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Can occur *hormesis* in guinea grass using glyphosate?

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Abstract: **Background:** Recent studies have demonstrated an increase in plant growth due to the application of low doses of glyphosate, which is part of the phenomenon known as *hormesis*. **Objective:** The present study aimed to evaluate the effects of glyphosate doses on the physiological and morphological parameters of *Panicum maximum* cv. Mombaça. **Methods:** The experiment was carried out in a greenhouse for 49 days after the application of glyphosate, in pots arranged in a completely randomized design, with four replications. The treatments consisted of ten doses of glyphosate (0; 3.78; 8.10; 16.64; 33.48; 67.7; 135; 270; 540 and 1,080 g a.e. ha^{-1}). Evaluations of growth, gas exchange, leaf temperature and plants' intoxication were carried out. The data were subjected to ANOVA and when significant, regression analysis was performed using the models for dose-response curves. **Results:** The physiological parameters, as well as the growth, were positively affected by the subdoses of glyphosate, with higher values than the control. The leaf temperature increased progressively according to the increase in doses. **Conclusion:** Subdoses of 3.78 to 270 g a.e. ha^{-1} of glyphosate promoted an increase in plant's growth and physiological parameters.

Keywords: dose-response; gas exchange; *Megathyrsus maximus*; *Panicum maximum*; subdose.

1. Introduction

Glyphosate is the most widely used herbicide in the world, due to several aspects related to the reduction of labor costs and its mode of action. Thus, as it has a broad spectrum of control and is highly effective, it is the main option for weed control in various agricultural production systems (Duke, Powles, 2008; Velini et al., 2009).

However, recent studies have shown that low doses of this herbicide can act stimulating the growth of several plant species (Velini et al., 2008; Cedergreen, Olesen, 2010; Carvalho et al., 2013; Pereira et al., 2013; Carbonari et al., 2014; Nascentes et al., 2018), possibly due to changes in the shikimic acid pathway (Velini et al., 2008), which is responsible for approximately 20% of the carbon assimilated by plants (Haslam, 1993). Such biphasic dose-response effect, in which low doses cause a stimulatory effect, followed by high doses causing a toxic effect, is known as *hormesis* (Calabrese, Baldwin, 2002).

Treating a crop with subdoses of a herbicide for a desirable phenotypic change can be valuable (Duke et al., 2006); for example, when glyphosate is applied in subdoses to stimulate the accumulation of sucrose and prevent flowering in sugar cane (Velini et al., 2009; Araldi, 2010). In a different way, it is also worth mentioning the work of Pereira et al. (2013), that when applying reduced doses (from 3.6 to 36.0 g a.e. ha^{-1}) of glyphosate in eucalyptus seedlings, reported increases of up to 22% in the dry matter of the crop. Doses within this range were also responsible for the increase of up to 20% in the dry matter of non-resistant soybean, compared to the control (Velini et al., 2008). For forage species (*Brachiaria brizantha*), Nascentes et al. (2015) observed a growth 31.8% greater than plants without application, for the dose of 10.5 g a.e. ha^{-1}. In spite of this, studies that aim to increase the vegetative mass of *Panicum maximum* for grazing, in response to a hormetic effect, are incipient in the literature.

The guinea grass (*P. maximum* syn. *Megathyrsus maximus*) is considered an important grass in pasture areas in several regions of Brazil, being one of the first forage species introduced in the country (Souza, 1999). Thus, the knowledge of their physiological and morphological characteristics is essential for the establishment of adequate management (Rodrigues, Reis, 1995). Thus, the study of the hormetic effect caused by low doses of glyphosate can be a viable alternative for the management of this species, aiming to an increase in plant growth and consequently production of green mass.

In addition to cultures varying in their responses due to the application of different glyphosate doses, as mentioned above, Belz and Duke (2014) pointed out

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that other factors can also interfere in the occurrence, or not, of the hormetic effect. They are: plant development stage (Carvalho et al., 2013), the final evaluation point (Cedergreen et al., 2009), environmental conditions at the time and after products' application (Belz, Cedergreen, 2010) and the clone or cultivar chosen (McDonald et al., 2001). Thus, studies are needed to understand how plant species respond to these variables not only to better understand this biological phenomenon, but also to obtain greater assertiveness in management aiming at increasing *P. maximum* green mass for pasture with the use of this herbicide.

