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Do Stimulate® and *Ascophyllum nodosum* seaweed promote the morphophysiological characteristics of *Cordia alliodora* seedlings?

Aline das Graças Souza[1], Oscar José Smiderle[2*], Sonicley da Silva Maia[3]

[1]Department of Agricultural Sciences, University Center of Maringá, Maringá, PR, Brazil
[2]Department of Seeds, Brazilian Agricultural Research Corporation, Boa Vista, RR, Brazil
[3]Department of Agricultural Sciences, Federal University of Roraima, Boa Vista, RR, Brazil

***Corresponding author: oscar.smiderle@embrapa.br**

Abstract

Biostimulants in native forest seedlings, when absorbed and assimilated by plants, can function as activators or inhibitors of their metabolic and physiological processes. In view of the above, the present study establishes the following research problem: Can doses of *Ascophyllum nodosum* seaweed extract and Stimulate® be effective in the promotion of initial growth and morphophysiological indices of *Cordia alliodora* seedlings? The experimental design used was completely randomized, in a 4 x 2 factorial scheme, corresponding to four doses (0, 0.2, 0.4 and 0.6 ml L^{-1}) and two hormones (*Ascophyllum nodosum* and Stimulate®), with five replicates, each of which composed of five seedlings (one in each container). The morphological variables evaluated were shoot height (H), stem diameter (SD), increments in stem diameter (ΔSD) and shoot height (ΔH), shoot dry mass (SDM, g $plant^{-1}$), root dry mass (RDM, g $plant^{-1}$), total dry mass (TDM, g $plant^{-1}$), and Dickson Quality Index (DQI). Physiological variables were: net assimilation rate (E_A, g.m^{-2}.day^{-1}), leaf relative growth rate (R_A, g.m^{-2}.day^{-1}), leaf area ratio (F_A, m^2.g^{-1}), specific leaf area (S_A, cm^2.g^{-1}) and leaf mass ratio (Fw, g.g^{-1}). The bioregulator *Ascophyllum nodosum* at dose of 0.2 ml L^{-1} promotes increments in height (ΔH) and stem diameter (ΔSD) of *Cordia alliodora* seedlings. *Ascophyllum nodosum* at dose of 0.2 ml L^{-1} has positive influence on the physiological indices studied in *Cordia alliodora* seedlings at 120 days after transplanting. The bioregulator Stimulate® at dose of 0.6 ml L^{-1} is not indicated to obtain *Cordia alliodora* seedlings with better quality and sturdiness and shorter nursery time. The bioregulator *Ascophyllum nodosum* at dose of 0.2 ml L^{-1} is recommended to obtain increments in height (ΔH) and stem diameter (ΔSD) in *Cordia alliodora* seedlings.

Keywords: Freijó; growth bioregulators; net assimilation rate; seedling quality index.

Introduction

Cordia alliodora is a species belonging to the family Boraginaceae, described by Ruiz and Pavon in 1799 as *Cendana alliodona*, and later grouped in 1941 within the genus *Condia* by 'Oken. Its most common synonyms are: *Cordia gerascanthus* Jacq. and *Cordia tricothoma*, but Johnston (1963), cited by Johnson and Morales (1972), prefers considering the latter as a variety.

Cordia alliodora stands out for being a pioneer species that produces a large amount of seeds annually. It regenerates in areas of open or natural clearings in the forest and easily occupies abandoned areas after agricultural activities, so it is a species with potential for implementation and establishment of reforestation in the northern region of Brazil (Smiderle and Souza, 2022a).

Despite the significant amount of seeds produced annually by *Cordia alliodora*, there is little information about its needs regarding the lack of adequate techniques and management within the seedling production sector, a scenario also associated with the scarcity of information and studies in the state of Roraima (Smiderle et al., 2022).

However, in the current scenario of production of seedlings of native forest species in northern Brazil, Smiderle and Souza (2022a) have adopted the practice of foliar application of biostimulants or phytohormones that have in their composition amino acids, humic substances (humic acids and fulvic acids), plant growth hormones, vitamins and various other elements, in addition to organic substances from seaweed extract.

