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Article

Glyphosate as Growth Regulator for Bahiagrass and Broadleaf Carpetgrass

Glyphosate como Regulador de Crescimento em Grama Batatais e São Carlos

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ABSTRACT - The objective of this work was to evaluate the effect of glyphosate herbicide as growth regulator on two turfgrasses bahiagrass and broadleaf carpetgrass. The experiments were conducted in a greenhouse, using a completely randomized design, with 10 glyphosate rates (0, 5.625, 11.25, 22.5, 45, 90, 180, 360, 720, and 1.440 g a.e. ha^{-1}) and four replicates. Bahiagrass and broadleaf carpetgrass plants were transplanted to 2 liter pots filled with a clayey soil. Phytotoxicity and green cover index (GCI) were evaluated through digital analysis; plant height at 7, 14, 21, and 28 days after application (DAA); and dry biomass of cuttings at 28 DAA. The glyphosate rates of 5.625 to 22.5 g a.e. ha^{-1} (for bahiagrass) and up to 90 g a.e. ha^{-1} (for broadleaf carpetgrass) reduced the plant growth, without affecting the plant visual quality phytotoxicity, GCI, and dry biomass in the evaluated periods. Contrastingly, rates equal to and above 45 g a.e. ha^{-1} (for bahiagrass) and equal to and above 180 g a.e. ha^{-1} (for broadleaf carpetgrass) caused phytotoxic effects in all evaluated periods and affected negatively plant height, GCI, and dry biomass, denoting the sensitivity of these grass species to these glyphosate rates. The digital image analysis allowed the verification and quantification of the effects of the herbicides on turfgrasses.

Keywords: *Axonopus compressus*, phytotoxicity, turfgrass, herbicide, *Paspalum notatum*.

RESUMO - Objetivou-se neste trabalho avaliar o efeito do uso do herbicida glyphosate como regulador de crescimento em grama Batatais e São Carlos. Os experimentos foram conduzidos em casa de vegetação no delineamento inteiramente casualizado com dez doses de glyphosate: 0; 5,625; 11,25; 22,5; 45; 90; 180; 360; 720; e 1.440 g a.e. ha^{-1} e quatro repetições. Tapetes de grama Batatais e São Carlos foram transplantados em vasos com capacidade de 2 L, preenchidos com solo de textura argilosa. Realizaram-se avaliações de intoxicação das plantas, taxa de cobertura verde (TCV) por meio de análise digital e altura das espécies de grama aos 7, 14, 21 e 28 dias após a aplicação (DAA), além da coleta das aparas para determinação da biomassa seca aos 28 DAA. Constatou-se que as doses entre 5,625 e 22,5 g a.e. ha^{-1} para Batatais e até 90 g a.e. ha^{-1} para São Carlos reduziram o crescimento, sem afetar a qualidade visual, tanto em termos de intoxicação quanto de TCV e biomassa seca, nas épocas avaliadas. Em contrapartida, as doses a partir de 45 g a.e. ha^{-1} para grama Batatais e 180 g a.e. ha^{-1} para São Carlos provocaram efeitos de intoxicação e redução de todos os parâmetros avaliados em todos os períodos, o quer demonstra sensibilidade dessas espécies de grama a essas doses. Adicionalmente, o uso da análise de imagem digital foi capaz de verificar e quantificar os efeitos de herbicidas em gramados.

Palavras-chave: *Axonopus compressus*, intoxicação, gramado, herbicida, *Paspalum notatum*.

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INTRODUCTION

Turfgrasses are used in different places, such as residential areas, sports areas, industrial parks, and public works. However, they regularly require proper management to achieve their aesthetic or functional purpose. According to Su et al. (2009), turfgrasses present a growing production, which uses various techniques and tools. Among these techniques, leaf cutting of turfgrasses by mowing procedures stands out for improvement of plant growth and quality. However, this operation has a high cost due to the frequency required to maintain the turfgrass quality during certain periods of the year, use in extensive areas, and difficulty of operation in areas with slopes that hinder machine operation (Maciel et al., 2007).

In addition, mowing in turfgrasses can reduce their tolerance to environmental stress due to high water loss, disease development, low carbohydrate storage, high sprouting intensity, and low root and rhizome growths (Maciel et al., 2011).

The use of underrates of herbicides as plant growth regulators has been an alternative for management of turfgrasses due to operational difficulties and high maintenance costs (Maciel et al., 2011). The low cost and high availability of active ingredients in the market for growth inhibition are factors that favor the use of herbicides as plant regulator (Velini et al., 2010).

Glyphosate is one of the herbicides that can be used for this purpose; it acts on the shikimic acid pathway by inhibiting the EPSPs enzyme (5-enolpyruvylshikimato-3-phosphate synthase), which is responsible for the formation of aromatic amino acids, tryptophan, tyrosine, and phenylalanine that are essential for plant growth and development (Velini et al., 2009). Low rates of this herbicide can be used as growth regulator for rice (Gitti et al., 2011) and sugarcane (Leite and Crusciol, 2008) crops; its low cost and high availability in the market are factors that favor its use for this purpose (Leite and Crusciol, 2008; Gitti et al., 2011). Other herbicides also have the potential to be used as plant regulators in grass species, such as imazapic, imazethapyr, imazaquin, and metsulfuron-methyl (March et al., 2013).

