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Promoting the growth of *Brachiaria decumbens* by humic acids (HAs)

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Abstract

The increasing demand for meat and milk has stimulated interest in the development and recovery of pastures. Although humic acids (HAs) have been shown to have a biostimulating effect on plant growth, their use in pastures remains relatively unknown. This study aimed to evaluate the growth of *Brachiaria decumbens* at different HAs concentrations and application times. This study was conducted in a greenhouse with pots containing 1.5 L of soil collected in a degraded pasture. A trial was carried out in a randomized block design in which five HAs concentrations (0, 7.5, 15, 30 and 60 mg L^{-1} C) and four application times were used as follows: leaf spray 15 days after emergence (LS 15 DAE); LS 45 DAE; LS 60 DAE; and successive leaf sprays (15, 45 and 60 DAE). Stem height, diameter, forage production and root development were evaluated. Height and stem diameter measurements were performed weekly. At 45 and 90 DAE, a cut was made at a height of 10 cm to simulate grazing and to measure forage production. At 90 DAE, the root mass was assessed. The HAs used at the concentration of 60 mg L^{-1} C applied at 15 DAE promoted increases of 44% in plant height and 196% in forage mass. After the cut, the optimum concentration was approximately 40 mg L^{-1} C. Although the use of HAs promoted root development, successive applications had deleterious effects on the plant. HAs could improve pasture biomass production at a low cost if applied at the ideal concentration.

Keywords: pasture, forage management, biofertilizers, plant development.

Introduction

Because a substantial portion of its cattle herds are pasture raised, Brazil has some of the lowest meat and milk production costs in the world (Ferraz and Felício, 2010). Brazil has 172 million hectares of planted and natural pastures (IBGE, 2007), most of which are covered by the *Brachiaria* genus, especially the *Brachiaria decumbens* species, because of its excellent vegetation coverage, drought resistance and dry mass production of up to 15 Mg ha^{-1} year (Alvim et al., 2002).

Due to the intensification of the milk and beef production systems, serious problems of pasture degradation have been observed, this contributes to a low production of forage, compromising the development of livestock and the sustainability of the productive system (Dias-Filho, 2015). It is estimated that 50% to 70% of pasturelands in Brazil present some level of degradation (Dias-Filho, 2014). Extensive pasture livestock production when limited advances on native biomes destroying biological diversity. Therefore, in recent years, cattle farming on pasture has undergone several transformations intended to reduce intrusion on fragile ecosystems. Through strategies that can recover these degraded areas greater efficiency of production per area is sought (Dias-Filho, 2011). In this context, the major challenge for pasture livestock production is the development of technologies that increase forage production in existing areas. These technologies will play a key role in the sustainable production of pastures, rendering them both agronomically efficient and environmentally suitable (Dias-Filho, 2011).

Humic acids (HAs) represent the most stable reactive fraction of humified organic matter (Canellas et al., 2001) and are products of not only the decomposition of different organic residues but also microbial resynthesis (Arancon et al. 2005). The use of HA-type humic substances (such as those extracted from vermicompost) has well-documented potential as plant growth promoters (Façanha et al., 2002; Baldotto et al., 2009, 2010, 2011; Baldotto and Baldotto 2015, 2016; Canellas et al., 2013, 2015a). This effect depends on the HA concentration, the application time and the plant species. Silva et al. (2015) observed optimal HA concentrations for the growth of *Cattleya warneri* seedlings *in vitro* of approximately 48 mg L^{-1} C. When evaluating the rooting of sugarcane micro-shoots, Marques Jr. et al. (2008) obtained optimum growth with 19 mg L^{-1} C. Lima et al.

(2011) observed increased tomato productivity when applying HAs eight days after transplanting tomato seedlings. Although most studies focus on the use of HAs either in seeds or for early plant development, it is important to observe the effects of their use on plants at later stages of the growth and development cycle. However, the use of HA as a technology to enhance forage production and increase pasture longevity remains unexplored.

For that reason, this study aimed to evaluate the development of *Brachiaria decumbens* with the application of HAs at different concentrations and times.

