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Paspalum notatum growth and pigment content in response to the application of herbicides[1]

*Crescimento e teor de pigmentos de **Paspalum notatum** em resposta a aplicação de herbicidas*

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Abstract - Bahiagrass (*Paspalum notatum*) is a poacea resistant to trampling, water restriction and low soil fertility; however, it requires frequent cutting to eliminate the biomass excess it produces. To inhibit the growth of turfs and to avoid or delay cutting, growth regulators and herbicides have been tested. The goal of this work was to evaluate the potential of three herbicides as growth regulators on bahiagrass, and their influence on growth and chloroplast pigment content. The experimental design was in randomized blocks, in split-plot scheme, with three herbicides (glyphosate, isoxaflutole and tembotrione) and five doses (0, 68, 136, 272, 544 g a.e. ha^{-1}; 0, 24, 48, 96, 192 g a.i. ha^{-1} and 0, 13, 26, 52, 104 g a.i. ha^{-1}), respectively, with four replications. The following characteristics were evaluated: phytointoxication, dry mass, grass height and chloroplast pigment content. Data were submitted to analysis of variance and, when significance was observed, the regression test was performed, up to the second degree, separately for each herbicide. Herbicides demonstrated effectiveness in controlling turf size; however, the phytotoxic effect was observed with the increase of the doses and a damage in the aesthetic aspect of the grass. Isoxaflutole has greater potential to be used as a growth reducer on bahiagrass, due to the low necessary dose to reduce height and to low phytointoxication. The content of chloroplast pigments is changed singularly for each used herbicide, and it has a direct relation with the dry mass of leaves.
Keywords: bahiagrass; chemical control; grass maintenance; post-emergent herbicides; size reduction

Resumo - A grama batatais (*Paspalum notatum*) é uma poacea, resistente ao pisoteio, restrição hídrica e baixa fertilidade do solo, porém, exige cortes frequentes para eliminar o excesso de biomassa produzido. Para inibir o crescimento dos gramados e evitar ou retardar a operação de corte, têm sido testados reguladores de crescimento e herbicidas. O objetivo do trabalho foi avaliar o potencial de três herbicidas como reguladores de crescimento em grama batatais, e sua influência

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no crescimento e teor de pigmentos cloroplastídicos. O delineamento experimental foi em blocos casualizados, em esquema de parcelas subdivididas, com três herbicidas (glyphosate, isoxaflutole e tembotrione) e cinco doses (0, 68, 136, 272, 544 g e.a. ha^{-1}; 0, 24, 48, 96, 192 g i.a. ha^{-1} e 0, 13, 26, 52, 104 g i.a. ha^{-1}), respectivamente, com quatro repetições. Foram avaliadas as características, fitointoxicação, massa seca, altura do gramado e teor de pigmentos cloroplastídicos. Os dados foram submetidos a análise de variância e, quando constatada significância, foi realizado o teste de regressão até segundo grau separadamente para cada herbicida. Os herbicidas demonstram eficiência no controle do porte do gramado, no entanto, constata-se efeito fitotóxico com o incremento das doses e o prejuízo no o aspecto estético do gramado. O isoxaflutole possui maior potencial para ser utilizado com redutor de crescimento para a grama batatais, devido à baixa dose necessária para reduzir a altura e a pouca fitointoxicação. O teor de pigmentos cloroplastídicos é alterado de maneira singular por cada herbicida utilizado, e têm relação direta com a massa seca das folhas.
Palavras-chaves: grama-batatais; controle químico; manutenção de gramado; herbicidas pós-emergentes; redução de porte

Introduction

A very much used grass species in central-southern Brazil, bahiagrass (*Paspalum notatum*) belongs to the Poaceae family; coming from the American continent, it is a long day plant (Marousky and Blondon, 1995). It blossoms between October and March, has green leaves concentrated in the base part, it easily covers all the ground and it helps protecting the soil (Kissmann, 1997). It resists to trampling and water deficits as well as adapting to low fertility soils; but it requires frequent cutting and grass maintenance (Goatley et al., 1998). On turfs, there is the need for constant cutting, due to the quick growth of plants; thus, for proper maintenance that provides harmony and reaches the ornamental purpose of the green roof, this management is vital (Marchi et al., 2013). Therefore, it is also the main maintenance cost factor of turfs, especially in great areas (Maciel et al., 2007).

