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EFFECTS OF 2,4-D = PICLORAM SUBDOSES ON COTTON CROP

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ABSTRACT: The aim of this work was to evaluate vegetative and reproductive characteristics of cotton using subdoses of 2,4-D + picloram herbicides at different phenological crop stages. The experiment was carried out in a randomized block design in a factorial scheme, with treatments consisting of the combination of four 2,4-D + picloram subdoses (0, 10, 25 and 50 ml of commercial product ha^{-1}) and application with spray volume of 200 L ha^{-1}, at different phenological crop stages (B1, B2, B3 and B5), with 4 replicates. The sowing of the 'IMA 5801 B2RF' cotton cultivar was carried out on December 6, 2018, with later evaluations of the vegetative and reproductive crop development. The application of 2,4-D + picloram subdoses resulted in decrease in its agronomic characteristics, with greater decrease with increasing subdoses, mainly applied at initial B1 and B2 stages. The application of 2,4-D + picloram subdoses does not promote the hormesis effect in cotton crop.

KEYWORDS: *Gossypium hirsutum*; herbicide management; phytotoxic effect; surface response methodology.

EFEITOS DE SUBDOSES DE 2,4-D E PICLORAM NA CULTURA DO ALGODOEIRO

RESUMO: O objetivo da pesquisa foi avaliar características vegetativas e reprodutivas do algodoeiro pelo uso de subdoses dos herbicidas 2,4-D + picloram em diferentes estágios fenológicos da cultura. Experimento conduzido no delineamento de blocos casualizados em esquema fatorial, com tratamentos constituídos pela combinação de quatro subdoses dos herbicidas 2,4-D + picloram (0, 10, 25 e 50 ml do produto comercial ha^{-1}) e aplicação com volume de calda de 200 L ha^{-1}, em diferentes estágios fenológicos do algodoeiro (B1, B2, B3 e B5), com 4 repetições. A semeadura da cultivar IMA 5801 B2RF em 06 de dezembro de 2018, posteriormente realizou-se avaliações de desenvolvimento vegetativo e reprodutivo no algodoeiro. A aplicação de subdoses de 2,4-D + picloram resultou na diminuição das suas características agronômicas, tendo maior decréscimo com o aumento das subdoses, aplicadas principalmente, nos estágios iniciais B1 e B2. A aplicação de subdoses de 2,4-D + picloram não promove o efeito hormese na cultura do algodoeiro.

PALAVRAS CHAVE: *Gossypium hirsutum*; manejo de herbicida; efeito fitotóxico; metodologia de resposta superficial.

INTRODUCTION

Agriculture is the main source of income for most of the country's economy, especially in developing countries, and cotton is one of the important crops (Abbas and Ahmad 2018), known as "white gold" for producing large amount of income and jobs (Ali et al., 2014). The main cotton producing countries are India, China, the United States, Pakistan and Brazil, with Brazil being the fourth world's largest cotton lint exporter (Khan et al., 2020)

Aiming at achieving high productivity, the use of substances that are initially considered toxic, their use at lower doses to induce hormesis, should be studied in order to maximize this effect. The term hormesis is used in the area of toxicology to describe a response that causes stimulation at low dose or beneficial effect and deleterious or toxic effect at high dose (Mattson, 2008). Some substances, toxic at high doses, may provide benefits at lower doses (Duke et al., 2006; Ferrari et al., 2021a; Ferrari et al., 2021b).

Several chemical products can induce the hormesis effect in plants, with herbicides being the most widely known and studied (Agathokleous et al., 2018). Responses to low doses of synthetic auxins have been studied by several researchers through plant development (Americo et al., 2016). Studies have demonstrated the positive response to the application of herbicide subdoses in cotton; however, most of these studies are carried out with glyphosate and 2,4-D (Américo et al., 2016; Marques, 2019; Furlani Junior et al., 2011).

Tordon is an auxin-mimicking herbicide having in its formulation compounds 4 - amino-3,5,6 - trichloro picolinic acid (Picloram) and 2,4 - Dichlorophenoxyacetic acid (2,4-D) (Adapar, 2018). Herbicides classified as synthetic auxins (HRAC group O) perform the same functions as the naturally occurring plant hormone, indole-3-acetic acid (IAA) (Busi et al., 2018). As it is an herbicide with hormonal action, when incorrectly used, even at low doses, has high potential to damage crops sensitive to its active ingredient (Godinho Junior et al., 2017).

