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Seedling growth of the invader *Calotropis procera* in ironstone rupestrian field and seasonally dry forest soils

Crescimento de plântulas da espécie invasora *Calotropis procera* em solos de campos rupestres ferruginosos e floresta seca sazonal

Silvia H. F. de Oliveira[1]
silviahfo@hotmail.com
Daniel Negreiros[1]
dnapbr@yahoo.com.br
G. Wilson Fernandes[1*]
gw.fernandes@gmail.com
Newton P. U. Barbosa[1]
newtonulhoa@gmail.com
Rafaella Rocha[1]
rafarocha.bh@gmail.com
Jarcilene S. Almeida-Cortez[2]
cortez_jarcy@yahoo.com

Abstract

The present study evaluated the growth, biomass allocation and nutrient content in seedlings of the invasive and exotic especies *Calotropis procera* (Aiton) W.T. Aiton (Apocynaceae), cultured in greenhouse, in soils from two different ecosystems: ironstone rupestrian fields (Canga) of Brumadinho, Minas Gerais; and seasonally dry forest (Caatinga), of Serra Talhada, Pernambuco. Seedlings from the Canga treatment were significantly higher in concern to stem length, leaf biomass and total biomass. In respect to nutrient content there were higher phosphorus, iron and zinc levels in the seedlings from the Canga treatment. The iron accumulation indicates the capacity of *C. procera* to tolerate high levels of iron, which is characteristic of Canga soils. In the Caatinga treatment there was a higher root/shoot ratio and a higher potassium accumulation in the plant tissues. The obtained results suggest that *C. procera* displays a good adaptation to the edaphic conditions of the Canga treatment, which indicates an invasive potential towards the Canga ecosystem.

Key words: Caatinga, *Calotropis procera*, Canga, early growth, invasion.

Resumo

O presente estudo avaliou o crescimento, a alocação de biomassa e os teores de nutrientes em plântulas da espécie exótica e invasora *Calotropis procera* (Aiton) W.T. Aiton (Apocynaceae), cultivadas em casa de vegetação, em solos provenientes de dois ecossistemas distintos: campo rupestre ferruginoso (Canga, Brumadinho, Minas Gerais) e floresta seca sazonal (Caatinga, Serra Talhada, Pernambuco). Plântulas do tratamento Canga foram significativamente maiores em relação ao comprimento do caule, biomassa de folhas e biomassa total. Em relação aos teores de nutrientes, houve maior teor de fósforo, ferro e zinco nos tecidos de plântulas do tratamento Canga. O acúmulo de ferro indica a capacidade de *C. procera* em tolerar os altos teores de ferro característicos dos solos de Canga. No tratamento Caatinga, houve uma maior razão raiz-ramo e maior acúmulo de potássio em seus tecidos. Os resultados obtidos sugerem que *C. procera* demonstra boa adaptação às condições dos solos do tratamento Canga, indicando um potencial para invasão no ecossistema de Canga.

Palavras-chave: Caatinga, *Calotropis procera*, Canga, crescimento inicial, invasão biológica.

[1] Ecologia Evolutiva e Biodiversidade, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. C.P. 486, 30161-970 Belo Horizonte MG, Brazil.
[2] Laboratório de Ecologia, Departamento de Botânica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco. Rua Nelson Chaves s/n, 50670-420 Recife PE, Brazil.
* Author for correspondence.

Introduction

Biological invasions are considered the second major cause of the world biodiversity loss (Lonsdale, 1999; Lake and Leishman, 2003) and, even more worrying, is the irreversibility of the impacts caused by invasion, which worsens within the time, as the exotic species disseminate (Lodge, 1993). Biological invasions occur when one species is established in an area beyond its normal distribution, remaining with a viable population within the time, usually generating impacts (Williamson, 1996; Rejmánek, 2000; Richardson *et al.*, 2000; Sakai *et al.*, 2001; Espínola and Junior, 2007). The occupation and transformation of the habitat, the alteration of ecological relations and evolutionary processes, the hybridization with native species and extinctions are some of the impacts due to biological invasions (Williamson, 1996). Biological, geographical, and environmental barriers, as well as resource competition with native species are some of the main hindrances for the establishment of exotic species (Parendes and Jones, 2000; Rejmánek, 2000; Sakai *et al.*, 2001). The success of an establishment depends on the exotic species features, as on the susceptibility of the invaded habitat, which is already disturbed most of the time (Williamson, 1996; Lonsdale, 1999).