Results

Growth of Brachiaria decumbens with different doses and application times of HAs

The application of HAs after the emergence of *Brachiaria decumbens* promoted plant growth. The increase in concentration was related to shoot growth, showing a 45% and 33% increase for LS 15 DAE and LS SLS DAE, respectively, at a concentration of 60 mg L^{-1} C at the time of the first cut (Figure 1a).

The HAs concentrations had different effects on plant height at leaf spraying times for the second cut (Figure 1b). There was no difference in the growth of *Brachiaria decumbens* when HAs were applied at 15 and 45 DAE, and LS 45 DAE was performed shortly after the first cut. For LS 60 DAE, which occurred 15 days after the first cut, the dosage of 30 mg L^{-1} C of HAs showed the highest plant growth, similar to LS 15 and 45 DAE.

For each HAs dosage evaluated, leaf spraying times had a significant effect on plant height. The periods that had HA application (15 and SLS DAE) differed from the periods that did not have HAs application until the first cut, demonstrating the positive effect of HAs application on initial plant growth (Figure 2a). There was a positive effect of HAs on the initial development of the plant and regrowth, and at the HA dosage of 60 mg L^{-1} C for LS 15 and 45 DAE, the plant height increased compared to other application times and HA concentrations.

Figure 3 shows the effect of HA concentration on *Brachiaria decumbens* stem diameter in the first cut. The HA concentration of 30 mg of L^{-1} C resulted in the largest mean diameter among the evaluated concentrations. There was an increase in mean stem diameter up to the HA dosage of 42 mg L^{-1} C, which is considered the optimum HA dosage to be applied for the maximum increase in plant stem diameter.

There was a significant difference in the mean stem diameter for the application times only for the second cut. There is a positive effect of HA application on the mean stem diameter when HAs are sprayed shortly after plant emergence and regrowth. Successive applications had a deleterious effect on stem diameter, with stem diameter decreases of up to 17% compared to other application times (Figure 4).

Shoot and root dry matter of Brachiaria decumbens after HAs application

The effect of HA concentration on shoot dry matter accumulation in the first cut can be seen in Figure 5a. The increase in the HA concentration influenced the plant's dry mass production, with a similar result for plant height (Figure 1a). The HA concentration of 60 mg L^{-1} C promoted a higher dry-matter yield for both application times (15 and SLS DAE), at 196 and 204% higher than that obtained in the control treatment.

In the second cut (Figure 5b), the incremental increase in HAs concentrations had a positive effect when applied at 15 and 45 DAE, resulting in a significant accumulation in the dry-matter yield (1.61 and 1.98 g), representing an increase of 38 and 74% of dry matter, respectively, compared to the control treatment. For LS 60 DAE and LS SLS, the 30 mg L^{-1} C dosage resulted in higher dry-matter yield. As the concentration increases, dry-matter yield decreases.

The HA application times promoted different results in the first cut (45 DAE) when analysed by concentration (Figure 6a). There was no difference between the leaf spraying times for the lowest concentrations (7.5 and 15 mg L^{-1} C HAs) and the leaf spraying times for the concentrations 30 and 60 mg L^{-1} C of HAs. In each case, *B. decumbens* showed an increase in the shoot's dry mass in the plants that received the initial application of HAs (15 and SLS DAE). For the HA dosage of 60 mg L^{-1} C, this increase may reach 254% when compared to plants that did not receive the initial application of HAs (45 and 60 DAE). In the second cut (90 DAE), the greatest effect of the HA application occurred on the regrowth of *Brachiaria decumbens*, with a higher shoot dry-matter yield than for the other application times (Figure 6b).

The effect of increasing HA concentration, at different application times, on root dry matter may be seen in Figure 7. The increased HA concentration increased the root dry-matter yield when applied soon after plant emergence (15 DAE). For this time of leaf spraying, the root dry matter showed an average increase from 5.15 to 7.65 g, which corresponds to 48% increase in root dry mass compared to the control treatment.

When applying HAs after cutting (45 and 60 DAE), the dry-matter yield of *B. decumbens* plants is higher at 34 and 35 mg of L^{-1} C, respectively. This increase reaches 192% and 211% for LS 45 and 60 DAE, respectively, compared to the control. Even with the decrease in the root's dry matter after the optimum dosage of 60 mg L^{-1} C, LS 15, 45 and 60 DAE did not show a positive effect on the plant's root growth in the initial development and regrowth (Figure 8).

