

IMPROVING SEED GERMINATION OF FOUR SAVANNA-WOODLAND SPECIES: EFFECTS OF FIRE-RELATED CUES AND PROLONGED SOAKING IN SULPHURIC ACID

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DAYAMBA SD, SANTI S & SAVADOGO P. 2014. Improving seed germination of four savanna-woodland species: effects of fire-related cues and prolonged soaking in sulphuric acid. A study was carried out to investigate the effects of fire related cues (heat and smoke) and sulphuric acid treatments on the germination of four woody savanna species, namely, *Terminalia avicennioides*, *Piliostigma thonningii*, *Piliostigma reticulatum* and *Prosopis africana*. The heat treatment consisted of putting seeds in a preheated oven at 100, 150 and 200 °C for 2.5 min. Seeds were then soaked in four concentrations of smoke solution (0 (tap water), 25, 50 and 100% smoke solutions). Parallel to smoke and heat treatment, seeds of the four species were each soaked in concentrated sulphuric acid for 2 hours and also according to the conventional soaking time of each species. Under experimental conditions, heat and smoke did not stimulate seed germination. For *P. thonningii* and *P. africana*, soaking seeds for 2 hours in sulphuric acid significantly increased germination capacity compared with conventional soaking time of 5 min. Longer soaking time reduced mean germination time. Results of this study will be useful in the establishment of these species.

Keywords: Heat, smoke, seed pretreatments, propagation by seeds, restoration, Sudanian savanna, West Africa

DAYAMBA SD, SANTI S & SAVADOGO P. 2014. Menambah baik percambahan biji benih empat spesies hutan cerang savana: kesan petunjuk api serta rendaman lama dalam asid sulfurik. Satu kajian dijalankan untuk menyelidik kesan petunjuk api (haba dan asap) dan asid sulfurik terhadap percambahan empat spesies savana berkayu iaitu *Terminalia avicennioides*, *Piliostigma thonningii*, *Piliostigma reticulatum* dan *Prosopis africana*. Dalam ujian haba, biji benih dimasukkan ke dalam ketuhar pada suhu 100 °C, 150 °C dan 200 °C selama 2.5 min. Biji benih kemudiannya direndam dalam larutan asap berkepekatan 0% (air paip), 25%, 50% dan 100%. Selari dengan ujian asap serta haba ini, biji benih keempat-empat spesies direndam dalam asid sulfurik pekat sama ada selama 2 jam atau mengikut tempoh lazim setiap spesies. Hasil kajian menunjukkan bahawa haba serta asap tidak merangsang percambahan biji benih. Bagi *P. thonningii* dan *P. africana*, rendaman selama 2 jam dalam asid sulfurik meningkatkan keupayaan percambahan dengan signifikan berbanding dengan tempoh rendaman lazim selama 5 min. Tempoh rendaman yang lebih lama mengurangkan min tempoh percambahan. Keputusan ini amat berguna dalam usaha penanaman spesies yang dikaji.

INTRODUCTION

The ability of a forest ecosystem to regenerate is crucial for its sustainable exploitation and conservation. Plant species regenerate either by sexual or asexual means. Germination (regeneration through sexual means) dominates in most savanna species (Ky-Dembele et al. 2007). However, recruitment process is often hindered by low germination rate and some species are only found in the adult stage. To

enhance rapid sustainable management of these savannas, there is a need for the understanding of basic silviculture requirements of plant species. Knowledge on seed germination is important for afforestation success.

In savanna environment, heat and smoke from forest fire trigger post-fire germination of some species (Keeley & Fotheringham 1998, van Staden et al. 2000, Dayamba et al.

2008). The brief exposure of seeds to elevated temperatures (heat shock) typically breaks the seed coat that act as barrier for uptake of water and diffusion of oxygen into the embryo. The physical barrier is broken either by cracking the cuticular layer or opening a strophliolar plug in the seed coat (Bell et al. 1993, Bell 1999). Heat shock may also influence physiological processes within the embryo (Bell & Williams 1998). Smoke acts on the embryo of the seed through a chemical compound known as karrikinolide (Chiwocha et al. 2009), which increases the level of expression of expansins (Jain et al. 2006). Expansins are proteins involved in the degeneration of cell wall during germination.

There are numerous studies of the effect of smoke on germination in other regions (e.g. van Staden et al. 2000, Light et al. 2002, Flematti et al. 2004). However, similar study in the Sudanian savannas of West Africa is scarce. For seeds known to possess physical dormancy, this barrier should be disrupted in some way before the seeds start to germinate. Several pre-sowing treatments such as hot water, sulphuric acid and mechanical scarification have been used and proven successful to overcome seed coat-imposed dormancy (Teketay 1996, Tigabu & Oden 2001). In Burkina Faso, acid treatment is usually recommended for most physically dormant seeds. However, under conventional acid scarification, germination of some species still needs investigation and improvement.

