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Establishment of *Cryptostegia madagascariensis* in the semiarid: what is the role of abiotic factors in germination and initial growth?

Potencial invasor de *Cryptostegia madagascariensis* no semiárido: qual o papel dos fatores abióticos na germinação e crescimento inicial?

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Studies on seed germination and seedling development in different environmental conditions help to understand the ability of exotic species to colonize new environments. *Cryptostegia madagascariensis* is an exotic invasive species in the semiarid Caatinga Phytogeographical Domain, in the northeastern region of Brazil. In this study, was evaluate how the abiotic factors interfere in germination and the impacts of a water deficit in the initial growth of seedlings. During germination, the seeds were exposed to different temperatures (constant and alternating) combined with two light conditions (white and dark light). In germination, we also evaluated the effects of water and saline stress. In initial growth, the seedlings were submitted to four levels of water availability (100%, 70%, 40% and 10% of field capacity). The milder (20 at 30°C) and alternate temperatures studied provided greater germination four days after sowing, independent of light. High temperatures (35°C) are a limiting factor for germination, as well as water and saline stress (>2 MPa). The water deficit reduces the growth of the seedlings, but these showed morphological adaptations to the different treatments. However, lower levels of water reduced its invading potential. Therefore, factors such as high temperature (> 30° C), saline and water stress, reduce its germination potential and the reduction of water availability affects its growth, but not its survival.
Keywords: Biological invasion, germination of seeds, seedling establishment.

Estudos sobre germinação de sementes e desenvolvimento de plântulas em diferentes condições ambientais ajudam a entender a capacidade de espécies exóticas colonizarem novos ambientes. *Cryptostegia madagascariensis* é uma espécie exótica invasora no domínio fitogeográfico da Caatinga, região semiárida do Nordeste do Brasil. Neste estudo, foi avaliado como os fatores abióticos interferem na germinação e os impactos do déficit hídrico no crescimento inicial de mudas. Durante a germinação, as sementes foram expostas a diferentes temperaturas (constante e alternada), combinadas com duas condições de luz (luz branca e escura). Na germinação, também foram avaliados os efeitos do estresse hídrico e salino. No crescimento inicial, as mudas foram submetidas a quatro níveis de disponibilidade de água (100%, 70%, 40% e 10% da capacidade de campo). As temperaturas mais amenas (20 a 30°C) e alternadas estudadas proporcionaram maior germinação quatro dias após a semeadura, independente da luz. Altas temperaturas (35 °C) são um fator limitante para a germinação, assim como o estresse hídrico e salino (>2 MPa). O déficit hídrico reduz o crescimento das mudas, porém estas apresentaram adaptações morfológicas aos diferentes tratamentos. No entanto, níveis mais baixos de água reduziram seu potencial invasor. Portanto, fatores como alta temperatura (> 30 ° C), estresse salino e hídrico reduzem seu potencial de germinação e a redução da disponibilidade de água afeta seu crescimento, mas não sua sobrevivência.
Palavras-chave: Invasão biológica, germinação de sementes, estabelecimento de plântulas.

1. INTRODUCTION

Invasive species cause impacts at different scales, altering soil properties, reproductive potential and thus causing losses in biological diversity [1, 2]. Therefore, studies are needed to investigate the ecological factors and inherent characteristics of each species that affect the naturalization process in new communities [3]. Thus, it is known that the invasiveness of exotic species may be associated with a number of characteristics, such as reproduction [4], phenotypic plasticity [5], and ability to colonize low resource environments [6].

Studies on seed germination and emergence of seedlings are important tools to help understanding the potential for colonization of exotic species in introduced areas [7], the optimal conditions for germination are generally similar to those required for growth [8]. In addition, seed germination is a key event for the propagation of species and can be regulated by environmental factors such as temperature, light, salinity, and soil moisture [9].

After germination, the success of an invasive species in different environmental conditions is also related to its ability to utilize limited resources efficiently [10]. In arid and semi-arid regions, rains occur irregularly and are alternated by periods of drought [6], making water availability one of the main limiting factors for the establishment of new species in these regions [6, 11]. Thus, in many arid regions exotic species invade areas of higher water availability [12]. Studies regarding invasive plants that occur in riparian or flooded areas of dry regions, show that these species are able adapt to the reduction in water availability [13, 14].