Negative effects of successive applications of HAs

At the time of the second cut (Figure 2b), successive spraying of HAs hindered the appropriate plant growth, causing a decrease in height when compared to other application times. This is more evident with the increase of the AHs dosage which had a negative effect on the development of *Brachiaria decumbens* shoots, resulting in a decrease of up to 29% in plant height between the dosage 7.5 and 60 mg of L^{-1} C of HAs. Figure 5b shows the decrease shoot's dry matter after successive applications and increase concentration of HAs. This decrease reached 53% with an increase in concentration from 7.5 to 60 mg L^{-1} C, that is, per-plant dry-matter yield fell from 1.18 to 0.63 g.

When applied successively, HAs reduced the growth of *Brachiaria decumbens* roots in degraded pasture soil, a result that might be related to the development and production of shoot dry matter (Figures 2b and 6b). The

Table 1. Chemical attributes of the soil used in the experiment.

pH	P	K^+	Na^+	Al^{3+}	H^++Al^{3+}	Ca^{2+}	Mg^{2+}	SB	CEC	BS
	-------mg kg^{-1}-------		-------------------cmol$_c$ dm^{-3}----------------							%
4.2	3.2	15.0	2.0	1.4	6.4	0.1	0.04	2.2	8.6	25.0

pH in H_2O; SB: sum of exchangeable bases; CEC: cation exchange capacity; BS: base saturation

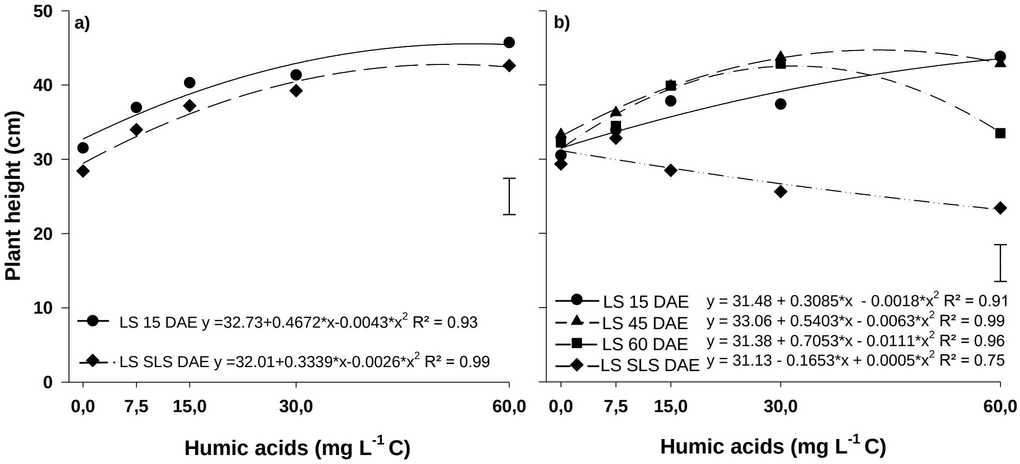

Fig 1. Height of brachiaria (*Brachiaria decumbens*) plants measured at 45 DAE (time of first cut, a) and 90 DAE (time of second cut, b), in a greenhouse, Alegre-ES. LS 15, 45 and 60 DAE, leaf spraying of HAs at 15, 45 and 60 days after emergence; LS SLS, successive leaf spraying of HAs (15, 45 and 60 DAE). * Significant at 5% according to the t-test. The vertical bar represents the least significant difference (LSD) between the times for all dosages.

