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Germinating Dynamics and Seedling Production of *Cordia trichotoma* (VELL.) Arrabida ex Steudel

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Abstract

Cordia trichotoma is an arboreal species with quick initial growth, also known as *Louro pardo*. The wood of this species is used to make furniture, it seeds have short viability and studies about the production of seedlings are scarce. Thus, the objective of this study was to evaluate seed germination at different temperatures, emergence from different substrates, and the seedling development from seeds with different diameters. To observe germination and seedling development seeds were distributed on filter paper with light and dark conditions at different temperatures. To evaluate emerging seedlings, seeds were distributed into trays with commercial substrate, sand, soil and sand, and soil, sand and dung. We evaluated the percentage and speed of germination and seedling emergence, and at the end of the experiment in the nursery, we determined the length of seedlings, roots, and stems, as well as the dry mass of roots, leaves, and stems. Seed germination on filter paper substrate started on day 3 and lasted until day 16. Regarding the diameter, we observed a significant difference in seed germination in different temperatures in the dark. In nursery conditions, the diameter of the seed also influenced plant emergence and development.

INTRODUCTION

Brazil has a large number of forest species with potential use for anthropogenic purposes and can also be used to recover areas of degraded forest. Therefore, information about initial seed development and production of seedlings is important in adapting methods to recover degraded areas [1], which aim to plant and establish these species in the field. The production of healthy seedlings depends on basic knowledge about the biology of the species, identifying basic needs, and ideas for germination, for seedlings to form and grow. Although there are a significant number of papers on various aspects of native Brazilian species, these works are often sporadic and discontinued, and as a whole, do not provide complete information on the biology of many species. Therefore, seedling production of various species still lacks basic information. Among the many native plants of Brazil, *Cordia trichotoma,* has a high commercial value due to its use in urban tree plantings and wood for construction [2].

This species belongs to the Boraginaceae family, with 130 genera and 2500 species worldwide [3,4], of which nine genera and about 150 species occur in Brazil. Representatives of this family are found both in the tropics as well as the subtropics, temperate, and arctic zones [3]. In Brazil can be found in the Northeast, Central West, Southeast and South, occurring in Brazilian savannah, gallery forests, and caatinga [5].

Many species of the genus have larvicidal activity presenting diterpenes, sequiterpenes, and flavonoids, and, according to folk medicine, have healing, anti-allergenic, anti-inflammatory, and diuretic properties. Due to the various uses and the ability to establish in degraded areas [6], the *Cordia* species can be an important component in the recovery of these disturbed areas.

Cordia trichotoma flowers between February and April, fruit matures between May and July, and after dispersal the seeds are viable for approximately three months, which hinders seed production throughout the year [2].

Considering the high level of species richness in the genus, there are relatively few species with studies that investigate the germination ecophysiology and the ideal conditions for producing seedlings. Studies present results on different storage types [7], seed stratification [8], germination at different temperatures and substrates [7], fruit morphology, seeds and seedling development [6], vegetative propagation [9] and tetrazolium test [10]. However, seed biometrics and their relationship to germination and seedling formation, have been little studied. Moreover, the understanding of seed physiological ecology of a species does not end with the study of a single collection in a given region, as seed production is variable according to conditions of the environment and the parent plant. In fact, to understand seed biology of a species requires a large number of studies, including many collections of seeds from different regions of occurrence.

From a physiological point of view, germination is the emission of the primary root, culminating in the formation of a seedling with all constituent parts [11]. In this process, several factors can affect the germination and formation of seedlings [12,13], and consequently, the seedling production. Among these factors, one can cite the initial seed quality, temperature and light for germination and substrate type for seedling development and Seed quality is influenced by several environmental, genetic and nutritional factors of the plant [11].

In regards to *C. trichotoma,* even though a species with high potential for anthropogenic use, the methods and processes to obtain high germination and quality of seedlings are still unknown. Thus, this study aimed at determining abiotic conditions that promote a high percentage of seed germination for *C. trichotoma*, verify if seed size affects germination and the production of seedlings, and if simple substrates, which can be obtained by producers of seedlings, can be used to produce high quality seedlings.

