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PLANT STIMULANT EFFECT ON BRASIL-BAHIA TOBACCO GROWTH AND PRODUCTION[1]

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RESUMO

EFEITO DE ESTIMULANTE VEGETAL NO CRESCIMENTO E PRODUÇÃO DE FUMO BRASIL-BAHIA

Objetivou-se avaliar o efeito do estimulante vegetal Stimulate®, via pulverização foliar, sobre o crescimento e produção de fumo (*Nicotiana tabacum* L.). Foram utilizados fumo Brasil-Bahia e Stimulate®, nas doses 0,0 mL L^{-1}; 1,0 mL L^{-1}; 3,0 mL L^{-1}; 5,0 mL L^{-1}; e 11,0 mL L^{-1}. A partir de 15 dias após a semeadura (DAS), foram aplicadas um total de seis pulverizações, uma vez por dia, a cada cinco dias. No viveiro convencional, após 43 DAS, avaliou-se o número de folhas; comprimentos da haste e raiz; massa de matéria seca de hastes, folhas e raízes; e área foliar. No campo, as plantas permaneceram por 64 dias (107 DAS) e avaliou-se o número de folhas; número de folhas viáveis; altura da planta; massa de matéria seca de folhas e hastes; e área foliar. No viveiro convencional, o Stimulate® diminuiu a massa de matéria seca da haste, raiz e folha e a área foliar do fumo, porém, foi eficiente no aumento do número de folhas e comprimento da haste, para o fumo Brasil-Bahia. No campo, o Stimulate® não foi eficiente no aumento da produção de folhas, porém, incrementou a altura e a massa de matéria seca do caule.

PALAVRAS-CHAVE: *Nicotiana tabacum* L.; pulverização foliar; regulador vegetal; Stimulate®.

ABSTRACT

The purpose of the study was to evaluate the effect of the Stimulate® plant stimulant, applied to leaves by spraying, on tobacco (*Nicotiana tabacum* L.) growth and production. The Brasil-Bahia tobacco and Stimulate®, at the doses of 0.0 mL L^{-1} (control - water); 1.0 mL L^{-1}; 3.0 mL L^{-1}; 5.0 mL L^{-1}; and 11.0 mL L^{-1}, in watery solution, were used. On the fifteenth day after sowing (DAS), the treatments were applied. A total of six sprayings were made, once a day, each five days. After forty-three DAS, the number of leaves; stem and root length; stem, roots, and leaves dry matter; and leaf area were evaluated, under nursery conditions. In the field, the plants remained for 64 days (107 DAS) and the number of leaves; number of viable leaves; plant height; stem and leaves dry matter; and leaf area were evaluated. Stimulate®, under nursery conditions, decreased stem, root and leaf dry matter and leaf area for tobacco. Stimulate® was also efficient to increase the number of leaves and stem length, under nursery conditions, for the Brasil-Bahia tobacco. Under field conditions, Stimulate®, applied during the vegetative stage, was not efficient to increase leaf production, however, it increased stem height and dry matter.

KEY-WORDS: *Nicotiana tabacum* L.; leaf pulverization; plant regulator; Stimulate®.

INTRODUCTION

Brazil is the second largest worldwide tobacco (*Nicotiana tabacum* L.) producer, as far as its quality is concerned. Furthermore, it is the largest tobacco exporter in the world. According to MAPA (2010), the national production, in 2007/2008, was 1,932 kg ha^{-1} and the harvested area covered 473 thousand hectares.

In Bahia, in 2007, the harvest process resulted in 11,122 ton, in Recôncavo da Bahia, a region where the tobacco crop is one of the main agricultural activities and important source of income for small farmers and large companies. Among the tobacco species produced in that region, the Brasil-Bahia tobacco, used for manufacturing cigars and cigarettes, stands out. The region accounts for less than 5% of the national production, and about 97% of what is produced is addressed to foreign markets. Thus, tobacco is responsible for the direct involvement of people across the region and, in 2007, reached a cultivated area of 12,485 hectares (Anuário... 2007).