Table 2. Average composition of the vermicompost used for the extraction of humic acids.

pH	C	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^+ + Al^{3+}$	SB	CEC	BS
	g kg^{-1}	---mg dm^{-3}---		-----------------------cmol$_c$ dm^{-3}----------------------						%
7.1	67	952	4.9	22.2	8.9	0	2.2	45.1	47.2	96

pH in H_2O; SB: sum of exchangeable bases; CEC: cation exchange capacity; BS: base saturation

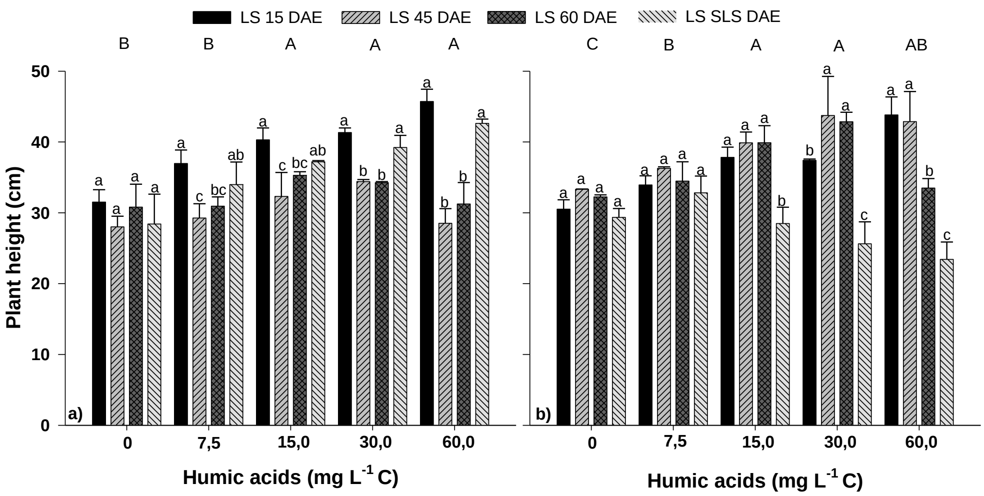

Fig 2. Height of brachiaria (*Brachiaria decumbens*) plants measured at 45 days after emergence (DAE) (time of first cut, a) and 90 DAE (time of second cut, b), in a greenhouse, Alegre-ES. Vertical bars represent the standard deviation of the mean. Means followed by the same capital letter do not differ among the evaluated concentrations. Means followed by lowercase letters do not differ among each other within each dosage of vermicompost humic acids (HAs), according to the LSD test (p ≤ 0.05).

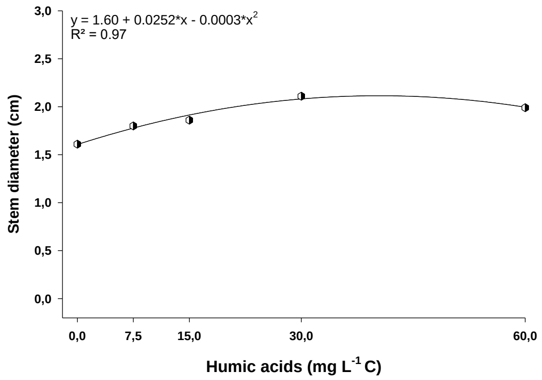

Fig 3. Average diameter of *Brachiaria decumbens* stem treated with humic acids 45 days after emergence (DAE), at time of first cut. Each dosage is an average value of the application times. * Significant at 5% according to the t-test.

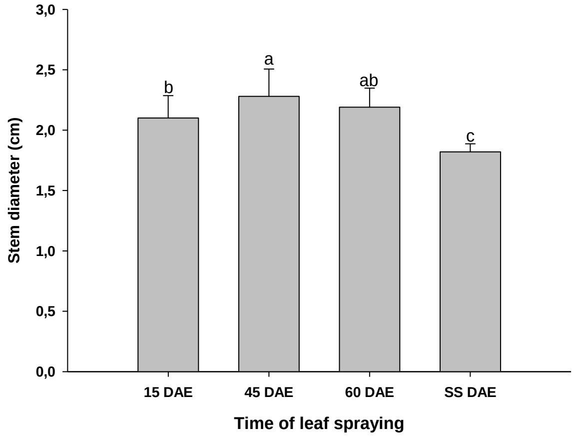

Fig 4. Average diameter of *Brachiaria decumbens* stem, treated with humic acids, 90 days after emergence (DAE), at time of second cut. Vertical bars represent the standard deviation of the mean. Means followed by the same letter do not differ from each other according to the LSD test ($p \leq 0.05$).