Works with applications of 2,4-D + picloram subdoses in cotton crops, taking into account the phenological stages of application, are scarce. Given the above, the aim of this work was to evaluate the vegetative and reproductive characteristics of cotton crop using 2,4-D + picloram subdoses at different phenological crop stages.

MATERIAL AND METHODS

The experiment was carried out during the agricultural year of 2018/19 on the premises of the Experimental Farm of the Faculty of Agrarian and Technological Sciences – UNESP, located in the municipality of Dracena. Before the experiment installation, a composite sample originated from 20 simple soil samples from the entire experimental area was collected and analyzed, which result was: P resin = 7 mg.dm^{-3}, S = 4 mg. dm^{-3}, O. M. = 21 g. dm^{-3}, pH $CaCl_2$ = 5.2, K = 2.3 mmolc. dm^{-3}, Ca = 5 mmolc dm^{-3}, Mg = 5 mmol. dm^{-3}, H+Al = 15 mmolc. dm^{-3}, Al^3+ = 0 mmolc dm^{-3}, CEC= 27.5 and V(%) = 45.5.

The experimental design used was randomized blocks arranged in a 4x4 factorial scheme. Treatments consisted of a combination of four Tordon subdoses (2,4-D; 240 g active ingredient (a.i.) L^{-1} + picloram; 64 g a.i. L^{-1}) (0, 10, 25 and 50 ml of the commercial product ha^{-1}, namely: dose 0 = control; dose 10 ml = 2.4 g a.i. 2,4-D + 0.64 g a.i. picloram ha^{-1}; dose 25 ml = 6.0 g a.i. 2.4 -D + 1.6 g a.i. picloram ha^{-1}; dose 50 ml = 12 g a.i. 2,4-D + 3.2 g a.i. picloram ha^{-1}), applied at different phenological crop stages (B1, B2, B3 and B5 – appearance of the 1st, 2nd, 3rd and 5th floral bud) with 4 replicates, totaling 48 plots. Plots consisted of 4 rows with 6.0 m in length and spacing between rows of 0.75 m. The useful area of each plot was constituted by the 2 central rows, neglecting 0.50 m at both ends of each row.

The experimental area had *Urocloa* cv. 'Marandú' pasture installed 15 years ago without soil correction or fertilization. The preparation of the experimental area began on 10/01/2018 with harrowing followed by leveling harrow and subsequently application of 1 t ha^{-1} of limestone to raise base saturation to 60%. The sowing of 'IMA 5801 B2RF' cotton cultivar occurred on December 6, 2018 together with the sowing fertilization with 500 kg ha^{-1} of NPK in the 04-14-08 formulation. Seedling emergence occurred on 12/12/2018 and after thinning, a population of 8 plants per m^{-1} was obtained.

Herbicide subdoses were applied in the form of a directed jet, with CO2 costal sprayer with constant pressure and volume of 200 l ha^{-1}. Applications were carried out in the morning between 8:00 am to 9:00 am, with no or little wind incidence, also using a screen to avoid drifting to other plots. On 01/18/2018, topdressing fertilization was performed at dose of 300 kg ha^{-1} of NPK in the 25-00-20 formulation.

During the conduction of the experiment, all necessary cultural treatments were carried out such as control of weeds, pests, diseases and irrigation by surface sprinkler based on Kc and Cc was carried out when necessary.

Parameters were evaluated in the useful area of each plot, and the Spad reading of Chlorophyll 1 (clofofiLOG Falker CFL1030, in F3, appearance of the 3rd flower) was performed on the third fully expanded leaf from the apex to the base in 10 plants; Spad reading of chlorophyll 2 (in F7, appearance of the 7th flower). At the physiological maturity of the cotton plant, the following evaluations were carried out: height (with measuring tape from the ground level to the last branch of the apical meristem); stem diameter (with caliper at 2 cm above the ground); number of nodes on the main stem (by direct counting); boll mass (collecting 20 bolls in the middle third of plants and obtaining the average weight per unit); number of bolls per plant (by direct counting) and seed cotton yield (manual harvest of all bolls).

Data were submitted to analysis of variance (ANOVA) using the SISVAR version 5.3 software (Ferreira, 2011) and subsequently the goodness-of-fit test was performed for response surface models of significant interactions between factors. Then, 3-dimensional graphs were plotted, considering the two analyzed factors and the response variable. Finally, linear correlation was performed between variables, which came from a correlation matrix with 1% significance level using the R software.

