soil surface did not survive after the burning events, since 0% germination was verified for all replications after 21 days in the incubator. This
Fig. 1. Temperatures taken every minute throughout 20 minutes of burning. Means and standard errors were calculated based on 60 readings in any given minute. Temperatures were recorded on the soil surface during burning of 300g and 450g of dry grass.

Fig. 1. Temperaturas medidas cada minuto durante 20 minutos de quema. Los promedios y errores estándares fueron calculados a partir de 60 mediciones en un minuto. Las temperaturas fueron medidas en la superficie del suelo durante la quema de 300g y 450g de pasto seco.

Fig. 2. Temperatures taken every minute throughout 20 minutes of burning. Means and standard errors were calculated based on 60 readings in any given minute. Temperatures were recorded 2 cm below the soil surface during burning of 300g and 450g of dry grass.

Fig. 2. Temperaturas medidas cada minuto durante 20 minutos de quema. Los promedios y errores estándares fueron calculados a partir de 60 mediciones en un minuto. Las temperaturas fueron medidas a 2 cm de la superficie del suelo durante la quema de 300g y 450g de pasto seco.
result shows that only those *M. bimucronata* seeds buried in the soil may survive, and then germinate, after a burning event.

Results from the split-plot ANOVA showed that the mean values of germination percentages (Table 1) and GSI (Table 2) differed significantly among the different quantities of dry grass. However, no statistical differences were found among the different moments of seed imbibition even when taking the interaction between effects into consideration (Tables 1 and 2).

**Table 1.** Results from ANOVA (split-plot design) comparing mean germination percentages among the different quantities of dry grass burned (0, 300g or 450g), and between the different moments of seed imbibition (0-h and 24-h). The error term was structured as the different moments of seed imbibition nested within trays (blocks). Interaction between effects was also compared.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>Dry grass quantity (1)</td>
<td>2</td>
<td>0.468</td>
<td>0.234</td>
<td>11.599</td>
<td>0.039</td>
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<tr>
<td></td>
<td>Seed imbibition moment (2)</td>
<td>1</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.006</td>
<td>0.943</td>
</tr>
<tr>
<td></td>
<td>Interaction (1) x (2)</td>
<td>2</td>
<td>0.074</td>
<td>0.037</td>
<td>2.162</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Percentage values were arcsin (√proportion) transformed prior to analysis. Los valores de los porcentajes fueron transformados por arcsin (√proporción) antes de analizar los resultados.

**Table 2.** Results from ANOVA (split-plot design) comparing mean values of the Germination Speed Indexes (GSI) among different quantities of dry grass burned (0, 300g or 450g), and between the different moments of seed imbibition (0-h and 24-h). The error term was structured as the different moments of seed imbibition nested within trays (blocks). Interaction between effects was also compared.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSI</td>
<td>Dry grass quantity (1)</td>
<td>2</td>
<td>46.214</td>
<td>23.107</td>
<td>30.271</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Seed imbibition moment (2)</td>
<td>1</td>
<td>0.102</td>
<td>0.102</td>
<td>0.086</td>
<td>0.789</td>
</tr>
<tr>
<td></td>
<td>Interaction (1) x (2)</td>
<td>2</td>
<td>4.310</td>
<td>2.155</td>
<td>1.814</td>
<td>0.305</td>
</tr>
</tbody>
</table>

GSI values were square root transformed prior to analysis. Los valores de IVG fueron transformados por raíz cuadrada antes de analizar los resultados.

**Fig. 3.** Paired comparisons of the mean germination percentages among different quantities of dry grass burned (0, 300g or 450g). Means with different letters differ significantly (p<0.05) by split-plot ANOVA.

**Fig. 4.** Paired comparisons of the mean Germination Speed Index (GSI) among different quantities of dry grass burned (0, 300g or 450g). Means with different letters differ significantly (p<0.05) by split-plot ANOVA.

When mean germination percentage values were compared, the control group did not differ from the 300g treatment (MS = 0.0006; F = 0.020; P = 0.900); but both, the control group (MS = 0.269; F = 29.441; P = 0.032) and the 300g treatment (MS = 0.393; F = 18.589; P = 0.0498), presented significantly higher germination percentages than the 450g treatment (Fig. 3). This shows that temperatures in the 450g treatment were harmful for seeds. It is interesting to note, however, that the GSI from the 300g treatment was significantly greater than...
the control group ($MS = 1.009; F = 18.858; P = 0.0492$) and the 450g treatment ($MS = 3.176; F = 65.684; P = 0.015$). This indicates that in the 300g treatment seeds had fast germination (Fig. 4). The GSI was not significantly different between the control group and the 450g treatment ($MS = 0.290; F = 14.749; P = 0.062$). With respect to data variability, the least variability (CV) was verified in the 300g treatment considering the germination and the GSI data (Fig. 5).

Fig. 5. Coefficients of variation (CV) calculated for the germination percentages and the Germination Speed Indexes (GSI) in the different treatments of dry grass burned (0, 300g or 450g).