However, for some species the reduction of water availability may have direct effects on invasiveness, as it reduces growth and productivity [15, 16]. Morais e Freitas (2012) [16], showed that moderate water stress did not severely affect the invasive *Acacia longifolia* (Andrews) Willd, but reduces growth and affects the physiological traits, which may limit the invasion of this species in an area of low water availability. In climbing plants, one of the groups that cause the most impacts, invasiveness is associated with rapid growth, so they tend to settle in areas of high humidity, such as riparian areas [17].

Cryptostegia madagascariensis Bojer ex Decne. (Apocynaceae) is a scandent shrub native to Madagascar (Africa), where it occurs in dry forests, disturbed areas, and river banks, growing as a shrub or as a climbing plant [18]. In the northeastern region of Brazil, it has become an invasive species, establishing dense populations mainly in areas near rivers [19]. One of the characteristics of this species is to produce many seeds in a single reproductive event which are dispersed by wind and germinate in great quantity [20]. One of the areas most invaded by *C. madagascariensis* in Brazil are the riverine “carnaubais” [19], areas characterized by high water availability part of the year and the conspicuous presence of the endemic palm C*opernicia prunifera* (Mill.) H. E. Moore, known as carnauba [21].

Therefore, understanding how abiotic factors (e.g., temperature, light, water, and saline stresses) affect germination and how water availability, the most limiting factor for the establishment of seedlings in dry regions [6], interferes with its initial growth may help to understand the characteristics that enable *C. madagascariensis* to invade the Brazilian semiarid regions. Additionally, this will help identify areas most susceptible to invasion and trace control strategies based on the factors that promote their establishment.

Our hypotheses are that: I- *C. madagascariensis* presents high germination rates, tolerating a wide range of temperatures; II- The availability of light increases its germination, as it is a species that invades mainly disturbed areas; III- Reduced water availability negatively affects the germination and its potential for invasion, as higher water availability environments concentrate the invaded areas. Thus, our objectives were to evaluate how abiotic factors interfere in germination and the impacts of a water deficit in the initial growth of seedlings of the invasive *C. madagascariensis*.

2. MATERIALS AND METHODS

2.1 Seed Collection

The seeds used in the experiment were collected at the Experimental Farm Vale do Curu, (latitude 3º49’25” S; longitude 39º20’20” W) which belongs to the Federal University of Ceará (UFC) and is located in Pentecoste County, Ceará State, Brazil. This farm is located in the Brazilian semiarid region with annual rainfall of 772.2 mm (local weather station). After collection, we packed the fruits in plastic bags that were transported to the Seeds Laboratory of the UFC. We collected plant material from this farm and took it to be identified by specialists the Herbarium Prisco Bezerra - EAC UFC and deposited under record number 54608 (EAC).

2.2 Germination

We evaluated germination at different temperatures, light conditions, and under water and saline stresses, through two experiments. In the first, we evaluated germination at different temperatures and light conditions. We used temperatures ranging from 20 to 40°C, constant (20, 25, 30, 35, and 40°C) and alternating (20/25, 20/30 and 20/35°C). Each temperature was combined with two light conditions: white and continuous dark, totaling 16 treatments, in a factorial arrangement in a completely randomized design (8 temperatures × 2 light). Constant temperatures we used 12/12 hours of light and dark, and for alternating temperatures, 12 hours of light corresponding to the highest temperature (day) and 12 hours of dark were used for the lowest temperature (night). In the absence of light, the Petri dishes were wrapped in aluminum foil and packed in a black plastic bag.

In each treatment, four replicates of 25 seeds were used, distributed in Petri dishes containing two sheets of 'germitest' paper as substratum, moistened with 3.5 ml of distilled water. The seeds were placed in germination chambers of the type B.O.D. and evaluations were performed daily for 10 days after sowing. Treatment dark the seeds were evaluated with green light of safety. A protruded radicle was the criterion established for the seeds to be considered germinated. On the tenth day, we calculated the germination (%) and the average germination time [22].