RESULTS AND DISCUSSIONS

According to the analysis of variance (Table 1), it was observed that there was no statistical difference between isolated factors and interaction only for Spad 1 and 2 reading. Regarding the other variables, statistical significance was observed between analyzed factors and the goodness-of-fit analysis of models was performed for these variables.

The results of the goodness-of-fit analysis (Table 2) showed that there was significance level of parameters for variables.

Table 1. Summary of the analysis of variance for the characteristics of cotton submitted to 2,4-D + picloram subdoses.

F test	Subdoses (S)	Application time (E)	Interaction (SxE)	CV %
Boll mass	0.0001**	0.0001**	0.0001**	4.74
Number of bolls	0.0001**	0.0001**	0.0001**	5.91
SPAD 1 reading	0.6202ns	0.2708 ns	0.5950 ns	3.68
SPAD 2 reading	0.6416 ns	0.3804ns	0.1029 ns	2.80
Yield	0.0001**	0.0001**	0.0001**	6.58
Plant height	0.0001**	0.0001**	0.0001**	2.24
Diameter	0.0001**	0.0001**	0.0001**	2.35
Number of nodes	0.0001**	0.0001**	0.0001**	4.65

*significant p<0.05 **significant p<0.01. ns p>0.05.

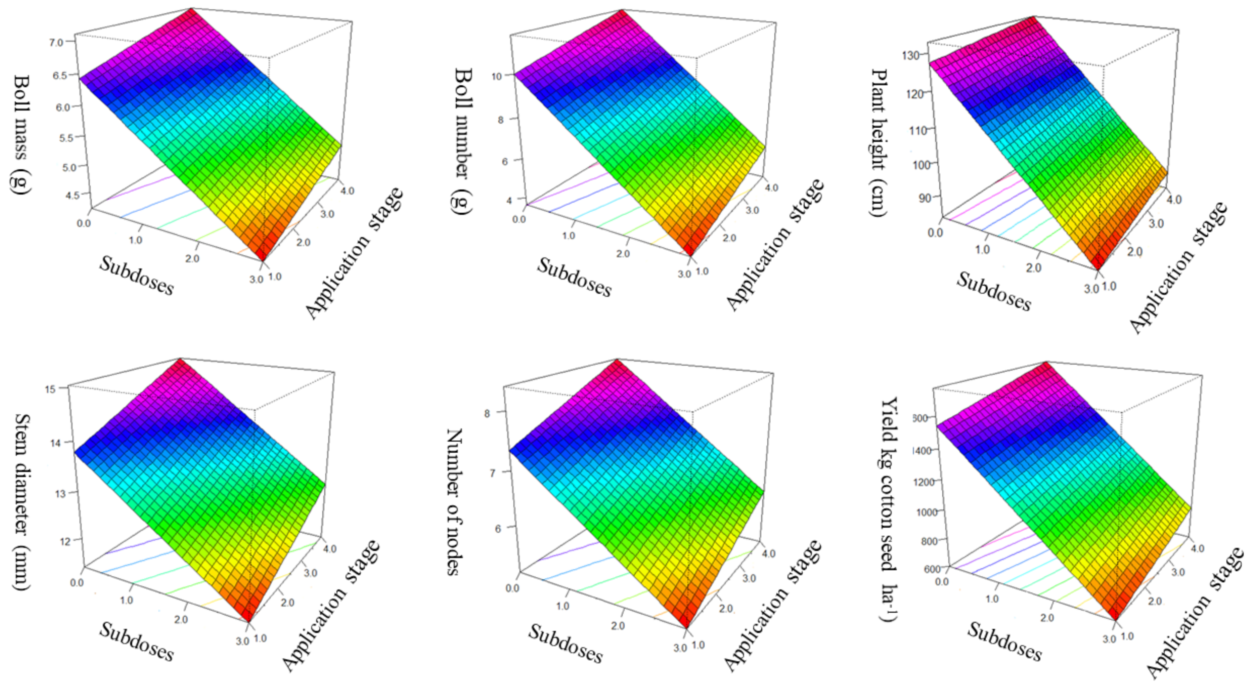
Table 2. Quality of fit analysis for surface response models of the interaction between 2,4-D + picloram subdoses applied at different stages of cotton development.