There is no official or safe rate recommendation of herbicides as plant regulators for management of turfgrasses in Brazil (Brasil, 2018). In the United States of America, the use of herbicides as plant growth regulators has been widely studied. However, Brazil lacks studies on this topic (Dinalli et al., 2015) due to low commercial interest of pesticide industries in maintaining or financing researches directed to this market. Thus, there is a need for studies evaluating new or existing molecules that may have growth regulation effect for different plants and purposes. In addition to the reduction in plant height, the aesthetic quality and natural color of turfgrasses should be maintained, without any symptoms of necrosis or chlorosis caused by these molecules after their application.

Digital image analysis has been used to evaluate effects of herbicides on turfgrasses due to its accuracy and easy conduction (Hoyle et al., 2013). Despite little studies have used this technique in Brazil, it can be an important tool to evaluate weed control effectiveness and herbicide symptoms in grass species.

Therefore, the objective of the present work was to evaluate the effect of glyphosate herbicide as a plant growth regulator on two grass species bahiagrass (*Paspalum notatum* L.) and broadleaf carpetgrass (*Axonopus compressus* (Sw.) P. Beauv).

MATERIAL AND METHODS

Two greenhouse experiments were conducted for each species (bahiagrass and broadleaf carpetgrass), during two periods (September to October 2017, and February to March 2018). The soil of the experimental units was classified as dystroferric Red-Yellow Latosol (EMBRAPA, 2013); it was collected from the arable layer (0.0 to 0.2 m) and fertilized at 40 days before transplanting, as recommended by Godoy et al. (2012a). When the grasses were transplanted, the soil presented the following physicochemical characteristics: 246 g kg^{-1} of sand, 533 g kg^{-1} of clay, and 201 g kg^{1} of silt; 23 g dm^{-3} of organic matter; pH ($CaCl_2$ 0.01 mol L^{-1}) of 6.8; 44 mg dm^{-3} of P (resin); 1.70, 41.00, 13.00, and 18.00 $mmol_c$ dm^{-3} of K, Ca, Mg, and H+Al, respectively, and base saturation of 81%.

The experimental design was completely randomized with four replications. The treatments consisted of nine glyphosate rates (5.625, 11.25, 22.5, 45, 90, 180, 360, 720, and 1,440 g a.e. ha[1]) and a control treatment (without glyphosate application). The grasses were transplanted to experimental plots consisting of 2 liter plastic pots (15 × 15 cm). Two days before the application of the treatments, they were cut at a height of 3 cm, using pruning shears.

The treatments were applied at 40 days after the transplanting of the grasses, using an indoor sprayer equipped with a spray boom containing four XR110.02VS tips, which were spaced 0.5 m apart and positioned at 0.5 m height in relation to the surface of the experimental units. The system was set to a spray speed of 3.6 km h^{-1}, using a spray volume of 200 L ha^{-1} and constant pressure of 150 kPa, pressurized by compressed air. The average air temperature and relative humidity at application were 28 °C and 55% for the first experiment, and 23 °C and 59% for the second experiment, respectively.

The pots were irrigated according to the need of the turfgrass during the experiments to maintain the necessary humidity for the good development of the plants. The irrigation was done through soil up to seven days after the application (DAA) of the herbicide to ensure no washing of the applied herbicide from the aerial part of the plants. After this period, irrigation was applied on the top of the plants.

Phytotoxicity, green cover index (GCI), and plant height of the grasses were evaluated at 7, 14, 21, and 28 DAA. Phytotoxicity was evaluated by a percentage scale, where 0% corresponds to plants showing no injury, and 100% corresponds to death of the plant (SBCPD, 1995).

The experimental units were photographed using a 3-megapixel digital camera for GCI evaluation; the camera was fixed in a similar light box structure to the one made by Peterson et al. (2011). The images were transferred to a computer and GCI was determined using the SigmaScan program (v.5.0, SPSS, Inc., Chicago IL 60611).

Evaluating GCI using digital image analysis can provide quantitative measures of the grass coverage by examining pixels. The GCI of each species was calculated by dividing the number of green pixels by the total number of pixels in each image (Karcher and Richardson 2005). The evaluations considered the hue, saturation, and brightness of the red, green, and blue (RGB) colors of each image (Karcher and Richardson 2003). A preliminary calibration of the photos was performed to identify specific ranges, since grass species may differ in color. The ranges were set to 32 to 100 for saturation and 43 to 113 for hue, for the two studied species. Calibration of saturation and hue ranges has also been performed in studies evaluating the effects of herbicides on turfgrasses (Henryet al., 2012; Mccullough et al., 2012; Hoyle et al., 2013).