According to Smiderle et al. (2022), biostimulants function as activators of cell metabolism in *Hymenaea courbaril* seedlings, reactivating physiological processes in different stages of development, as well as stimulating root growth and increasing the size of the assimilatory apparatus.

This panorama is determined through growth analysis by the physiological indices, which indicate the capacity of the assimilatory system (source) of plants to synthesize and allocate organic matter in the various organs (sinks) that depend on photosynthesis, respiration and translocation of photoassimilates from fixation sites to use or storage sites (Chrysargyris et al., 2018).

Therefore, physiological indices express the physiological conditions of the plant and quantify the net production derived from the photosynthetic process. This performance is influenced by biotic and abiotic factors (Shukla et al., 2019; Menegatti et al., 2022).

In view of the above, the present study establishes the following research problem: Can doses of *Ascophyllum nodosum* seaweed extract and Stimulate® be effective in the initial growth and morphophysiological indices of *Cordia alliodora* seedlings?

Results and discussion

Analysis of variance

The analysis of variance revealed significant effects ($p<0.05$) for the interaction between bioregulators and doses for the variables shoot dry mass and total dry mass of the plants. There were also significant simple effects of bioregulator doses on shoot height, stem diameter, height and diameter increments, Dickson quality index (DQI) and root dry mass.

***Relations between bioregulator doses and seedlings of** Cordia alliodora*

Fig 1 (A) shows that, for shoot height, Acadian® at the dose of 0.2 ml L^{-1} results in a 17.07% increment compared to the same dose of Stimulate® at 120 DAT.

Seaweed extract is a natural source of cytokinins, which in addition to promoting cell division, directly influence the photosynthetic rate of forest seedlings, promoting greater plant growth, which explains the promotion of height with foliar application of Acadian® at 120 DAT at dose of 0.2 m L^{-1} (Smiderle et al., 2022)

The superiority achieved for stem diameter was also obtained with foliar application of Acadian® at dose of 0.2 ml L^{-1}, which led to a gain of 20.63% compared to the same dose of Stimulate®, so the use of bioregulators for the promotion of biomass and growth of *Cordia alliodora* is feasible.

For Smiderle et al. (2022), the application of seaweed extract (Acadian®) at the dose of 0.2 ml L^{-1} caused gain of 12.5% in stem diameter in *Hymenaea courbaril* seedlings in northern Brazil, which was also found in the present study with *Cordia alliodora* seedlings (Fig 1B).

The increments in height (ΔH) and stem diameter (ΔSD), evaluated at 120 DAT, for *Cordia alliodora* seedlings are presented in Fig 2A and 2B, respectively. Increase in ΔH with the bioregulator Acadian® was also found at the dose of 0.2 ml L^{-1}, with a maximum increment of 35.5 cm, while for ΔSD the maximum increment was 5.02 mm.

In addition, the maximum ΔH for the bioregulator Stimulate® was 30.6 cm (Fig 2A), while the maximum ΔSD for Stimulate® was 3.82 mm (Fig 2B).

In this context, it should also be noted that doses above 0.6 ml L^{-1} of both Acadian® and Stimulate® did not influence the increments in height (ΔH) and stem diameter (ΔSD), with similar results to those found in the control (Fig 2A and 2B).

These results are of great relevance because they show that foliar application of plant bioregulators at the appropriate dose and at the moment when plants require a greater amount of phytohormones readily available for the maintenance of morphophysiological processes promotes the conversion of energy into biomass increments, as reported in the studies conducted by Leal et al. (2020) and Smiderle et al. (2022) with native forest seedlings and in the present study, with *Cordia alliodora* seedlings (Fig 2A and 2B).

In turn, shoot dry mass (SDM) increased by 55.0% with foliar application of 0.2 ml L^{-1} of Acadian® compared to the control, showing a significant and positive effect. In addition, the dose of 0.4 ml L^{-1} of Stimulate® promoted a 43.0% gain in SDM compared to the control (Table 1).