Parameters	Estimated	Standard error	T value	p> |t|	R adj^2
			Boll mass, g		
Intercept	6.20	0.24	24.96	<0.01**	0.59
Subdoses, g a.i. ha^{-1}	-0.73	0.07	-9.27	<0.01**	
Application stage	0.22	0.07	2.82	<0.01**	
			Number of bolls		
Intercept	9.44	0.40	23.50	<0.01**	0.82
Subdoses, g a.i. ha^{-1}	-2.13	0.12	-16.55	<0.01**	
Application stage	0.56	0.12	4.41	<0.01**	
			Yield, kg cotton seed ha^{-1}		
Intercept	1467.12	51.605	28.42	<0.01**	0.85
Subdoses, g a.i. ha^{-1}	-315.20	16.527	-19.07	<0.01**	
Application stage	75.26	16.527	4.55	<0.01**	
			Plant height, cm		
Intercept	125.70	3.32	37.85	<0.01**	0.75
Subdoses, g a.i. ha^{-1}	-14.78	1.06	-13.86	<0.01**	
Application stage	1.78	1.06	1.67	<0.05*	
			Stem diameter, mm		
Intercept	13.36	0.35	37.28	<0.01**	0.49
Subdoses, g a.i. ha^{-1}	-0.80	0.11	-7.02	<0.01**	
Application stage	0.41	0.11	3.64	<0.01**	
			Number of nodes		
Intercept	7.00	0.25	27.17	<0.01**	0.61
Subdoses, g a.i. ha^{-1}	-0.75	0.08	-9.09	<0.01**	
Application stage	0.35	0.08	4.24	<0.01**	

*significant p<0.05 **significant p<0.01

Assessing the boll mass (Figure 1), it was observed that as subdoses increased, there was a decrease in this variable. Furlani Junior et al. (2011) evaluated boll mass as a function of the application of 2,4-D subdoses in cotton and found no statistical difference for this variable, unlike results obtained in this study.

Figure 1. Response surface plots in three dimensions of the significant interaction between 2,4-D + picloram subdoses applied at different stages of cotton development. Subdoses 0- control; 1-2.4 g a.i. 2,4-D + 0.64 g a.i. picloram ha-1; 2- 6.0 g a.i. 2,4-D + 1.6 g a.i. picloram ha-1; 3-12 g a.i. 2,4-D + 3.2 g a.i. picloram ha-1. Application stages: 1 - B1 2- B2, 3- B3, 4- B5 (appearance of the 1st, 2nd, 3rd and 5th flower bud, respectively).



According to results obtained for the number of bolls, there was a decrease in values as herbicide subdoses increased in the four application times (Figure 1). Rosa et al. (2011) found that there was no statistical difference in the amount of reproductive structures in cotton submitted to glyphosate subdoses. Americo et al. (2016) and Furlani Junior et al. (2011) observed that the application of 2,4-D subdoses increased cotton reproductive structures at subdoses of 2.72 g a.i. ha^{-1}. According to the authors, the application of subdoses of synthetic auxin caused changes in cotton development, promoting greater amount of reproductive structures.

Regarding the average yield (Figure 1), it was observed that as subdoses increased, there was a decrease in the values of this variable at all application times proposed in this work. Americo et al. (2016) and Furlani Junior et al. (2011) found increase in cotton yield at 2,4-D concentrations of 1.90 and 2.72g a.i. ha^{-1}, respectively. Marques (2019) found that 2,4-D subdoses of 0.855 and 1.71g a.i. ha^{-1} promoted hormesis effect for all cotton production variables at stage B4. Rosa et al. (2011) reported maximum increase in the yield of this crop submitted to glyphosate subdoses of 65g a.i. ha^{-1}.

However, Constantin et al. (2007) evaluated the effect of 2,4-D subdoses on simulated drift in cotton and found significant reduction in yield from dose of 6.72 g a.i. ha^{-1} and according to these authors, cotton is sensitive to drift of 2,4-D at the beginning of flowering (F1) and, at this stage, it tolerates maximum of 3.36 g a.i. ha^{-1} and from stage C1, cotton sensitivity to 2,4-D is reduced, and the crop starts to tolerate up to 13.44 g a.i. ha-1 (2.0%) of drift (0.50%).