This study was therefore conducted to determine the effects of heat, plant-derived smoke solution and acid on seed germination of some woody species of savanna in order to improve their germination and allow the establishment their plantation on degraded areas. The objectives this study were to determine (1) the concentration of liquid smoke for optimum rate of germination of savanna species, (2) optimum temperature for germination of these species and (3) optimum soaking time of the seeds in acid that can yield good germination.

MATERIALS AND METHODS

Seed sources

Seeds of four woody species, dominant in the study area, all used as fuelwood and some (e.g.

Piliostigma species) as fodder, were considered in the present study. The species were selected because, under conventional seed treatments, they still have low and/or slow germination. Seeds of *Terminalia avicennioides* (Combretaceae) and *Piliostigma thonningii* and *Piliostigma reticulatum* (Caesalpiniaceae) were collected directly from plants at the time of natural dispersal of each species in January–February 2008 in Laba State forest (11° 40' N, 2° 50' W). Seeds were kept in ambient room condition (mean daily minimum and maximum temperatures were 16 and 32°C respectively) until the time of germination tests. Seeds of the fourth species *Prosopis africana* (Mimosaceae) were purchased from the National Tree Seed Centre in Ouagadougou, Burkina Faso. These seeds were originally collected in February 2008 in Kangounadéni (10° 29' N, 4° 37' W) and kept at 18–22 °C and 44% relative humidity.

Seed treatments

Heat treatment

Four levels of heat shock (no heat, incubating seeds at 100, 150 or 200 °C) were used in completely randomised factorial design with four replications, each replicate comprising 25 seeds. Seeds were exposed to heat shock by putting them in a preheated oven for 2.5 min which represented the mean residence time of lethal temperature during fires in savannas (Savadogo et al. 2007, 2012, Dayamba et al. 2010a). The levels of heat shock in this study were considered after taking into account results of other studies (Zida et al. 2005, Dayamba et al. 2008, 2010b) and temperatures observed in the first centimeter below ground during the passage of bushfires (Dayamba et al. 2010a). For each temperature level, seeds of each replicate were placed in an aluminium plate and heated in the oven independently. The oven was allowed to stabilise to the specified temperature before a new set of seeds was placed. This way of heating the seeds avoids problem of pseudoreplication in germination studies (Morrison & Morris 2000).

Smoke solution treatment

Aqueous smoke solution was produced following the procedure described by Dayamba et al. (2010b). A mixture of the fuel from the most

dominant native herbaceous and ligneous species in the Tiogo and Laba State forests was burned in a barrel and the deriving smoke was forced through a pipe to bubble in a canister containing 3 L of water. Native species were used as fuel to simulate natural smoke produced during annual burning in a real savanna environment. A smoking time of 4 hours was chosen to allow trapping of substantial amount of active compound by the water. However, this time is shorter than the time adopted by Dayamba et al. (2010b) to avoid obtaining a high concentration of crude smoke that may have inhibitory effect on germination of some species (Light et al. 2002). Smoke solution obtained at the end of the 4 hours smoking was the stock solution (100% smoke solution) and was then diluted with tap water. Four concentrations of smoke solution were prepared, namely, 0 (tap water), 25 (¼ smoke solution + ¾ tap water), 50 (½ smoke solutions + ½ tap water) and 100% smoke solution (v/v). For smoke treatment, all seeds (heated and control) of each species were soaked in each smoke solution for 24 hours.

Chemical scarification

For acid treatment, seeds of each species were soaked in sulphuric acid following conventional soaking times prescribed by the National Tree Seed Centre, namely, 30 min for *T. avicennioides* and *P. reticulatum*, and 5 min for *P. thonningii* and *P. africana*. Parallel to these conventional times, seeds of the four studied species were soaked in sulphuric acid for a longer time, i.e. 2 hours to try to improve germination. After soaking, seeds were thoroughly rinsed with tap water and soaked for 24 hours in tap water before being transferred to Petri dishes on filter paper for germination tests. The number of repetitions and seeds per replication were the same as those for smoke and heat shock treatment.

Germination test

Seeds were placed in Petri dishes on two layers of filter papers moistened with distilled water. For heated and smoked seeds, germination trial was performed at different temperatures to determine optimum germination conditions (Zida et al. 2005): (1) constant temperature of 25 °C, (2) constant temperature of 35 °C and (3) 30 °C from 6.00 a.m. till 6.00 p.m. and 25 °C from 6.00 p.m. till 6.00 a.m. Germination test

for acid treatment was performed under the last temperature combination.