An important point to consider in this variable is that higher SDM production generally indicates higher production of photoassimilates that are essential for the greater growth of *Cordia alliodora* seedlings, which was evidenced by the leaf relative growth rate (R_A, $g.m^{-2}.day^{-1}$) described in Table 2. This reinforces the importance of the appropriate choice of plant-growth bioregulators and their doses, which could favor the success of the enterprise, whether commercial or for ecological purposes.

The higher root dry mass (RDM) with the Acadian® dose of 0.2 ml L^{-1}, compared to the control (Table 1), contributed significantly to the higher total dry mass (TDM) and to the Dickson quality index (DQI). According to Smiderle et al. (2022), the index is a good indicator of the quality of seedlings of forest species native to the Northern region of Brazil, because it considers for its calculation the sturdiness and balance of biomass distribution among organs, and both parameters are considered important for a reliable recommendation of seedling quality. According to these same authors, the value considered ideal for DQI is equal to or greater than 1.00.

Based on the results obtained, Acadian® dose of 0.2 ml L^{-1} led to DQI of 1.67 and Stimulate® dose of 0.4 ml L^{-1} led to DQI of 1.21, while the value obtained in the control was 0.70, which is below the value considered ideal by Smiderle et al. (2022) (Table 1). It is worth pointing out that, for DQI, there were no significant differences between the applied doses of the bioregulator Stimulate®.

Physiological indices

It should be highlighted that the values obtained for net assimilation rate (E_A) considering the tested doses of Stimulate® did not differ, as occurred with the doses of Acadian® (Table 2). In general, *Cordia alliodora* seedlings that received foliar applications of Stimulate® and Acadian® at the different doses showed an increase in E_A compared to the control (Table 2), confirming the positive performance in relation to the net assimilation rate.

The specific leaf area (S_A) and leaf area ratio (F_A) (Table 2) of *Cordia alliodora* seedlings showed significant differences between bioregulators and doses evaluated at 120 DAT. In addition, for specific leaf area (S_A), the Acadian® dose of 0.2 ml L^{-1} differed from and outperformed the other doses tested, proving to be adequate for the initial growth stages of *Cordia alliodora* plants.

It is known that, after investing in the root system and still in the initial growth period, plants tend to allocate a large amount of energy for leaf production (Menegatti et al., 2020), in order to maximize light capture and energy production, ensuring the maintenance of sufficient leaf area to keep photosynthetic rates higher than respiratory rates (Saeger et al., 2020).

In general, foliar application of the bioregulator Stimulate® in *Cordia alliodora* plants at dose of 0.4 ml L^{-1} led to higher leaf area ratio (F_A) compared to the other doses of Stimulate® and to the dose of 0.4 ml L^{-1} of Acadian® (Table 2). These results are consistent with those recorded in *Hymenaea courbaril* by Smiderle et al. (2022) for F_A, which

Table 1. Mean values for the interaction between plant bioregulators (Stimulate® and Acadian®) and doses for the growth variables shoot dry mass (SDM, g plant^{-1}), root dry mass (RDM, g plant^{-1}), total dry mass (TDM, g plant^{-1}) and Dickson quality index (DQI) of *Cordia alliodora* seedlings, at 120 days after transplanting.

	SDM		RDM		TDM		DQI	
DOSE	Acadian®	Stimulate®	Acadian®	Stimulate®	Acadian®	Stimulate®	Acadian®	Stimulate®
0	2.92cA	2.93bA	2.30bA	2.00bA	5.22bA	4.92bA	0.70bA	0.70bA
0.2	6.53aA	4.88aB	4.98aA	4.05aA	11.50aB	8.92aA	1.67aA	1.18aA
0.4	4.60bA	5.14aA	4.08abA	4.16aA	8.68abA	9.20aA	1.05abA	1.21aA
0.6	2.94cB	4.46abA	2.16bA	3.48aA	5.30bB	7.94abA	0.68bB	1.13aA
CV	9.21		10.32		12.10		12.23	

[1] Lowercase letters (a, b, c) compare the means for the variables between the doses of bioregulators, and uppercase letters (A, B) compare the means for the variables between the bioregulators, by Tukey test at 5% probability level.