In the second experiment, we simulated water and salt stresses during germination using an osmotic solution of polyethylene glycol (PEG 6000) and sodium chloride (NaCl), respectively. The solution of water stress was prepared according to the methodology described by Michel e Kaufmann (1973) [23], and for the salt stress the van't Hoff equation was used [24]. We used concentrations of -0.2, -0.4, -0.6, -0.8, and -1.0 MPa for each of the stress treatments, besides the concentration of 0.0 referring to control treatment where we used only distilled water.

Thus, in this experiment seeds were submitted to six water stress treatments and six salt stress treatments with four replicates of 25 seeds each treatment. The substrate was moistened with 3.5 ml of the solution corresponding to each treatment. The substrate was moistened with 3.5 ml of the solution corresponding to each treatment and were placed in germination chambers at 25°C with a photoperiod of 12/12 hours of light and dark.

After 15 days of evaluation the seeds that did not germinated in the stresses evaluated were transferred to Petri dishes moistened with distilled water and evaluated for a further 10 days to verify the ability to recovery after a period under stress conditions, according to Gorai et al. (2009) [25]. With the obtained data, we calculated germination (%), mean germination time, and germination recovery (%).

2.3 Initial growth in water deficit

The seedlings for the experiment were produced after sowing in trays containing soil and humus (3: 1 ratio). After 30 days, 160 seedlings were randomly selected and transplanted to 32 cm high pots containing 8 kg of soil, standardized by weighing. The soil used in the experiment was previously dried open air, sieved, and determined the field capacity (FC) through the amount of water retained by the soil after saturation and natural drainage according to Souza et al. (2000) [26]. Water retention in 8 kg of soil was 1.4 L, determined as 100% of the field capacity. A water availability gradient was simulated with four levels: absence of stress (100% of FC), low stress (70% of FC), moderate stress (40% of FC), and severe stress (10% of FC).

The pots were arranged in four replicates, 10 plants for each treatment arranged in a completely randomized design. The seedlings underwent a period of acclimatization, during which for two days they continued to receive 100% of the FC and then started to receive water levels according to each treatment. To maintain the four water levels, eight vessels of each treatment was weighed every other day and the lost volume supplemented. During the experiment, the temperature and relative humidity was monitored by means of a thermohygrometer with a maximum temperature of 37.1 °C and a minimum of 24.2 °C, and a mean ambient relative humidity of 63.5%.

At 75 days, morphological measurements of the plants of each treatment were taken to verify the effects of water stress. Initially the following variables were measured: height, diameter of the stem base, and number of leaves. Then, the plants were removed from the pots using running water and the length of the root was measured and also measured the total leaf area. The plants were divided in leaves, stems, and roots and placed in the drying oven at 80°C for 48 hours to determine the dry mass. With the data obtained, we calculated the specific leaf area (SLA), root / shoot mass ratio (R/S ratio), relative growth rate (RGR) [27], and root specific growth (RSG) [28] for each treatment.

2.4 Data analysis

Analyzed the temperature × light interaction data using a two-way ANOVA. The salt and water stress variables in the germination was analyzed through a simple ANOVA. To use the ANOVA, we tested the normality and homogeneity of the data through the Kolmogorov-Smirnov and Levene tests, respectively.

The germination data when not normal was transformed into sine arc √x / 100. The means were compared by the Tukey test at 5% probability. The effects of water stress on the seedlings were analyzed by ANOVA. Data that were not normal were transformed by log (x +1). The means were compared by the Tukey test at 5% probability. Finally, we performed a regression analysis among the variables related to growth: leaf area, total dry mass, and RGR. All statistical Analyses and graphs was performed using software Sigma Plot 12.00 and Assistat.