Thus, according to what has been reported, we hypothesize that *P. maximum*, as observed for other species, including grasses, may respond positively to glyphosate subdoses, showing an increase in its growth. Thus, the present study aimed to evaluate the effects of increasing doses of glyphosate on physiological and morphological aspects of *Panicum maximum* cv. Mombaça, in order to identify doses that cause a stimulatory effect on the plants.

2. Materials and Methods

The experiment was carried out in greenhouse conditions, in the municipality of Vitória da Conquista-BA, with geographical coordinates of: 14°53'32.2"S, 40°48'23.9"W and altitude of 923 m. The study was conducted for 49 days after the application (DAA) of the treatments, when an average temperature of 22.7 °C (maximum of 28.7 °C and minimum of 17.9 °C) and relative humidity of 67.5% were registered.

The plant species *Panicum maximum* (syn. *Megathyrsus maximus*) cv. Mombaça (guinea grass), acquired from Sementes Rancharia®, located in the municipality of Rancharia-SP, was used. Sowing was carried out in polypropylene trays, previously filled with agricultural substrate.

At 20 days after sowing, three seedlings were transplanted to pots with a capacity of 2 L, containing soil removed from the arable layer of a soil classified as Red-Yellow Alic Oxisol. Pre-transplant fertilization was carried out with amounts equivalent to 48 kg of KCl ha^{-1}, 40 kg of simple superphosphate ha^{-1} and 100 kg of urea ha^{-1}.

Irrigation was performed with deionized water, with 200 mL applied per plot, per day, distributed in one application in the morning and another in the afternoon, until 25 DAA of the treatments. After this period, the amount of water per pot was doubled.

The experimental treatments consisted of ten doses of glyphosate (Crucial®): 0; 3.78; 8.10; 16.64; 33.48; 67.7; 135; 270; 540 and 1,080 g a.e. ha^{-1}. A completely randomized design was used, with four replications.

The herbicide's application was carried out when the plants had two to three leaves fully expanded, with height less than 10 cm (40 days after sowing). The application was made using a CO_2-pressurized backpack sprayer (2 kgf cm^{-2}), coupled to a bar containing four 110-02 flat jet tips, spaced at 0.5 m, regulated for a tank volume of 220 L ha^{-1}. At the time of application, the soil was moist, and the temperature and relative humidity were 28 °C and 56.5%, respectively.

Twenty four hours after herbicide's application, the rate of net CO_2 assimilation, stomatal conductance, transpiration rate, intercellular CO_2 concentration and leaf temperature were determined using an infrared gas analyzer (IRGA, mod. LCpro-SD, ADC BioScientific®). The evaluations were conducted on the second fully expanded leaf, in the basipetal direction, with a minimum length of 2.5 cm. For this purpose, a chamber for dicotyledonous leaves adapted to monocotyledons was used, with a dichroic light source in the upper part of the chamber. The working conditions were: 400 μmol CO_2 mol^{-1}; 19 mmol H_2O mol^{-1}; air temperature was set at 25 °C and the photosynthetically active photon flow of 1200 μmol m^{-2} s^{-1}.

In addition, at 7, 14, 21, 28, 35 and 42 DAA of glyphosate, the number of tillers per plant was also evaluated, as well as the degree of intoxication, using the visual scale proposed by Sbcpd (1995), in which 0% corresponded to the absence of injuries and 100% to the plants' death.

At the end of the experimental period (49 DAA), the guinea grass plants were cut close to the soil, and their leaves were detached to the leaf area determination (mod. LI-3100, LiCor®, USA). Then, leaves and culms were placed into a forced air circulation oven, at a temperature of 70 °C, for 120 hours, to determine the dry matter mass, after weighing on a precision scale.

The data obtained were subjected to ANOVA by the F test (AgroEstat® software) and when significant, regression analysis was performed using the models for dose-response curves (Origin® 8.5, OriginLab®), using the equation proposed by Brain and Cousens (1988).