The germination room was illuminated from 6.00 a.m. till 6.00 p.m. with 20 $\mu\text{Em}^{-2}\text{s}^{-1}$ light from a fluorescent lamp (F40 W/33 RS cool white light) mounted on the germination table to simulate conditions of day and night. Four replications, each with 25 seeds, were used per treatment. The experiment was run for 30 days for all species. Germination was monitored on a daily basis and seed showing a radicle of at least 2 mm long was considered as germinated and was recorded and discarded. Watering was done once or twice daily with tap water to avoid drying of the medium.

Statistical analysis

Germination capacity (GC) and mean germination time (MGT) were calculated for each species and treatment as:

$$\text{GC}(\%) = \left(\frac{\sum n_i}{N} \right) \times 100$$

$$\text{MGT (days)} = \frac{\sum (t_i \times n_i)}{\sum n_i}$$

where n_i = number of seeds germinated each day, N = total number of seeds sown and t_i = number of days starting from the date of sowing (Bewley & Black 1994). Analysis of variance (ANOVA) was performed to determine the significance of the effects of liquid smoke, heat and time of soaking with acid on seed germination. Data set for germination capacity was arcsine-transformed (Zar 1996) in order to improve homogeneity of variance and normality before analysis. When significant differences were found between treatments, a pair-wise comparison was made using Tukey's test at 5% level of significance. Results were considered significant when $p < 0.05$ and showing tendency when $0.05 < p < 0.1$.

RESULTS

Irrespective of the temperature used, seeds of the four species subjected to the different treatment combinations of heat and liquid smoke did not germinate or showed very low germination rate after 30 days of monitoring. However, a cutting test performed on a few seeds of *T. avicennioides* showed that most of them still had viable embryos after 30 days of test.

On the other hand, soaking time of seeds in sulphuric acid had significant effect on germination capacity for *P. thonningii* ($F_{[1,6]} = 26.736, p = 0.002$) and *P. africana* ($F_{[1,6]} = 294, p < 0.001$). Increasing soaking time of seeds in acid increased germination capacity for *P. thonningii* ($94 \pm 1.15\%$ for 2 hours versus $21 \pm 5.51\%$ for 5 min soaking) and *P. africana* ($86 \pm 4.76\%$ for 2 hours versus $2 \pm 1.15\%$ for 5 min soaking) (Figure 1). There was also significant effect of soaking time on mean germination time for *P. thonningii* ($F_{[1,6]} = 81.89, p < 0.001$) while

only a tendency was observed for *P. reticulatum* ($F_{[1,6]} = 5.636, p = 0.055$). Mean germination times for *P. thonningii* were 1.4 and 7.1 days for 2 hours and 5 min soaking respectively while for *P. reticulatum*, 1.3 and 2.1 days for 2 hours and 30 min soaking respectively and for *P. africana*, 2.5 and 3.8 days for 2 hours and 5 min soaking respectively. The effect of increased soaking time of seeds in acid was statistically significant on *P. thonningii* and *P. africana* and their germination capacity increased more than three folds while their mean germination time