Calotropis procera (Aiton) W.T. Aiton (Apocynaceae) is a shrub 2.5 m to 6 m tall (Ismail, 1992). The root system is very developed, in which the pivotant main root can reach from 1.7 to 3 m depth on sandy desert soils (*e.g.* Barbosa *et al*., 2007). Flowering and fruiting endures all year. *C. procera* is an invasive species which has been disseminated in Brazil. The species comes from tropical Africa, India and the Middle East (Brandes, 2005), and has reached Brazil as an ornamental plant in the northeast coast in the early 20th century (Kissmann and Groth, 1992). In Brazil it has gained several popular names such as *flor-de-seda, leiteira, algodão-de-seda*, *queimadeira* or *ciúme*. *C. procera* has a large medicinal use in its original areas, due to its anti-inflammatory, analgesic, antimicrobial and abortive properties (Kumar and Sehgal, 2007). This species is also used as ornamental and forage in the Brazilian northeast region, due to its resistance to water stress during the dry season (Andrade *et al*., 2008).

C. procera has some features considered common among invasive plants, such as fast growth and dissemination, high seed production efficiently dispersed by the wind, non specialized pollination system and high tolerance to poor soils. Therefore, it is difficult to eradicate, just like successful invasive plants (Barreto *et al*., 1999; Melo *et al*., 2001). Because of its original occurrence in desert regions (Obeid and Mahmoud, 1971; Abbassi *et al*., 2003), where water deficit, high thermal amplitude and nutritionally poor and sandy soils are some of the natural traits, some morphological and physiological adaptations are found in *C. procera*. These traits lead to an improved establishing capacity in arid, degraded and deficient soils, like road edges, pastures and abandoned areas (Ferreira, 1973; Kissmann and Groth, 1992; Colombo, 2007). In Brazil, *C. procera* has been successfully established in some Cerrado areas and mainly in the northeast seasonally dry forest (Ferreira and Gomes, 1976), especially on areas subjected to anthropogenic disturbances.

Seasonally dry forest (heretofore called "Caatinga") is the only exclusively Brazilian biome. It exhibits a rich biological diversity (Pereira *et al*., 2001; Albuquerque and Andrade, 2002; Franca-Rocha *et al*., 2007). Its vegetation is xeromorphic and highly adapted to the arid conditions, like the hydric deficit, uneven rains, high temperatures, and unequal soils differing in deepness, fertility, salinity and mineralogical constitution (Albuquerque and Andrade, 2002; Costa and Araújo, 2003). The main impacts to this ecosystem are the extensive cattle raising, agriculture, and vegetal and wood extraction. These activities have been conducted in a predatory and inadequate way, generating impacts such as overgrazing, deforestation, and disordered burnings practiced to open space for the agriculture. All together, these impacts have led to erosive processes, degraded areas, and desertification (Drumond *et al.*, 2000; Pereira *et al*., 2001). It is likely that the successful establishment of *C. procera* in the Brazilian Caatinga owns to the physical similarity of this biome with the arid environments where it comes from (Mares, 1999).