3. Results and Discussion

Analyzing the gas exchange parameters evaluated 24 hours after glyphosate's application, it was found that the net CO_2 assimilation rate (*A*) obtained a constant increase, from the dose of 3.78 to 270 g a.e. ha^{-1}, with a sharp drop in subsequent doses (Figure 1a). On this occasion, the greatest increase in CO_2 assimilation was obtained by plants that received a dose of 270 g a.e. ha^{-1}, reaching values approximately 92% higher than the control without application (Figure 1a).

For the transpiration rate (*E*) of guinea grass plants, the response pattern was the same as that observed for *A*, in which doses from 3.78 to 270 g a.e. ha^{-1} provided a constant increase in this characteristic, followed by a sharp drop caused by the two highest doses (Figure 1b). The spraying of 270 g a.e. ha^{-1} of glyphosate was the one that provided the greatest increase in the plants' transpiration rate, with an increase of 114% compared to the control (Figure 1b).

As for stomatal conductance (g_s), it was noted that the three lowest doses did not cause an increase in the values of this variable, equaling the control (Figure 1c). Again, the dose of 270 g a.e. ha^{-1} of glyphosate provided the highest value of g_s, being 56% higher than the control, possibly because it is a variable directly related to the transpiration rate (Figure 1c).

As for the intercellular carbon concentration (C_i), the response pattern was the opposite of the net CO_2 assimilation rate, in which there was a continuous drop in values, up to the dose of 270 g a.e. ha^{-1}, followed by a recovery in the two highest doses (540 and 1080 g a.e. ha^{-1}) (Figure 1d). These results are directly related to the use of carbon in the photosynthetic process, being inversely proportional. Thus, the carbon molecules present in the leaf mesophyll were used in the CO_2 assimilation process, in which the plants that obtained the highest *A* also obtained the lowest C_i (Figures 1a and 1d).

Regarding leaf temperature, measured at the same time as gas exchange, there was a continuous increase directly related to the increase in glyphosate doses (Figure 2).

It was possible to observe that at 7 days after application (DAA), plants intoxication started from the dose of 135 g a.e. ha^{-1}, causing 35% of toxicity compared to the control and reaching up to 47% in the highest dose (1,080 g a.e. ha^{-1}) (Figure 3). At 14 DAA, it was noted that from the dose of 67.7 g a.e. ha^{-1} there was intoxication in the treated plants, which remained until 28 DAA, always with values below 11%. In general, the highest intoxication scores were observed at 14 DAA. At 21 DAA there was a reduction of symptoms in plants, which led to a total recovery of intoxication levels at 42 DAA, except for the highest dose of glyphosate (1,080 g a.e. ha^{-1}), which maintained 8.61% grades at the end of the experimental period (Figure 3).

