

## Influence of some environmental factors on the seed ecology of *Stryphnodendron obovatum* Benth.

Paulo Roberto de Moura Souza Filho  
Camila Kissmann\*

Universidade Estadual Paulista, Rio Claro, SP, Brazil.

\*Corresponding author: camilakissmann@hotmail.com

### Workshop Information

I Workshop of Plant Biology (I Workshop de Biologia Vegetal) was held in the Bioscience Institute – UNESP, campus of Rio Claro, Brazil, during August 20 and 21, 2012. Workshop was a scientific event organized by Post-graduate students from that Institute aiming to integrate Post-graduate and Graduate students from different areas related to Plant Biology (Anatomy, Ecology, Evolution, Morphology, Physiology, and transitional areas) from different Universities. Workshop Organization offered a large number of speaking activities, scientific discussions, and extra short-courses to improve the knowledge and formation of students in Plant Biology.

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### INTRODUCTION

The Cerrado (Brazilian savanna) is the largest, richest, and possibly most threatened tropical savanna in the world. It comprises a wide variety of physiognomic forms that vary from open grassland to closed-canopy forested savanna and supports a rich flora, with high endemism level (Klink and Machado 2005).

Germination is influenced by intrinsic (seed maturation stage, dormancy and seed age) and environmental factors (water availability, light, temperature) (Bewley and Black 1994). Seeds of most savanna trees exhibit dormancy that consists in barrier that prevents germination even in the presence of otherwise favorable conditions for it. Physical dormancy is the most common type in this environment (Baskin and Baskin 2001). The seed coat impermeability of to water, or hard-seededness, is a common mechanism of dormancy in Leguminosae (Rolston 1978), although it is also found in several other botanical families. In nature, the fire, high or fluctuating temperatures, or biological actions such as gut passage or microorganisms, is the ways by which

physical dormancy can be overcoming (Bewley and Black 1994, Baskin and Baskin 1998).

The regeneration strategies of *cerrado* species depend on a large extent of germination, which in turn is influenced by physiological characteristics of the seeds such as dormancy. With the aim of proving information about how *S. obovatum* seeds overcoming the dormancy in their natural habitat we attempted to simulate biotic and abiotic factors to which they are subjected to and hypothesized that: (1) Physical dormancy of this seeds can be overcoming by action of fungus that were find colonizing seeds in fallen fruits on the ground; (2) Alternate temperatures, simulating the natural fluctuations which occur in Cerrado areas, is effective in alter the *S. obovatum* hard seed coats, allowed water imbibition; (3) High temperatures, as those caused by fire, are able improve its seed germination.

### MATERIAL AND METHODS

Three experiments were conducted simultaneously. The first experiment was conducted with seeds of *S. obovatum* collected from fallen fruits, while the other

two experiments were performed with seeds obtained from fruits collected directly from the tree. Fruits (fallen or not) were collected from six mother plants in a savanna fragment (22° 13' S e 47° 53' W) in Itirapina, SP, Brazil. Seeds were removed manually from the fruits. Only before the experiments II and III, the seeds were surface sterilized in a 10% sodium hypochlorite solution for one minute.

Experiment I. Seeds from fallen fruits were separated in two groups according to visible fungus infestation (characterized by white spots): (1) seeds with low degree of fungus infestation (less than half of the seed) (FI); and (2) seeds with high degree of fungus infestation (more than half of the seed) (FII). Each group of seeds was placed to germinate in acrylic boxes (gerbox) filled with sterilized vermiculite moistened with distilled water.

Experiment II. Seeds were placed in gerboxes filled with wet (wet treatment) or dry vermiculite (dry treatment) and then, placed in BOD chambers regulated at alternating temperature of 10 °C-30 °C (12 hours each temperature) and constant temperature of 45 °C, during 7 days. After these period the gerbox were transferred to a climatized room at 25 °C ± 2.

Experiment III. The exposure of seeds to fire were simulated by placing seeds either an oven at 60°C for 40 minutes or at 100°C for 5 minutes duration (adapted of Schmidt et al. 2005). Immediately after heat treatment, seeds were sown in gerboxes filled with moistened vermiculite and placed in a climatized room at 25°C ± 2 under continuous white light. At the same time, five repetitions of 30 seeds were mechanically scarified (at the side opposite to the embryo using a sandpaper number 120) and placed to germinate in the climatized room at 25°C ± 2 under white light, and was used as a control treatment. In all experiments, the number of germinated seeds was verified each other day during the first month of the experiment, and, subsequently, each 30 days over a period of 430 days. Data were used to calculate the final percentage of germination. For each treatment, in all experiments, it was used 5 replicates with 25 seeds in each one and all the experiments were carried out in a completely randomly design.

The experiments I and III data were submitted to ONE-Way ANOVA and data of experiment II were submitted to a Two-Way ANOVA. Means were compared by Kruskal-Wallis or Tukey test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

Only partially fungi infected seeds presented similar germination than control seeds while seeds that present infestation high degree of fungus exhibited low percentage of germination (Figure 1A). It suggests the

interaction between seed coat and these fungi was not efficient in softening the seed coat. Interestingly, a different response was observed for seeds that were completely or that have the major party of seed coat infected by fungus, which presented low germination percentage.