MATERIALS AND METHODS

The diaspores were collected from 10 individuals in the natural environment of the species occurring in the rural municipality of Aquidauana (19 ° 29'12,2 "at 19 ° 30'49,8 'S and 55 ° 35'28,5" the 55 ° 42'37,9 "W) in Mato Grosso do Sul, Brazil. The collection was carried out considering size, vigor, and health of individuals who were located at a minimum distance of 50 meters from one another.

After collection, the diaspores were placed in paper bags and stored at an average temperature of ± 25°C during the research project. In the laboratory, the collected seeds were manually cleaned, eliminating impurities and damaged or deformed diaspores. Any remaining fruit was also removed from seeds. Then, the weights of 1,000 seeds were determined, using the methodology described in [11], and, using a caliper, the biometrics (length and diameter) of 3,200 seeds were measured. Length was measured from the base of the apex and diameter from the midline of the largest portion. From the knowledge of seed biometrics, the group was subdivided into classes according to diameter.

To evaluate seed germination in different conditions, seeds were evaluated at temperatures of 25°C and 30°C and 20-30°C (photoperiod with alternating temperatures, 12 hours at 20°C without light and 12 hours at 30°C with light) presenting the same moisture condition in all the experiment. Seeds from each class were spread on paper towels moistened with 2.5 times their weight in water [11], and kept in a growth BOD chamber until stabilization of germination. In the no light treatment, paper towel rolls were placed in black polyethylene bags.

Monitoring of germination was done every 24 hours, with germination being considered when seeds presented primary roots greater than 2 mm in length. Counts of normal seedlings were also performed every 24 hours, being considered those with fully expanded cotyledons, undamaged hypocotyl which is proportional to the size of the seedling, and vigorous primary root or a set of vigorous secondary roots. The percentage of germination was calculated 16 days after sowing and germination speed index (GSI) was calculated according to the formula proposed by [14]. The evaluation of germination in the absence of light was conducted in a darkroom under green light. The experimental design was a completely randomized block design, with four replications of 50 seeds for each light regime, dark and diameter class.

During the germination tests, seed morphology and development was described, from radicle protrusion until the formation of a normal seedling, considering the group of seedlings in each condition and the abnormalities that arose.

- Seedling emergence test was conducted in the greenhouse with 50% shade, in which seeds were distributed in trays with 200 cells containing red soil, sand, and chicken manure (ratio of 1:1:1), sand mixture and red earth (1:1), and commercial substrate Tropstrato®. These substrates were chosen to evaluate the emergence and production of seedlings in simple substrates that are prepared and used in plant nurseries. By comparing two mixtures with a commercial substrate, the producer may have alternatives to their commercial substrates. Emergence percentage was calculated daily, seedlings were considered emerging when any part protruded above the substrate surface. The calculation of percentage, average time, speed, and the index of emergence synchronization were done according to formulas city by [15]: Emergencepercentage = n/a.100,

In which: n= # of emerging seedlings; a= total number of seeds sampled

- Average time (t)

$t=\sum ni.ti/ \sum ni$ in which: ni = # of emerging seedlings between observations t-1 and ti

- Average velocity (v)

$V=1/t$

- Synchronization Index (E)

$E=-\sum fi. Log_2 fi$ in which: fi= relative emergence frequency

The emergence observation period was extended until stabilization, which occurred 60 days after sowing. After emergence, plant growth was monitored at intervals of 15 days, measuring the diameter of the stem base using a digital caliper, shoot height (using a scale in centimeters), and counting the number of leaves present on each plant.

At the end of the experiment, seedlings were removed from their containers, the total length of seedlings, roots, and stems, as well as the number of leaves were determined. Vegetative parts were dried in an oven at 70°C for 24 hours to determine dry mass and respective fractions. Root/shoot ratio also calculated. Data was submitted to analysis of variance and means were compared using a Tukey test ($p \leq 0.05$) for different times of observations, percentage data was transformed using arcsine root x +1.