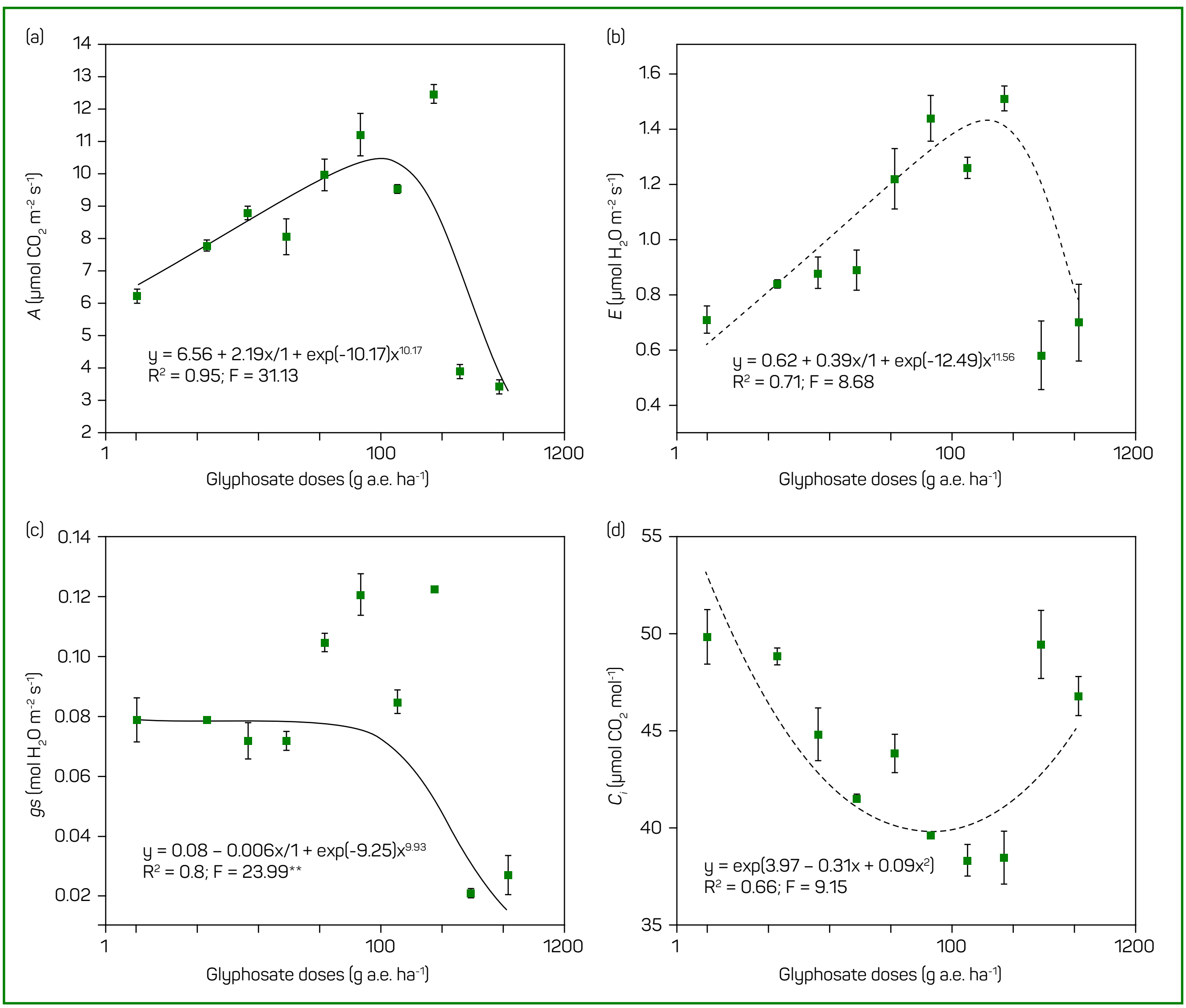

Figure 1 - Net CO_2 assimilation rate (a), transpiration rate (b), stomatal conductance (c) and internal carbon concentration (d) of *Panicum maximum* plants 24 hours after application of increasing doses (0; 3.78; 8, 10; 16.64; 33.48; 67.7; 135; 270; 540 and 1080 g a.e. ha^{-1}) of glyphosate. The bars indicate the standard error of mean (*N* = 4).

For the dose that caused the greatest stimulatory effect on guinea grass (270 g a.e. ha^{-1}), the highest intoxication scores was observed at 14 DAA, with values of 35%. After this period, there was a progressive decrease in symptoms, until their absence, at 42 DAA (Figure 3).

As for the tillering of *P. maximum* plants, at 7 DAA, a reduction of 75% in the number of tillers was observed, compared to the control, when the plants were exposed to the dose of 1,080 g a.e. ha^{-1} of glyphosate (Figure 4). At 14 DAA, the dose of 135 g a.e. ha^{-1} provided a 38% increase in the number of tillers, compared to the control (Figure 4). From 21 DAA, it was observed that the highest dose (1,080 g a.e. ha^{-1}) of glyphosate stimulated tillering, a fact that was maintained throughout the evaluations, with 116% increments at 28 DAA, 117% at 35 DAA and 56% at 42 DAA (Figure 4). Despite this, this increase in the number of tillers did not increase any growth variable at the end of the experimental period (Figures 5, 6a and 6b).