The application of plant regulators/stimulants, aiming to improve yield standards, has presented promising and significant results, mainly for crops which have achieved high technology and

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management levels (Vieira & Castro 2004). According to Castro & Vieira (2001), the mixture of two or more plant regulators with other substances (amino-acids, nutrients, or vitamins) is known as biostimulants or plant stimulants. These substances, however, due to their composition, concentration and proportion, may increase plant growth and development, stimulating cell division, differentiation and elongation, favoring the plant hormonal equilibrium, and also increasing absorption and water and nutrient use by plants (Vieira & Castro 2003). These substances act directly in root growth, whereas auxin (Cato 2006), along with gibberellin and cytokinin, assure adequate plant growth and development, and consequently good production. For tobacco, the use of plant regulator, in order to evaluated growth parameters and yield, is uncommon, and that is what makes this research important.

The objective of the present study was to evaluate the effect of the application, via leaf spraying, of the Stimulate® plant stimulant on the Brasil-Bahia tobacco growth and production.

MATERIAL AND METHODS

The experiments, under conventional nursery and field conditions, were carried out in the Capivari Farm (12º37'28,9''S and 39º03'41,1''W), in Governador Mangabeira, Bahia State, Brazil, owned by Danco - Tobacco Commerce and Industry Limited, and the analyses were carried out in the Plant Physiology Laboratory of the Universidade Federal do Recôncavo da Bahia, located in Cruz das Almas, Bahia State, Brazil, from May to September 2006.

The experiments were performed in a randomized design, with five treatments and four replications. Treatments consisted of Brasil-Bahia tobacco and five concentrations of Stimulate® in watery solution, as it follows: 0.0 mL L^{-1} (control - water); 1.0 mL L^{-1}; 3.0 mL L^{-1}; 5.0 mL L^{-1}; and 11.0 mL L^{-1}.

Stimulate® is a plant stimulant produced by Stoller do Brasil and contains 0.09 g L^{-1} of Kinetin (cytokinin), 0.05 g L^{-1} of Gibberellic acid (gibberellin), 0.05 g L^{-1} of Indolebutyric acid (auxin), and 99.981% of inert ingredients (Stoller do Brasil 1998).

Under conventional nursery conditions, the pelletized seeds were sown in expanded polystyrene trays containing PlantMax® substrate, humidified with water. According to Hutchens (1999), pelletized seeds are used because, besides making the handling of individual tiny tobacco seeds easier, they can be precisely inserted in determined places and correct depths, reaching better results, when compared to naked seeds.

Trays containing 256 cells were divided in four quadrants, corresponding to one repetition each. In order to keep the substrate humidity rate, the trays were covered with non-woven fabric, according to the seedlings production system of the company, until full seed germination (which took place around the seventh day).

Fifteen days after sowing (DAS), when the small plants had already enough leaf area, the treatments were applied by spraying the leaves. A total of six sprayings were made, once a day, every five days, by using a hand sprayer, applying 100 mL in each treatment, always in the early morning (between 5:30 and 6:30 a.m.). After three hours, the small plants were irrigated with tap water, in order to keep the substrate humidity, throughout the experimental period. After 33 DAS, when the six sprayings were over, the plants were irrigated only with tap water, until transplantation to the field.

When the seedling stage was concluded (under conventional nursery, at 43 DAS), with plants reaching around 15 cm in height, part of them was used to evaluate growth under conventional nursery, while the others were transplanted to the field, for yield evaluation. No fertilization was used during this period.

Growth evaluation

A plant from each quadrant was collected, corresponding to the four replicates of each treatment. Then, they were taken from the substrate, washed with tap water, and had their aerial and root areas measured with a millimeter ruler. The number of leaves was determined by direct counting. The determination of leaf area was made by means of the ratio of the disc dry matter and full leaf dry matter. The discs were obtained with the help of a known area perforator, avoiding the central nervure, taking four leaf discs from each plant (replicates).