Growth regulators and herbicides have been tested at subdoses with the goal to inhibit the growth of some grass species, in order to avoid or delay the cutting operation (Monquero et al., 2012; Marchi et al., 2013). However, there are some difficulties in inhibiting the grass growth without affecting the green color of the species. Plant growth regulators are becoming important in grass management programs, since they suppress plant growth without damaging its visual quality and without causing leaf phytointoxication, discoloring or thinning (Costa et al., 2009; Maciel et al., 2013; Dinalli, et al., 2015).

The suppression of growth without damaging the grass green color with the use of growth regulators may reduce the frequency of the hoeing operation, providing financial and time saving (Queiroz, 2016). It is worth highlighting dosage tests of herbicides as plant growth regulators are still a newly experienced area, and there is a lack of information to understand the physiologic implications affecting the phytometric and aesthetic performance of grass (Marques, 2012; Velini et al., 2010).

Thus, the goal of this work was to evaluate the potential of three herbicides as growth regulators on bahiagrass, and their influence on growth and chloroplast pigment content.

Material and Methods

The experiment was conducted on an area that was completely covered in bahiagrass (*Paspalum notatum*), in the city of Londrina, Paraná state, in 2015. The area coordinates are (23º 20’ 32” S and 51º 12’ 32” O), with an average altitude of 540 m. The soil was characterized as Distroferric Red Latosol (dRL), (EMBRAPA, 2013).

The climate of the region is Cfa according to the classification of Köppen. The meteorological data were obtained by the automatic station of the Universidade Estadual de Londrina, and are exposed in Figure 1.

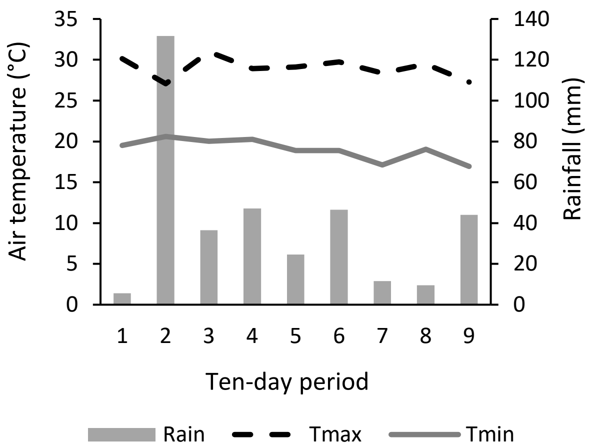

Figure 1. Rainfall (Rain), maximum (Tmax) and minimum (Tmin) average temperature during the experimental period (February 1st to April 30th 2015). Londrina, Paraná state, 2015.

The chemical analysis of the soil in the 0-20 cm layer presented the following data: pH ($CaCl_2$) = 5.50; P = 8.55 mg dm^{-3}; K = 1.60 cmol$_c$ dm^{-3}; Ca = 5.30 cmol$_c$ dm^{-3}; Mg = 2.20 cmol$_c$ dm^{-3}; Al = 0 cmol$_c$ dm^{-3}; H+Al = 4.28 cmol$_c$ dm^{-3}; CTC = 13.38 cmol$_c$ dm^{-3} and V = 68.01%.

The adopted experimental design was the randomized block one, in split-plot scheme, with three herbicides: glyphosate (Atanor 48, 480 L^{-1} g a.e., CS, ATANOR S.A.), isoxaflutole (Provence 750 WG, 750 L^{-1} g a.i., GD, Bayer Crop Science S.A.) and tembotrione (Soberan, 420 L^{-1} g a.i., SC, Bayer Crop Science S.A.) and five doses (0, 68, 136, 272, 544 g a.e. ha^{-1}; 0, 24, 48, 96, 192 g a.i. ha^{-1} and 0, 13, 26, 52, 104 g a.i. ha^{-1}), respectively, with four replications. Each plot was constituted by 10 m^2 and sub-plots of 2 m^2; one central m^2 from each subplot was considered as usable area.

The application of the treatments was performed on 02/23/2015, one week after the grass cutting, using a CO_2 pressurized backpack equipment, with a 50 cm T-shaped bar, containing two AXI 11002 spraying nozzles, with a working pressure of 207 kPa for a 200 L ha^{-1} flow. At the time of application, air relative humidity was around 70%, with wind speed of 3 m s^{-1} and average air temperature of 28°C.