Regarding leaf area, an average increase of 24% was observed for the doses from 3.78 to 540 g a.e. ha^{-1}, when compared to the control without application (Figure 5). Specifically for the dose of 540 g a.e. ha^{-1}, it is worth mentioning that although the plants in this treatment obtained a larger leaf area than the control, this increase did not result in a greater production of dry matter of leaves (Figure 6b), which suggests the formation of thinner and lighter leaves.

On the other hand, the highest dose of the product caused a reduction in the leaf area of 27% compared to the control (Figure 5), even whit this treatment obtaining the largest amount of tillers (Figure 4), which shows the toxic effect of this dose to the plant growth.

For the dry matter of culms (DMC), it was possible to observe that the lowest dose (3.78 g a.e. ha^{-1}) of glyphosate was the one that provided the greatest growth of guinea grass culms, being approximately 21% higher than the control (Figure 6a). In the following doses, there was a gradual decrease in the values of DMC as the doses of glyphosate increased. Thus, as well as occurred for the net CO_2 assimilation rate (Figure 1a), the doses of 540 and 1,080 g a.e. ha^{-1} were the only ones that caused a deleterious effect on plant growth.

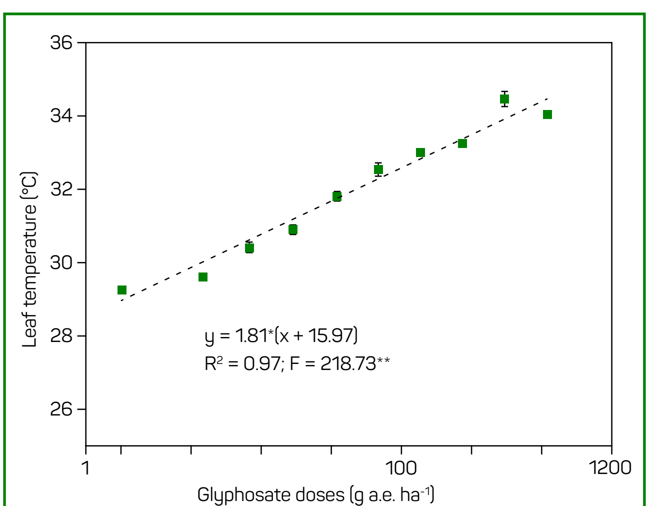

Figure 2 - Leaf temperature (°C) of *Panicum maximum* plants 24 hours after application of increasing doses (0; 3.78; 8.10; 16.64; 33.48; 67.7; 135; 270; 540 and 1080 g a.e. ha^{-1}) of glyphosate. The bars indicate the standard error of mean (*N* = 4).

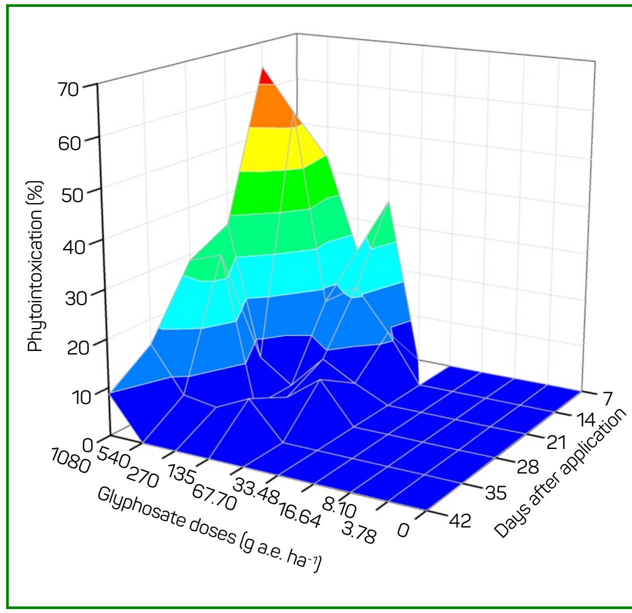

Figure 3 - Phytointoxication percentage (SBCPD, 1995) of *Panicum maximum* plants, evaluated at 7, 14, 21, 28, 35 and 42 days after application (DAA) of increasing doses of glyphosate.