Plant height (mm) was measured by the average height in two points of the turfgrass in each pot, using a ruler, considering the vertical distance between the soil surface and the leaf tips at natural inclination. Samples of cuttings were collected at 28 DAA to determine the dry biomass; all plants were cut close to the soil surface and dried in a forced-air circulation oven (70±2 °C) until constant weight.

The data were subjected to analysis of variance and, when significant, to nonlinear regression analysis ($p<0.05$), using the SISVAR program, as shown in Equation 1 for plant height, GCI, and dry biomass, and by Equation 2 for phytotoxicity:

$$Y = y0 + ae^{-bx} \qquad \text{(eq. 1)}$$

$$Y = y0 + a(1-e^{-bx}) \qquad \text{(eq. 2)}$$

where: $y0$ = minimum value estimated for the response variable; a = maximum and/or minimum value estimated for the response variable or parameter estimated by the model; b = slope of the curve; x = herbicide rate (g a.e. ha^{-1}); e = constant. Graphics were developed using the Sigmaplot v.12 program (SPSS Inc., United States).

RESULTS AND DISCUSSION

The green cover index (GCI) and the phytotoxicity of bahiagrass and broadleaf carpetgrass of the glyphosate rate on these grasses were inversely proportional (Figures 1 and 2). Thus, as

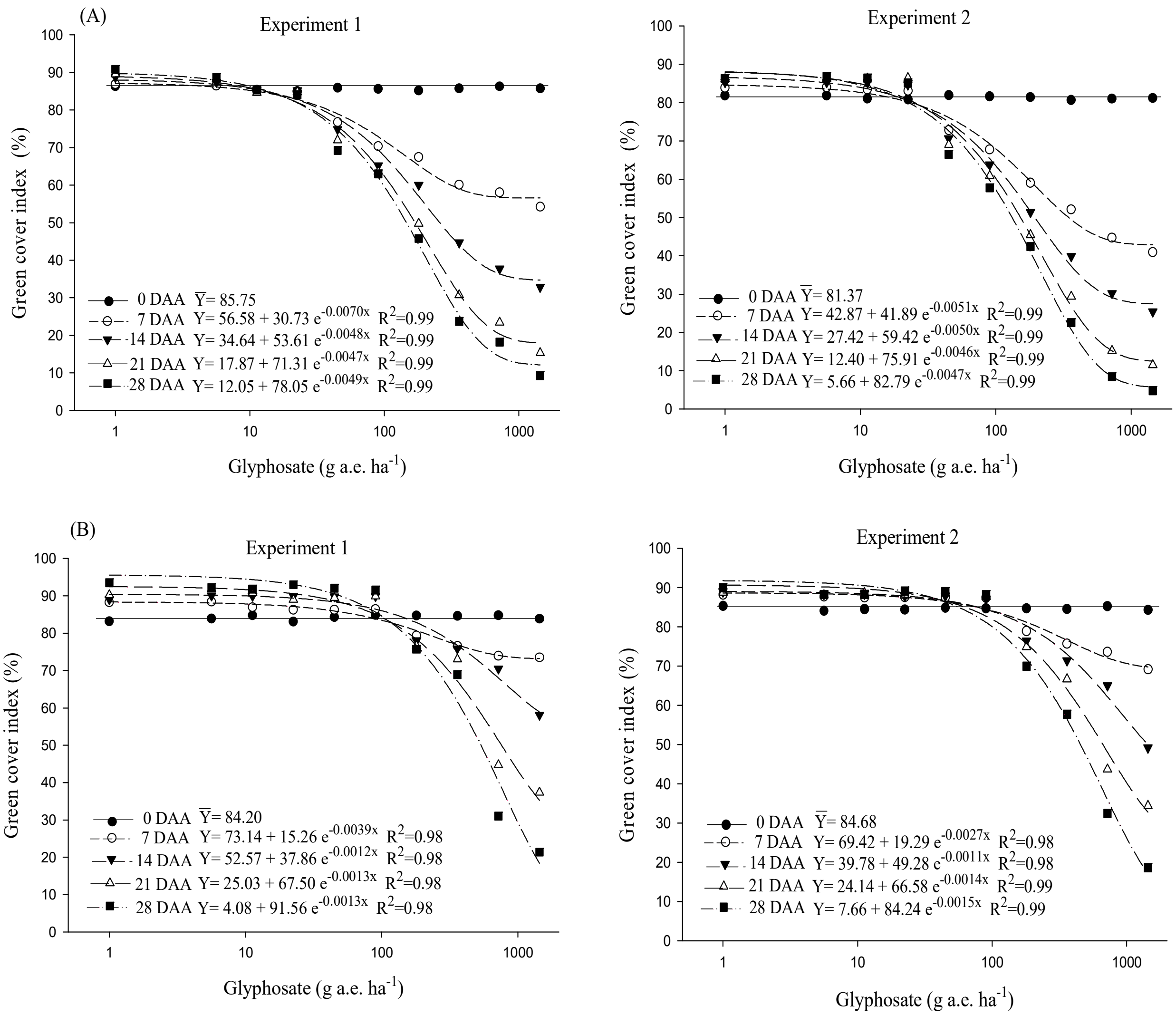

Figure 1 **-** Green cover index (%) in experiments 1 and 2 as a function of glyphosate rates applied to bahiagrass (*Paspalum notatum*) (A) and broadleaf carpetgrass (*Axonopus compressus*) (B).

glyphosate dose caused injuries, there was a reduction in GCI, indicating the importance of evaluations by digital image analysis to assess effects of herbicides on turfgrasses.

