



Journal of Environmental Analysis and Progress

ISSN: 2525-815X

Journal homepage: www.jeap.ufrpe.br/
<http://dx.doi.org/10.24221/jeap.2.3.2017.1453.258-265>



Rainfall events, high CO₂ concentration, and germination of seeds in Caatinga

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ARTICLE INFO

Received 29 Jun 2017
Accepted 10 Jul 2017
Published 31 Jul 2017

ABSTRACT

In semi-arid environments, the distribution of rainfall over time is decisive in emergence in a soil seed bank. The objective of this study was to evaluate the effect of rainfall events on the soil seed bank emergence of Caatinga species under high CO₂ concentration. The experiment was carried out in an experimental area of Embrapa Semi-arid in a randomized complete block design, with subdivided plots. Each plot consisted of different environmental conditions (open top greenhouse with an injection of 550 ppm CO₂; open top greenhouse and environmental CO₂; natural environment). The subplots consisted of the depth at which the seeds of *Poincianella pyramidalis* and *Myracrodruon urundeuva* were sown (superficially and buried at 0.02 m and 0.06 m depth). Seedling emergence was monitored daily after the first rains. During the experiment, weather data showed a rainfall volume of 83 mm, an average air temperature of 28.7°C, average soil temperature of 35.4 and 34.9°C, at depths of 0.02 and 0.06 m, respectively. Seedling emergence started 56 days after sowing and four days after the first rains. After 154 days of the onset of the experiment, drip irrigation was performed. The greenhouse environment, regardless of the addition of CO₂ or not, allowed higher emergence percentage of *P. pyramidalis*. The seeds of this species sowed on the soil surface only emerged when irrigation started. *M. urundeuva* seeds showed low germination even after irrigation. **Keywords:** Emergence, *Poincianella pyramidalis*, *Myracrodruon urundeuva*, soil seed bank.

Introduction

Caatinga Biome covers an extensive area of the Brazilian Northeast, characterized by a semi-arid climate, with stochastic rainfall events, 300-1000 mm/year concentrated in three to five months during the year. Its main vegetation is tree and shrubs, with specific adaptations to its harsh habitats, such as loss of leaves in the dry period, small leaves, thorns and xerophytic adaptations (Queiroz, 2009). In this ecosystem is found a diversity of vegetal species of considerable economic and biological value, as aroeira-do-sertão (*Myracrodruon urundeuva* Allemão) and

catingueira-verdadeira (*Poincianella pyramidalis* (Tul.) L.P. Queiroz).

Myracrodruon urundeuva is a native species of Brazil, occurring in Northeast, Southeast and Central-West regions, occurring in Caatinga, Cerrado, and Atlantic Forest biomes. It flourishes between June and July, and the fructification is between August and December. This Anacardiaceae is considered one of the native species that presents the most resistant wood because its core practically doesn't rot. This species can be used in reforestation, forage, ornamental and medicinal purposes (Maia, 2012). Due to this characteristic and its medicinal

properties, it has been widely explored and included in the official list of endangered species of Brazilian flora in the vulnerable class, according to the Ministry of the Environment (Brasil, 2008).

Seed germination of *M. urundeuva* occurs over a temperature range of 20 a 35°C (Virgens et al., 2012; Oliveira et al., 2014). However, 40% of freshly harvested seeds germinate at 40°C (Oliveira et al., 2015). Seeds of *M. urundeuva* are more tolerant to osmotic stress induced by PEG 6000 than induced by NaCl with germination thresholds at -0,9 MPa and -0,56 MPa, respectively (Dantas et al., 2014; Oliveira et al., 2014b; Oliveira, 2015).

Poicianaella pyramidalis is an endemic Leguminosae from Caatinga, with extensive use in the Brazilian semi-arid region due to its potential for reforestation, forage, timber, and medicinal. It is a medium-sized tree, of four to 12 meters height with an open and irregular canopy. Its trunk has about 50 cm in diameter, light gray bark, with rhytidome that is detached in elongated and irregular blades. Its flowers are yellow and arranged in racemes. Flowering is from October to February and fructification from December to June. It adapts to the most varied types of soil, mainly stony ones (Siqueira-Filho et al., 2009; Maia, 2012).