The roots, leaves, stem and leaf discs were conditioned separately in identified paper bags and placed in a forced air circulation oven, with temperatures of 65ºC ± 5ºC, for 72 hours, in order to determine the dry matter content.

Yield evaluation

After the growth evaluation, twelve plants from each treatment were transplanted to the field, and planted using a distance of 1.0 m between rows and 0.45 m between plants, and treatments were distanced by 1.50 m. The fertilization used was based on NPK, following the company production system (the industry uses different concentrations in tobacco fertilization, thus, these data and soil analysis were not disclosed by it).

When the leaves destined to the manufacturing of cigars and cigarettes reached the harvest stage, totaling 64 days in the field (107 DAS), four plants from each treatment were selected, corresponding to the four repetitions.

The number of leaves was determined by direct counting and classified, with the help of the company employees, as full leaves and viable leaves (leaves for filling, green, big, less fragile, thicker, and consistent). The height of the plants was defined by using a measuring tape. Twenty leaf discs were taken, in order to determine the leaf area.

Stems, leaves and leaf discs were separately conditioned in identified paper bags and placed in a forced air circulation oven, with temperatures of 65ºC ± 5ºC, until reaching constant weight, for determination of dry matter.

The collected data were submitted to the variance analysis, at 5% and 1%, according to the F Test. The polynomial regression analysis was performed for those variables, where the test indicated significant discrepancies in the performed treatments.

RESULTS AND DISCUSSION

Under conventional nursery conditions, only root length (RL) was not significantly influenced by Stimulate® doses (Table 1). This result could be explained by the greater sensitivity of roots not completely developed yet, once this product has the ability, due to its composition, properties and chemical characteristics, to favor growth and development of the root system. Reghin et al. (2000) reported favorable results for root length in assays with arracacha (*Arracacia xanthorrhiza*). According to them, this plant stimulant can be used to promote rooting. Castro & Vieira (1999), in soybean assays, also reported plants with more developed and vigorous root systems, with growth and total length superior to the ones reported for plants not treated with Stimulate®.

For the number of leaves (LN), results showed significant effect ($p \leq 0.05$), in relation to Stimulate® applied via spraying. This biostimulant promoted an increase in the number of leaves, along with the increase in the dosage, according to the equation $\hat{Y} = 0{,}154x + 4{,}631$, with a determination coefficient of 85.4%. It was verified that the highest value obtained was 6.25 leaves, for the 11 mL L^{-1} Stimulate® dosage, reporting an increase of 39%, in comparison to the control (Figure 1). The positive results are in agreement with the experiment carried out by Castro et al. (2001), with ´Pêra` oranges, where the application of 1 L ha^{-1} of that stimulant increased the number of branches and leaves. These results can be attributed to plant regulators present in that biostimulant, in balanced and favorable proportions (Sampaio 1998). So, cytokinins, in equilibrium with auxins and gibberellins, promoted a positive effect on

Table 1. Variance analysis for the Brasil-Bahia tobacco growth, under conventional nursery conditions, in response to leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

VS	LD	Mean square						
		LN	SL (cm)	RL (cm)	SDM (g)	RDM (g)	LDM (g)	LA (cm^2)
Treatment	4	2.1250*	12.5750*	3.1967 ^{ns}	0.0335*	0.0115*	0.1815*	86.7166**
Error	15	0.4833	1.0878	1.403	0.0038	0.0040	0.0230	4.2014
VC (%)		13.24	10.72	14,24	5.88	5.44	20.21	9.58
General mean		5.25	9.72	8,32	1.06	1.16	0.75	21.40

^{ns}: non-significant; * and **: significant at 5% and 1%, respectively; LN: leaf number; SL: stem length; RL: root length; SDM: stem dry matter; RDM: root dry matter; LDM: leaf dry matter; and LA: leaf area.