RESULTS

Seed and seedling descriptions

The average weight of 1000 seeds of *C. trichotoma* was 367 grams, indicating that in 1 kg of seeds there are 2,724 seeds.

The seeds had an average length of 8.69 ± 0.62 mm and a mean diameter of 4.49 ± 2.27 mm. Considering the biometrics and the amount of seeds, the group was divided into two diameter classes, 1) less than 4 mm and 2) larger or equal to 4 mm.

The first visual signs of germination start with the swelling of the seed that increases in volume and after the third day after initial sowing the whitish protrusion of the radicle emerges from some seeds, by breaking the integument in the seed apex. On the 9th day, the radicle was observed with 2 cm (Figure 1a). On the 11th day the root already was 4 cm and the cotyledons began to expand until they opened fully, with light green leaves (Figure 1b). On the 19th day the seedling was complete, with primary and secondary roots and a hypocotyl of 3 cm. The pair of cotyledons is dark green with several ribs and had various ribs extending from the base (Figure 1c).

Following criteria established by the Rules for Seed Analysis [11], the normal seedling showed green coloration, well developed shoots with simple and alternated leaves that presented a ribbed tap root system, which is the standard when considering the evaluation of germination. Figure 1

Germination in laboratory

At all temperatures and light conditions evaluated, the emergence of the first root protrusions occurred on the third day after sowing and lasted until the 16th day with germinating stabilizing on the 27th day.

With the presence of light, the percentage of germination and normal seedlings did not differ between classes, at all temperatures (Table 1). In the absence of light, a higher percentage of germination was observed in seeds of class 2, from 20°C and 20-30°C, indicating that the diameter of the seed in this case influenced the germination (Table 1).

The count of normal seedlings was held from the 8th day after the beginning of germination. Not all seeds that released the primary root gave rise to normal seedlings (Table 1).

In the absence of light a smaller percentage of germination was observed at 25°C, in relation to 30 °C and 20-30°C. There was no significant difference in the percentage of seedlings after planting in relation to diameter of the seed, the absence or presence of light, and temperature, although seeds from class 2 had a higher percentage of seedlings at the end of the count, this difference was not significant (Table 1).

The average time, average speed, and synchronization index showed no significant difference between the treatment temperature and the presence or absence of light (Table 2).

Emergence in nursery

Seedling emergence began on the 10th day after sowing and continued until the 28th day. The biggest emergence percentage occurred 24th days after sowing in commercial substrate.

Emergence was higher in class 1 seeds, in all substrates, which influenced the average length and the synchrony recorded for this treatment (Table 3).

The emergence percentage in the commercial substrate was higher than in other substrates (Table 3).

There was no significant difference in the average emergence time and synchronization index between the evaluated treatments. But the emergence rate was higher in commercial substrate and the mixture of soil, sand and dung for the two seed classes. In general terms, the emergence percentage was always