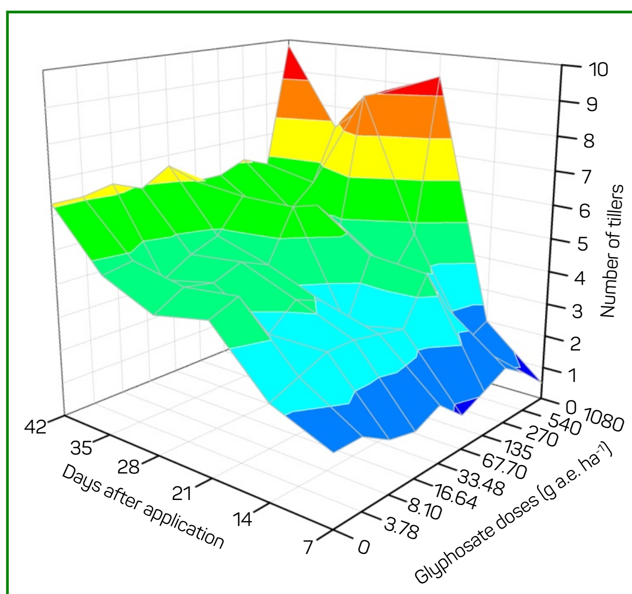

Figure 4 - Number of tillers of *Panicum maximum* plants, evaluated at 7, 14, 21, 28, 35 and 42 days after application (DAA) of increasing doses of glyphosate.

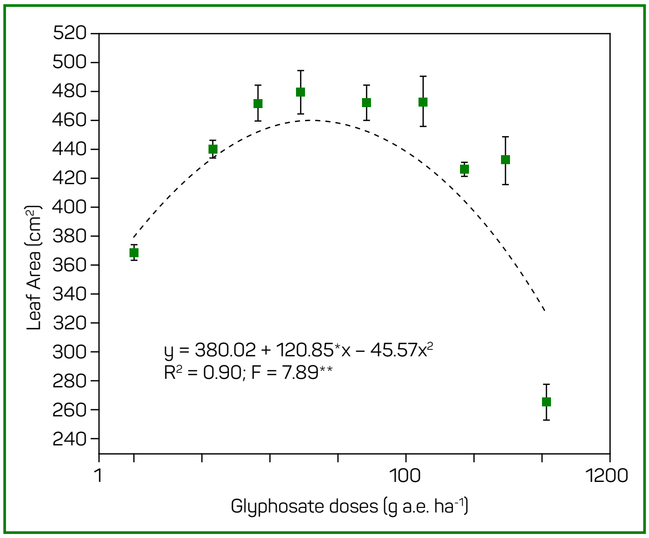

Figure 5 - Leaf area (cm²) of *Panicum maximum* plants at 49 days after application of increasing doses (0; 3.78; 8.10; 16.64; 33.48; 67.7; 135; 270; 540 and 1080 g a.e. ha⁻¹) of glyphosate. The bars indicate the standard error of mean (*N* = 4).