The southern portion of Espinhaço range divides two worldwide biodiversity hotspots, the Cerrado and Mata Atlântica Rain Forest. This range is predominantly composed of quartzitic rocks and reaches up to approximately 2,000 m a.s.l. Above 900 m a.s.l. in the range, the vegetation is called rupestrian field, where the highly sclerophyllous species predominate. At the southern area of these mountains, the rupestrian vegetation is formed by ironstone rupestrian fields because it grows on soils rich in iron (heretofore called "Canga"). This vegetation is under a severe habitat destruction caused primarily by mining activities (Jacobi and Carmo, 2008). The Canga vegetation is extremely rich in species and endemism. Plants possess some fine adaptations to the severe habitat conditions imposed by this environment. The species are xeromorphic and adapted to high solar exposure, high thermal amplitude, and strong wind incidence. The soils are shallow, acid, and nutritionally poor, containing toxic levels of aluminium and heavy metals, and a low capacity of water retention (Jacobi *et al.*, 2007; Viana and Lombardi, 2007; Jacobi and Carmo, 2008). The vast and intense mining activities practiced in this rupestrian field ecosystem, completely destroys the vegetation, leaving behind highly degraded areas, from which all the superficial soil layer, containing organic matter, nutrients and the seed bank, is taken away (Jacobi and Carmo, 2008). These areas are abandoned and can not be recovered naturally, becoming favourable to invasion by exotic species (*e.g.* Pivello *et al.*, 1999; Medina and Fernandes, 2007). Hence, considering the fact that Canga is under potentially exotic species invasion, in view of the vast habitat alteration, and the fact that *C. procera* has colonized several environments which have distinct biotic and abiotic features, it becomes urgent to test the capacity of *C. procera* to grow in an ecosystem of Canga. A first step is the conduction of a growth performance evaluation of this species' seedlings into substrates provided from ecosystems in which it has already been established and substrates provided from ecosystems it can potentially colonize. Furthermore, populations of *C. procera* are already reaching the regions dominated by the rupestrian fields in the northern Espinhaço range, hence posing a severe threat to its biodiversity and ecosystem services.

The goal of this study was to evaluate the growth performance, biomass allocation and nutrient content in seedlings of *C. procera*, sown in soils proceeding from two different places: Caatinga, a soil already colonized by the species, and Canga, a soil showing a strong potential of colonization by the species.

Material and methods

Seeds of *Calotropis procera* were collected in areas of seasonally dry forest in the Parque Estadual da Mata Seca – Itacarambi, in north Minas Gerais State (14°53'08"S - 44°00'05"W). Seeds were obtained in the field, through manual collection of mature fruits, in thirty adult individuals, in March 2007. Seeds without scars of pathogens, predation or bad formation were selected for the experiment. Seeds were kept on paper bags on a dark place, under ambient temperature, for eight months. *C. procera* seeds exhibit high percentage of germination after long periods of time (Labouriau and Valadares, 1976).

Soils were collected from two places in the present study: (i) ironstone rupestrian field soil (Canga) – Typic Hapludult (Soil Survey Staff, 1999), Serra da Calçada (20°04'49"S - 43°59'28"W), Brumadinho, located in the Quadrilátero Ferrífero, Minas Gerais, Brazil; (ii) seasonally dry forest (Caatinga) – Typic Dystrustepts (Soil Survey Staff, 1999), Fazenda Experimental do Instituto Agronômico de Pernambuco (IPA) (7°59'00"S - 38°19'16"W), Serra Talhada, Pernambuco, Brazil. The soils collected in the species occurrence area (Caatinga) were obtained below the individuals of *C. procera*. In each sampled locality, the substrate was obtained through 20 soil samples with 20 cm diameter x 20 cm depth, in a randomized design, according to Dick *et al*. (1996). A soil sample from each area was submitted to chemical analysis, according to Silva *et al.* (1999) and granulometric analysis, according to EMBRAPA (1997).

The experiment was conducted in a greenhouse covered with canvas with 30% reduction of the luminosity, situated in the Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, in Belo Horizonte, Brazil. The plants were arranged in a complete randomized design (2 treatments × 15 replications). Because of the differences in germination, the comparisons were made using only 12 plants in the Canga treatment and 11 in the Caatinga treatment. They were sown directly into each soil substrate (Canga and Caatinga), on plastic bags of 6 cm wide x 16.5 cm long (0.5 L). Each soil substrate was sieved and mixed until complete homogenization before filling the plastic bags. The germinated seedlings grew for eight weeks from the moment of the sowing. The plots were thinned to leave one plant per pot. Seedlings were irrigated by sprinkler device for 8 minutes, at intervals of 8 hours with a total of 17.5 mm of water per/day, following Negreiros *et al.* (2009).