Table 2. Mean values for the interaction between plant bioregulators (Stimulate® and Acadian®) and doses for the physiological indices net assimilation rate (E_A, g.m^{-2}.day^{-1}), leaf relative growth rate (R_A, g.m^{-2}.day^{-1}), specific leaf area (S_A, cm^2.g^{-1}), leaf area ratio (F_A, m^2.g^{-1}) and leaf mass ratio (Fw, g/g^{-1}) of *Cordia alliodora* seedlings, at 120 days after transplanting.

		0	0.2	0.4	0.6	CV
E_A	Stimulate®	0.000047bA	0.000057aA	0.000058aA	0.000056aA	6.11
	Acadian®	0.000049bA	0.000059aA	0.000057aA	0.000056aA	
R_A	Stimulate®	0.0037723cA	0.0045427abB	0.0047592aA	0.0042206bcA	7.08
	Acadian®	0.0041413bcA	0.0049734aA	0.0045974abA	0.0040398cA	
S_A	Stimulate®	218.93cA	252.18bB	271.05aA	239.29bB	10.42
	Acadian®	212.36cA	355.98aA	258.79bA	253.16bA	
F_A	Stimulate®	116.45cA	139.41bB	192.50aA	145.56bB	14.28
	Acadian®	115.40cA	201.07aA	136.25bB	111.82cA	
F_W	Stimulate®	0.53782aA	0.57369aA	0.58328aA	0.56628aA	14.12
	Acadian®	0.52711aA	0.57433aA	0.55853aA	0.56793aA	

[1] Lowercase letters (a, b, c) compare the means for the variables between the doses of bioregulators, and uppercase letters (A, B) compare the means for the variables between the bioregulators, by Tukey test at 5% probability level.

Table 3. Chemical characteristics of the substrate in the production of *Cordia alliodora* seedlings.

	pH	K	P	Ca	Mg	Al	H+Al	CEC	SB	OM	Zn	Fe	Mn	Cu	B	S
		--------cmol/dm³-------								dag/kg	----------mg/dm³----------					
Substrate	6.7	0.31	0.87	11.0	0.7	0.0	1.1	13.31	12.01	3.5	16.5	13.5	88.6	0.3	0.5	17.2

Substrate composed of sand + soil + carbonized rice husk + organic compost (1:1:1:1).

Table 4. Physical composition of the substrate formulated for the production of *Cordia alliodora* seedlings.

	Particle size		
Substrate	Clay	Silt	Medium Sand
	----------dag kg^{-1}----------		
	22.99	11.78	67.23

Substrate composed of sand + soil + carbonized rice husk + organic compost (1:1:1:1).

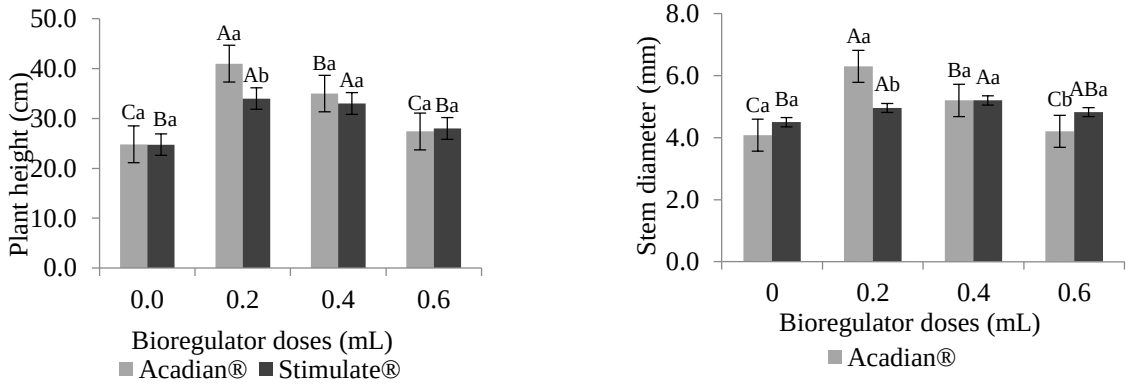

Fig 1. Mean values of plant height (A) and stem diameter (B) obtained with foliar application of two bioregulators at four doses (Stimulate®: 0; 0.2; 0.4 and 0.6 ml L^{-1} and Acadian®: 0; 0.2; 0.4 and 0.6 ml L^{-1}), respectively, in *Cordia alliodora* seedlings in a greenhouse. Lowercase letters (A, B, C) compare the means for the variables between the doses of bioregulators, and uppercase letters (a, b, c) compare the means for the variables between the bioregulators, by Tukey test at 5% probability level.