Evaluations on phytointoxication and grass height were performed on day 7, 14, 21 and 28 after the application (DAA) of the herbicides and the dry mass and pigment content were determined on day 28 DAA.

Phytointoxication was determined according to the adaptation of the methodology proposed by the E.W.R.C. - grades based on the scale proposed by the European Weed Research Council (1964), where “grade 1” means absence of symptoms and “grade 9” means death of 100% plants; all subplots received 3 grades from different evaluators. As for the evaluation on grass height, the distance between soil and the edge of the highest leaf was measured in five places within each subplot, with the help of a centimeter ruler.

Dry mass was determined by harvesting all the aerial part of the grass in 1 m^2 of each subplot; these samples were taken to a drying oven with forced ventilation, at the temperature of 65°C for 72 hours. After that, the dry mass of the samples was measured on an analytical scale.

In order to determine the content of chlorophyll and carotenoids, 0.2 g samples of fresh leaf tissue were macerated in liquid nitrogen, and subsequently placed in 50 mL falcon tubes; 10 mL of a solution with 80% acetone and 20% water was added after a period of 7 days for the complete extraction of pigments. The extracts were filtered, and data were read on a spectrophotometer at the wave lengths of 663, 645 and 434 nm for chlorophyll a, b and carotenoids, respectively (Meschede et al., 2011). The determination of pigment levels (mg dm^{-3}) were based on the equations reported as follows, according to Whitham et al. (1971).

In addition, the sum of chlorophyll a, b and carotenoid contents was performed, obtaining the total pigment content.

$$C\,a = (11.24\ x\ A663 - 2.04\ x\ A645)$$
$$C\,b = (20.13\ x\ A645 - 4.19\ x\ A663)$$
$$Carotenoids = (1000\ x\ A434 - 1.90\ x\ C\,a - 63.14\ x\ C\,b)/214$$

Caption: A - absorbance in spectrophotometry
Caption: C – chlorophyll

The results were submitted to analysis of variance by F test at 5% probability and, in case of significance, the regression test was performed up to the second degree, separately for each herbicide.

Results and Discussion

Glyphosate

Starting from the analysis of variance, glyphosate (Figure 2) presented differences among the tested doses for all the analyzed characteristics, except for the carotenoid content, on day 28 after application (28 DAA).

As for the phytointoxication variable (Figure 2A), it was possible to verify that, with the increase in the applied dose, there was an increase in the damage to plants; the increase rate was 0.5% for each g of added glyphosate. There was a change in the grass color, with later senescence, which reduced the aesthetic aptitude of the turf. As for height, the lowest plant size was obtained with the dose of 246 g a.i. ha^{-1} (Figure 2B). Similar results were found for the content of chlorophyll a and b, where the minimum point was obtained with doses of 263 and 249 g a.e. ha^{-1}, respectively (Figure 2D and 2E).

These responses are due to the action mechanism of glyphosate, whose active site is the EPSPsynthase (5-enolpyruvylshikimate-3-phosphate synthase); it paralyzes the aromatic amino acid biosynthesis route (phenylalanine, tyrosine and tryptophan) and it deregulates the route of the shikimic acid, which is responsible for the use of 20% of the carbon fixed by plants (Velini, 2009). It is worth highlighting that these amino acids are precursor of most aromatic compounds in plants, that glyphosate reduces the synthesis of phytoalexins and that there is an increase in the concentration, at toxic levels, of nitrate, ethylene, cinnamic acid and other compounds that accelerate the death of plants, due to the herbicide effect over the aforementioned route (HRAC, 2017).

This is different from what was observed by Dinalli at al. (2015) who, when applying 200 g a.e. ha^{-1} of glyphosate on Korean lawngrass (*Zoysia japônica* Steud), did not observe phytointoxication on the turf. However, these authors verified size reduction without, nonetheless, observing alterations in the chlorophyll content. Similarly, Brighenti et al. (2012) observed size reduction and phytointoxication increase on African star grass (*Cynodon nlemfuensis*) with the increase in glyphosate doses, starting from 720 g a.e. ha^{-1}.

When analyzing the total pigment content, it presented the lowest value at the dose of 239 g a.i. ha^{-1} (Figure 2F). Costa et al. (2010) observed significant alterations in the size and dry mass of bahiagrass plants with the use of herbicides during post-emergence; the effect of these compounds over the growth and development of the turf. As for the content of photosynthetic pigments, Gazola et al. (2016) when working with Korean lawngrass, observed a similar content as the one in this work, in relation to glyphosate.