Primary shoot length, diameter and leaf pairs number of each plant were recorded on the second and eighth week after germination. Primary shoot length was measured with a ruler (mm) while shoot diameter was measured with a digital caliper (0.01 mm precision). Weeding herbs were manually controlled weekly. The relative growth rate (RGR) was calculated according to Hunt (1982):

- RGR for shoot length: $RGR = (\ln L_2 - \ln L_1) / (t_2 - t_1)$;
- RGR for diameter: $RGR = (\ln D_2 - \ln D_1) / (t_2 - t_1)$;
- RGR for leaf number: $RGR = (\ln N_2 - \ln N_1) / (t_2 - t_1)$;

where: L = shoot length; D = shoot diameter; N = total leaf pairs number; t_1 = second week after germination; t_2 = eighth week after germination.

The leaf, stem, and root biomass production were recorded at the end of the experiment. A destructive biomass sampling was done by splitting the aerial part into leaves and shoots. For root collection samples, a careful washing with running water was carried out until complete removal of substrate, using a 2 mm sieve. To determine dry biomass production, each component was bagged, frozen and then dried at 70°C until constant weight was obtained. Biomass was weighted in an analytical scale (±0.001 g precision) (Chiariello *et al*., 1989).

To evaluate biomass partitioning among plant organs, the root/shoot ratio was calculated according to Hunt (1982):

- Root/shoot ratio = $W_R / (W_L + W_S)$;
where: W_R = total root dry weight; W_S = total stem dry weight; W_L = total leaf dry weight.

For the analysis of the nutritional content of plant tissues, leaf, stem, and root biomass were gathered into three subsamples for each treatment. Each subsample containing one third of the total biomass (all tissues) was sent for chemical analysis. The analysis quantified the percentage of P, K, Ca, Mg, S, Zn, Fe, Mn e Cu. The level of each element was determined after the nitric-perchloric digestion of the samples. The phosphorus was determined by colorimetry; potassium by flame photometry; calcium, magnesium, sulphur, zinc, iron, manganese and cooper by atomic absorption spectrophotometry (Sarruge and Haag, 1974).

The growth, biomass partitioning and nutrient content in the seedlings data were analysed by the non parametric Mann-Whitney test (Conover, 1980), since the residuals did not fit the assumptions of homoscedasticity after transformation attempts (Montgomery *et al.*, 2006).

Results

In a general Canga substrate was less fertile than Caatinga substrate (Table 1). Canga substrate exhibited high acidity (pH = 4.1), while Caatinga substrate was considered alkaline (pH = 6.8). The base saturation is considered very low in Canga (5.5%) and medium in Caatinga (59.9%). The organic matter level was medium in Canga (2.8%) and very low in Caatinga (0.1%). The soil texture of Canga is loamy-sand and it is sandy-loam in Caatinga (Table1).

Seedlings of Canga treatment exhibited significantly higher values of leaf dry weight ($p = 0.036$), total seedling dry weight ($p = 0.027$), shoot length ($p = 0.010$) and shoot diameter ($p = 0.008$) (Figures 1C, D, F, G). The root/shoot ratio, the seedlings of Canga treatment trended to allocate more biomass in the aerial part of the plant ($p = 0.012$) (Figure 1H).

Canga seedlings exhibited significantly higher levels of phosphorus (44% higher; $p = 0.050$), iron (approximately 4 fold higher; $p = 0.050$) and zinc (approximately 2 fold higher; $p = 0.050$), while Caatinga seedlings exhibited higher levels of potassium (approximately 2 fold higher; $p = 0.050$) on their tissues (Table 2).

The roots of Caatinga seedlings had a particular trait regarding its morphology, as they grew around the soil sample, leaning against the plastic bag. They were thicker and darker than the Canga roots that displayed a branched growth, occupying equally the entire soil mass.

Table 1. Chemical and textural properties of the Canga and Caatinga soils. In italic, the classification of the average values, according to Alvarez *et al.* (1999): *VLw*: very low; *Lw*: low; *Med*: medium; *Gd*: good; *VGd*: very good; *H*: high.