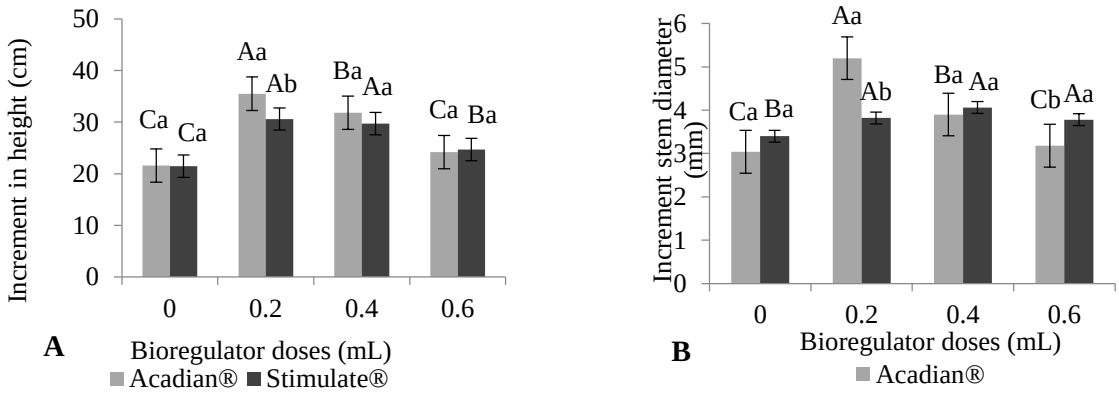

Fig 2. (A) Increment in height (ΔH) and (B) increment in stem diameter (ΔSD), at 120 DAT, of *Cordia alliodora* seedlings, as a function of doses of bioregulators (Stimulate®: 0; 0.2; 0.4 and 0.6 ml L^{-1} and Acadian®: 0; 0.2; 0.4 and 0.6 ml L^{-1} in a greenhouse. Lowercase letters (A, B, C) compare the means for the variables between the doses of bioregulators, and uppercase letters (a, b, c) compare the means for the variables between the bioregulators, by Tukey test at 5% probability level.