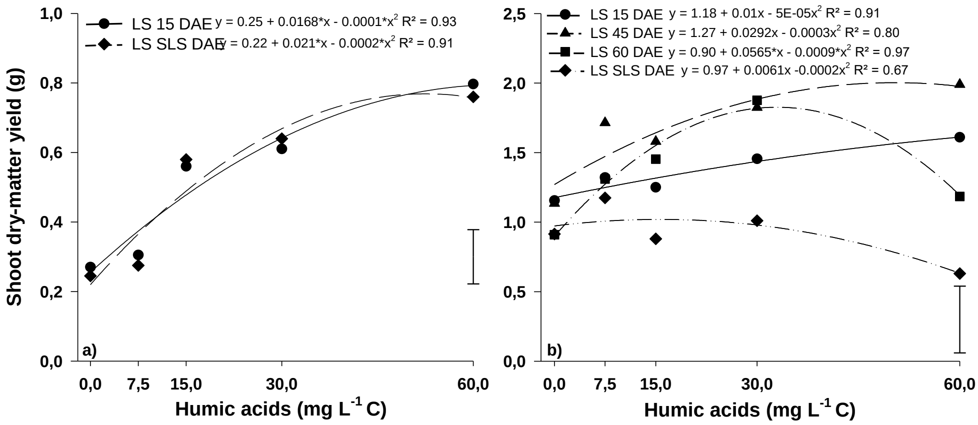

Fig 5. Dry matter of *Brachiaria decumbens* shoot 45 days after emergence (DAE), at time of first cut (a) and 90 DAE, at time of second cut (b). LS 15, 45 and 60 DAE, leaf spraying of HAs at 15, 45 and 60 days after emergence; LS SLS, successive leaf spraying of HAs (15, 45 and 60 DAE). * Significant at 5% according to the t-test. The vertical bar represents the least significant difference (LSD) among the times for all dosages.

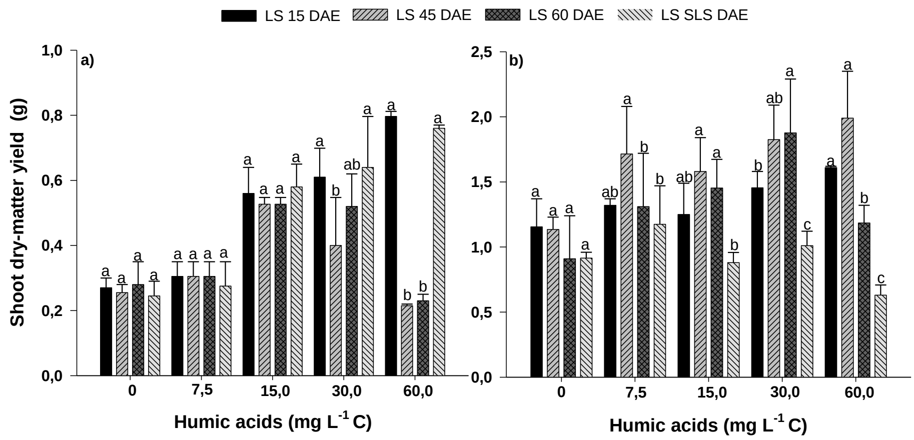

Fig 6. Dry matter of *Brachiaria decumbens* shoot treated with HAs 45 days after emergence (DAE), at time of first cut (a) and 90 DAE, at time of second cut (b), in a greenhouse, Alegre-ES. Vertical bars represent the standard deviation of the mean. Within each dosage of HAs, the means followed by the same letter did not differ among themselves according to the LSD test (p ≤ 0.05).