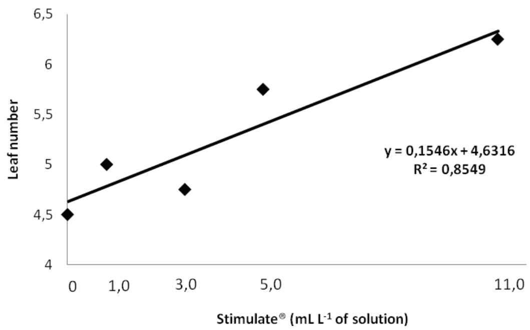

Figure 1. Leaf number (LN), in plants of Brasil-Bahia *Nicotiana tabacum* L., under conventional nursery conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

leaf growth. Cytokinin promotes lateral bud elongation and can also initiate the transport of growth substances, favoring increases in the number of leaves.

The quadratic equation $\hat{Y} = 0{,}070x^2 - 0{,}466x + 9{,}378$, with determination coefficient of 98%, was significant ($p \leq 0.05$) for representing stem length (SL). The highest value found (12.85 cm) refers to the 11 mL L^{-1} Stimulate® dosage, 37% superior to the control, while the minimum value (8.6 cm) refers to the 3.33 mL L^{-1} Stimulate® dosage. This value is 8.5% lower than the control and 33.07% lower than the largest stem length found. From the minimum point, the growth in stem length, in regard to the dosages of the stimulant, can be observed in Figure 2. Santos & Vieira (2005) reported similar results, when the 17.5 mL L^{-1} Stimulate® dosage was used for cotton, and also observed an increase of 29.7%, in comparison to the control.

Stem dry matter (SDM), represented by the linear equation $\hat{Y} = -0{,}019x + 1{,}137$ ($R^2 = 0.855$), showed to be significant ($p \leq 0.05$). The highest value for dry matter (1.15 g) was obtained at the 1 mL L^{-1} Stimulate® dosage, surpassing in 5.8% the control treatment. The highest dosage (11 mL L^{-1} of Stimulate®) was 21.26% inferior, in regard to the highest value found for stem dry matter, and 16.7% inferior to the control. Therefore, with the increase of the dosages up to 11 mL L^{-1}, a significant ($p \leq 0.05$) reduction of this variable occurred (Figure 3). Similar results for stem dry matter were found by Cato et al. (2005), for soybean, when Stimulate® was applied during the V_5 stage. In this assay, a root growth rate higher than that observed for stem and leaves occurred, suggesting a possible interaction among auxins, cytokinins, and gibberellins, favoring root growth and promoting less stem dry matter.

The positive effect for stem height and the negative effect for dry matter (Figures 2 and 3) could be possibly explained by the higher cells stretching rate. This effect, regardless of GA and IBA, is present in the Stimulate®. Indolbutiric acid promotes a breakage process, due to enzymes present in the links among microfibers of the cell wall, which would promote an increase in plasticity and decrease in the reflection coefficient, whereas the low relative value of the osmotic potential in the interior of the vacuole would promote the influx of water, resulting in the increase of cell dimensions. Gibberellic acid promotes α-amylase synthesis and, under its action, there is the formation of sugars in the starch cells, whereas the osmotically active product would promote decrease in the osmotic potential of the cell, causing influx of water and increasing cell dimension (Taiz & Zeiger 2009).

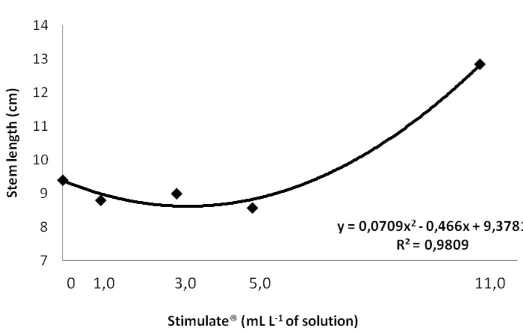

Figure 2. Stem length (SL) in plants of Brasil-Bahia *Nicotiana tabacum* L., under conventional nursery conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