This species presents a wide range of environmental tolerance, reaching 10m height when water is available during its development and a shrub size (2 m) when the water supply is restricted (Antunes, 2012). These shrubby-trees are also adapted to different types of soils, including the poorest and stony soils (Maia, 2012), and presents high-density populations throughout Caatinga. Adult plants and seeds of *P. pyramidalis* have very high tolerance to water deficit (Antunes et al., 2011) and saline and sodic environments (Matias et al., 2013).

Soil seed banks represent a stock of regeneration potential and an important component of resilience in plant communities. Soil seed bank also plays a key role in vegetation dynamics in degraded ecosystems and some stressful environments (González-Alday et al., 2009; Bossuyt & Honnay, 2008). Knowledge mechanisms that maintain natural community dynamics may result in an understanding of soil seed banks and its relation to vegetation (Ooi, 2015), which in turn can contribute to improving land management practices (Hopfensperger, 2007). The ability of plant species to produce seeds remaining viable in the soil allows them to overcome temporally unsuitable stressful environmental conditions for germination and establishment, spreading germination risk in time

and conserving population genetic variation in the long term (Bossuyt & Honnay, 2008).

Caatinga soil seed bank presents a high diversity of species (Ferreira et al., 2014; Fabricante et al., 2016; Paz, Silva & Almeida-Cortez, 2016), remaining in the soil during an unfavorable period for emergency (Mamede & Araújo, 2008). However, Caatinga soil seeds bank must complete its life cycle within the short rainy season to maintain a viable population.

Brazilian northeast is, both environmentally and socially, the most vulnerable region to climate change in Brazil (Stocker, 2013). Increasing temperatures up to 4°C and decreases in rainfall events can lead to desertification, implying not only climatic but phytogeographical, economic and social changes (Nóbrega, Santiago & Soares, 2016). However, these variations are not limited only to water deficit and temperature increase, but also to the increase in the atmospheric concentration of carbon dioxide [CO₂] (Araújo et al., 2015).

In recent years several studies have analyzed the potential effects of increased concentrations of greenhouse gases in the atmosphere on seeds, seedlings and adult plants of various native and cultivated species (Dantas et al., 2017; Costa et al., 2017). However, an almost unexplored but important feature of global climate change is changing patterns of rainfall, with likely increases in rainfall variability (Stocker, 2013).

This study aims to evaluate if the [CO₂] in the atmosphere can influence the seedling emergence and recruitment of soil seed banks; how small rainfall events onset seeds germination and emergence of seedlings of two native Caatinga species sowed at different depths of the soil, by simulating different conditions of a soil seed bank of these species at Caatinga.

Material and Methods

Seeds of *P. pyramidalis* were harvested from 17 trees, in the district of Massaroca, at Juazeiro-BA (9°52'09"S, 40°16'42"W, 469 m of altitude), in July 2016. *Myracrodruon urundeuva* seeds were harvested from seven trees in the district of Jutai, at Lagoa Grande, PE (08°33'33"S, 40°12'10"W, 409 m altitude), in September 2016.

The original vegetation of the areas is characterized as hyper xerophilic Caatinga (Amaral & Fernandes, 2007), with shrubs and scattered trees. The soil is yellow dystrophic Oxisol (IBGE, 2006), with hot and dry climate, classified as BSh, according to Köppen, which is, semi-arid with average temperatures above 18°C (SEI, 1998; Alvares et al., 2013).

After harvested, *P. pyramidalis* fruits were dried under a plastic canvas in a shadowed protected environment. Afterward, fruits were beaten and seeds collected. In the laboratory, by visual evaluation, damaged seeds and other impurities (fruit debris, branches, seedlings and other species, among others) were discarded to homogenize and purify the lot (Matias, Oliveira & Dantas, 2014).

Processing of *M. urundeuva* seed was carried out by removal of the branches and wings

(chalice). Then, seeds were submitted to a seed blower to separate the impurities, based on size differences, weight and density (Matias, Oliveira & Dantas, 2014).

For evaluation of the effect of rainfall events and environmental [CO₂] on the soil seed bank, this trial was carried out in the rainy season, between October 25, 2016, and April 20, 2017, at the Experimental Field of Caatinga, Embrapa Semi-Arid, Petrolina-PE.



Figure 1. Experimental field of Caatinga, Embrapa Semi-arid, in Petrolina-PE. A. Overview of the experimental area; B. Aluminium circular structure with PVC rigid film, CO₂ injection hoses placed laterally; C. Seeds sowed on the soil surface and different depths, with different infrared gas analyzer- IRGA sensor at center; D1. Dead; D2. Live *Poincianella pyramidalis* seedlings after 45 days with no rain. Photos: Angelotti, A.; F.; B-C. Silva, F. F. S.; D. Dantas, B. F. (2017).