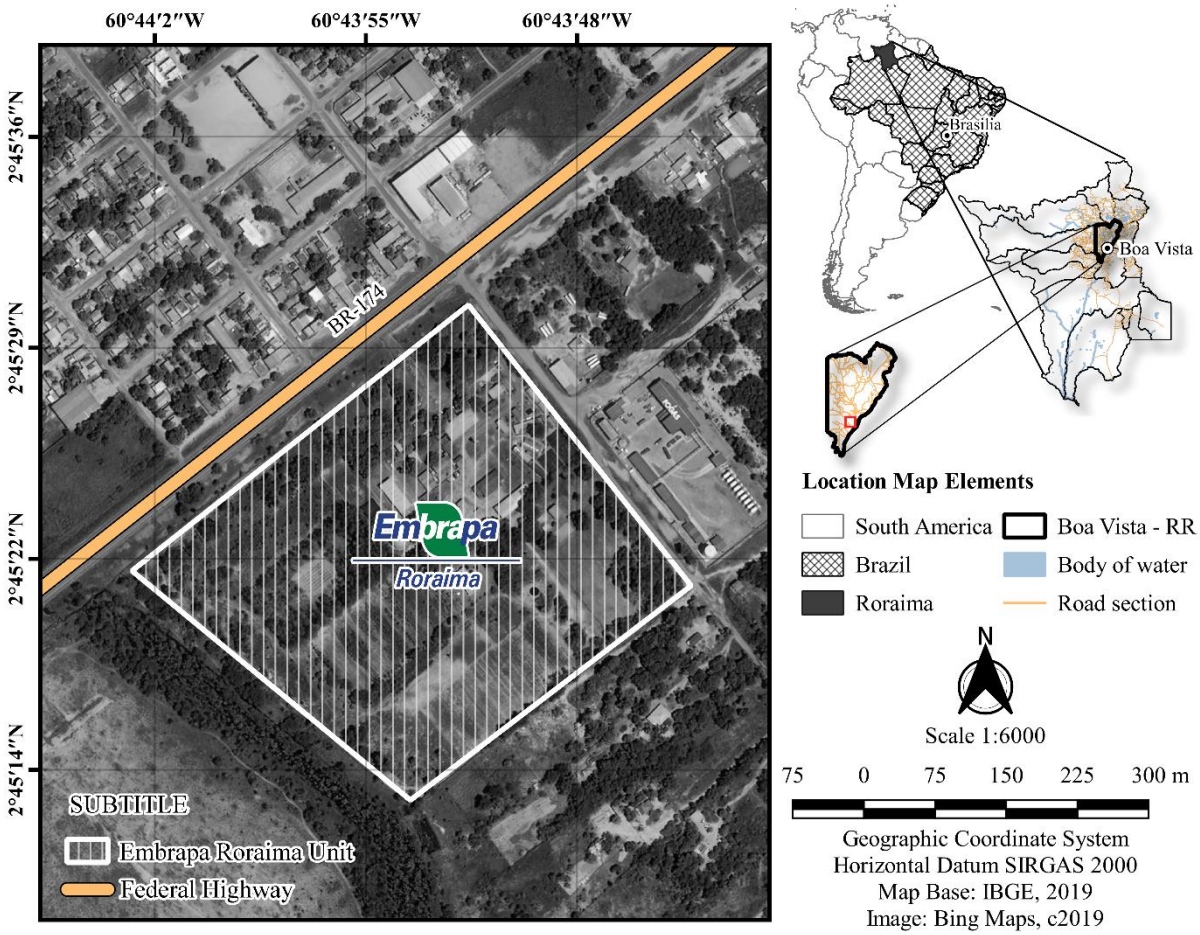

Fig 3. Location map of *Cordia alliodora* tree, municipality of Boa Vista, Roraima, Brazil.

expresses the useful leaf area for photosynthesis. The vast majority of plants show better performance than forest species, i.e., they have high F_A at the beginning of the cycle, a period in which their leaves develop for a greater light uptake, which subsequently decreases due to the interference of upper leaves over the lower ones, characterizing self-shading (Hönig et al., 2018), hence reducing the useful leaf area for photosynthesis. In general, increments in S_A, F_A and Fw are followed by a decrease in E_A due to the mutual shading of leaves (Katiyar et al., 2021; Smiderle et al., 2022), which was observed in the present study (Table 2).

Among the components F_A and S_A, the latter is more plastic than the leaf mass ratio (F_W), especially with regard to the factors of the medium. F_W is undoubtedly a conservative growth index regarding environmental conditions, which was observed by Smiderle et al. (2022) and in the present study in *Cordia alliodora* seedlings for the plant bioregulator Acadian®, mainly at the dose of 0.2 ml L^{-1}.

Thus, the improvement in the techniques of production of *Cordia alliodora* seedlings addressed in this work may positively contribute to the production sector of native and/or exotic forest seedlings, since the data make it possible to infer about the subsequent behavior of this combination to the point of significantly increasing the biomass, sturdiness and quality of the seedlings.

Material and methods

Plant material used in the experiment

The study was conducted in a greenhouse at Embrapa Roraima from September 2021 to January 2022. The species used in the present study was *Cordia alliodora*. The seeds were collected manually from trees present at Embrapa Roraima headquarters (2°45'22" N latitude, 60°43'55" W longitude and 80 m altitude), located alongside the BR-174 Highway, km 8, in the municipality of Boa Vista, state of Roraima, Brazil (Fig 3).