**DISCUSSION**

Seed germination of some plant species is increased either after the passage of fire or after treating seeds with high temperatures; this positive response is expected to occur in fire-prone inhabiting species because fire increases the availability of safe sites, which diminishes nutrient competition and enhances seedling establishment (Maret & Wilson, 2000; Jutila & Grace, 2002; Bossuyt & Honnay, 2008; Morbidoni et al., 2008). In addition, heat shock may break seed dormancy of several plant species, promoting a positive effect on seed germinability (Hanley et al., 2001; Luna et al., 2007; Bossuyt & Honnay, 2008). In this study, the germination percentage of heat-treated seeds dropped significantly under the 450g treatment in the fire-simulated experiment, when compared to the control and the 300g treatment. Therefore, high temperatures did not enhance the overall germination of *M. bimucronata* seeds. However, the velocity of seed germination was significantly increased in the 300g dry grass burning treatment.

Although seed germination was not increased by high temperatures, the variability of germination percentage and the GSI data decreased in the 300g treatment (Fig. 5). It is known that the germination capacity (germination and velocity) of chemically scarified *M. bimucronata* seeds is higher and less variable than that on non-scarified seeds (Ribas et al., 1996; Fowler & Carpanezzi, 1998; Tomaz et al., 2007; Kestring et al., 2009). Thus, similar to chemical scarification, reduced variability was verified in the present study after burning 300g of dry grass. *M. bimucronata* seeds present a hard seed coat that constitutes a physical barrier to water absorption (Kestring et al., 2009). Therefore, the most likely mechanism that could explain the higher GSI of seeds exposed to the burning of 300g of dry grass is that temperatures above 60 °C (Fig. 2) would augment seed coat permeability. This would allow water to enter the seed at higher rates than on untreated seeds (Baskin & Baskin, 1998), thus increasing the seed germination velocity with concomitant reductions in overall germination variability.

The GSI and germination percentages were not enhanced in the 450g treatment, suggesting that the temperatures around 100 °C (Fig. 2), lethal to most seeds, must have damaged the embryos but still allowed approximately 28% germination (Fig. 3). Even though the ability of *M. bimucronata* seeds to germinate after exposure to high temperatures may suggest fire adaptation, this issue should be interpreted with caution. For those plant species inhabiting areas where fire is prevalent, like savannas and some woodland ecosystems (Goldammer, 1993; Schelin et al., 2004), the role of fire may favor selection of those fire-adapted seeds (Clarke & French, 2005). However, given that *M. bimucronata* plants usually grow in damp soils and/or in areas adjacent to watercourses, fire becomes a transient event, which makes this environmental factor less likely to drive seed selection. Transient fire events around *M. bimucronata* populations are more frequently linked to anthropogenic disturbances than to natural causes.

Some studies have shown that the duration of exposure to high temperatures is also an important variable to be considered. For example, Mucunguzi & Oryem-Origa (1996) verified that dry heat treatments triggered germination of *Acacia gerrardii* Benth. and *A. sieberiana* DC. seeds after varying the intensity and duration of exposure. Schelin et al. (2004) also observed that heat treatments had a stimulatory effect on *A. macrostachya* seed germination, and the exposure duration was also important in determining seed germination capacity. In our fire–simulated experiment with buried seeds, it was verified that temperatures were highly variable during the burning process and did not stabilize at a given temperature for more than three minutes (Fig. 2). This variability may be the rule because during a natural fire, temperatures may not be uniform in space and time, and depend on factors such as litter quantity, quality and distribution, and wind (Vivar-Evans et al., 2006). Our results showed that the burning of 450g of dry grass was injurious to *M. bimucronata* seeds due to high temperatures; it was also observed that temperature increased gradually while burning 300g of dry grass. Conversely, temperature
increased rapidly under the 450g treatment, and seeds probably experienced a high heat shock in this case. Thus, the high rate of temperature increase may also have caused deleterious effects on *M. bimucronata* seeds.

The role of fire in stimulating germination of species occurring in fire-prone areas is well documented (Dixon et al., 1995; Roche et al., 1998; Tieu et al., 2001; Schelin et al., 2004) in contrast to the scarce data on the germinative response of plant species native to areas where fire events are uncommon. Therefore, we encourage more germination response studies with plant species that present seeds with hard coats and do not inhabit historically fire-prone sites; interesting results may emerge from these studies.

**CONCLUSIONS**

Our study showed that although temperatures around 100 °C significantly decreased the germinability of *M. bimucronata* seeds when they were buried, germination percentages did not differ between the control group (seeds without heating treatments) and the 300g treatment. Further, the velocity of germination was enhanced after burning 300g of dry grass; therefore, although one may tend to assume that seeds from plants living near damp areas would not withstand high temperatures from fire events, it may not be the rule. This could be the case, for example, for those species presenting hard seed coats, which eventually could take advantage of the burning events.

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