3. RESULTS

3.1 Germination

The interaction between temperature and light had a significant effect only on the percentage of germinated seeds ($p < 0.05$). Only temperature significantly affected average germination time ($p < 0.01$). The highest percentages of germination, approximately 90%, occurred at the constant temperatures of 20 and 25°C and at alternating temperatures 20/25, 20/30 and 20/35°C. However, at 35°C, germination was less than 10% and at 40°C there was no germination. The seeds do not depend on light to germinate, although under temperatures of 25 and 30°C and dark continuous caused a reduction in germination (Figure 1a). Was observed that at 25, 20/25, and 20/30°C, most of the seeds germinated until the fourth day. Alternatively, at temperatures of 20, 30, and 35°C, the germination time was seven days (Figure 1b).

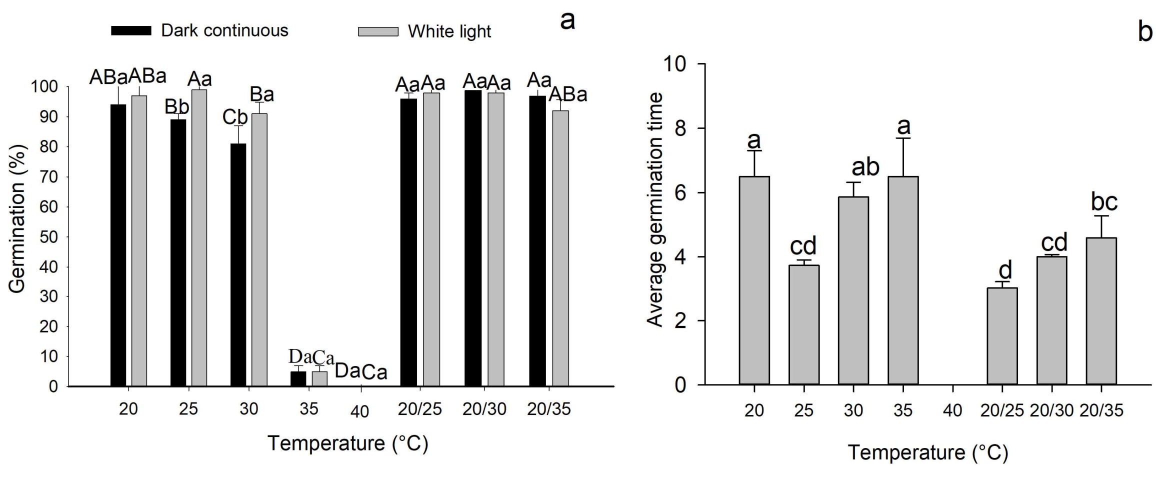

Figure 1: Germination (a) and average germination time (b) of Cryptostegia madagascariensis *submitted to different temperatures and light intensity cycles. Columns represent the averages and bars represent error. a: The temperatures are followed by the same lowercase letter and the same upper case between temperatures indicate no difference by the Tukey test (p<0.05). b: Means followed by the same lowercase letters do not differ by the Tukey test (p<0.05).*

The two stress conditions, water and salt, significantly affected the variables studied in germination ($p < 0.01$). The percentage of germination was not affected until -0.2 MPa. However, in salt stress the germination was completely inhibited at -0.8 MPa (Figure 2a) and in the water stress only at -1.0 MPa (Figure 2b). We also observed that for both types of salt concentration the seeds showed a high recovery capacity after 15 days when transferred to distilled water (Figure 2, a and b).

The germination time was very sensitive to stress, as the addition of salt (PEG 6000 or NaCl) to the substrate increased germination time at the lowest concentration (-0.2 MPa). In the control treatment, germination occurred at four days, while in the presence of salt, germination time was greater than seven days (Figure 2, c and d).