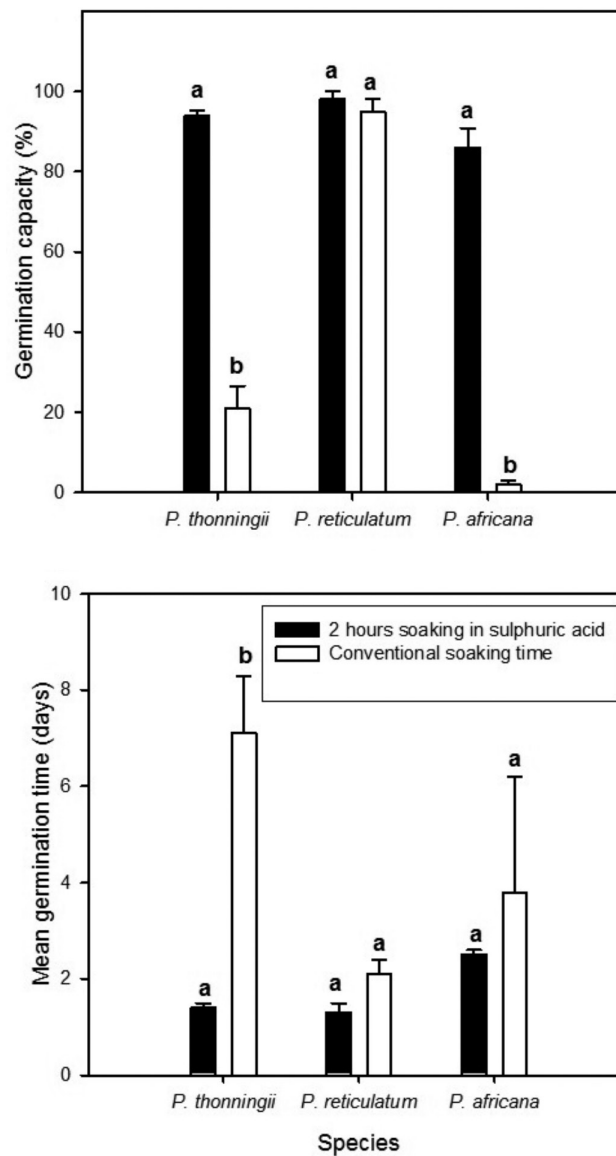


Figure 1 Effect of soaking time in sulphuric acid on germination capacity and mean germination time of three Sudanian savanna species; conventional time = 5 min for *Piliostigma thonningii* and *Prosopis africana* and 30 min for *Piliostigma reticulatum*

was reduced to one third. *Terminalia avicennioides* seeds did not germinate irrespective of soaking treatment.

DISCUSSION

Under conditions of this experiment, smoke and heat did not stimulate germination of the species tested. Probably, heat treatment could not release the seeds from their physical dormancy due to the short heating time. It could also be that heat treatment released the physical dormancy of seeds but induced a temporary heat-induced physiological dormancy (Baskin & Baskin 1998, Dayamba et al. 2010b) that could not be released within the 30 days of germination trial. Since no substantial germination occurred, the effect of varying germination temperatures could not be assessed.

For smoke treatment, particularly concerning *T. avicennioides*, results did not corroborate findings by Dayamba et al. (2008) whereby smoke had positive effect on germination, although the overall germination was low. Dayamba et al. (2008) used aerosol smoke which was more efficient than smoke water (Flematti et al. 2004). Smoke and heat did not have any additive effect. Although seed coat could be cracked by heat, the dose of liquid smoke used might not be sufficient to stimulate germination (Flematti et al. 2004, Dayamba et al. 2008). However, taking all germinated tested species (*P. africana*, *P. thonningii* and *P. reticulatum*) into account, these results were consistent with those obtained by Razanamandranto et al. (2005) and Dayamba et al. (2008) who found that smoke treatment under laboratory conditions was not very efficient in stimulating germination in seeds with physical dormancy such as those tested in the present study. We had hoped that heat treatment would have cracked the seed coat and released the physical dormancy, allowing smoke to have a stimulating effect through action on the embryo. However, heat levels tested did not produce successful results.

Scarification with concentrated sulphuric acid has been widely used to improve germination of several hard-seeded species (Tigabu & Oden 2001, Veasey & de Freitas 2002, de Villiers et al. 2002, Zida et al. 2005). Scarification disrupts the barrier for the uptake of water and oxygen by the seed and permits radicle emergence by weakening the seed coat structure. The disintegration of the seed coat as well as the

micropylar plug is the reason for increased imbibition and subsequent germination of seeds treated with concentrated sulphuric acid (Baskin & Baskin 1998). However, for better use of acid in scarification, an optimum soaking time must be determined to allow substantial removal of the physical barrier without destroying the embryos. In our experiment, all species treated with sulphuric acid, with the exception of *T. avicennioides*, showed better germination capacity after soaking for 2 hours compared with the conventional soaking time of either 5 or 30 min set by the National Tree Seed Centre. These results were consistent with previous studies by Zida et al. (2005) who reported increased rate of germination of some savanna species with increased soaking time in acid. Nevertheless, extended soaking time in acid may disintegrate embryo of the seed and inhibit germination (Baskin & Baskin 1998, Zida et al. 2005). In this study, *T. avicennioides* did not germinate even under conventional soaking time. This species seemed to have a complex dormancy ecology that would need further study. Based on our results, we recommend that for reforestation purpose, seeds of the species *P. africana*, *P. thonningii* and *P. reticulatum* be soaked in concentrated sulphuric acid for 2 hours instead of their conventional times before sowing.

CONCLUSIONS

This study was aimed at assessing how seed germination of savanna species might be improved by subjecting them to fire-related cues, namely, heat and smoke as well as concentrated sulphuric acid. However, neither heat nor smoke stimulated germination of the tested species. Lack of significant effect may well be linked to other factors of dormancy that must be removed prior to treatments using fire-related cues. In addition, seeds of savanna species can also respond to other effects of fire such as increased light conditions, increased nutrients, heat shock or any combination of factors. Soaking seeds in sulphuric acid for 2 hours increased germination capacity and reduced mean germination time for some of the tested species compared with conventional soaking times. Further research is needed to understand germination ecology of savanna species and for better use of treatments (such as sulphuric acid) to release dormancy and assist restoration.

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