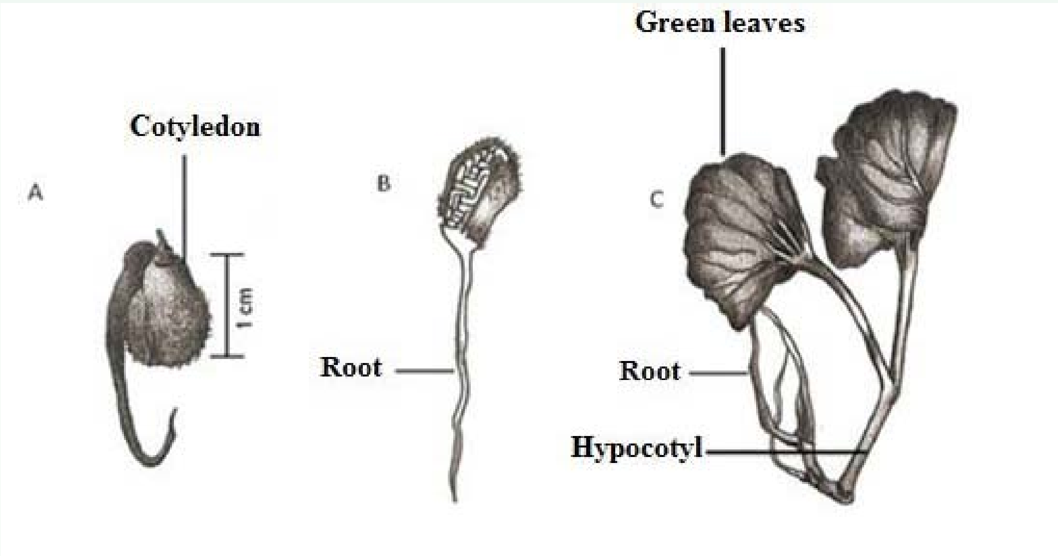

Figure 1 Stages of germination and seedling formation of *C. trichotoma*.1a) radicle was observed with 2 cm; 1b) root already was 4 cm and light green leaves; 1c) seedling was complete, with primary and secondary roots and a hypocotyl of 3 cm (Author of figure: Francielli Bao).

Table 1: Percentage of seed germination and normal seedlings of *C. trichotoma* with different diameters (less than 4mm diameter class1, class 2 equal diameter 4mm) subjected to different temperatures and light condition.

	Percentage of germination			Percentage of normal seedlings		
	Light					
Class	**25ºC**	**30ºC**	**20-30ºC**	**25ºC**	**30ºC**	**20-30ºC**
1	34.5 ± 4.9aA	39.5 ± 4.2aA	34 ± 3.7aA	28 ± 4.5aA	30 ± 4aA	20.5 ± 3.0aA
2	37 ± 6.1aA	44 ± 4.9aA	45.5 ± 4.1aA	33.5 ± 5.3aA	39 ± 4.3aA	38 ± 1.2aA
	Dark					
Class	**25ºC**	**30ºC**	**20-30ºC**	**25ºC**	**30ºC**	**20-30ºC**
1	32 ± 5.3bB	36.5 ± 2.7aA	36 ± 5.4aB	20.5 ± 5.1aA	28 ± 2.1aA	25.5 ± 1.3aA
2	39.5 ± 3.7bA	40.5 ± 3.8bA	55 ± 3.1aA	33 ± 5.7aA	32 ± 2.1aA	37.5 ± 2.8aA

Lower case letter: comparison of means in each row. Upper case: comparison of means in each column (Tukey at 5% probability).

Table 2: Average time, average speed and synchrony index for *C. trichotoma* seedlings arising from seeds with different diameters (less than 4mm diameter class1, class 2 equal diameter 4mm) kept at different temperatures and light condition, and dark.

Seed Class	Treatment	Average time (days)	Average velocity (days^{-1})	Synchronization Index (bits)
1	**25**	5.10a	0.19a	-2.52a
2		4.97a	0.20a	-2.53a
1	**30**	5.29a	0.20a	-2.53a
2		5.18a	0.19a	-2.15a
1	**20-30**	5.98a	0.16a	-2.66a
2		5.11a	0.19a	-2.40a
Dark				
1	**25**	5.53a	0.18a	-2.49a
2		5.00a	0.20a	-2.23a
1	**30**	5.68a	0.17a	-2.62a
2		5.25a	0.19a	-2.30a
1	**20-30**	4.45a	0.22a	-2.22a
2		5.62a	0.17a	-2.41a

a, b, c Tukey's HDS test in relation to Temperature (df = 31,000, α = 0.05)
* Tukey's HDS in relation to size (df = 31,000, α = 0.05)

Table 3: Emergence, average time, average speed and synchrony index for *C. trichotoma* seedlings arising from seeds with different diameters (less than 4mm diameter class1, class 2 equal diameter 4mm) seeded on different substrates.

Seed Class	Treatment	Emergence (%)	Average time (days)	Average velocity (days^{-1})	Synchronization Index (bits)
1	Commercial substrate	34a	15.33a	0.06a	2.88a
2		40a	14a	0.07a	2.18a
1	Sand	9b	15.17a	0.04b	2.72a
2		13b	14.89a	0.04b	2.87a
1	Sand and red earth	8b	20.33a	0.05ab	2.50a
2		11b	19.08a	0.05ab	2.84a
1	Soil, sand, and manure	9b	13.83a	0.07a	2.2a
2		11b	14.4a	0.07a	2.73a

Lower case letter: comparison of means in each column (Tukey at 5% probability).

greater for seeds with larger diameters (class 2).