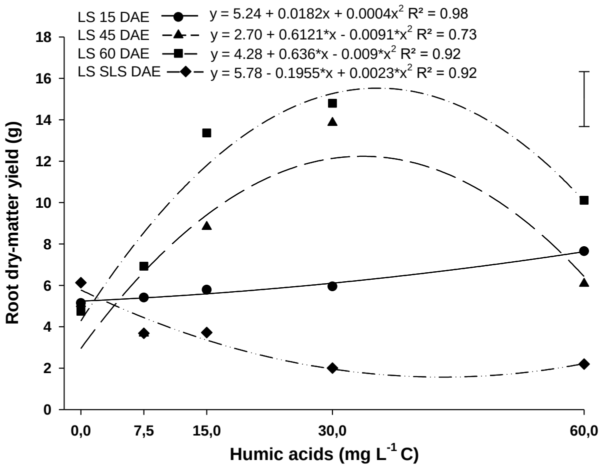

Fig 7. Dry-matter yield of *Brachiaria decumbens* roots, treated with HAs, 90 days after emergence (DAE), at time of second cut, in a greenhouse, Alegre-ES. LS 15, 45 and 60 DAE, leaf spraying of HAs 15, 45 and 60 days after emergence; LS SLS: successive leaf spraying (15, 45 and 60 DAE). * Significant at 5% according to the t-test. The vertical bar represents the least significant difference (LSD) between the times for all dosages.

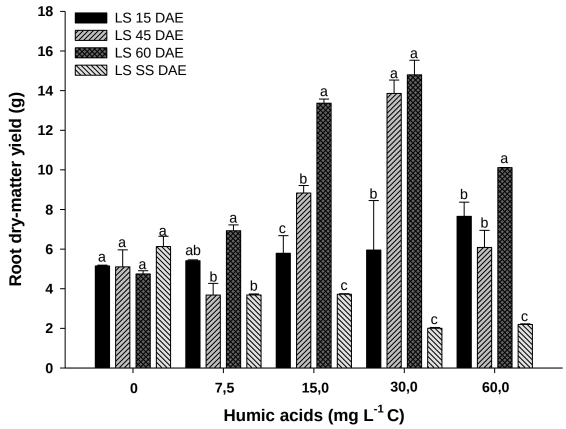

Fig 8. Dry matter of the *Brachiaria decumbens* root treated with HAs 90 days after emergence (DAE), at time of second cut, in a greenhouse, Alegre-ES. Vertical bars represent the standard deviation of the mean. Within each dosage of HAs, the means followed by the same letter did not differ among themselves by the LSD test (p ≤ 0.05).

decrease in dry-matter yield for the dosage of 60 mg L^{-1} C can reach 41% when compared to the HA dosage of 7.5 mg L^{-1} C.

Discussion

In this study, the biostimulating effect of HAs on the initial development and re-growth of *Brachiaria decumbens* observed resulted in greater shoot growth, stem diameter, forage production and root system growth. Pastures are the support base of Brazilian cattle farming, given that they provide the most practical and economic way of feeding cattle. For the Brazilian economy, the logical implication of the results is as important as livestock.

Among the physiological effects of the application of humic substances on the growth and development of plants, the biostimulating effects stand out. These effects are related to the fact that biostimulation activity is similar to that of plant hormones such as auxins, gibberellins, ethylene and cytokinins; (Canellas et al., 2002; Nardi et al., 2002; Canellas and Olivares, 2014; Canellas et al., 2015b). Several studies have demonstrated the positive effect of HAs on plant growth. For example, Jannin et al. (2012) observed the growth and accumulation of nutrients in the shoots of *Brassica napus* plants that received HAs. A similar result was observed by Mora et al. (2010), who evaluated the action of HAs on cucumber growth, and by Baldotto et al. (2009), who evaluated pineapple growth.

For pasture longevity, forage regrowth capacity represents a decisive factor in the production of biomass. In addition to studying the best HA dosages for the growth and development of *Brachiaria decumbens*, evaluating the time of HA application can provide answers about pastures' productive capacity and increase the efficiency of biostimulant technology based on humic substances and their integration with different pasture management practices. When HAs were applied after the cut (45 DAE), the incremental increase in HA concentrations had a similar effect when applied shortly after emergence, and the best dosage response was found at 60 mg L^{-1} C. When applied at 15 days after the cut (60 DAE), the best response was for 30 mg L^{-1} C (Figures 1b and 2b). This effect should be associated with plants' differential responses in the different phases of their ontogenic cycle in response to HA application. Although it is widely recognized that there is a clear modulation of plant response at different stages of growth and development from germination to grain filling, the molecular basis of the range of responses to humic substances is not yet known. Canellas et al. (2015b) observed higher grain yield when HAs were applied in the V6 and V8 stages of vegetative development when applying HAs in the form of leaf spray at different phenological stages of maize under field conditions.