	Canga		Caatinga	
pH (H2O)	4.12	*VLw*	6.81	*H*
Organic matter (%)	2.48	*Med*	0.13	*VLw*
P (mg/dm3)	4.30	*VLw*	13.20	*Lw*
K (mg/dm3)	27.00	*Lw*	113.00	*Gd*
Ca2+ (cmolc/dm3)	0.39	*VLw*	2.30	*Med*
Mg2+ (cmolc/dm3)	0.05	*VLw*	1.15	*Gd*
Al3+ (cmolc/dm3)	0.19	*VLw*	0	*VLw*
H+Al (cmolc/dm3)	8.70	*H*	2.50	*Lw*
Efective CEC (cmolc/dm3)	0.70	*VLw*	3.74	*Med*
Cation saturation (%)	5.50	*VLw*	59.90	*Med*
Al saturation (%)	27.10	*Lw*	0	*VLw*
Zn (mg/dm3)	9.16	*H*	1.22	*Med*
Fe (mg/dm3)	160.70	*H*	33.90	*Gd*
Mn (mg/dm3)	15.80	*H*	17.50	*H*
Cu (mg/dm3)	0.60	*Lw*	1.17	*Med*
S (mg/dm3)	10.80	*Med*	7.60	*VLw*
Coarse sand (%)	65.00		49.00	
Fine sand (%)	8.00		32.00	
Silt (%)	19.00		10.00	
Clay (%)	8.00		9.00	

Discussion

The low levels of phosphorus in the Canga soil can be explained by the high levels of iron. In general the Brazilian soils, especially in Cerrado and Canga, have low natural levels of P combined with low pH that are complexed to Fe and Al, further reducing the availability of these elements to plant uptake (Mesquita Filho and Torrent, 1993; Fontes and Weed, 1996). According to Troeh and Thompson (2005), the iron can bind to a significant portion of phosphate whenever in intemperized soils, which are not very soluble in acidic pH, then contributing to a low phosphorus availability. However, higher levels of P (44% higher) were recorded in the tissues of Canga seedlings (Table 2). Indeed, the characteristics of the roots of plants in Canga soil are typical of plants adapted to environments poor in phosphorus, increasing the area of soil exploited to absorb more phosphorus (Kochian *et al*., 2004). Ohira (1995), in an experiment with rice plants, noticed that the plants growth was higher (leaf biomass and root and shoot length) in the substrates exhibiting higher concentrations of Fe. Besides that, the P absorption by plants that grew in solutions containing higher Fe concentrations was considered high. However, the rate of translocation to the new leaves was smaller when there were high levels of Fe, suggesting the occurrence of antagonic effects between Fe and phosphate transfer to the tissues. The achieved results in the