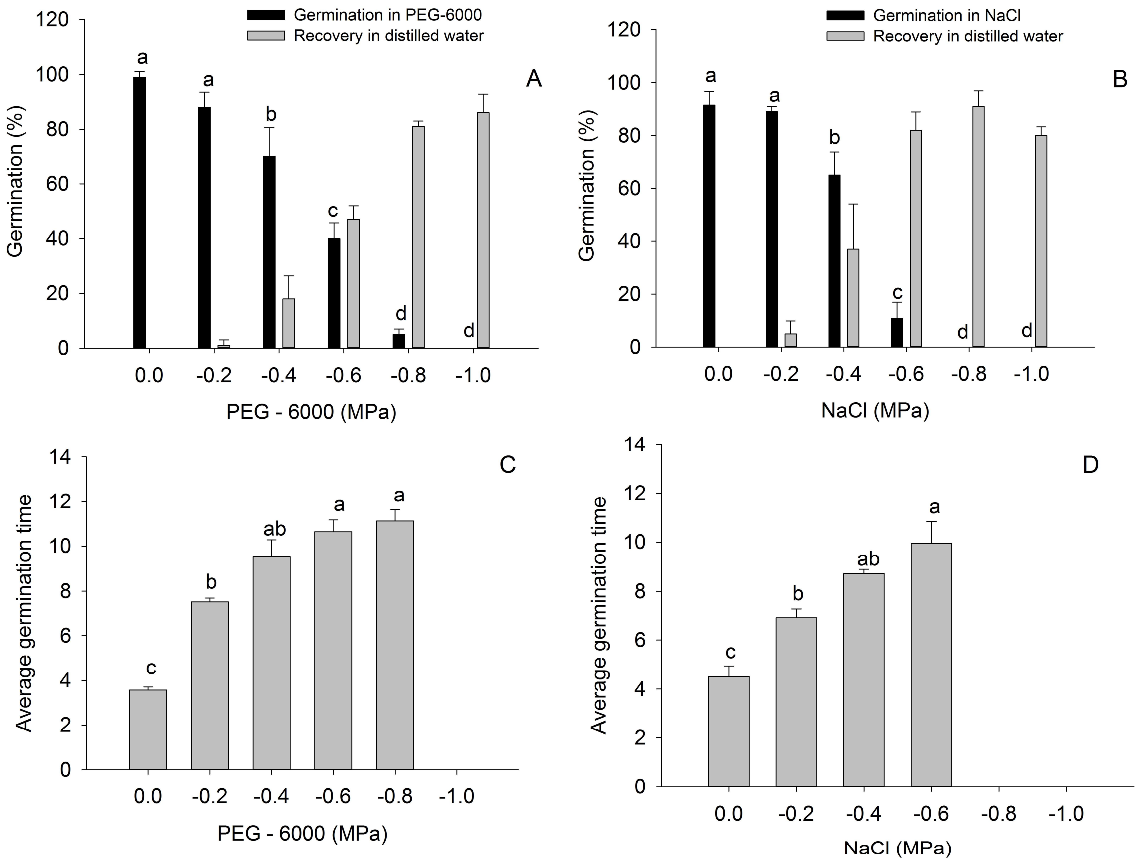

Figure 2: Germination (a and b), recovery (a and b) and average germination time (c and d) of Cryptostegia madagascariensis *submitted to water stress (PEG-6000) and salt (NaCl). Columns represent averages and bars represent error. Means followed by the same lowercase letters do not differ by the Tukey test (p<0.05).*

3.2 Initial growth in water deficit

During the initial growth, none of the seedlings died in the water deficit treatments. The results also showed that the reduction of water availability had a significant effect on all variables studied ($p < 0.05$) except for the root length, which was not affected (Table 1).

Table 1: Averages and F values for morphological characteristics of Cryptostegia madagascariensis *growing at different water levels. RGR- relative growth rate; SLA- specific leaf area; SRG- specific root growth; R/S- mass Root/ shoot ratio; TDM- total dry mass.*

Features	**Stress Level**				
	Absence (100%)	**Low (70%)**	**Moderate (40%)**	**Severe (10%)**	***F* values**
Height (cm)	56.15a	57.02a	57.02a	18.87b	581.64**
Diameter (mm)	4.87a	4.58a	4.61a	2.58b	13.49**
RGR ($g\ g^{-1}$ dia)	0.03a	0.03a	0.02b	0.006c	32.22**
Number of leaf	21.75a	20.16b	19.16b	3.99c	484.01**
Leaf area (cm^2)	360.26a	314.27b	238.07c	73.08d	450.02**
SLA ($cm^2\ g^{-1}$)	236.07b	159.46c	198.47bc	289.94a	10.76**
Root length (cm)	64.62a	58.50a	64.43a	60.63a	3.10^{ns}
SRG (cm g)	43.04b	35.08b	50.29b	90.56a	10.00*
R/S (g)	0.38b	0.42b	0.49b	1.28a	128.97**
TDM (g)	4.71b	5.60a	3.88c	1.18d	218.81**

Means followed by the same letter do not differ by Tukey test. * (p<0.05); ** (p<0.01); ^{ns} Not significant.