The experimental design was in randomized blocks, in split-plots. The main plots were the environments in which the seeds were sown, and subplots were sowing depths of each species evaluated. Two blocks with three main plots and three subplots were evaluated, totaling nine treatments for each species.

One hundred seeds of *P. pyramidalis* and 200 of *M. urundeuva* were sown superficially and buried at depths of 0.02 m and 0.06 m in each evaluated environment. The three environments of soil seed banks were characterized as an open-top greenhouse and 550 ppm CO₂ injection; open-top greenhouse and environmental CO₂; natural Caatinga environment (no greenhouse and no CO₂ injection). Open top greenhouses were small free air CO₂ enhancement (Mini-FACE) structures with modifications. The circular aluminum structure of 2.0 m diameter and 1.20 m height was sided by 0.40 mm PVC rigid film.

Two CO₂ injection hoses were placed along the structure circumference at 0.6 and 0.8 m (Figure 1). Infrared gas analyzers (IRGA), positioned at 0.5 m from the soil, monitored [CO₂] and information obtained was sent to a controller which regulated the opening of valves for CO₂ injection in the greenhouses. Pure CO₂ was injected against a blower to ensure a proper mixing and to achieve the required concentration when necessary.

Daily monitoring of emergence and seedling survival was conducted as of the first rainfall event after the trial setup (November 13, 2016).

The evaluation of the condition and viability of non-germinated seeds after rainfall events were made using plastic trays (0.2 x 0.3 x 0.1m) filled with the same soil from the trial site, in which the seeds of the two species were sown at the same depths (0.02 m and 0.06 m) and kept at the natural environment.

After 154 days from the beginning of the trial, drip irrigation was performed to verify the viability of the seeds, of each species, which had not germinated from the soil seed bank.

Soil temperature measurements were performed with sensors connected to the CR1000 data acquisition system (Campbell Scientific Inc., Logan, UT, USA), programmed to perform measurements at every 60 s with averages at each 30 min. The sensors CS107 (Campbell Scientific Inc., Logan, UT, EUA) were inserted into the soil at 0.02 and 0.06 m depth. Rainfall data (mm) was acquired from climatological stations at a 2000m distance from the experimental area.

Results

During six months in which the soil seed bank was evaluated, there was a pluviometric volume of 83 mm, average air temperature of 28.7°C, average soil temperature of 35.4 and 34.9°C, at depths of 0.02 and 0.06 m, respectively (Figure 2).

Twenty days after initiating the trial there were four days of small rainfall events, which accumulated 10.3 mm. During or after these

rainfall events there was no emergence of *P. pyramidalis* seedlings (Figure 2).

The emergence of *P. pyramidalis* seedlings started four days after a second rain event, at 56 days after sowing. On this occasion, a 9.5 mm rainfall event occurred, which resulted in an emergence of 5% of *P. pyramidalis* seedlings of this species in greenhouses with CO₂ injection (Figure 2BC) and 2.5% of seedlings in greenhouses without CO₂ (Figure 2EF) in the treatments in which the seeds were buried.

After this small rainfall event, few seedlings, which were approximately 0.05 m high, survived without additional rainfall for 24 days in greenhouses with CO₂ injection and 40 days in greenhouses without CO₂ injection. In this last condition, a single *P. pyramidalis* seedlings survived until the third rainfall event, 45 days with no rain. (Figure 2F).

In early February, a 17 mm rainfall event occurred, inducing the emergence of additional 6.5% and 4.5% of *P. pyramidalis* seedlings in greenhouse environments with and without CO₂ injection, respectively (Figures 2BCE).