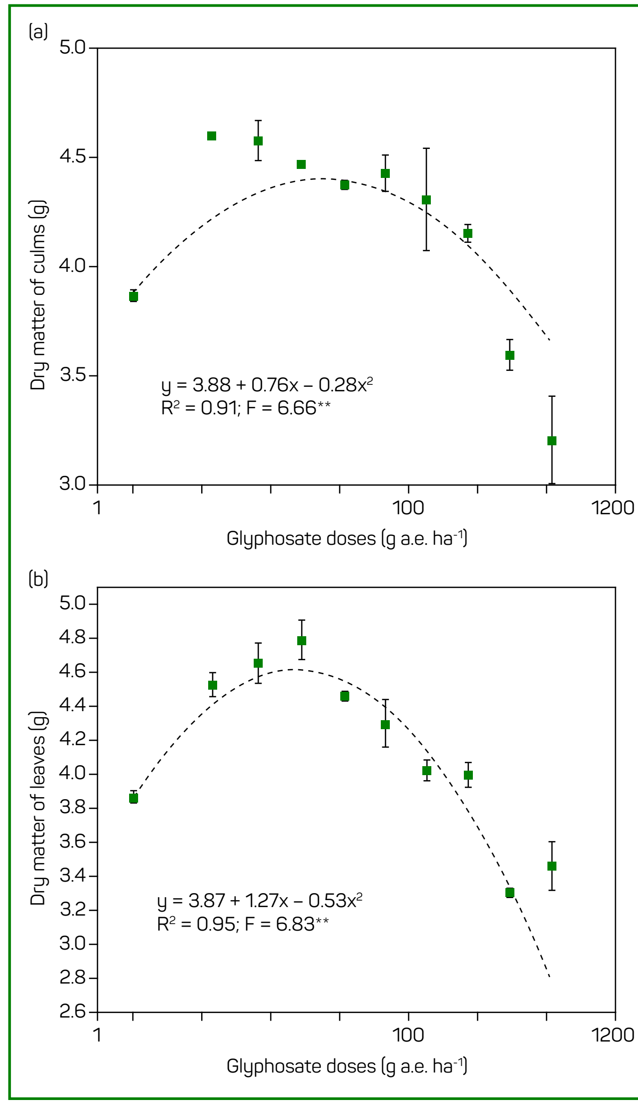

Figure 6 - Dry matter of culms (a) and dry matter of leaves (b) of *Panicum maximum* plants at 49 days after application of increasing doses (0; 3.78; 8.10; 16.64; 33.48; 67, 7, 135, 270, 540 and 1080 g a.e. ha⁻¹) of glyphosate. The bars indicate the standard error of mean (*N* = 4).

Regarding the dry matter of leaves (DML), there was a progressive growth in response to the increase in doses up to 16.64 g a.e. ha⁻¹, when the plants obtained DML 26.3% higher than the control. From this dose on, again a gradual decrease in values was noticed according to the increase in the herbicide doses (Figure 6b). It is important to note that, as with DMC, doses of up to 270 g a.e. ha⁻¹ did not decrease the growth of *P. maximum* plants, when compared to control (Figures 6a and 6b).

The increase in dry matter of guinea grass observed in the present study, caused by the application of low doses of glyphosate, is part of the phenomenon defined as *hormesis*. This term consists of a dose-response with a biphasic effect, caused by a stimulus due to the application of a chemical product that is toxic in high concentrations (Calabrese, Baldwin, 2002; Belz, Duke, 2014).

Glyphosate is the most used product with the intention of promoting the hormetic effect in plants (Brito et al., 2018). Thus, in recent years, several studies have been conducted in order to understand this phenomenon, both in dicotyledonous species [*Eucalyptus urograndis* (Pereira et al., 2013), *Coffea arabica* (Carvalho et al., 2013) and *Glycine max* (Velini et al., 2008)] as in monocot species [*Brachiaria brizantha* (Nascentes et al., 2015); *Brachiaria decumbens* (Moraes et al., 2020); *Saccharum officinarum* (Silva et al., 2009); *Zea mays* (Wagner et al., 2003)].

This increase in plant growth in response to subdoses of glyphosate has not yet been fully clarified, but it is likely to be related to changes in the shikimic acid pathway (Velini et al., 2008; Cedergreen, Olesen, 2010). Velini et al. (2008) observed that glyphosate-resistant soybean showed no changes in growth and concentration of shikimic acid when exposed to the herbicide. Conversely, soybean plants susceptible to glyphosate had a mass increase of approximately 20% compared to the control, as well as an increase in the concentration of this substance.

Once at the site of action, glyphosate inhibits the enzyme EPSPs (5-enolpyruvylchiquime-3-phosphate synthase), which is responsible for catalyzing the reaction of phosphoenolpyruvate and chiquimate-3-phosphate, forming the phosphated enolpyruvatochiquite-3-phosphate compound. Enolpyrivulchicamate-3-phosphate is a precursor to chorisic acid, which will produce the amino acids phenylalanine, tyrosine and tryptophan (Fedtke, Duke, 2005). Thus, a reduction in the synthesis of these compounds, will result in less production of essential amino acids in the route of shikimic acid, being that approximately

20% of the carbon fixed by the plant is destined to this route (Haslam, 1993), altering, with this, the synthesis of proteins, vitamins, alkaloids and also several important phenolic compounds, such as flavonoids and phytohormone auxin (Velini et al., 2009).