Seedling height was affected only by severe stress treatments. During the experiment, plants exposed to low or moderate stress levels reached more than 55 cm in height, and reached less than 19 cm under severe stress. Stem diameter was lower in the high stress treatment group but did not differ between the other treatments. We observed that the RGR of the seedlings was negatively affected in all treatments with water reduction, being lower under severe stress (Table 1). Was observed that the seedlings that received severe stress treatments experienced a loss of leaves. We also found no difference between leaf numbers at low and moderate stress levels. In relation to the leaf area, it was decreasing with the reduction of water availability, resulting from both the formation of smaller leaves and the loss of leaves (Table 1).

The SLA of *C. madagascariensis* was higher in the treatment of severe stress and lower in low and moderate stress. For root investment, we observed that root length was not affected by the availability of water. However, the seedlings that received the severe stress treatment showed a higher RSG and R/S ratio. The total dry mass of seedlings was lower in severe stress and higher in the treatment of low stress (Table 1).

In relation to the biomass allocation to the different organs, we observed that the seedlings that received severe stress allocated a greater proportion of biomass to the roots (50%). Considering the two intermediary stress levels, we found a similar allocation ratio between root and stem. On the other hand, in the absence of stress the largest mass was allocated to the stem, 41% (Figure 3).

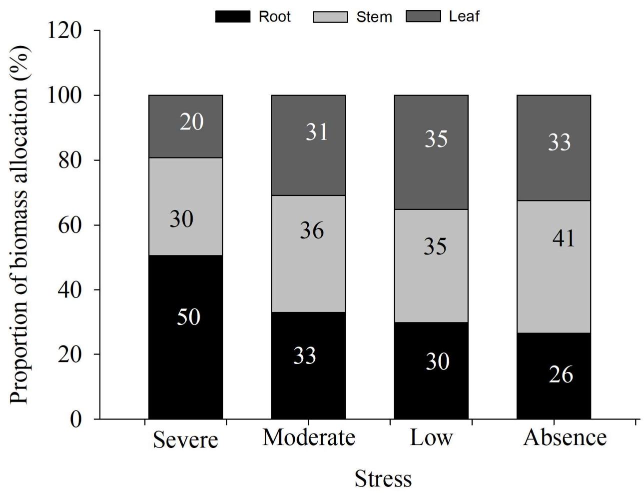

Figure 3: Proportion of biomass allocation between the different organs of Cryptostegia madagascariensis *growing at four levels of stress.*

The regression analysis of the growth variables showed that the increase in leaf area explains at least 86% of the accumulation of dry matter in the seedlings (Figure 4a). The increase of the leaf area is also related to a higher RGR, 74% (Figure 4b).