The monitoring of plant development showed an increasing pattern of height growth so that, after 60 days of planting, plants reached an average of 3.17 cm with three leaves.

Mean root dry mass surpassed the other fractions (leaf and stem) in all treatments and seed classes in the nursery, reflected significantly in the shoot ratio: root, clearly showing a greater investment in training the root system (Table 4). The average final length of plantlets (root and stem) showed a slight advantage for the seedlings from the seed class 2, showing that seed diameter influences the development of seedlings and young plants. Seedlings with larger average size, shoot length, and root, were cultivated in soil, sand, and manure (class 2) and the seedlings that had the lowest average height were grown in soil and sand (class 1).

Table 4: Mean shoot length, root length and number of leaves of seedlings of *C. trichotoma* obtained in different substrates from seeds with different diameters, being class 1 with a diameter smaller than 4mm and class 2 greater equal 4mm.

Seed Class	Treatment	Mean shoot length (cm)	Mean root length (cm)	Number of leaves	Weight (stem) (g)	Weight (roots) (g)	Weight (leaves) (g)
1	Commercial substrate	2.09c	10.23a	2.55c	0.01b	0.07ab	0.02ab
2		3.13c	8.76a	1.97c	0.01b	0.03b	0.01b
1	Sand	1.57b	9.21a	1.54ab	0.01b	0.03b	0.01b
2		1.38b	7a	1.28ab	0.01b	0.03b	0.01b
1	Sand and red earth	2.04bc	8.4a	1.81bc	0.01b	0.03b	0.01b
2		2.75c	6.87a	1.87bc	0.01b	0.04b	0.01b
1	Soil, sand, and manure	4.52a	9.94a	4.54a	0.07a	0.13a	0.13a
2		5a	11.25a	5a	0.06a	0.13a	0.13a

Lower case letter: comparison of means in each column (Tukey at 5% probability).

The average size of the roots was greater than mean stem length in all treatments and different seed classes.

DISCUSSION

Similar biometric measurements were identified by Felippi M et al., in seeds of *C. trichotoma* collected in Rio Grande do Sul, which had an average length of 0.8 - 0.9cm and width of 0.4 - 0.5 cm. However, *C. trichotoma* seeds collected in the valley of the Parana River region, located in the southeast of the State of Tocantins, showed an average size of 0.6 x 0.2 cm (length x width) and weight of 0.04 g [17], while the seeds of this study were collected from the city of Aquidauana-MS, in the Pant anal region were much larger and heavier (average size of 8.69 x 4.49mm and average weight of 0.367g). This variation occurs between seeds collected from conspecifics and often between seeds of the same plant [18]. Size variations of existing seeds of the same plant result from environmental effects during development [19]. In addition, the genetic component may also be related to the distinct difference in average size and weight of seeds collected in the Pant anal and those collected in Tocantins. In fact, the environmental conditions are very different between these two areas of collection, which also influences the formation and quality of seeds.

The formation of the seedling and the knowledge of morphological structures of native seeds and seedlings help in the identification and differentiation of species in the field, facilitating its recognition in the early stages of development [20]. The study of the seedling morphology also has a key role in the interpretation of germination under the Rules for Seed Analysis [21]. Barroso IC et al., Found early primary root emission in *Cordia sellowiana* after 18-32 days and seedlings formed 5 days after the release of the primary root, when the taproot was 2.5 cm long [6]. The same authors found that the onset of *Cordia myxa* germination occurs after 20 to 40 days, three days after the primary root reaches 3 cm and 8 days after sowing the cotyledons reach their final size. *C. trichotoma* seeds in this study germinated faster with primary root emission on the 3rd day after sowing. Felippi M et al., Already reported primary root in *C. trichotoma* between 6 and 10 days after sowing and seedling formation at about 25 days after sowing [16].