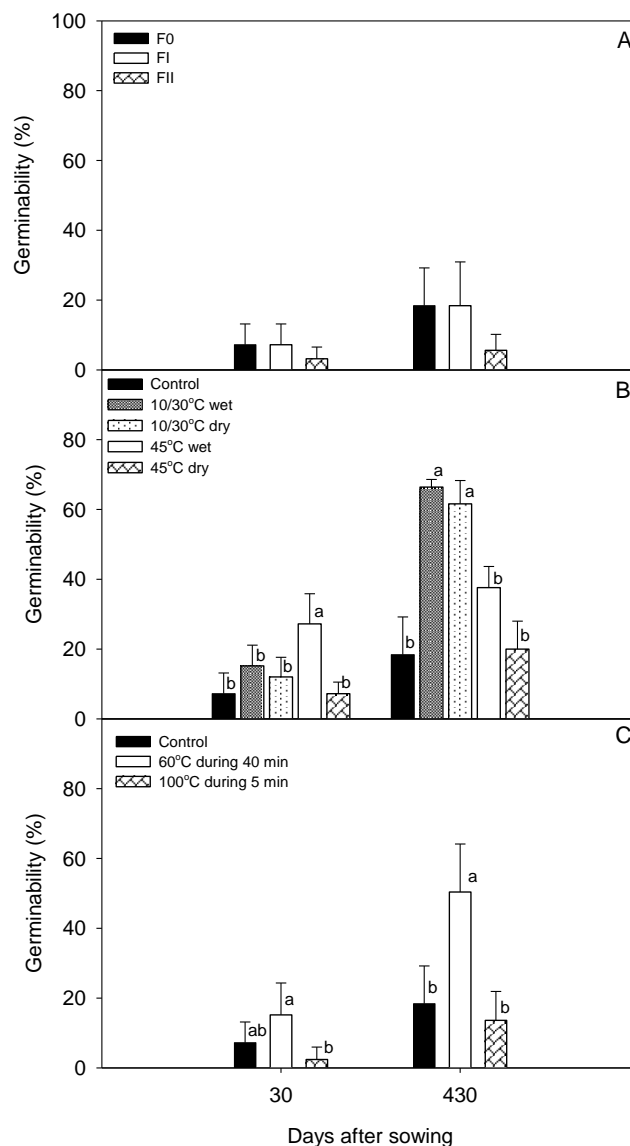


Figure 1. Comparisons of germinability of *S. obovatum* naturally infested by fungus (A) and germinability of un-infected seeds in response to alternate and high constant temperatures for a week (B) and high temperatures for some minutes (C). Absence of letters indicates a lack of significant differences among species and distinct letters indicate significant differences ( $P < 0.05$ ).

One advantage of the physical dormancy is that it provides an effective barrier against microbial access to nutrient-rich seed contents (Dalling et al. 2010). However, as we did not analyze the seed coat or the infected tissues, we cannot conclude if our results are associated with structural changes. These could allow the access to seed embryo and, consequently, loss of viability. Even because not all fungi and bacteria inhabiting seeds are associated with seed death or damage (Gallery et al. 2007).

Both exposures to alternating or high constant temperature had positive effects on germination of *S. obovatum* seeds, being that alternating temperature exhibited the highest germination. High constant temperature of 45 °C induced a faster germination, demonstrated by higher germination percentage at 30 days after sowing. The germination of seeds submitted to alternating temperature was slowly but, after some time, it surpassed the germination of the seeds submitted to high constant temperature (Figure 1B). No difference between wet and dry conditions was observed for seeds submitting to alternating temperature or 45 °C (Figure 1B). The beneficial effects of high temperatures associated to dry or dry-wet conditions in overcoming the physical dormancy was already related many years ago (Baskin and Baskin 1974).

Heat temperature treatments suggested that *S. obovatum* seeds can be benefited by some degree of fire, exhibiting an increasing germination. However, elevated temperatures can cause injury to the seeds (Figure 1C). The responses of seeds to high temperatures depend mainly on the interaction of the temperature and time exposure. Thus, another temperatures during different times need to be tested to determine the tolerance of the species to fire. Besides the considerable amount of studies concerning seed germination of Cerrado species, they usually focused on the responses of seeds to light and/or temperature, methods of dormancy breaking and seed storage in the soil (Zaidan and Carreira 2008) and studies about the germination ecophysiology of cerrado species still are scarce. Although the germination begins few days after start the experiment (data not shown), seeds of *S. obovatum* require long time to express all their germinative potential.

## CONCLUSIONS

Our first hypothesis failed in demonstrated that fungus presented in the seed coat of *S. obovatum* improve seed germination. The exposure of seeds to alternating temperature was highly effective in overcoming physical dormancy of the seeds, resulting in the highest seed germination percentage and thus, validating our second hypothesis. The third hypothesis was partially corroborated, since extremely high temperatures reduced the germination percentage.

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