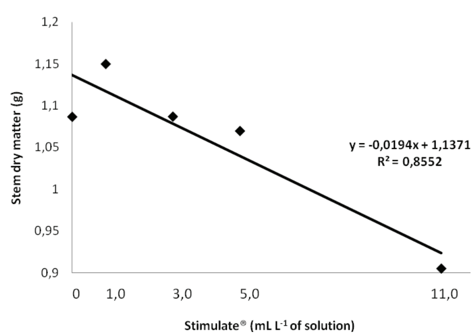

Figure 3. Stem dry matter (SDM) in plants of Brasil-Bahia *Nicotiana tabacum* L., under conventional nursery conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

Root dry matter (RDM) was significant ($p \leq 0.05$) and had a negative linear correlation with Stimulate® doses, according to the equation $\hat{Y} = -0{,}012x + 1{,}213$, with determination coefficient of 96.5% (Figure 4). Decreases occurred with the increase of the concentration of the biostimulant, whereas, in the highest Stimulate® dosage, the dry matter production was 10% inferior to the control and 11.2% lower than the highest value found for this variable. For higher dosages, lower increments were found. Dario et al. (2005) explain that the combined action of cytokinin and gibberellin can decrease the effects of gibberellin. According to Taiz & Zeiger (2004), the ideal growth balance of different plant organs is variable, whereas an endogenous concentration can favor growth in one organ and growth inhibition in another. Echer et al. (2006) observed that the 4 mL L^{-1} Stimulate® dosage showed greater efficiency, since it positively increased root dry matter for passion fruit.

Leaf dry matter (LDM) was significant ($p \leq 0.05$) and also had a negative linear correlation with Stimulate® doses, according to the equation $\hat{Y} = -0{,}046x + 0{,}938$ ($R^2 = 0.92$, Figure 5). Dosages higher than the control were incapable of stimulating leaf dry matter, negatively contributing for this variable. None of the dosages used were superior to the control treatment (0.93 g) and the highest Stimulate® dosage was 56.54% lower.

Klahold et. al. (2006) did not find significant differences for leaf dry matter, working with soybean, when dosages between 0.0 mL L^{-1} and 5.0 mL L^{-1} of Stimulate®, and also between 0.0 mL L^{-1} and 0.225 mL L^{-1}, were used. However, Echer et al.

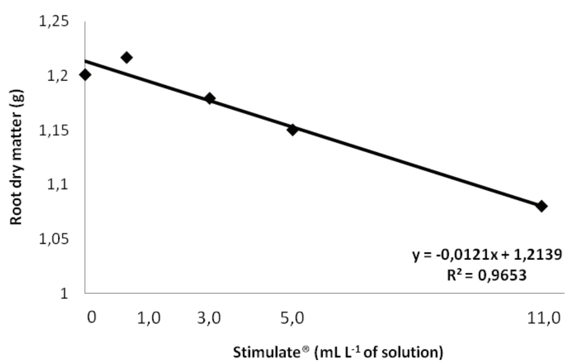

Figure 4. Root dry matter (RDM) in plants of Brasil-Bahia *Nicotiana tabacum* L., under conventional nursery conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

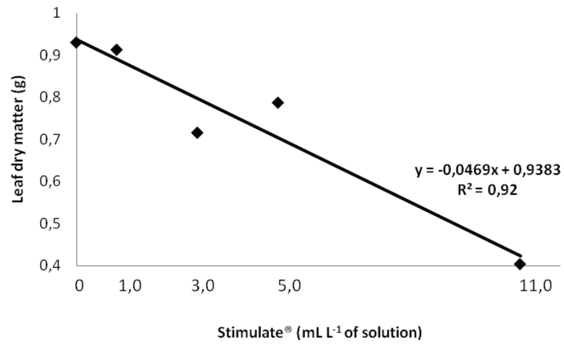

Figure 5. Leaf dry matter (LDM) in plants of Brasil-Bahia *Nicotiana tabacum* L., under conventional nursery conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