The climate of the municipality of Boa Vista, RR, is type Am (tropical monsoon climate), with average temperatures of 27.2 °C in the hottest month and 23.3 °C in the coldest month, with an annual average of 25.4 °C. The average annual rainfall is 1808 mm, with average values of 365 mm and 26 mm for the months of highest (June) and lowest (February) precipitation, respectively.

Experimental area

After obtaining the seeds, they were processed and then sown in a bed, which contained medium sand. Moisture was kept in the sand substrate through irrigation with four daily waterings.

Approximately twelve days after sowing, the seedlings began to emerge and, as soon as they homogeneously reached an approximate height of 5.0 cm, they were transplanted to polyethylene bags (15 x 35 cm) containing substrate composed of medium sand + soil + carbonized rice husk + organic compost (1:1:1:1). The average temperature within the greenhouse during the experimental period was 25 ± 5 °C, with relative humidity of 60% to 70%. Irrigation was performed manually according to field capacity.

The results of chemical and physical analysis of the substrate (Tables 3 and 4) were obtained using the methodology described by the Official Network of Soil and Plant Tissue Analysis Laboratories of RS and SC - ROLAS (SBcS/cQFS, 2016).

Experimental design and conduction of study

Foliar applications of the bioregulators based on *Ascophyllum nodosum* seaweed extract and Stimulate® were carried out using a manual sprayer with capacity for 100 mL, applying 20 mL of the solution (bioregulator and water) per plant, in the afternoon period from 16:30 h, according to the doses established for each bioregulator. The *Ascophyllum nodosum* and Stimulate® doses used were: 0, 0.2, 0.4 and 0.6 ml L^{-1}. The experimental design used was completely randomized, in a 4 x 2 factorial scheme, corresponding to four doses (0, 0.2, 0.4 and 0.6 ml L^{-1}) and two hormones (*Ascophyllum nodosum* and Stimulate®), with five replicates, each of which composed of five seedlings (one in each container). At 120 days after transplantation (DAT), the plants were evaluated for shoot height (H), with a graduated ruler, and stem diameter (SD), with a digital caliper. The increments in stem diameter (ΔSD) and shoot height (ΔH) were obtained from the data collected during the plant growth period until the end of the experiment.

Then, the plants were dried in a forced air circulation oven at 70 ºC for 72 hours, until reaching constant weight, for individual determination of shoot dry mass (SDM, g $plant^{-1}$), root dry mass (RDM, g $plant^{-1}$) and total dry mass (TDM, g $plant^{-1}$), obtained by the sum of SDM + RDM. Dickson quality index was determined using the formula DQI=TDM/[(H/SD) + (SDM/RDM)], according to Dickson et al. (1960). The values of net assimilation rate (E_A, $g.m^{-2}.day^{-1}$), leaf relative growth rate (R_A, $g.m^{-2}.day^{-1}$), leaf area ratio (F_A, $m^2.g^{-1}$), specific leaf area (S_A, $cm^2.g^{-1}$) and leaf mass ratio (Fw, $g.g^{-1}$) were determined from instantaneous values of Af, Wf and Wt, used in the equations F_A=Af/Wt, S_A =Af/Wf and Fw=Wf/Wt, according to Radford (1967) and Richards (1969).

Statistical analysis

All variables were subjected to comparison of means by Tukey test, at 5% probability level, and quantitative variables were subjected to regression analysis to assess plant growth response as a function of time. Data analysis was performed in the Sisvar statistical package (Ferreira, 2014).

Conclusions

The bioregulator *Ascophyllum nodosum* at dose of 0.2 ml L^{-1} is recommended to obtain increments in height (ΔH) and stem diameter (ΔSD) in *Cordia alliodora* seedlings.

Ascophyllum nodosum at dose of 0.2 ml L^{-1} has positive influence on the physiological indices studied in *Cordia alliodora* seedlings at 120 days after transplanting.

The bioregulator Stimulate® at dose of 0.6 ml L^{-1} is not indicated to obtain *Cordia alliodora* seedlings with better quality and sturdiness and shorter nursery time.

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