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Effect of substrates on the emergence of a Cerrado grass

Efeito de substratos na emergência de uma gramínea do Cerrado

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ABSTRACT

The large-scale production of non-tree species seedlings is an obstacle for advancing the ecological restoration of grasslands and savannas worldwide. We tested different substrates to verify the seedling emergence of the species *Aristida jubata* (Arechav.) Herter - Poaceae. We collected seeds in a "campo sujo" site – a grassland physiognomy of the Cerrado biome in São Paulo state, Brazil. Four substrates were tested in a random blocks experimental design: sand; sand+vermiculite; vermiculite and clayey soil. We used four replicates of 25 seeds –100 seeds per treatment. For statistical analyses we adopted ANOVA followed by Tukey test (p=0.05). We found positive significant effect in vermiculite substrate, where the average emergence reached 70%; the emergence rates in sand+vermiculite, sand and clayey soil tended to lower values.

Keywords: Brazilian savanna, ecological restoration, seedling production, *Aristida jubata* (Arechav) Herter.

RESUMO

A produção de mudas em larga escala de espécies não-arbóreas é um obstáculo para o avanço na restauração ecológica de ecossistemas campestres e savânicos em todo o mundo. Testamos diferentes substratos para verificar a emergência de plântulas da espécie *Aristida jubata* (Arechav.) Herter - Poaceae. Coletamos sementes em uma área de "campo sujo" - fitofisionomia do bioma Cerrado no estado de São Paulo, Brasil. Quatro substratos foram testados, em delineamento experimental de blocos ao acaso: solo argiloso, areia; areia+vermiculita, vermiculita. Usamos quatro repetições de 25 sementes cada - 100 sementes por tratamento. As análises estatísticas foram baseadas em ANOVA, seguida do teste de Tukey (p = 0,05). Encontramos efeito positivo significativo do substrato vermiculita, onde a emergência média chegou a 70%; as taxas de emergência em areia+vermiculita, areia e solo argiloso tenderam a menores valores.

Palavras-chave: produção de mudas, restauração ecológica, savana brasileira, *Aristida jubata* (Arechav) Herter.

INTRODUCTION

The Cerrado is the second largest Brazilian biomein territorial extension. It houses the richest savanna flora in the world, around 12,700 species (Forzza et al. 2012), being the vast majority in the herbaceous layer (The Brazil Flora Group 2015). Due to this high richness of plant species, being a great part of them endemic, and given its high level of threat, the Cerrado is ranked as one of the global *hotspots* for biodiversity conservation (Strassburg et al., 2017). The Cerrado current critical stage of conservation comes from its intense agricultural use and substitution by crop cultures and planted pastures with African grasses. Such grasses have brought another huge ecological problem to the Cerrado: biological invasions (Pivello et al., 1999; Martins et al., 2012; Vieira et al., 2019). Today, huge extensions of the Cerrado are degraded areas and fragments of secondary vegetation in need of ecological restoration (Durigan et al., 2007; Strassburg et al., 2017).

In São Paulo state, few, and small fragments of the Cerrado remain (Durigan and Ratter, 2006), and most of them are invaded by African grasses, represented mainly by species of the genera *Urochloa* and *Melinis* (Matos and Pivello, 2009). It is urgent to find alternatives to promote the ecological restoration of the Cerrado, especially by introducing native herbaceous species (Durigan and Ratter, 2006), a fundamental component of Cerrado grassland and savanna physiognomies (Passaretti et al., 2020). However, there are no efficient technical alternatives to promote the restoration of the herbaceous layer in tropical savannas yet (Buisson et al., 2019), which are being searched by science. An alternative for the herbaceous restoration of the Cerrado is through the planting of seedlings, but it is necessary to find ways to produce native seedlings on a commercially viable basis, allowing high availability for ecological restoration efforts. The existence of knowledge gaps has still hampered the production of Cerrado native species seedlings, especially herbs, to supply restoration projects (Kolb et al., 2016; Oliveira et al., 2020).

Grasses may be used to carry out an initial stage of herbaceous coverage in non-forest ecosystems, as they constitute a large part of its herbaceous diversity and can cover the soil relatively quickly (Chapman, 1996). The genus *Aristida* L. includes about 280 species present in warm temperate and tropical regions of the world, including woodlands, savannas, glades, grasslands, and deserts (Longhi-Wagner, 1990; Freeman, 2001). In Brazil, Longhi-Wagner (2015) reported 39 species and 7 varieties. The main characteristic of the genus *Aristida* L. is the presence of single-flower spikelets associated with tri-lemma awns preceded by a helical column (Longhi-Wagner et al., 2001). Kolb et al. (2016) found the fourth highest percentage of germinated seeds (28.00 to 14.4%) in the *Aristida* genus among seven other Cerrado genera. In studies conducted by Carmona et al. (1998) *Aristida* genus reached 88% of germination, and the seeds also showed to be resistant to storage, keeping the same percentage of germination after 17 months of storage in kraft bags and laboratory conditions. Dairel and Fidelis (2020) report no seed dormancy for this species. Other studies on the germination of *Aristida* L. grasses – most of them carried out in Australia – have focused on their response to different temperatures (Brown, 1987), period of storage and removal of the seed coating (Mott, 1974; Forsyth and Brown, 1982), seed permeability and viability (Mott, 1972), resistance to fire, defoliation and competition (Russell et al., 2013; Strong et al., 2013; Silcock et al., 2018), photoperiod and thermoperiod (Bhatt and Santo, 2018), and showed that *Aristida* seems to be a genus to scale the production of Cerrado grass seedlings, due to its high germination potential, seed storage resistance, perennial life cycle, large production of seeds, relatively abundance in grassland ecosystems, cosmopolitan distribution, and wide occurrence area (Passaretti et al., 2020).