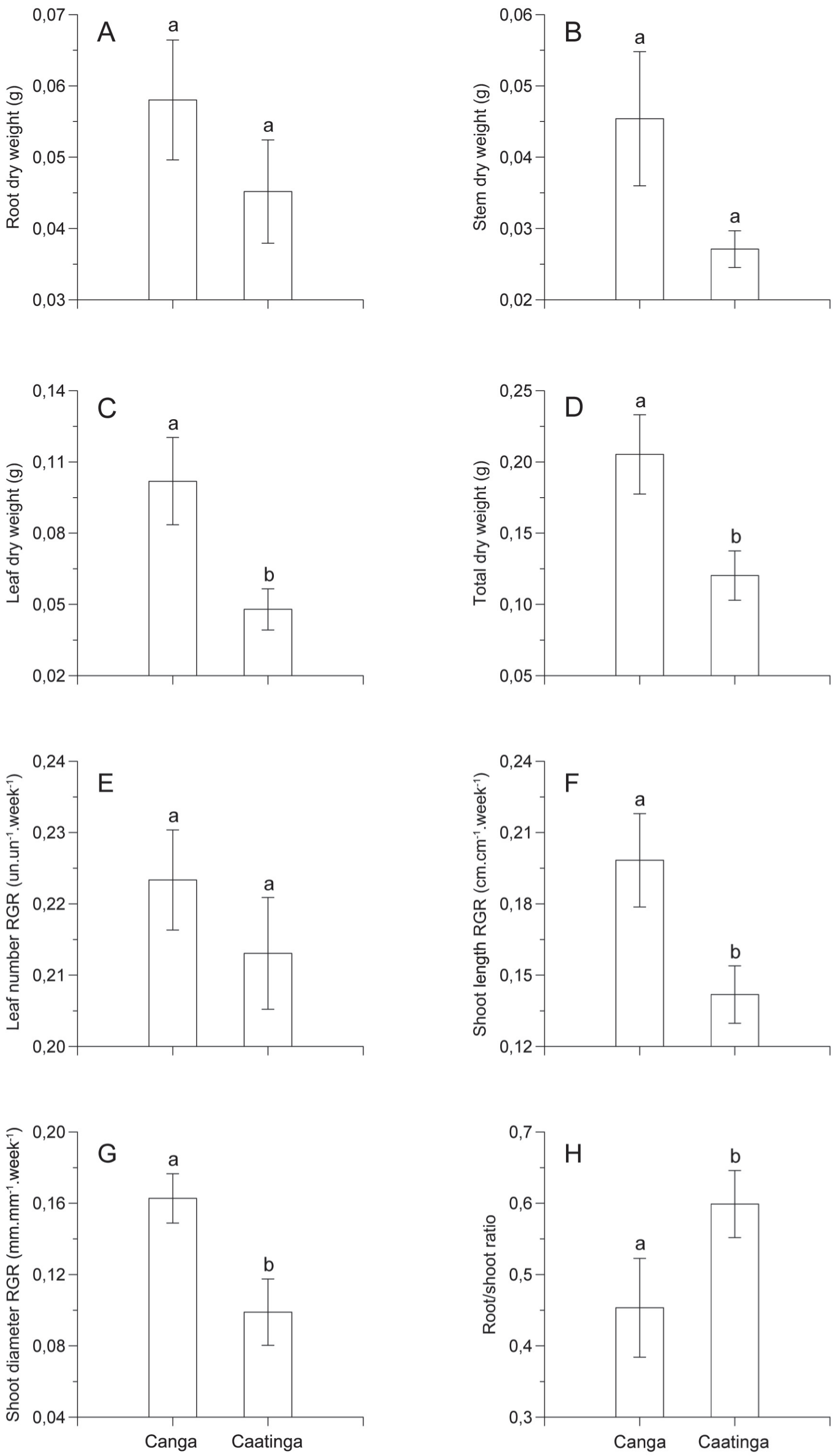

Figure 1. Seedling biomass, relative growth rate (RGR) and root/shoot ratio of *C. procera* grown for eight weeks in two different substrates. Canga (n=12), Caatinga (n=11). Values are represented by average ± standard error . **A**. Root dry weight (g); **B**. Stem dry weight (g); **C**. Leaf dry weight (g); **D**. Total seedling dry weight (g); **E**. RGR for leaf pairs number (un.un^{-1}.week^{-1}); **F**. RGR for primary shoot length (cm.cm^{-1}.week^{-1}); **G**. RGR for shoot diameter (mm.mm^{-1}.week^{-1}); **H**. Root dry weight/Shoot dry weight ratio. For each element, treatments followed by different letters are statistically different at the probability level ≤ 0.05, according to Mann-Whitney test.

present study corroborate to Biddulph and Woodbridge (1955), who postulate that part of the plant's absorbed Fe, or it is fixed in the roots, precipitates with some portion of the P, leaving it unavailable for the metabolic use. As a result, the tissues become relatively rich on total P, at the same time that growth can be impaired by the absence of metabolizable P. Therefore, despite the results in the present study indicate high concentrations of P and Fe in the plant tissues, there was no evidence that the plants were, actually, metabolizing these nutrients. The samples sent to tissue analysis contained all the parts of the plant, which means that the high levels of P and Fe could have been held in the roots, and not available to the tissues. Nevertheless the seedlings had larger growth in the Canga treatment. The organic matter, very important for soil stabilization and gradual P discharge to the plant (Brady, 1974; Barber, 1995), associated with a better soil aeration and increased efficiency of absorption or efficiency of P use, could have contributed to the higher growth on this soil. Future work on nutrient allocation and dynamics among plant organs is needed.

Al and Mn toxicity, and the Ca, Mg, N and P deficiency are probably the most restricting factors for plant growth on acidic soils (Baligar *et al.*, 1991). However, even though Canga substrate exhibited very low P, Mg and Ca levels, and very high Mn, Fe and Zn levels, these conditions did not seem to be restricting or toxic to *C. procera* development, which has a well known capacity of accumulating heavy metals (Rao and Dubey, 1992; Diaz and Massol-Deya, 2003).