In general, visual damages were found at seven days after application (DAA), with glyphosate rates equal to and above 45 g a.e. ha^{-1} for bahiagrass, and equal to and above 180 g a.e. ha^{-1} for broadleaf carpetgrass. Phytotoxic effects (Figures 3 and 4) were mainly characterized by suppression of vegetative development in both evaluated species, at different intensities, followed by yellowing or chlorosis of leaves. The yellowing found is one of the most common symptoms found after glyphosate application, which can be related to degeneration of chloroplast and inhibition of chlorophyll formation (Castro and Meschede, 2009).

Herbicide selectivity in grasses is dependent on the species used (Christoffoleti and Aranda, 2001), which explains the different responses of bahiagrass and broadleaf carpetgrass to the use of glyphosate herbicide as growth regulator.

According to Fry (1991), the application of 600 g a.e. ha^{-1} of glyphosate as growth regulator to centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) caused yellowing followed by severe chlorosis, which is unacceptable for good-quality lawns. Johnson (1990) found up to 43% phytotoxic effect at 4 DAA of 200 g a.e. ha^{-1} of glyphosate to bahiagrass. Barbosa et al. (2017) found a linear phytotoxic effect on bahiagrass as the glyphosate rates were increased, starting at 68 g a.e. ha^{-1}.

