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Overcoming dormancy and light requirements in seeds of *Heteropogon contortus,* a target species for savanna restoration

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ABSTRACT

As observed at a world scale, in Reunion Island *Heteropogon contortus* savannas face major pressure and need to be preserved and restored. However, the use of this species in restoration projects is hampered by the limited knowledge about the seed germination ecology and more specifically about dormancy and light requirements. Dormancy state and light requirements for germination of *H. contortus* seeds were assessed over a 3-year after-ripening period (*i.e.* storage). Dormancy loss was observed after one year of storage. Between one and two years of after-ripening, *H. contortus* seeds exhibit significantly higher mean germination percentages in daily light than in darkness, demonstrating their positive photoblasty (*i.e.* requires light for germination). Smoke solutions significantly enhanced germination in both daily light and darkness during the first two years of dry storage. This study also demonstrated that smoke-infused water with beekeeping smoker fuel could be an efficient method to enhance germination of *H. contortus* seeds. Overall, this method could be adopted for large scale restoration projects due to its efficiency, its low cost and its reproducibility.

1. Introduction

Savannas are most commonly found throughout tropical and temperate portions of the world and may cover up to 20% of the global land surface (Grace et al., 2006). Loss of savannas has been globally widespread and is often attributed to changes in land use and in climate, grazing and fire regimes (Scholes and Archer, 1997). These changes have led to the loss of species and genetic diversity as well as altered ecosystem functions (Breshears, 2006; Scholes and Archer, 1997). Ecological restoration could allow reversing land degradation and recovering biodiversity to counter severe human-caused extinctions (Clewell and Aronson, 2013; Wortley et al., 2013). Identifying factors controlling seed germination is essential in order to improve restoration success using native species (Alday et al., 2010; Bochet et al., 2007; Merritt and Dixon, 2011; Oliveira et al., 2013). Limited knowledge about seed dormancy (Alday et al., 2010; Marty and Kettenring, 2017; Oliveira et al., 2013), light requirements which regulate the timing of germination and seedlings survival (Baskin and Baskin, 2014; Pons, 2000), hinders the use of many species targeted for restoration.

Heteropogon contortus (L.) P. Beauv. Ex Roem. & Schult. also known as black speargrass, tanglehead, steekgras (in Afrikaans) and pili (in Hawaiian) is a Poaceae species, especially found throughout dry tropical and subtropical grasslands (Wagner et al., 1999). Among others, this species is one of the targeted species for restoration in several regions in the world including Hawaii (Daehler and Goergen, 2005), China (Peng et al., 2013), South Africa (van den Berg and Zeng, 2006) and Australia (Fensham et al., 2016).

In Reunion Island, savannas dominated by *H. contortus* are increasingly threatened by urbanization, high fire frequencies and introduction of exotic species for cattle forage (Lacoste and Picot, 2014). Several projects were undertaken in the island in order to conserve and/or restore its unique habitats. For instance, restoration trials using grass species was one of the main actions carried out to limit biodiversity loss within dry lowland forests of the West of the island (LIFE + Forêt Sèche, 2014). In this project, restoration trials using *H. contortus* was not successful due to the lack of knowledge in ecology of the species, especially in germination ecology.

Germination strategies of *H. contortus* have only received low interest. A physiological dormancy (Baskin and Baskin, 2004) was observed and is naturally broken within 6–12 months of dry storage (Baldos et al., 2014; Daehler and Goergen, 2005; Tothill, 1977). Dormancy can also be artificially broken by gibberellic acid treatments (1% m/v) (Tothill, 1977). However, due to the high cost of this artificial method, studies have focused on faster, easier and reproducible ways to enhance germination. Smoke-infused water derived from burning plant materials has widely been reported as a dormancy breaking tool for several species (Flematti et al., 2011, 2013; Ghebrehiwot et al., 2009; Grace et al., 2016; Nelson et al., 2012; van Staden et al., 1995). These

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include both fire adapted and non-fire adapted species (Flematti et al., 2013) concerning all dormancy classes (Dixon et al., 2009). Recently, Baldos et al. (2015) used different smoke-infused water formulations (xylose, *H. contortus* and food-grade liquid smoke) to enhance *H. contortus* seeds germination stored at 5 °C. However, storing seeds at low temperature could influence physiological processes and cause changes in germination requirements (Baskin and Baskin, 2014). Besides, the results presented in Baldos et al. (2015) came from experiments carried out on seeds after a single storage period. But physiological changes (such as dormancy state) can occur over storage periods (Née et al., 2017; Probert, 2000) suggesting that interpretation of dormancy breaking methods should also involve a time component in order to assess their efficiency.

The light requirements for germination of *H. contortus* remain largely unknown. Nevertheless, some studies suggest that seeds of this species are not positive photoblastic (*i.e.* do not require light for optimal germination) (Grace et al., 2016; Tothill, 1977). However, these studies failed to evaluate the importance of this parameter in germination of seeds of this species. Indeed, in these studies one light condition was associated to one temperature and compared to another light/temperature combination. Such experimental design did not allow testing light as a single factor (Tothill, 1977).