Greater shoot development and forage production in regrowth may be associated with one of the main physiological effects described for HAs (Silva et al., 2011), namely, root development, which depends on both the concentration and the time of application on the plant. In this study, the increase in root dry-matter yield occurred when HAs were applied in a single dose (15, 45 and 60 DAE). Higher root dry-matter yield is associated with morpho-anatomical and biochemical changes that result in the increased formation of lateral roots (Canellas et al., 2002) and root hairs (Silva et al., 2011), which increases the root surface area and mass, favouring the absorption of water and nutrients. Higher nutrient absorption is caused by the stimulation of plasma membrane H+-ATPase enzyme activity and the promotion of plasma membrane H+-ATPase enzyme synthesis. This effect is similar to that of the auxin hormone, which favours cell expansion through apoplast acidification and energy generation linked to the secondary systems for translocating ions and other solutes across the plasma membrane (Canellas et al., 2002; Façanha et al., 2002; Sondergaard, 2004). In a study with cucumber whose roots were treated with HAs, Mora et al. (2010) verified an increase in the activity of the H^+-ATPases of plasma membranes, causing an increase in the absorption and distribution of nitrate (NO_3^-) between the root and the shoot. In addition, Ramos et al. (2015) observed changes in the H^+ exudation profile along the root of HA-treated rice seedlings. The zone of greater exudation corresponded to the zone of greater absorption of calcium. Increased expression of calcium transporters in the presence of HAs was also observed. Jindo et al. (2016) observed that tomato plants treated with HAs increased the expression of high-affinity phosphate transporters in the root. Taken together, it is possible to conclude that HAs act both on the regulation of the hormonal balance responsible for plant growth and on the absorption of nutrients through induction of the transporters.

These growth-promotion characteristics tend to be reflected in low-fertility soils, favouring the potential of regrowth and forage production by the plant caused by the application of HAs after the cut (Figures 5b to 8). The effects of HAs on promoting Brachiaria growth justify the interest in its use for the better utilization of pasture areas. However, as shown in Figures 2b and 8, successive HA applications (SLS DAE) reduced the growth of *Brachiaria decumbens.* This effect is typical of the exogenous application of plant hormones and is well documented in scientific literature (Atiyeh et al., 2002; Baldotto and Baldotto, 2014; Bettoni et al., 2016).

This study indicates the importance of HAs for plant growth, representing a potentially alternative to reduce pasture production costs. The production and application costs of HAs are considered low because HAs are easily obtained from organic residues. The extraction procedures are relatively simple and may be adapted to a rural or commercial company, with no major impacts on the production process (Baldotto and Baldotto, 2014). Thus, the application of HAs could improve pasture biomass production through more efficient nutrient absorption, reducing the advance to native biomes.

Materials and methods

Experimental site and treatments

The study was conducted in a greenhouse of the Department of Plant Production of the Centre of Agrarian Sciences and Engineering of the Federal University of Espírito Santo (Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo - CCAE-UFES). The experimental design was a randomized complete block design with a 5x4 factorial scheme with three replicates. The first factor represents five concentrations (0, 7.5, 15, 30 and 60 mg of carbon (C)) of the HAs. The second factor represents four application times: leaf spraying (LS) at 15 days after emergence (DAE); LS 45 DAE; LS 60 DAE; and successive leaf spraying (SLS) at 15, 45 and 60 DAE in sequence. The treatments of 45 DAE and 60 DAE are the applications that occurred after the first cut, representing the physiological effects of the application of HAs on the regrowth of *Brachiaria decumbens*.

The *Brachiaria decumbens* species was used, with sowing in pots containing 1.5 L of a dystrophic Yellow Red Latosol material collected at a 0-20 cm depth of a degraded pasture in the city of Alegre, located in Southern Espírito Santo State.