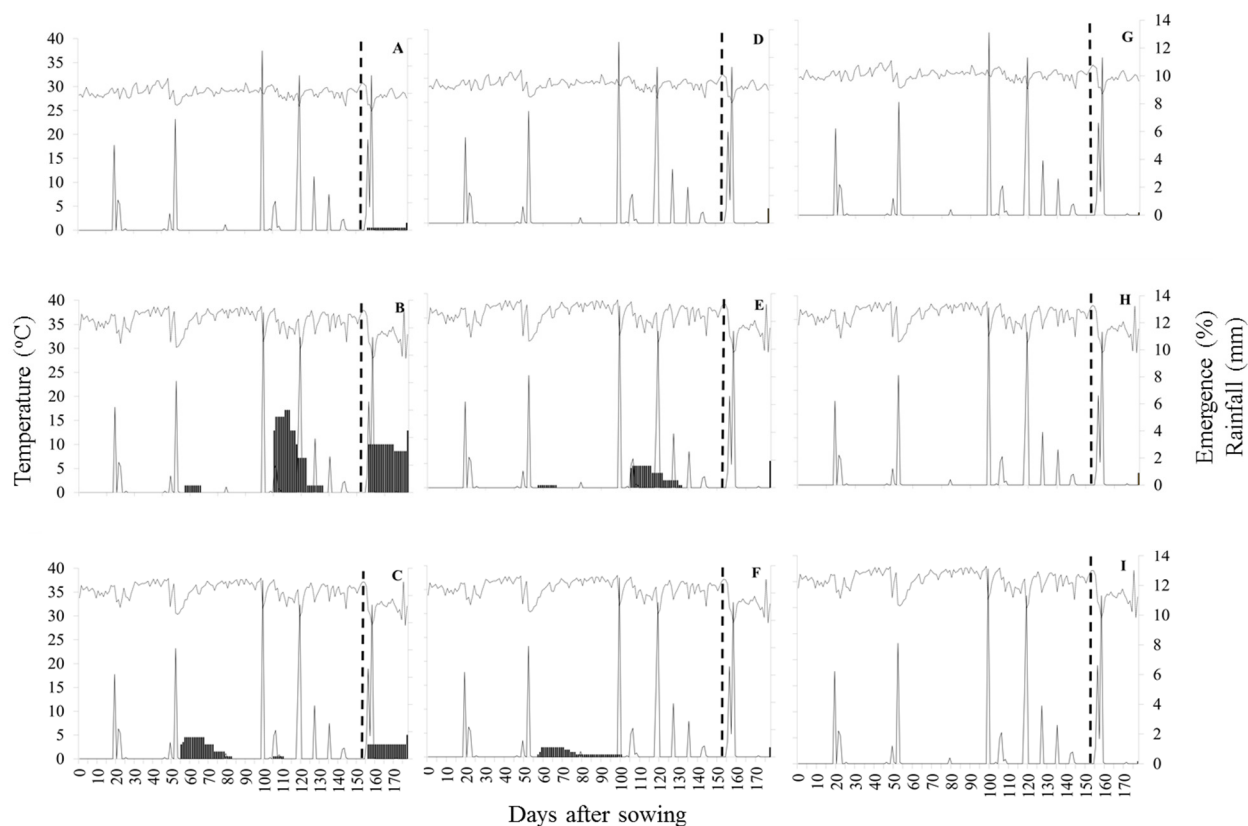


Figure 2. Emergence of *Poincianella pyramidalis* seeds (columns) at soil surface (ADG); buried at 0.02 m (BEH) and buried at 0.06 m (CFI) in open top greenhouse with side plastic and injection of 550 ppm CO₂ (A-C); open top greenhouse and environmental CO₂ (D-F); and natural environment. Dotted lines refer to daily average temperature (°C) at seed depth, and full lines refer to rainfall (mm) from 10/25/2016 to 04/25/2017. Dashed vertical lines indicate irrigation onset. Embrapa Semiárido, Petrolina-PE.

Greenhouses favored the emergence of *P. pyramidalis* seedlings, probably by blocking dry winds and maintaining soil moisture. Seeds of this species when placed on the soil surface only emerged once drip irrigation started, after 154 days of trail setup (Figure 2ADG).

For all evaluated conditions, only 0.5% of *M. urundeuva* seeds emerged (data not shown), even after irrigation. However, when in plastic trays maintained in a natural environment where irrigated at the end of the field trial, we found 5% emergence *M. urundeuva* of seedlings.

Discussion

Establishment of species in the field can be facilitated by adult individuals close to the seedlings, can be hindered by competition with nearby plants (Miranda, Padilla & Pugnaire, 2004). In addition to water availability, interspecific competition seems to be one of the most influential factors in the recruitment of *M. urundeuva* seedlings, since seedlings of this species that emerged during the trial were not established.

Constant rains are usually crucial to establishment of species of fast germination (Vieira et al., 2008), However, this does not always occur in semi-arid regions where environmental stochasticity, which is random variation in environmental conditions (Kreyling, Jentsch & Beierkuhnlein, 2011), is much more pronounced than in other environments (Sala & Laurenroth, 1982).