During after-ripening changes can occur in germination barriers such as dormancy (Bewley, 1997; Leubner-Metzger, 2003) as well as light requirements for germination (Zhou et al., 2005). This work aims to assess the effects of storage, light and dormancy breaking treatments on seed germination of *H. contortus*. Our goal is to define cost-effective ways to enhance seed germination of *H. contortus* after different periods of storage. We specifically investigated:

1. Changes in dormancy state of *H. contortus* seeds after different periods of storage;
2. The importance of light for optimal germination of *H. contortus* seeds;
3. The potential of smoke infused water treatments to break dormancy and overcome light requirements for seed germination after different periods of storage.

2. Materials and methods

2.1. Seed collection

Freshly matured seeds were collected and used during the experiments. The seeds were harvested in Reunion Island (South-West of Indian Ocean), in Cap La Houssaye (Saint-Paul) on the West coast of the island (21°01′05″ S; 55°14′12″ E). Pellets (seeds, hulls, and awns) were hand-picked in April 2013, April 2014 and April 2015. After the harvest, seeds from each collection were transported to CIRAD (Centre de Coopération Internationale de Recherche Agronomique pour le Développement) laboratories in the South of the island (21°19′16″ S; 55°29′06″ E), air-dried and stored, in triplicate, in brown paper bag at room temperature. Although germination data were collected for 3 consecutive years (see Appendix A), only results of 2014 collection are further presented, as similar trend was observed for the two other years.

2.2. Smoke water solutions preparation

Before each germination test, beekeeping smoker fuel was used to prepare smoke infused water solutions. The preparation process was similar to the one described by Coons et al. (2014). In their process, the smoker fuel was burned inside a stainless steel bee smoker. The smoke flows from the smoker through a heat-resistant rubber hose. The end of the rubber hose is placed in a 1000-mL side-arm flask containing 300 mL of water. A water aspirator is attached to the side arm of the flask. The vacuum generated by the aspirator is used to help draw the smoke from the smoker into the water. In order to prepare the high volumes needed (4L per preparation), our process was carried out using an aquarium pump instead of running water. During our process, 150 g of smoker fuel (CHARM'COMBUST, Thomas Apiculture) are burnt. The combustion is carried out until total plant material combustion (150 min). After cooling down, the smoke water solution was stored at 5 °C until being used. Between each preparation, all the material was cleaned up in order to retrieve all the smoke residues and ashes. Three diluted solutions (undiluted, 1/2, 1/100 (v/v)) were prepared for each germination test. To detect cyanide compounds in the smoke water solutions, a qualitative colorimetric test was performed using a Cyantesmo test paper (MACHEREY-NAGEL, GmbH & Co. KG). The development of a blue color indicated the presence of cyanide derived compounds in the freshly prepared smoke water solution.

2.3. Pre-sowing treatments

Prior to sowing, caryopses were removed from awns with pliers. In order to break dormancy and to overcome light requirements, seeds were soaked in one of the smoke water solutions (undiluted, 1/2 and 1/100 (v/v)) for 16 h, under continuous stirring to ensure a complete contact of the seed with the solutions.

2.4. Germination tests

For each of the pre-sowing treatments and for control, 5 replicates of 20 seeds were placed in Petri dishes (60 × 15 mm, Greiner Bio-One International GmbH), filled with 15 mL of white pool filter sand which was moisturized until saturation. To ensure limited pathogens growth during the test, the sand was heated to 140 °C for 2 h in a drying oven, cooled down and aseptically poured into each plate. To determine light requirements for seed germination of *H. contortus*, all the germination tests were carried out in 2 light conditions: daily photoperiod (12 h/12 h) and constant darkness (0h/24 h) (Baskin and Baskin, 2014). The darkness was induced by placing the Petri dishes in a black box at the beginning until the end of each test. The tests were carried out at a constant temperature of 25 °C ± 0.3 °C in order to simulate the average annual temperature recorded for savannas of the West of Reunion Island.

Germination for seeds placed in darkness was only checked at the end of each experiment, when seeds placed in light stopped germinating (*i.e.* 8–9 days after sowing). A seed was considered germinated at the emergence of the radicle. Seeds that have completed germination were retrieved from the dishes. During the observation, moisture was also visually checked and adjusted with filtered water if necessary. Each test was ended after 9 days (all treated seeds completed germination within this period). In addition, smoke-water-stimulated germination was compared to GA_3-stimulated germination under dark conditions. In order to check the remnant viability of stored seeds, a tetrazolium test was conducted at the end of this study (38 months after collection).

2.5. Statistical analysis

Germination percentages were analyzed by fitting generalized linear models (GLMs) with a binomial distribution. Binomial distribution is appropriate for proportion-based germination data (Hay et al., 2014). Germination percentages of control seeds placed in daily light were compared after different periods of storage in order to evaluate dormancy changes. Germination percentages of control seeds placed in both light conditions were compared after different periods of storage in order to assess light requirements of *H. contortus* seeds for germination. Germination percentages were then compared among smoke water treatments (control, 1/100, 1/2, undiluted) after different periods of storage for seeds placed in daily light, and the same analysis was conducted for seeds placed in darkness. These two analyses were done in order to assess the effects of smoke treatments on both dormancy and light requirements, and to determine changes of these effects over time.