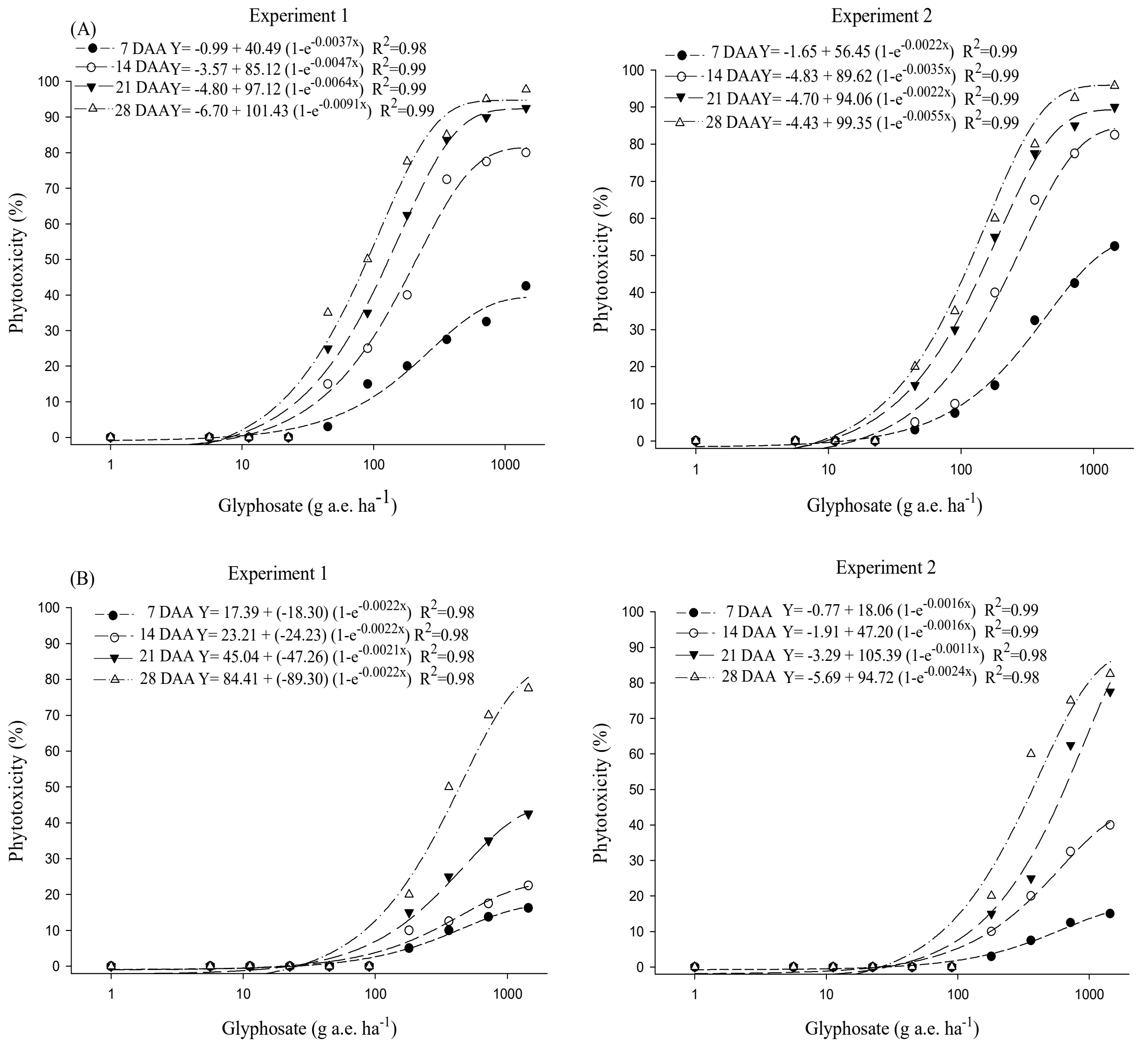

Figure 2 - Phytotoxic effect (%) on bahiagrass (*Paspalum notatum*) (A) and broadleaf carpetgrass (*Axonopus compressus*) (B) as a function of glyphosate rates, in experiments 1 and 2.

Dinalli et al. (2015) and Gazola et al. (2016) evaluated herbicides as growth regulators for emerald grass and found mild phytotoxicity symptoms (<10%) with the application of 200 g a.e. ha^1 of glyphosate; the plants presented yellowing, but the turfgrass recovered without compromising its aesthetic quality.

The use of herbicides as growth regulators must inhibit plant growth without affecting its characteristic green color, density, and aesthetic quality; thus, reducing the number of mowing operations and, indirectly, contributing to the reduction of labor, fuel, and equipment costs (March et al., 2013). The glyphosate rates of up to 22.5 g a.e. ha^{-1} (for bahiagrass) and up to 90 g a.e. ha^1 (for broadleaf carpetgrass) did not cause phytotoxic effects. The effects of herbicides as growth regulators are dependent on plant species, herbicide rate, application time, number of applications, and environmental conditions at application.

The plant height (Figure 5) of both grass species decreased as the glyphosate rates were increased. The glyphosate rates equal to and above 45 g a.e. ha^{-1} (bahiagrass) and equal to and above 180 g a.e. ha^{-1} (broadleaf carpetgrass) suppressed the plant growth. The glyphosate rate from 5.625 to 22.5 g a.e. ha^{-1} (for bahiagrass grass) and up to 90 g a.e. ha^{-1} (for broadleaf carpetgrass) reduced plant growth without affecting the turfgrass quality regarding phytotoxicity and GCI over the evaluated periods. These results denote the feasibility of application of low rates of glyphosate