As many factors can affect the formation of seedlings, for example, the presence of damage to the embryo and inadequate temperature [12], the primary root emission does not guarantee that the seedling will be formed. In this study, 44% of seeds issued their primary root at 30°C with light, however only 39% formed normal seedlings, and may succeed in establishment in the field.

Despite the lower percentage of germination at 25°C compared with other temperatures, this difference did not persist to the formation of normal seedlings. In fact, germination varies according to the temperature and lower temperatures lead to slower rates of imbibing and emission of the primary root [12,21]. However, this slight delay was eliminated when comparing the percentage of normal seedlings between different temperatures, where there was no statistical difference.

Donadio N et al., Found germination percentages of *C. trichotoma* exceeding 74%, higher values than found in this study, in which the largest percentage was 55%. These authors claim that the best substrate for germination of *C. trichotoma* seed was filter paper. Donadio N et al., found 40% germination and 29% seedling emergence, results slightly lower than the present work [22]. The differences observed in seeds collected in various locations show the influence of climatic conditions, of the soil, genetics and inheritance from mother plant, such as the physiological maturity at the time of collection, on the quality of seeds [16,21].

Observing the percentage of germination and normal seedlings, the average time, average speed, and synchronization index obtained at three temperatures, verifies that the range of 25°C to 30°C and alternating temperatures are suitable for *C. trichotoma* seeds. The germination rates reported by Donadio N et al., indicate that *C. trichotoma* germinates more slowly than other species of the genus; however has higher germination rates for newly harvested seeds. The light did not affect seed germination, which can be considered a neutral photoblastic feature that allows the germination to occur in both clearings with seeds deposited on the ground, and in more confined areas, with the seeds deposited under the litter or even buried.

The substrate serves to provide the seed with an environment in which it can germinate and the plantlet can develop into a new seedling. This has a great influence on the germination process, because factors such as structure, aeration, water holding capacity, and degree of infestation by pathogens, may favor or hinder the germination of seeds [23]. The commercial substrate showed best conditions for germination and seedling emergence, which may be related to greater water retention by the substrate.

However, despite the commercial substrate being the most effective for seedling emergence, for the formation of seedlings the soil substrate, sand, and manure was most efficient with larger shoot length averages, number of leaves, and weight of stem, roots and leaves. In turn, the commercial substrate was inadequate for the formation of seedlings, because the shoot length and number of leaves were the lowest of all evaluations; stem, root, and leaf weight were also lower than plants grown in soil, sand and manure. For the production of seedlings, considering growth and development, the presence of manure in the substrate provided the formation of higher quality seedlings, which presents the best conditions for success in establishing the field. Thus, for efficient production of *C. trichotoma* seedlings, seeds can be distributed in substrates suitable for emergence, and then seedlings can be transplanted into substrate containing manure.

Investment in the root system, in detriment to the aerial part, shows a kind of strategy to ensure a root volume that withstands environments and drier periods. The ability of plantlets to adapt the root architecture, producing deep main roots and high-density secondary roots can allow them to access available water sources after the upper soil layers have dried, allowing them to maintain their growth in periods of low rainfall, often making more competitive plants in natural environments [24]. According to Crawley MJ, Lambers H et al., some individuals may invest more in roots to increase nutrient uptake in environments where the soil resource availability is low [25,26].

To optimize the seedling production, using larger seeds may provide more plantlets which can subsequently be transplanted into the growth environment and develop into seedlings. Seed diameter influenced germination in both the laboratory and the nursery. The seeds of class 2 showed superior germination, seedling emergence, seedling length, and number of leaves in the dark at 25°C. The size of the seed does not always affect the germination itself, but affects the strength of the resulting seedling [27]. According to these authors larger seeds provide more vigorous plantlets that, under adverse field conditions, can result in different stands in favor of larger ones.

CONCLUSION

The diameter of seeds, in both laboratory and nursery conditions, influenced germination, emergence, and plant development, where larger seeds resulted in greater germination, emergence, and plant size. Of the nursery treatments the commercial substrate provided better quality seedlings.

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