Therefore, the targeted size reduction was verified around the glyphosate dose of 250 g a.e. ha^{-1}; it was possible to observe few alterations in the aesthetic aspect of the turf up to this limit, which allows considering the use of the herbicide to reduce the growth of bahiagrass.

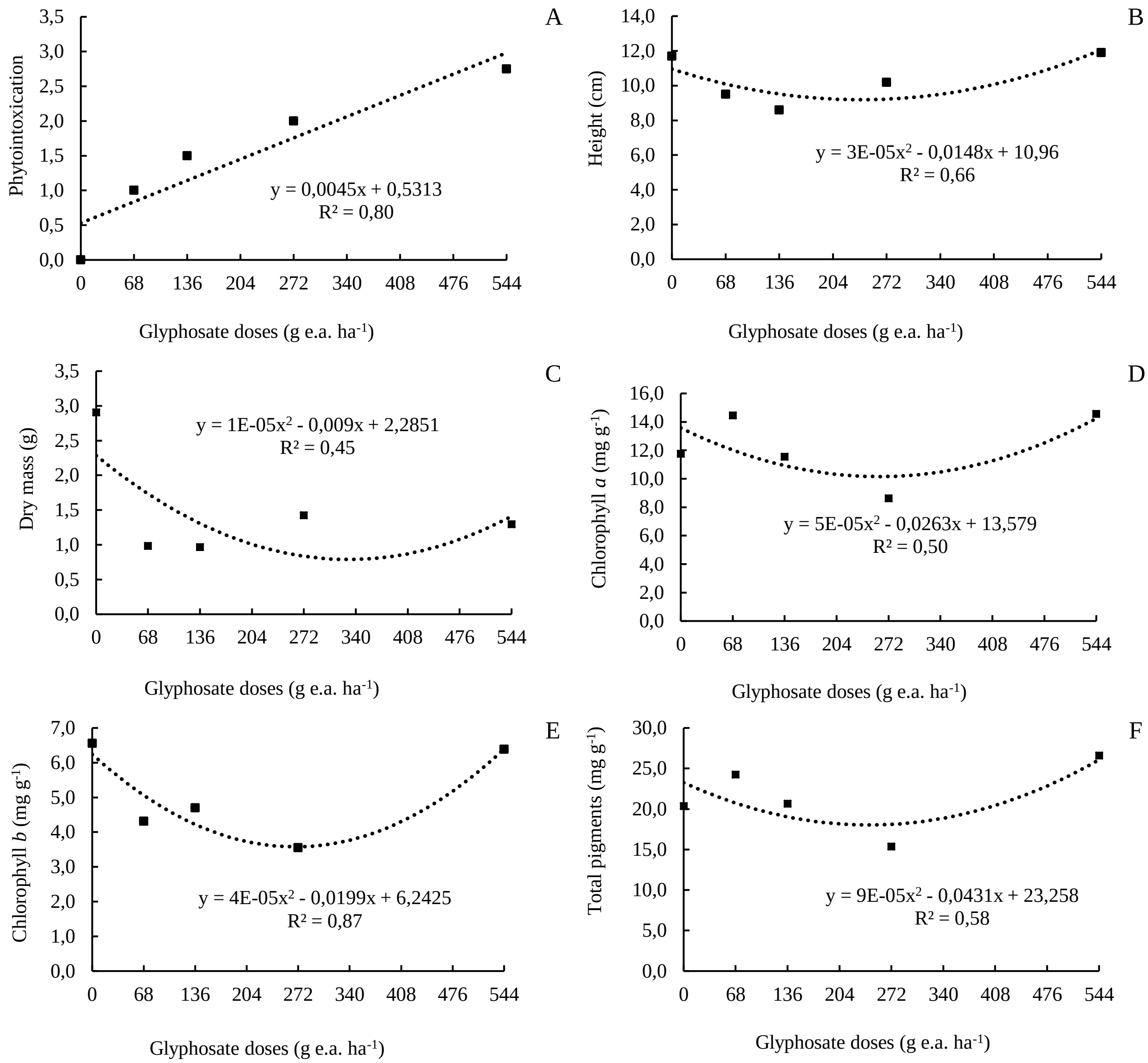

Figure 2. Percentages of phytointoxication (A), height (B), dry mass (C), content of chlorophyll a (D), content of chlorophyll b (E) and content of total pigments (F) of bahiagrass (*Paspalum notatum*) in response to glyphosate herbicide doses. Londrina, Paraná, 2015.