Chemical characterization of soil

The collected soil was air dried and sieved with 2.0 mm mesh to obtain air-dried fine soil (ADFS). For soil chemical characterization (Table 1), the following analyses were performed: (1) pH in water, in 1:2.5 soil: liquid suspension; (2) exchangeable Al, Ca, Mg and Na, extracted with 1 mol L^{-1} KCl, in the 1:10 ratio, with Al determined by titration with 0.025 mol/L NaOH, Ca and Mg by atomic absorption spectrometry (AAS) and Na by flame photometry; (3) available K and P by extraction with $Mehlich^{-1}$ (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}), in the 1:10 ratio, measured by flame photometry and colourimetry, respectively; and (4) H+Al using 0.5 mol L^{-1} $Ca(OAc)_2$, adjusted 7.0 pH, in the 1:15 ratio, titrated with 0.0606 mol L^{-1} NaOH (EMBRAPA, 1997).

Chemical composition of the vermicompost and obtaining the HAs

The vermicompost used to obtain HAs was produced with a mixture of filter cake from sugarcane and cattle manure 5:1 (v/v). The organic residues were mixed and earthworms (*Eisenia foetida*) were added at a ratio of 5 kg worms per m^3 of organic residue. This procedure was performed at the Centre for the Development of Biological Inputs for Agriculture (Núcleo de Desenvolvimento de Insumos Biológicos para a Agricultura - NUDIBA) at the State University of Northern Rio de Janeiro (Universidade Estadual do Norte Fluminense - UENF), Campos dos Goytacazes - RJ. It was air-dried and sieved (2 mm mesh sieve) and chemically characterized (Table 2).

The humic acids were extracted as described by Canellas et. al (2002), with minor modifications. In brief, 10 volumes of 0.1 mol L^{-1} NaOH was mixed with 1 volume of vermicompost, under N_2 atmosphere. After four hours of stirring, the material was centrifuged (2,657 *g*, 20 min) and acidified to 1.5 pH with 6 mol L^{-1} HCl. The material was centrifuged again and the supernatant was discarded. HAs were solubilized, precipitated two more times and treated for 16 hours with dilute HCl:HF (1:20, v:v). After centrifugation, the HAs were titrated at 7.0 pH with 0.1 mol L^{-1} KOH and subjected to dialysis on 10 mL dialysis membranes (cut-off 14 KDa, Thomas Scientific, Swedesboro, NJ). After dialysis, the HAs were lyophilized and stored. The C content measurement present in the HA samples was performed by dry combustion on a PerkinElmer 2400 II elemental analyser.

Cultivation conditions and evaluated parameters

Ten seeds of *Brachiaria decumbens* were sown per pot. Five plants per pot were selected 15 DAE. The parameters used for the choice of seedlings were homogeneity, vigour and size.

The experiment was performed for 90 days, and stem height and diameter were measured using a ruler and digital calliper. To simulate grazing, two cuts were made 10 cm from the substrate surface. These cuts were performed at 45 and 90 DAE. At 45 and 90 DAE, the shoot's dry matter production was evaluated, and at 90 DAE, the root's dry matter was obtained. The volume of humic suspension applied for each treatment was 200 mL. The humic suspension was applied with a Pre-Compression Spray device 1.25 Liters PCP-1P.

Statistical analysis

The data were submitted to analysis of variance and when significant, the least significant difference (LSD) test ($p \leq 0.05$) was used for the application time factor and regression analysis was used for the HA dosage factor, employing SISVAR software (Ferreira, 2011). The models were chosen based on the significance of the regression coefficients, using Student's t-test at 5% probability and the coefficient of determination (R^2).

Conclusion

HAs isolated from vermicompost were effective in promoting the growth of *Brachiaria decumbens*. The results depended on both the concentration and the time of application and emphasize this technology's potential for the formation and rotational management of pastures. In addiotion, this experiment provided results to justify further study in a field situation. Foliar application at 15 days after emergence (LS 15 DAE) was most effective in both planting and regrowth, but for planting, the best HA concentration was 60 mg L^{-1} C, whereas for regrowth, it was 40 mg L^{-1} C. The same behaviour was found in the stimulation of the pasture root system, that is, the highest root growth was obtained with AF 15 DAE in both planting and regrowth with HA concentrations of 60 and 35 mg L^{-1} C, respectively. Successive application of HAs on Brachiaria is not recommended since it reduces the growth of both shoot and root system.

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