Substrate types also seem to affect the germination and emergence of plants. For example, Pacheco et al. (2006) verified vermiculite as the best substrate for germination of *Astronium urundeuva* (M.Allemão) Engl.; Pagliarini et al. (2014) found superior results for germination of *Hymenaea courbaril* L. in vermiculite compared to sand; Gonçalves et al. (2013) obtained better germination results for *Enterolobium contortisiliquum* (Vell.) Morong on vermiculite-based substrates. On the other hand, Alves et al. (2002) obtained higher germination of *Mimosa caesalpiniifolia* Benth. in sand; Emerson et al. (2003) carried out germination tests on *Phoenix roebelenii* O'Brien in different substrates and found lower germination in vermiculite, having considered an unsuitable substrate for that species. The studies, therefore, are still incipient and species specific.

Under this scenario, the present study aimed to verify the effect of substrates in the emergence of *Aristida jubata* (Arechav.) Herter (Poaceae), a species frequently found in the southern part of Cerrado (Longhi-Wagner, 1990), to support management, conservation, and restoration efforts.

MATERIAL AND METHODS

Seed Harvesting

Seeds of *Aristida jubata* (Arechav.) Herter were harvested in November 2019 in a fragment of Cerrado "campo sujo" at Mairinque (São Paulo state, Brazil; 23°32'14'39"S and 47°11'15'94"W; ALT 894 m). Local soils are classified as dystrophic Red Yellow Argisols and dystrophic Haplic Cambisols, according to the Brazilian System of Soil Classification -SiBCS (Oliveira et al., 1999; Rossi 2017). The climate is Cfa, subtropical with hot summer (Köppen classification), with temperatures over 22 °C and precipitation over 30 mm in the driest month of the year (Alvares et al., 2013). The manual harvesting of seeds was carried out by walking through imaginary transects to cover the maximum of the area, with the samples being collected randomly. The seeds were stored in kraft bags, dried in the shade, and manually processed in the sequence (full and empty spikelets selection).

Experimental Design

We compared the emergency of *Aristida jubata* under the same experimental conditions (source and period of seed collection, management of seeds, temperature, water supply and recipient size), except for the substrate. Four substrates (clay soil, medium sand, medium sand+ vermiculite (1:1), vermiculite) were allocated in 3 litter polystyrene trays (30.3 x 22.1 x 7.5 cm) in a randomized block design with four replicates per block containing 25 seeds in each one.

The experiment was carried out from Nov/11/2019 to Dec/09/2019, and coleoptile emergence was evaluated every three days. The emergence was considered when seedling coleoptile protrusion reached at least 2 mm above substrate surface. The experiment was installed in a shade place protected from rain; water supply was provided through irrigation every three days. During the experiment, the mean, maximum and minimum average temperatures were respectively 22.4 ºC, 23 ºC and 13.8 ºC (National Institute of Meteorology - INMET).

For each replicate, final emergence percentage and mean emergence time (MET) were calculated following Labouriau (1983) (Equation 1): $MET = \sum(nt) / \sum n$, in which: n is the number of emerged seedlings; t is the number of days.

Data Analysis

ANOVA was used for statistical analyses followed by post hoc Tukey test to compare treatments (p=0.05). Percentage of emergence was treated as dependent variable, and substrates as factors. Normality and homogeneity of variance assumptions were checked by using Shapiro-Wilk and Levene tests, respectively. Analyses were carried out in R (R Core Team, 2019).

RESULTS

The percentage of emergence varied among treatments, and the substrate vermiculite showed significant effect when compared to sand and clay soil substrates (Figure 1, Table 1). Besides, the mean emergence time (MET) was similar across substrates (Table 1).

The effect of vermiculite in *A. jubata* seedling emergence was evident since the first evaluation: 56% of emergence 10 days after the beginning of the experiment in vermiculite treatment against 27% in sand and clay soil treatments (Figure 1). Although the mean emergence times in different substrate treatments were similar (Table 1) the emergence curve for sand treatments (sand and sand+vermiculite) took longer to stabilize when compared to the other treatments, as seen in the emergency rates throughout time (Figure 1).