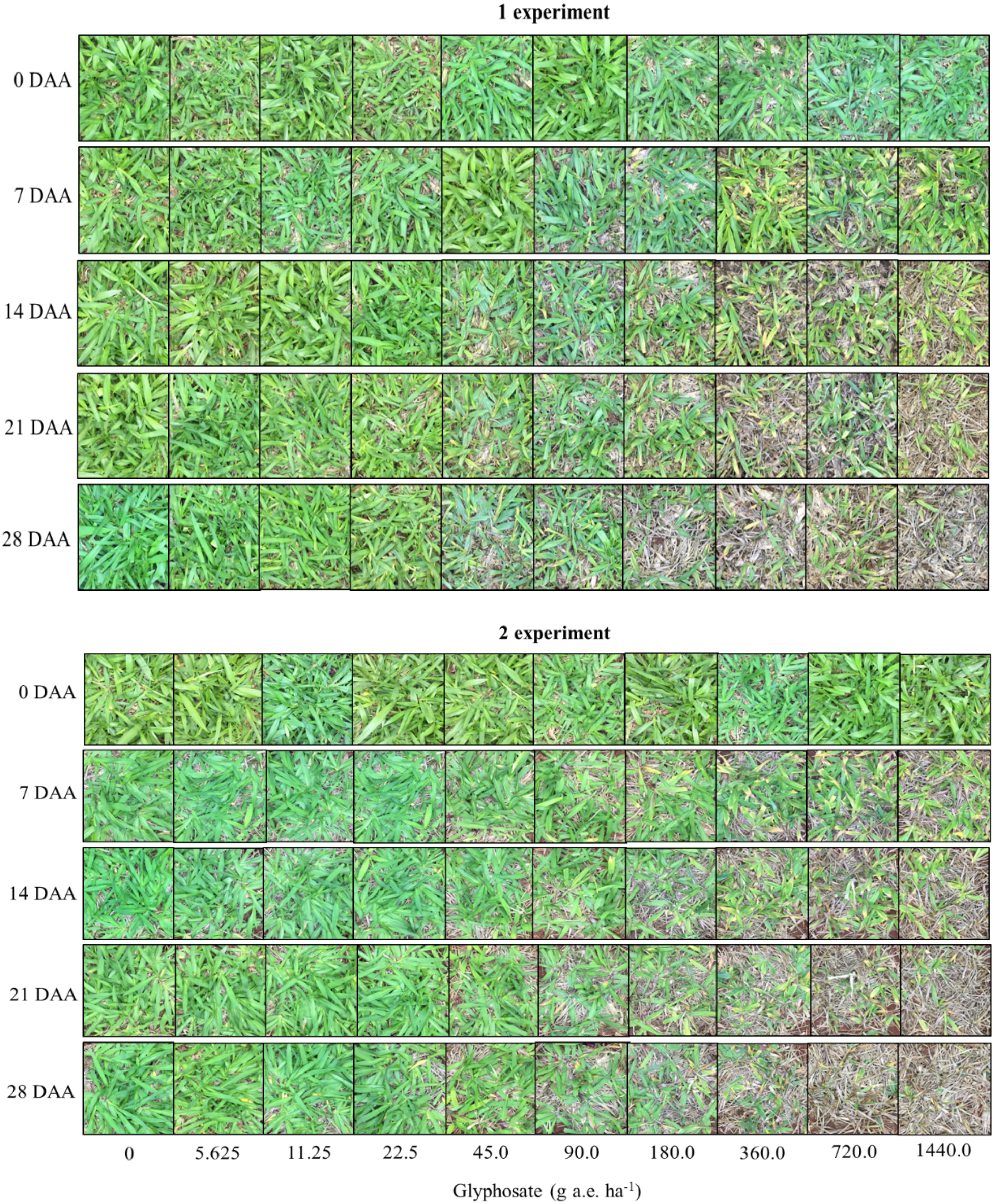

Figure 3 - Phytotoxic effect on bahiagrass (*Paspalum notatum*) caused by glyphosate rates. DAA = days after application.

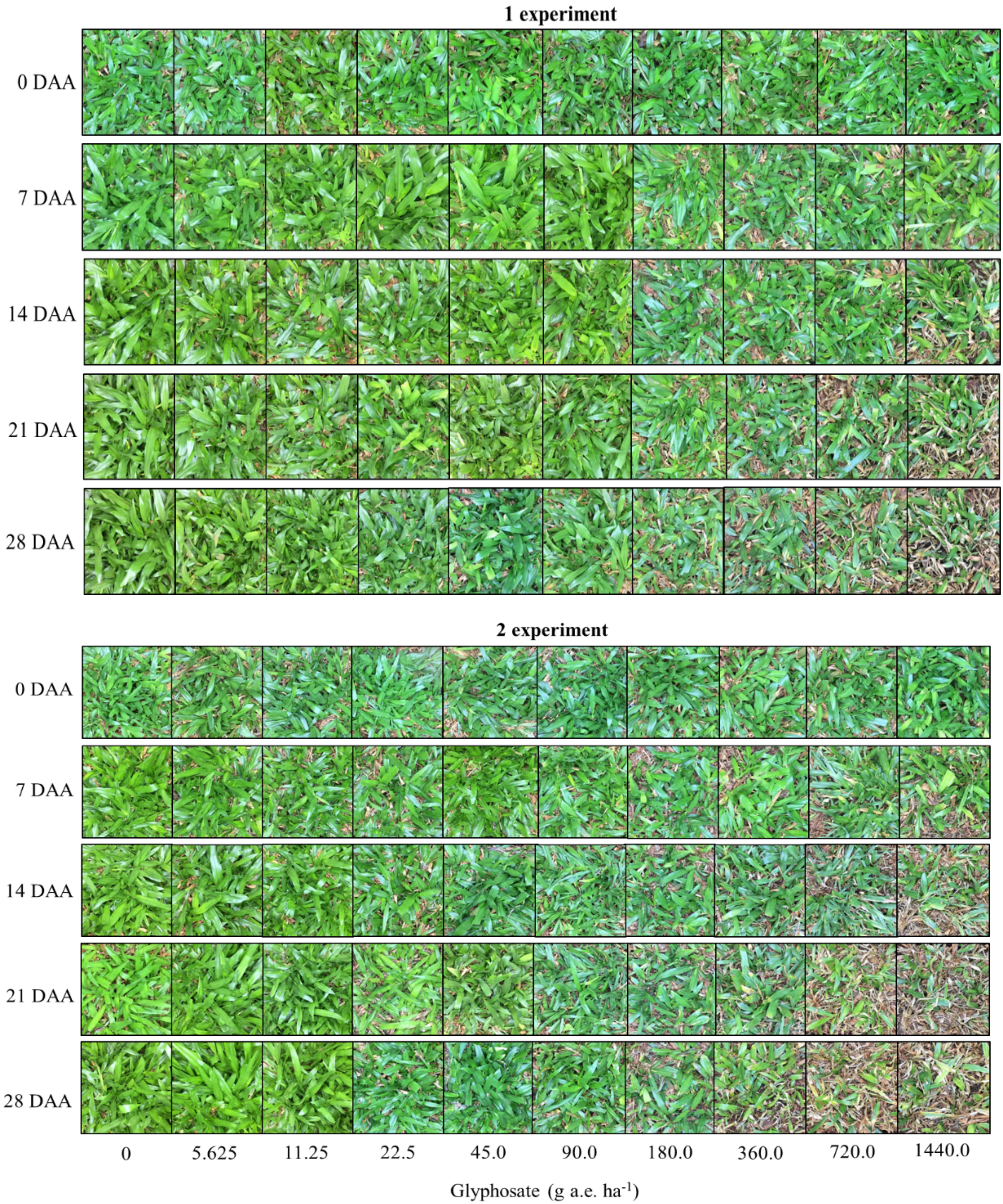

Figure 4 - Phytotoxic effect on broadleaf carpetgrass (*Axonopus compressus*) caused by the glyphosate rates. DAA = days after application.