(2006), using higher dosages of the stimulant, observed a positive response for leaf dry matter, for yellow passion fruit, when 0 mL L^{-1}, 4 mL L^{-1}, 12 mL L^{-1}, 16 mL L^{-1}, and 20 mL L^{-1} Stimulate® dosages were used. In the same study, the authors observed a decrease in leaf area, for passion fruit, with significant reductions among treatments, reaching up to a 19.7 % decrease, if compared to the control, when 3.0 mL L^{-1} and 0.150 mL L^{-1} of Stimulate® were used, presenting similar behavior for tobacco.

For leaf area (LA), the highest dosage had an average of 13.31 cm^2 and was 45.9% inferior to the control. According to the quadratic model $\hat{Y} = -0{,}081x^2 - 0{,}100x + 24{,}326$ ($R^2 = 0.96$), the point of maximum was reached at 0.62 mL L^{-1} of Stimulate®, with leaf area of 24.2 cm^2, which was 1.53% lower than the control (Figure 6). Rodrigues et al. (2002) attributed the leaf area increment, for soybean (30 days after sowing), to plant regulators present in Stimulate®.

Decreasing results in stem length and in stem, root, and leaf dry matter can be explained by effects caused by some internal hormonal disequilibrium, that, during the developmental stage, requires efficiency in the physiological processes. The action of these substances depends on environmental conditions and plant characteristics and genetic potential (Vieira & Monteiro 2002).

Under field conditions, the application of Stimulate® doses had no effect on number of leaves (NL), number of viable leaves (NVL), leaf dry matter (LDM), and leaf area (LA) (Table 2).

The non-significant results for leaf number and dry matter were similar to the ones found by

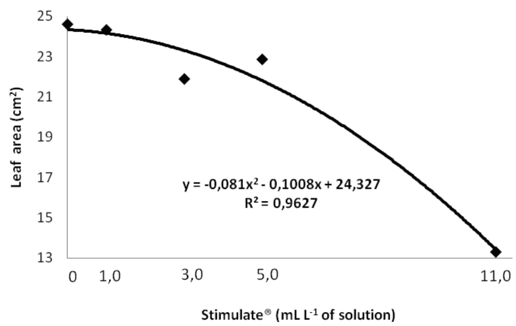

Figure 6. Leaf area (LA) in plants of Brasil-Bahia *Nicotiana tabacum* L., under conventional nursery conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

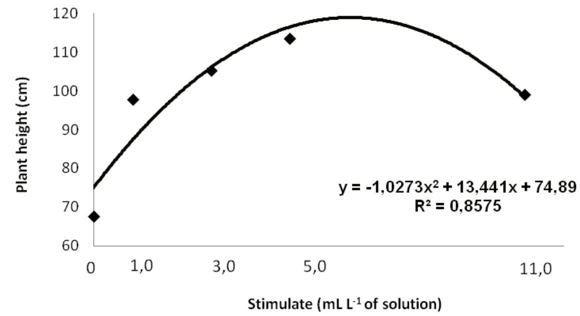

Figure 7. Plant height (PH) in plants of Brasil-Bahia *Nicotiana tabacum* L., under field conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

Dourado Neto et al. (2004), for maize assays, where the effect of the exogenous application of gibberellin and cytokinin was not observed too. The combined application of these regulators led to the decrease of the gibberellins effects. Despite the proven efficiency of this product, in favoring an adequate hormonal equilibrium, increasing growth and acting effectively under several physiological processes, such as leaf growth (Vieira & Castro 2004), new studies and determination of new concentrations, in order to prove the results, are necessary, since, according to Alleoni et al. (2000), plant regulators are more active during the vegetative stage.