The final emergence rates in the clay soil, sand+vermiculite (1:1) and sand treatments did not show significant difference. Vermiculite – a material suitable for aerating the soil and retaining water and nutrients, in its expanded form is chemically active, biologically inert and has low density (Ugarte et al., 2005) – showed to be the best substrate for the emergency of *Aristida jubata* seedlings when compared to the other substrates tested.

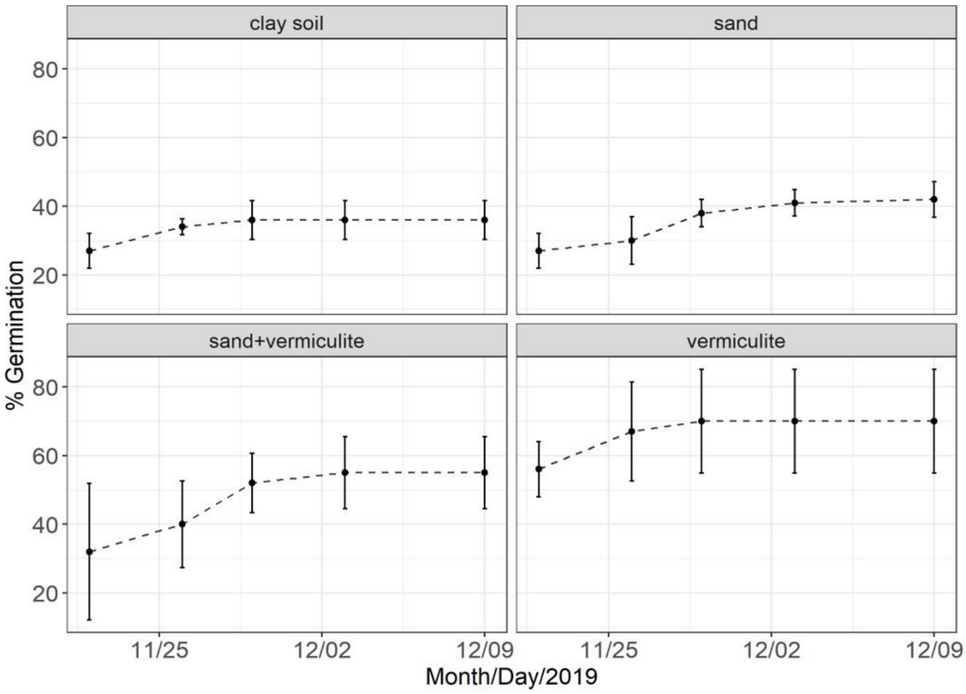

Figure 1. Mean percentage and standard deviation of emerged seeds of *Aristida jubata* (Arechav.) Herter in four substrates.

Table 1. Mean and standard deviation of the final emergence percentage and mean emergence time (MET) of the *Aristida jubata* (Arechav.) Herter in four substrates. Means followed by the same letter are not significantly different according to Tukey test (p>0.05; n=4; 25 seed per replicate).

Substrate	Final Emergence (%)	Standard Deviation (%)	MET (days)	Standard Deviation (days)
Vermiculite	70a	5.2	5.17a	0.84
Sand+vermiculite	55ab	10.5	5.1a	1.97
Sand	42b	5.6	5.2a	1.02
Clay soil	36b	15.1	5.2a	0.84

DISCUSSION

This study brings a contribution to the production of *A. jubata* seedlings, a native Cerrado grass, on a larger scale. In the germination studies of Cerrado shrubs and herb species the germination rates obtained are low and/or very variable according to treatment and species (Carmona et al., 1998; Lima et al., 2014; Musso et al., 2014; Kolb et al., 2016; Paredes et al., 2018). In this study we show a high emergence rate – around 70% – for *A. jubata* in vermiculite substrate and a short mean emergence time, around five days.

The use of fresh seeds may also have been important for obtaining emergence values above 50% in the vermiculite substrate. Moreover, vermiculite has a uniform chemical and granulometric composition, with low density and good water retention capacity (Martins et al., 2012), providing suitable conditions for the germination and development of grasses (Pellizzaro et al., 2017; Vieira et al., 2019). The selection of substrate guarantees better results for germination, emergence, and seedling production (Fanti and Perez, 1999).

Despite the notable resilience of neotropical savannas, that favors their natural regeneration, studies have shown the need for active restoration to reach high plant diversity (Cava et al., 2017). However, studies also point to a lack of knowledge concerning the biodiversity restoration of these ecosystems (Buisson et al., 2019).

Thus, the introduction of lower strata species to accelerate Cerrado restoration and increase its species richness is essential, especially perennial fast-growing herbs, which would provide conditions for restablishing the herbaceous layer structure.

Facing the limited knowledge on the production of Cerrado herbs (Zaidan and Carreira, 2008), further studies are needed in the search for suitable species, adequate substrates, and efficient techniques to restore the Cerrado herbaceous layer on an ecological basis and to conserve the biome biodiversity.

CONCLUSION

The emergence of a native Cerrado grass, *Aristida jubata*, is higher on vermiculite substrate, while no difference was found in sand, sand+vermiculite, and clayey soil. The mean emergence time was not affected by the substrate type.

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