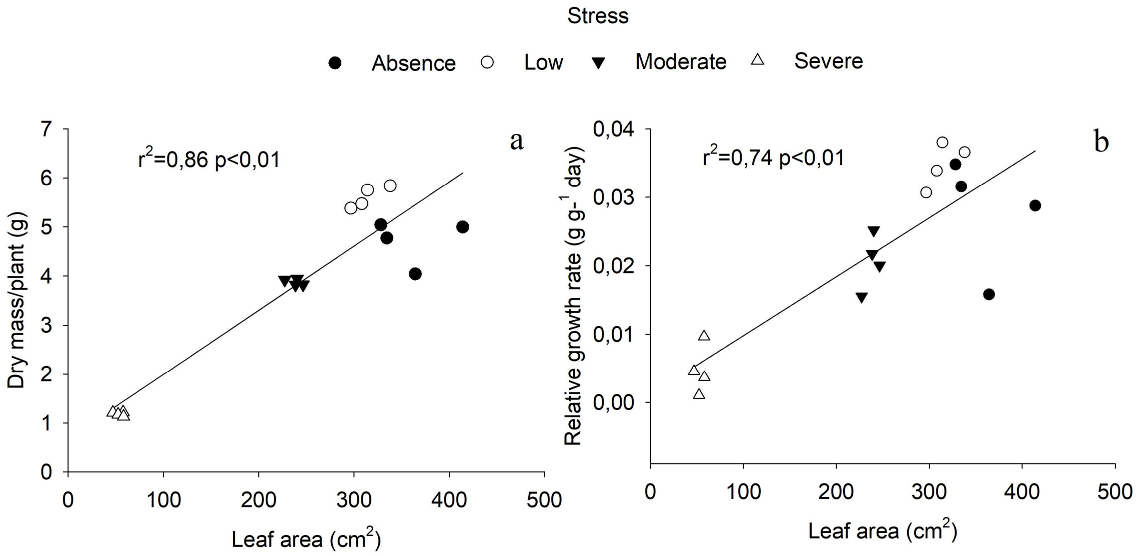

Figure 4: Regression analysis between leaf area and total dry mass (a) and leaf area and relative growth rate (b) of Cryptostegia madagascariensis *growing at four stress levels.*

4. DISCUSSION

The invasive potential of *C. madagascariensis* can be directly associated with high reproduction rates [20] and rapid and high germination, in addition to germination independent of light. We also observed that adaptations to declines in water availability can guarantee the survival of seedlings of this invader in low water levels, which can occur even in riverine areas of the semiarid [29]. On the other hand, high temperatures, and saline and water stresses can inhibit their germination. In addition, reducing water availability reduces seedling growth during the initial establishment, and can be a determining factor for the invasiveness of this species in the semiarid region of Brazil.

Seeds of *C. madagascariensis* germinate rapidly when water availability and temperatures are adequate, with an average germination time of four days at temperatures of 25, 20/25 and 20/30°C. According to Abreu e Garcia (2005) [30], rapid germination is an important ecological strategy, enabling seedlings to take advantage of favorable environmental conditions. These authors also affirm that the average germination time also indicates the speed with which a species is able to colonize a new environment. Therefore, the rapid germination of *C. madagascariensis* allows it to rapidly colonize a new area when it offers suitable abiotic conditions.

On the other hand, high temperatures constitute a barrier to germination, becoming very low or absent above 30°C. In addition, when germination occurred, the mean time was higher at about 7 days. According to Baskin e Baskin (2014) [9], the absence of germination at elevated temperatures may be a survival strategy, so that seedlings are not exposed to unfavorable conditions.

Light is not a fundamental resource for the germination of *C. madagascariensis*, although at temperatures of 25 and 30°C the absence of light reduced germination. Viera et al. (2004) [20], also studied the germination of *C. madagascariensis,* light reduction percentages, and the formation of normal seedlings. Thus, environments with more light availability should increase their germination, which may help to explain the higher rate of occurrence of this invader in disturbed areas as observed by Sousa et al. (2016) [31]. However, the germination of *C. madagascariensis* occurs at different temperatures and light availability, which shows that this species is able to colonize environments with different climatic conditions, such as under the canopy and in open areas.

The seeds of *C. madagascariensis* are very sensitive to water and saline stress. Small concentrations of the salts PEG 6000 and NaCl reduced the percentage and increased the time to germination. According to McDonald (2007) [32] and Ramirez et al. (2014) [33], decreased water availability may inhibit the mobilization of reserves, respiration, and enzymatic activity in seeds. Although water and saline stress may inhibit the germination of *C. madagascariensis*, the seeds maintain viability and high germination rates when transferred to distilled water, reaching similar percentages to those observed in the control treatments. Pujol et al. (2000) [34] found that low osmotic potential is a factor that can induce dormancy (inhibition of germination until favorable conditions) in seeds. According to Khan et al. (1998) [35], this may occur through compounds that inhibit germination.