Isoxaflutole

In relation to isoxaflutole (Figure 3), characteristics of phytointoxication, contents of chlorophyll a and carotenoids increased according to the increase in the herbicide dose (Figure 3A, 3D and 3E), with increase rates of 0.63% for phytointoxication, 0.045 mg g^{-1} for content of chlorophyll a and 0.012 mg g^{-1} in the content of carotenoids. Grass height had its lowest values at the dose of 90 g a.i. ha^{-1} (Figure 3B); however, all tested herbicide doses resulted in lower grass height in relation to its absence. The content of chlorophyll b did not present significant differences for the used doses. Dry mass results showed a decrease according to the increase in the isoxaflutole dose; at every used 1 g a.i. ha^{-1}, there was a 0.0033 g reduction in the dry mass (Figure 3C).

The action mechanism of this herbicide consists in blocking the synthesis of carotenoids in an indirect way; at the beginning, the enzyme p-hydroxyphenylpyruvate is blocked, resulting in the blockage of the synthesis of plastoquinone. Thus, consequently, the biosynthesis of carotenoids is paralyzed (Senseman, 2007). However, it is worth highlighting that for the used doses of the herbicide on bahiagrass, it was not possible to verify effectiveness in the paralysis of the carotenoid biosynthesis route, which is related to the extremely low reported phytointoxication, as opposed, on day 28 DAA when leaves treated with higher doses presented higher pigment contents.