Stimulate® was effective regarding plant height (PH), presented significance ($p \leq 0.05$), and is represented by the quadratic equation $\hat{Y} = -1{,}027x^2 + 13{,}44x + 74{,}89$, with determination coefficient of 85.7%. At the point of maximum, a height of 118.86 cm, at the 6,54 mL L^{-1} Stimulate® dosage, was observed, being 76.1% higher, in comparison to the control treatment (Figure 7). All values encountered were superior to the control, and even the highest Stimulate® dosage (11 mL L^{-1}) was 46.67% higher than it. Dourado Neto et al. (2004) found similar behavior, for their experiment with maize, when Stimulate® was applied via leaf spraying. They observed an increase in plant height that, according to them, was due to the application of exogenous gibberellin, component of the biostimulant. On the contrary, when Albuquerque (2004) applied the same regulator, for experiments with castor beans (*Ricinus communis* L.), at the 0 mL L^{-1}, 7 mL L^{-1}, 14 mL L^{-1}, 21 mL L^{-1}, and 35 mL L^{-1} Stimulate® dosages, no significant differences, regarding this variable, were observed.

The quadratic model $\hat{Y} = -0{,}703x^2 + 9{,}503x + 34{,}99$ ($R^2 = 93{,}4\%$) was found for the stem dry matter (SDM) variable. The point of maximum was 67.12 g, for the 6.7 mL L^{-1} Stimulate® dosage, which promoted an increase of 93.8%, in comparison to the control. Regardless of the decrease, all treatments, according to the averages observed, were superior to the control (Figure 8). The highest Stimulate® dosage (11 mL L^{-1}) was 56.19% superior to the control. Height and stem dry matter, under field conditions, reached higher values between the dosages of 6.5 mL L^{-1} and 6.7 mL L^{-1} of Stimulate®. For stem dry matter, despite the decrease of 6.76 mL L^{-1} of Stimulate®,

Table 2. Variance analysis for the Brasil-Bahia tobacco yield, under field conditions, in response to leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

VS	LD	Mean square					
		LN	NVL	PH (cm)	LDM (g)	SDM (g)	LA (cm^2)
Treatment	4	0.2000^{ns}	1.9500^{ns}	1214.32*	88.862^{ns}	589.737*	48524.4^{ns}
Error	15	1.3166	1.7300	317.700	89.203	239.873	64283.3
VC (%)		10.58	16.25	18.45	17.63	30.34	17.88
General mean		10.85	8.10	96.60	53.54	51.05	1417.78

^{ns}, *: non-significant and significant at 5%, respectively; LN: leaf number; NVL: number of viable leaves; PH: plant height; LDM: leaf dry matter; SDM: stem dry matter; and LA: leaf area.

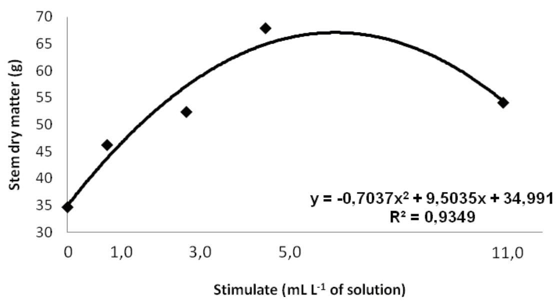

Figure 8. Stem dry matter (SDM) in plants of Brasil-Bahia *Nicotiana tabacum* L., under field conditions, for leaves sprayed with five Stimulate® doses (Governador Mangabeira, Bahia State, Brazil, 2006).

all treatments were superior to the control. Alleoni et al. (2000) explain that plant regulators are more active in producing dry matter from the vegetative stage until flowering.

CONCLUSIONS

1. Stimulate®, under conventional nursery conditions, decreases stem, root and leaf dry matter, and leaf area for tobacco. The application of Stimulate®, via leaf spraying, is efficient in increasing the number of leaves and stem length, under nursery conditions, for the Brasil-Bahia (*Nicotiana tabacum* L.) tobacco.
2. Under field conditions, Stimulate®, applied during the vegetative stage, is not efficient for increasing leaf production. However, it increases stem height and dry matter for the Brasil-Bahia (*Nicotiana tabacum* L.) tobacco.

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