Therefore, the ability of *C. madagascariensis* seeds to remain viable under conditions of high stress and to germinate when these conditions are overcome shows that these factors do not affect their viability. Gorai et al. (2009) [25], observed that *Diplotaxis harra* (forssk.) boiss, an invader from the arid region of Tunisia, also presents a high capacity for recovery of germination after stress. Thus, this characteristic may be a strategy to colonize and invade semi-arid regions, which are more subject to drought and saline conditions [36].

As expected, water is one of the most important resources for the invasive potential of *C. madagascariensis*, since it negatively affects the germination and initial growth of the seedlings. Low water availability resulted in changes in most of the morphological characteristics assessed during initial growth, many related to adaptations to stress conditions. The reduction in the number of leaves and leaf area observed in *C. madagascariensis* reduces perspiration surfaces and the loss of water [37, 15]. Our results showed that the reduction of leaf area occurred during low stress conditions, and in severe stress we observed the loss of leaves in seedlings. Brown et al. (1998) [38] verified that the invasive congener *C. grandiflora* is a decidua optional according to the availability of water. Therefore, the loss of leaves by *C. madagascariensis* under conditions of higher water stress may indicate that the formation of a dense canopy on native species is related to areas with increased water availability.

The reduction of RGR under moderate stress conditions can be an advantageous strategy for invasive plants in arid environments, as according to Rejmánek (2011) [4], high growth rates demand higher water consumption. In addition, the treatment of low stress induced an increase in productivity (TDM) of the seedlings of *C. madagascariensis*. According to Morais e Freitas (2012) [16], this higher growth in low levels of stress can occur so that seedlings can take advantage of the available water. In the seedlings that grew under moderate stress levels, we observed only leaf changes (leaf area and SLA lower) and shoot growth. While during severe stress the seedlings presented more extreme signs of dehydration, such as loss of leaves and changes in roots. Even with lower water availability, the seedlings showed growth of the root system. According Farooq et al. (2009) [15] and Drenovsky et al. (2008) [28], the seedlings can increase the uptake of soil resources through a higher root / shoot ratio. In addition, under severe stress, the seedlings presented a high RSG, which indicates the production of thinner roots at a lower cost to the plant and also increases the area of absorption [39, 40].

The observed morphological changes contribute to the survival of *C. madagascariensis* under conditions of a water deficit. However, they may conflict with the invasiveness of this species, since the invading potential of climbing plants is associated with high productivity [17] through large leaf areas and high photosynthetic rates [41]. We observed that RGR and the total dry mass of *C. madagascariensis* are directly related to the increase of leaf area. Therefore, the production of smaller leaves and the loss of leaves when water availability is low reduces the growth capacity of this invader. Thus, it is expected that the formation of dense coverings of *C. madagascariensis*, high growth, and the ability to scale the nearby vegetation are associated with areas of high humidity, such as the rivers and areas of *C. prunifera*.

Finally, we can say that the high germination rates under different abiotic conditions is a factor associated with the invasive potential of this species. Therefore, control measures must be taken to prevent its reproduction. In addition, during initial growth the seedlings tolerate different water availabilities. However, we believe that *C. madagascariensis* seedlings can be successfully established in environments with a moderate degree of water stress, and conditions of greater stress should limit their expansion. From these results, mechanical measures of control for this invader should be adopted during the driest periods, as it may be most vulnerable. We also recommend more studies that include other abiotic variables and the tolerance of plants to drought over longer periods of time.

5. CONCLUSION

High temperatures (> 30 °C), salt and water stress reduce their germination potential. However, germination is not affected by light availability. During initial growth, reduced availability of water affects its growth and leaf development, which may reduce its competitive capacity.

Finally, we can say that the high germination rates under different abiotic conditions is a factor associated with the invasive potential of this species. Therefore, control measures must be taken to prevent its reproduction. In addition, during initial growth the seedlings tolerate different water availabilities. However, we believe that *C. madagascariensis* seedlings can be successfully established in environments with a moderate degree of water stress, and conditions of greater stress should limit their expansion. From these results, mechanical measures of control for this invader should be adopted during the driest periods, as it may be most vulnerable. We also recommend more studies that include other abiotic variables and the tolerance of plants to drought over longer periods of time.

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