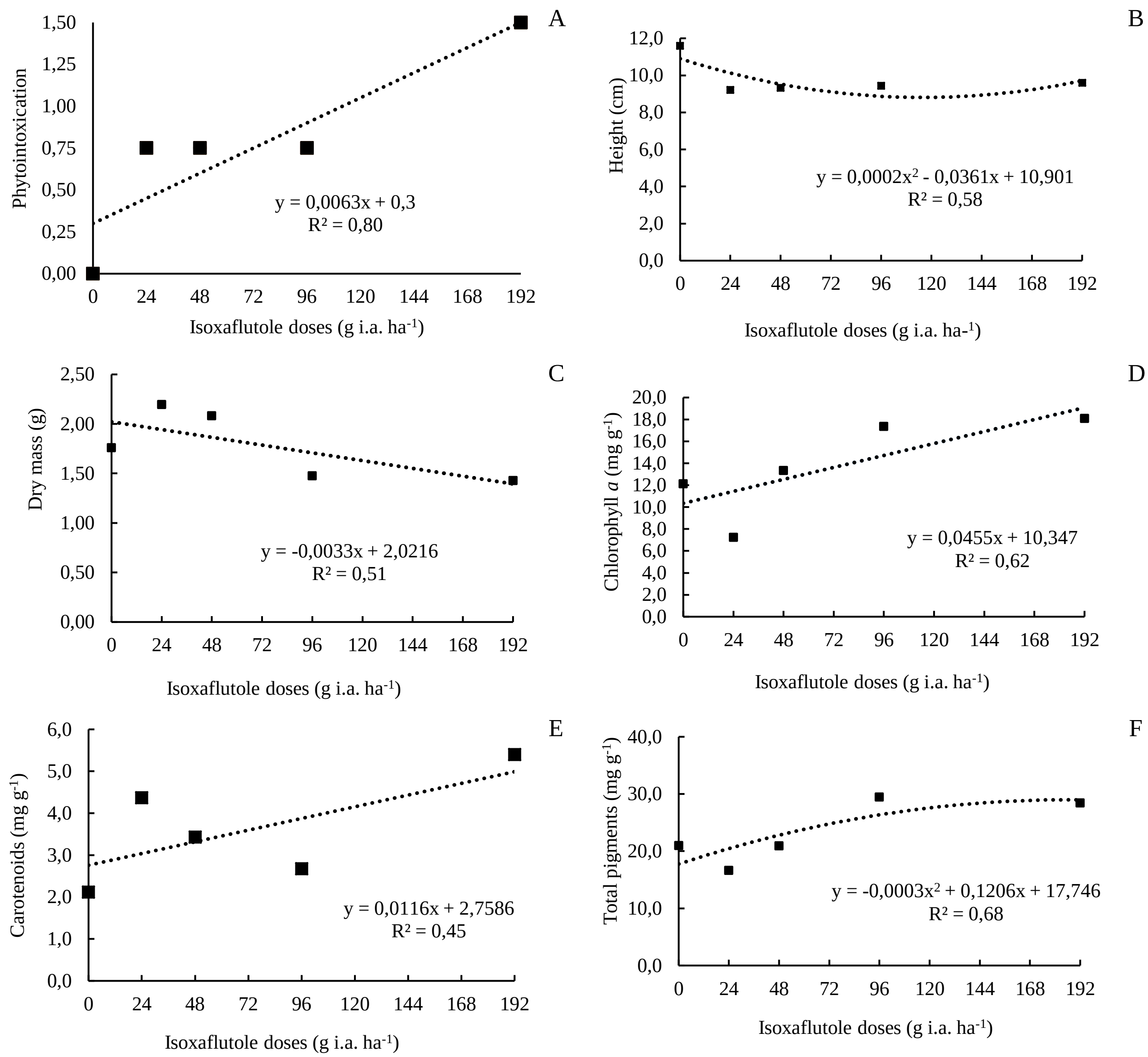

Figure 3. Percentages of phytointoxication (A), height (B), dry mass (C), content of chlorophyll a (D), content of carotenoids (E) and content of total pigments (F) of bahiagrass (*Paspalum notatum*) in response to isoxaflutole herbicide doses. Londrina, Paraná, 2015.

However, data referring to the total pigment content had polynomial adequacy, with the maximum point at 201 g a.i. ha^{-1} of isoxaflutole, and this is justified by the reduction in the grass mass, that is, there was a reduction in leaf growth and pigment concentration according to the increase in the isoxaflutole dose.

Results demonstrated that there was a reduction in the grass height around the isoxaflutole dose of 100 g a.i. ha^{-1}, with little alteration in the visual aspect up to this limit, which allows using the herbicide as a growth reducer on bahiagrass.

Tembotrione

Thus, similarly to glyphosate and isoxaflutole, the phytointoxication percentage increased according to the increment in the tembotrione dose (Figure 4A). A similar response was obtained by Dan et al. (2010) with the culture of pearl millet, where the phytointoxication was higher according to the increase in the tembotrione dose, causing damages to the growth of plants.

Unlike the other herbicides, the use of tembotrione did not obtain a standard for the accumulation of dry mass with the dose increase (Figure 4B).