The granulometric analysis of the substrates used in the present work desconsidered fractions over 2 mm diameter. Therefore, although the Caatinga substrate has been classified as more sandy (sandy-loam) in relation to the Canga substrate (loamy-sand), the textural effect in the water accumulation and soil compaction, usually largeron clay soils, was different in this case. Canga substrate exhibited much larger particle fragments, like gravels and rocks, demonstrating, visually, a smaller soil compaction and higher aeration. The Caatinga substrate exhibited more compaction and a deficient drainage. The aeration is correlated to the humidity, since waterlogged soils present low oxygen diffusion (Brady, 1974; Marschner, 1995). Therefore, the aeration was far lower in Caatinga substrate, when comparing with Canga substrate. This condition is probably pertinent with the morphological contrasts recorded by the seedling roots between the two treatments.

The better growth performance displayed by Canga seedlings could also be correlated to a better nutrient absorption by abundantly branched roots that should be able to cover a higher extent of the substrate, and consequently, absorb more nutrients.

The root/shoot ratio on both treatments was smaller than one, indicating a higher biomass allocation to the aerial parts, contradicting the expectations for species from desert and nutritionally poor soils, which normally present a higher biomass allocation to the root (Chapin, 1980; Linch, 1995). However, as the seedlings grew on a well irrigated environment, with reduced luminosity incidence, the seedlings may have invested less on root biomass due to these less stressful conditions (Bloom *et al.*, 1985). The lower root/shoot ratio values found on Canga treatment results from the proportionally higher leaf biomass production.

The present study suggests that *C. procera* may have the capability to initiate the colonization process on Canga ecosystems. Of course, *in situ* conditions are much different than greenhouse conditions, especially if we consider that plant growth on Canga's ironstone outcrops are restricted to the microhabitats grounded in the rock fissures where organic matter and water are assembled, and where the plant interaction inside the community contributes for their mutual survival (Benites *et al*., 2003; Jacobi *et al.*, 2007; Viana and Lombardi, 2007). At the same time, environments featuring high solar incidence, thermal amplitude, strong winds and nutritionally poor and shallow soils are well tolerated by *C. procera*, in view of the resemblance of these features to the ones in *C. procera* native habitats (Sharma, 1934), just as similar to the environmental stressful traits of Canga (Jacobi *et al.*, 2007; Jacobi and Carmo, 2008). As shown in this study, *C. procera* is capable of tolerating high levels of Fe and Mn in the soil. Other studies have corroborated its tolerance and capacity of accumulating Fe (Rao and Dubey, 1992). Considering that high Fe levels on Canga' soils is an important restricting trait to plant growth in this ecosystem (Jacobi *et al.*, 2007), the tolerance of *C. procera* to the Fe is even more supportive to its invasion potential into this fragile ecosystem.

Calotropis procera is already found in the northern portion of the Espinhaço range (Barbosa *et al.*, 2007), and is able to outspread. Recently designated as biosphere reserve by UNESCO, the Espinhaço range has been suffering several antropic disturbances, caused by agriculture and livestock, mining activities, roads buildings and tourism. These disturbances can favor the establishment of this species, not only in the ironstone outcrops areas, but all over the Espinhaço range, including quartizitic rupestrian fields and cerrado areas. New studies must elucidate if this species is capable of establishing into these areas, under field conditions, so then invasion fighting programs can be carried out in the still preserved areas.

Table 2. Chemical characterization of the plant tissues of *C. procera* seedlings grown for eight weeks in two different soils. Canga (n=3), Caatinga (n=3). Values are mean ± standard error. For each element, treatments followed by different letters are statistically different at the probability level ≤ 0.05, according to Mann-Whitney test.

	Canga	Caatinga
P (%)	0.36±0.01 a	0.25±0.04 b
K (%)	1.39±1.55 a	3.15±0.32 b
Ca (%)	1.47±0.05 a	1.53±0.10 a
Mg (%)	0.61±0.03 a	0.54±0.04 a
S (%)	0.73±0.24 a	0.52±0.07 a
Zn (ppm)	122.00±8.74 a	68.50±10.04 b
Fe (ppm)	19,896.27±3,058.93 a	5,139.60±369.16 b
Mn (ppm)	269.93±17.03 a	218.40±9.00 a
Cu (ppm)	30.63±1.85 a	21.80±6.06 a

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