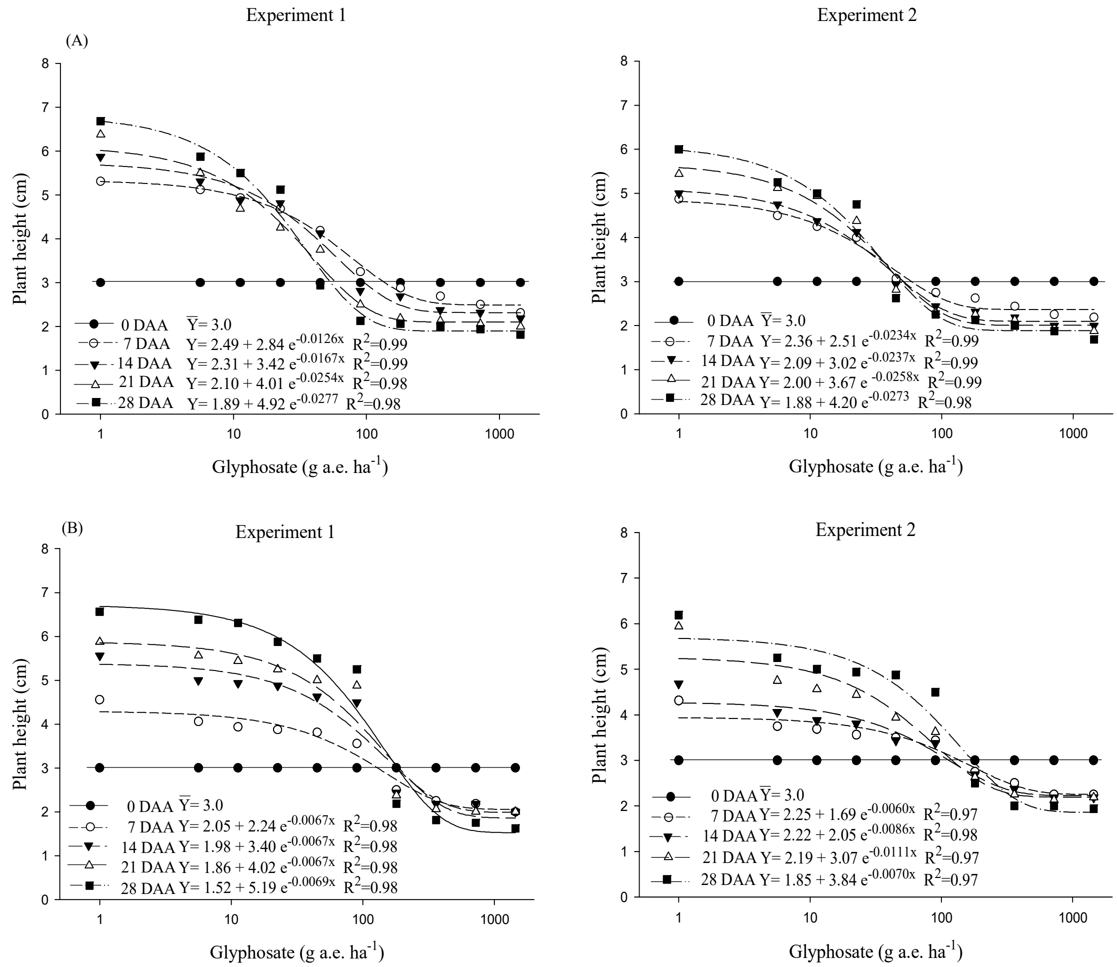

Figure 5 **-** Plant height (cm) as a function of glyphosate rates applied to bahiagrass (*Paspalum notatum*) (A) and broadleaf carpetgrass (*Axonopus compressus*) (B), in experiments 1 and 2.

herbicide as growth regulator for bahiagrass and broadleaf carpetgrass grasses without deleterious effects.

Glyphosate inhibits the EPSPs enzyme, which catalyzes the key reaction in the shikimic acid pathway, which is a specific metabolic pathway of plants that is required for the production of many secondary compounds, including auxin and essential aromatic amino acids for plant growth and development (Velini et al., 2009; Taiz and Zeiger, 2013). In addition, the inhibition of these enzymes affects the production of several compounds that are involved in plant growth regulation, such as phenolic compounds, including tryptophan, which is the precursor of the indolylacetic acid (IAA) synthesis. Therefore, the reduction in plant height is due to lower IAA production, which impairs cell elongation (Velini et al., 2009). Similarly, several studies have shown reduction in plant height due to the use of glyphosate in turfgrasses (Johnson, 1990; Fry, 1991; Dinalli et al., 2015; Barbosa et al., 2017), which corroborate the results found in the present study.