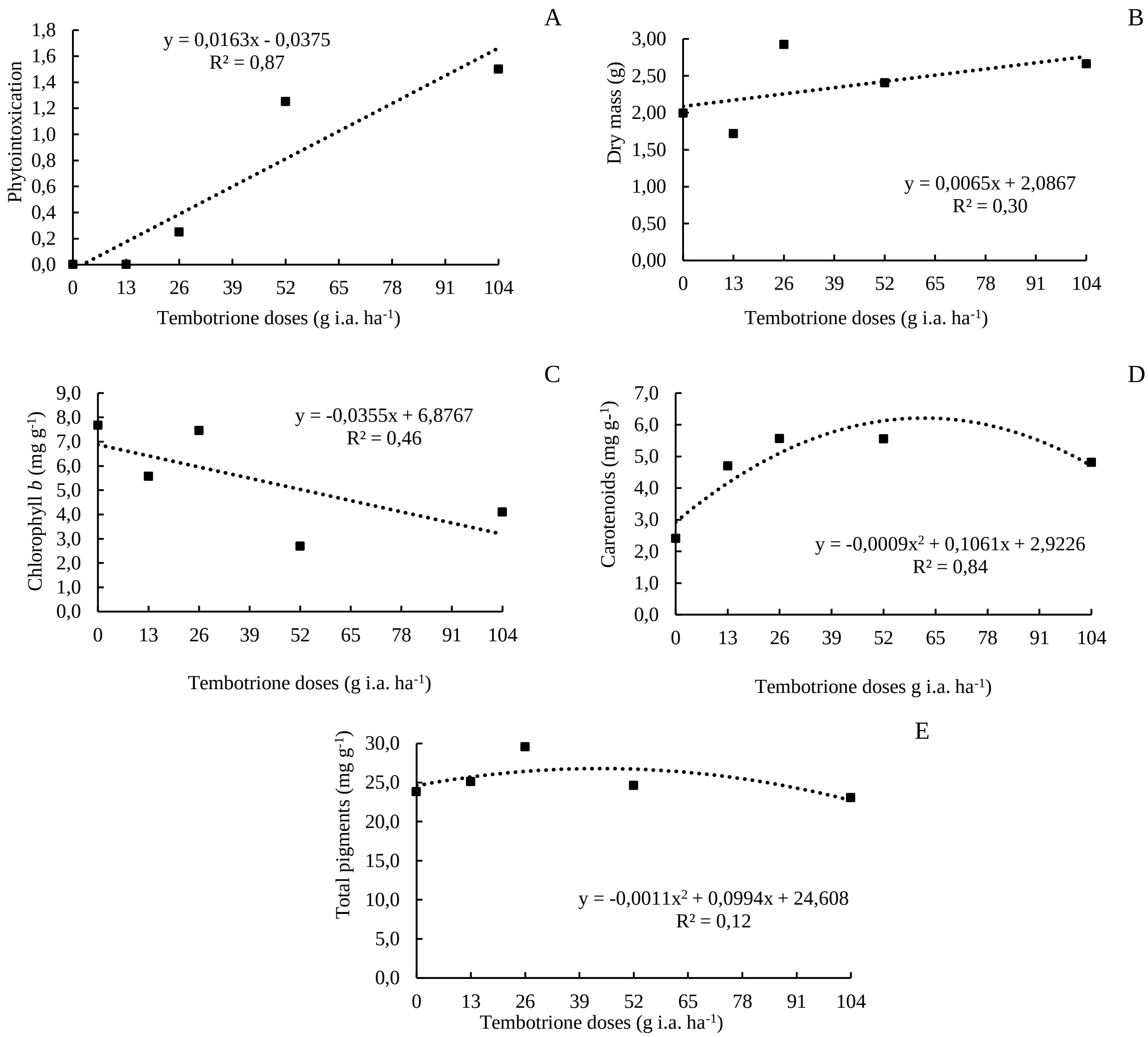

Figure 4. Percentages of phytointoxication (A), dry mass (B), content of chlorophyll b (C), content of carotenoids (D) and content of total pigments (E) of bahiagrass (*Paspalum notatum*) in response to tembotrione herbicide doses. Londrina, Paraná, 2015.

This contrasts with what was observed for the culture of sorghum, where, with the increase in the tembotrione doses, the dry mass of the plants presented a reduction (Dan et al., 2009). Dry mass reduction of *Urochloa ruziziensis* was also observed by Adegas et al. (2011), due to the use of tembotrione.

The application of tembotrione resulted in a 0.036 mg g^{-1} reduction of chlorophyll b for each 1 g a.i. ha^{-1} applied (Figure 4C). Contents of chlorophyll a and the height parameter did not present effects on tembotrione doses at 5% significance by F test.

Tembotrione doses of 59 and 45 g a.i. ha^{-1} resulted in higher carotenoid and total pigment contents for the turf (Figure 4D and 4E). Carotenoids are the main pigments that are responsible for the dissipation of light energy. This excessive energy collected by the photosystem II is passed from chlorophylls to some carotenoids from the xanthophyll cycle, and the release occurs in the form of heat, which protects plants from photo inhibition (Demmig and Adams, 1992).

The increase in the tembotrione dose may promote alterations in the content of carotenoids, through transformations in the biosynthesis route, which begins in geranyl-pyrophosphate farnesyl-pyrophosphate (with the participation of the enzymes prenyltransferase and farnesyltransferase) and subsequently transforms phytoene into phytofluene. This corresponds to the metabolic pathways that alter carotenoid contents in the plant, since the electrons given for the transfer come from plastoquinone (Meschede et al., 2011).

It was not possible to identify a growth reduction pattern for bahiagrass with the application of increasing doses of tembotrione up to 104 g a.i. ha^{-1}; however, the herbicide changed positively the pigments and caused low phytointoxication on the grass. Nonetheless, more doses and environmental conditions must be tested to reach conclusions about the use of this herbicide as a growth reducer on turfs.

Conclusions

Herbicides demonstrated effectiveness in controlling turf size; however, the phytotoxic effect was observed with the increase of the doses and a damage in the aesthetic aspect of the grass.

Isoxaflutole has greater potential to be used as a growth reducer on bahiagrass, due to the low necessary dose to reduce height and to low phytointoxication.

The content of chloroplast pigments is changed singularly for each used herbicide, and it has a direct relation with the dry mass of leaves.

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