Yamashita and Guimarães (2006) analyzed the effects of simulated glyphosate drift on cotton cultivars and development stage and found that the application of glyphosate affected seed cotton production, mainly for dose of 360 g and ha^{-1}, in which when compared to control, production reached 75% for 'ITA-90' and 73% for 'BRS-Facual' cultivars.

Regarding plant height (Figure 1), it was observed that there was a decrease in the value of this variable as herbicide subdoses increased in the four application times. Americo et al. (2016) reported that the use of growth regulator (mepiquat chloride) at dose of 50 g a.i. ha^{-1} showed efficiency in controlling cotton growth, not interfering with other vegetative and reproductive characteristics. Neves (2009) evaluated the effect of applying glyphosate subdoses ranging from 0 to 72 g a.i. ha^{-1} in 'FMT 701' cotton cultivar and concluded that glyphosate application increased plant height by 7% at subdose of 16.2 g a.i. ha^{-1} in relation to control and in 'BRS Cedro' cultivar, this increase reached 15% at subdose of 27.4 g a.i. ha-1 in relation to control. Allender (1997) found that cotton plants applied with 5 µg/L of 2,4-D showed growth stimulation compared to controls. Yamashita and Guimarães (2005) reported that the application of glyphosate at subdose of 540 g ha^{-1} caused a more drastic decrease in cotton plant height when compared to subdose of 270 g ha^{-1}.

For stem diameter (Figure 1), in general, no hormesis effect was observed, given that as herbicide subdoses increased, there was a decrease in the value of this variable. Rosa et al. (2011) found no influence from the application of glyphosate subdoses and Americo et al. (2016) also reported no influence of the application of 2,4-D subdoses on cotton stem diameter. Meleiro (2016) studied the application of glyphosate subdoses in cotton crop and found that fifteen days after the application of glyphosate subdoses of 0, 26, 52, 78, 104 and 130 g a.i. ha^{-1}, this variable was not influenced by application of glyphosate subdoses, showing no statistically significant differences in the first two agricultural years and for the third year under study, it was observed that this variable fit into a linear model, that is, the diameter increased proportionally with increasing subdoses.

According to the number of nodes (Figure 1), there was a decrease in values as a function of increasing subdoses and application time. However, when observing the result from the application of subdoses of 12 g a.i. of 2,4-D + 3.2 g a.i. picloram ha^{-1} at stage B5, it was observed that there was an increase in this characteristic compared to the previous dose, but based on results obtained for the other variables, the use of higher subdoses in cotton becomes unfeasible. Rosa et al. (2011) verified in their work that there was no influence of the application of glyphosate subdoses on the number of nodes.

Although most results in literature demonstrate the hormesis effect of herbicide application in cotton, Cedergreen et al. (2007) found that herbicide mecoprop did not induce hormesis, as it is a synthetic auxin and has been shown to induce root elongation, increase in specific leaf area and biomass growth at low doses in other studies (Morré, 2000). The subdoses used in this experiment were high, not promoting the hormesis effect, but rather deleterious effects on plants.

In this work, the combination of two active ingredients with synergistic effect described by Gazziero (2015) at higher subdoses in order to simulate drift in weed control, made the toxic effects of herbicide application greater, promoting deleterious effects on the reproductive and vegetative characteristics of cotton, differing from results mentioned above, which used only one active ingredient and at low subdose. Similar results were found by Ferrari et al. (2021c) with glufosinate-ammonium low doses application in upland rice.

In this study, the use of high 2,4-D + picloram subdoses is due to the fact that the effects of the combination of two active ingredients applied to cotton are not known. In general, subdose of 12 g a.i. 2,4-D + 3.2 g a.i. picloram ha^{-1} applied at stages B1 and B2 were the treatments that presented the worst values. Stage B1 is when the first flower bud of the cotton plant becomes visible and stage B2 is when the first flower bud of the second fruit branch becomes visible. In the phase that comprises the first flower until the opening of the first boll, some apples are already in the maturation phase. As a result, in the second half of this phase, any factor, whether biotic or abiotic, that may cause stress and reduce the photosynthetic rate will result in damage caused by the increased incidence of immature fibers (Rosolem, 2001).