Caatinga biome presents a remarkable seasonality, with rainy period concentrated between 3-5 months (Maia, 2012). Additionally, within this gap, rainfall is sparse and limited, favorable for rapid germination, but crucial to the success of the seedling establishment. It tends to aggravate in drought years (from 2010 to 2017) and with scenarios predicted for future climates (Marengo, 2014; Marengo, Torres & Alves, 2016).

Highest historical averages of precipitation in the Caatinga region, in Petrolina-PE, are concentrated between November and April. Within this range, the smallest records occurred between the years of 2011-2012, with accumulated rainfall of 114.5 mm and in the period evaluated in this study. In the last 40 years, the average annual precipitation is around 510 mm (Embrapa Semiárido, 2017), approximately 85% more than it rained during the present study.

Low soil moisture retention capacity, high evaporation rates, and evapotranspiration are aggravating the problem of water deficit (Galvêncio & Moura, 2005). This, in turn, can reach up to 70% per year (Marengo et al., 2011). Thus, events of low rainfall (<10mm) can account for up to 80% of

total rainfall during the year (Sala & Laurenroth, 1982). At a time when rains become scarce, due to climate changes, little that rains in rare events manage to promote germination onset, but the recruitment of seedlings is compromised (Ooi, 2015).

Poincianella pyramidalis is endemic from Caatinga biome and is classified as a pioneer, emerging within four days after the first rains (Figure 2). However, seeds of *P. pyramidalis* did not emerge when sown on the soil surface (Figure 2ADG). Direct sowing, with subsequent burial, protects the seed both by the risks of desiccation, as well as predation (Sovo, Tigabu & Odén, 2010). Also, water is not retained in Caatinga soil surface. Thus, it is required cover on *P. pyramidalis* seeds, since at the depths of 0.02 and 0.06 m seedlings emerged after the rains. In Caatinga, dispersion of *P. pyramidalis* fruits occurs in the dry season, a period of greatest deposition of litter (Souza et al., 2014, 2016).

The diversity of species in the Caatinga is maintained through processes that allow perpetuation, such as seed dispersal and its accumulation in the soil, forming a soil seed bank that remains viable for years (Baskin & Baskin, 2014). Soil seed bank is formed from a balance, where seeds enter through various dispersion mechanisms and exit through recruitment (seed germination and the establishment of young plants), seed predation and mortality (Gasparino et al., 2006).

In dry forest areas of southern Spain (Caballero et al., 2003, 2005) and desert areas in China (Wang et al., 2005). The reduction in the number of seeds in the soil is related to death and emergence period of seedlings. The risk of mortality becomes more evident when these seeds are exposed to rainfall in early summer, providing moisture only to germinate and having interrupted water supply in the initial growth phase (Mesgaran et al., 2017). However, some species that occur in Caatinga have post-germinative tolerance to desiccation and can resume its initial growth even after a short period without water as *Handroanthus impetiginosus* (Mart. Ex DC.) Mattos (Vieira et al., 2010).

Studies aiming to determine the minimum water requirement for germination of seeds that present high tolerance to water stress, such as *P. pyramidalis* (Antunes et al., 2011; Matias et al., 2015), are important tools to evaluate the ecological behaviour of these species, such as aspects of pre- and post-germination desiccation tolerance, and recruitment and establishment of seedlings in response to current and future environmental conditions. Therefore, a greater

amount of research should be developed evaluating the germination ecology of Caatinga seeds.

Conclusion

Environment [CO₂] did not influence the germination of *P. pyramidalis* and *M. urundeuva*. Sowing depth influences seeds of these species in the soil seed bank.

Recruitment of *M. urundeuva* seedlings is restrained by both low precipitation and interspecific competition.

Small and stochastic rainfall events, as predicted in future climate scenarios and drought years, does not completely inhibit *P. pyramidalis* seed germination. However, it compromises the seedlings survival.

Acknowledgements

The authors would like to acknowledge the funding for this research by Embrapa (MP3 03.12.12.004.00) and CAPES/Embrapa (Capes-Embrapa – Edital 15/2014 Proposta 171). Graduate scholarships were provided by Coordination for the Improvement of Higher Education Personnel (CAPES) and National Counsel of Technological and Scientific Development (CNPq).

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