The dry biomass of cuttings (Figure 6) decreased in 24% (experiment 1) and 39% (experiment 2) when using rates equal to and above 45 g a.e. ha^{-1} for bahiagrass; and 59% (experiment 1) and 57% (experiment 2) when using rates equal to and above 180 g a.e. ha^{-1} for broadleaf carpetgrass.

The reductions in dry biomass of cuttings caused by glyphosate may be related to its mechanism of action (Velini et al., 2009; Taiz and Zeiger, 2013). The shikimic acid pathway, which is affected by glyphosate, is responsible for the formation of phenolic compounds, which can represent up to 35% of the dry biomass (Kruse et al., 2000). The mowing frequency is determined by the growth rate of the turfgrass; thus, high growth rate and high biomass production are not desirable (Godoy et al., 2012b).

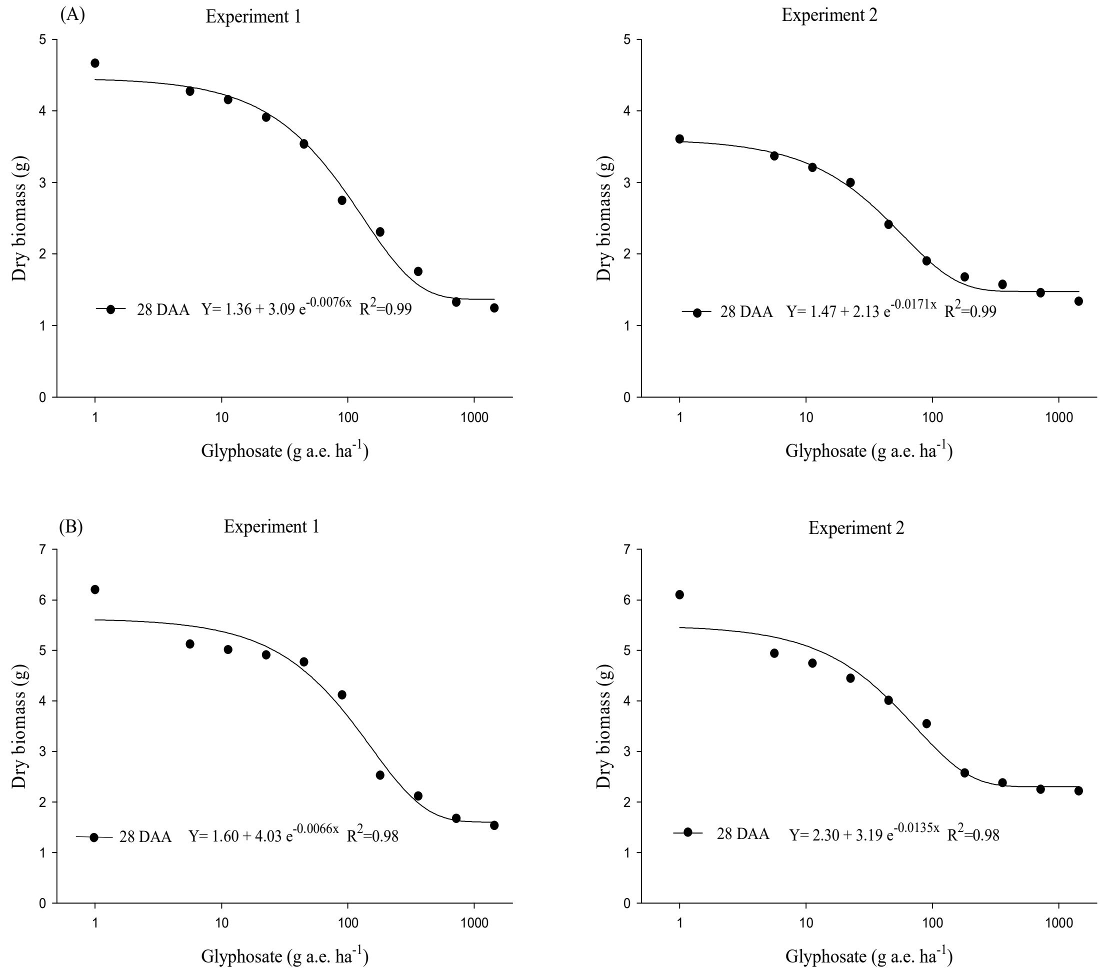

Figure 6 **-** Dry biomass of cuttings from the mowing of bahiagrass (*Paspalum notatum*) (A) and broadleaf carpetgrass (*Axonopus compressus*) (B) as a function of glyphosate rates, in experiments 1 and 2.

Considering the results found in the present work, the glyphosate herbicide can be used as a plant growth regulator when applied at rates of up to 22.5 g a.e. ha^{-1} for bahiagrass and up to 90 g a.e. ha^{-1} for broadleaf carpetgrass, without affecting the quality of the turfgrass, regarding phytotoxicity and GCI, in the evaluated periods. The use of digital image analysis is important for the verification and quantification of effects of herbicides on turfgrasses by assisting on recommendations regarding their selectivity and weed control effectiveness.

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