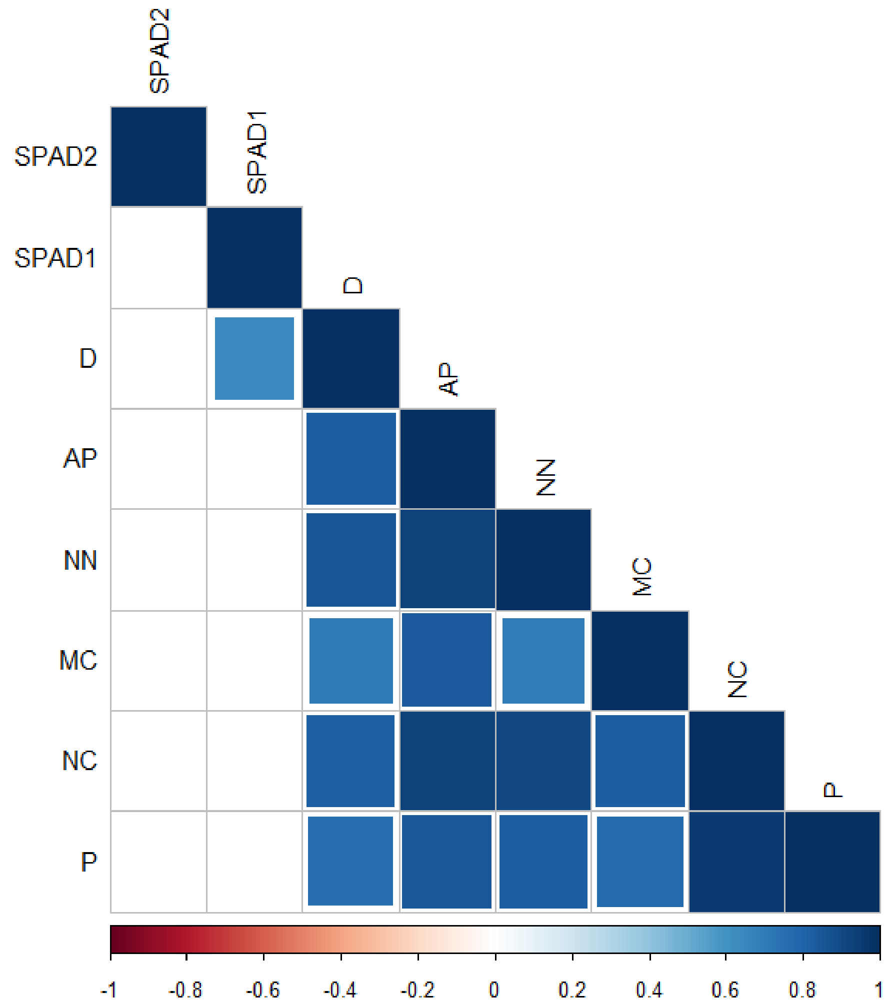
The fall of reproductive structures is a natural phenomenon in cotton and is regulated by the balance between tissue sugars and ethylene content. Thus, factors that influence the decrease in photosynthesis, or increase in metabolic expenditure, will cause a decline in reproductive structures (Rosolem, 2001). Auxin-mimicking herbicides act in the division and elongation of cells due to the hormonal imbalance caused in cells, with increase in ethylene synthesis (Marques, 2019). This explains the smaller number of reproductive structures of cotton submitted to Tordon application.

Spad 1 and 2 readings were not influenced by the application of the herbicide nor by application times. Melero (2016) reported that the Spad index can be an aid to explain vegetative and reproductive growth variables;

however, the indices obtained here do not follow the observed vegetative and reproductive growth trend.

According to the linear correlation of variables (Figure 2), it was observed that the stem diameter had a positive relationship with the Spad 1 index. Plant height was positively correlated with stem diameter. The number of nodes was positively correlated with stem diameter and plant height. Boll mass was positively correlated with stem diameter, plant height and number of nodes. The number of bolls had positive relationship with stem diameter, plant height, number of nodes and boll mass. Finally, yield was directly related to stem diameter, plant height, number of nodes, boll mass and number of bolls.

Figure 2. Linear correlation of vegetative and reproductive characteristics of cotton submitted to 2,4-D + picloram subdoses. MC = boll mass, NC = number of bolls, P = yield, AP = plant height, D = diameter, NN = number of nodes, SPAD1 = Spad 1 reading and SPAD2 = Spad 2 reading. non-significant interaction p<0.01.



The application of 2.4D + picloram subdoses results in decrease in agronomic characteristics, with greater decrease with increasing subdoses applied mainly at initial stages B1 and B2, and its use is not recommended to obtain the hormesis effect.

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