In addition, low doses of this herbicide inhibit lignin synthesis, making cell walls more elastic for a longer period and resulting in greater longitudinal growth (Duke et al., 2006), but not justifying the increase in dry matter observed in present experiment (Figure 6). Thus, an increase in the CO_2 assimilation rate is also one of the main factors in the increase in the production of vegetable mass (Figure 1).

In the present study, an increase in the net CO_2 assimilation rate (*A*) of 92% was verified for the 270 g a.e. ha^{-1} dose. Barley plants, on the other hand, obtained *A* approximately 20% higher than the control, when they were sprayed with 45 g a.e. ha^{-1} of glyphosate (Cedergreen, Olesen, 2010). In a similar way, Nascentes et al. (2018) observed an increase of about 60% in sugarcane plants with doses of up to 36 g a.e. ha^{-1}.

Cedergreen and Olesen (2010) observed that the increase in photosynthetic rates of barley treated with low doses of glyphosate remained for up to 7 consecutive days. The authors attributed the greater growth of plants to the increase in the efficiency of carbon assimilation, and not to the greater efficiency in the use of light. Thus, this increase in photosynthetic activity is related to the increase in rubisco activity, in the rate of use of triose phosphate and/or in the regeneration of RuBP (Cedergreen, Olesen, 2010).

Regarding the leaf temperature, it was observed that there was a gradual increase as the doses of glyphosate also increased (Figure 2). We hypothesize that the increase in leaf temperature is related to the greater metabolic activity in the cells of this tissue, caused by the herbicide. Thus, at doses from 3.78 to 270 g a.e. ha^{-1}, the result of these reactions was observed in the increase in mass (Figure 6), while at doses considered toxic, the metabolic activity was directed to the plant detoxification through the processes of inactivation or compartmentalization of the herbicide (Cole, 1994; Reddy et al., 2008). In this sense, it is worth mentioning that through measurements on respiration rates, Cedergreen and Olesen (2010) found greater metabolic activity in barley plants one day after exposure to low doses of glyphosate. The authors observed that the higher the dose, the greater the respiratory activity on the first day, which tended to gradually decrease on subsequent days.

Thus, the management of pastures with low doses of this chemical can become a viable alternative in order to increase the vegetal mass to be consumed by the animals, and also considering that the phytointoxication evaluations for the doses that stimulated the plants growth were not higher than mild or null (Figure 3). Nascentes et al. (2015) observed an increase of 21% in the dry matter of the aerial part of *Brachiaria brizantha* at 30 days after the application of 12.62 g a.e. ha^{-1} of glyphosate. In the present study, an average increase of 21% was found for DMC and 26.3% for DML, at 49 DAA. Such increment may favor the increase in the stocking rate of animals in the grazing area, which is directly influenced by the amount of vegetal mass offered (Barioni, Ferreira, 2007).

Despite this, Belz et al. (2011) highlighted that the use of herbicides in order to increase crop productivity should be widely studied, since the plant's development stage itself can influence the occurrence, or not, of the hormetic effect. In this sense, Cedergreen (2008) reported not having observed productivity gains when applying glyphosate to barley in a two-leaf stage. However, when the herbicide was applied at a grain filling stage, Cedergreen et al. (2009) found an increase in production of 12 to 15%. Additionally, it is important to note that the occurrence of *hormesis* is also dependent on the correct association of the plant's development with the dose used. Velini et al. (2008), working with *Commelina benghalensis* in the four-leaf stage, had to apply a dose five times higher than that used in the two-leaf stage, so that the greatest stimulus to plant growth could be observed. In addition to these, other factors can also influence the occurrence of *hormesis*, as previously mentioned, namely: environmental conditions at the time and after product application (Belz, Cedergreen, 2010), clone or cultivar chosen (McDonald et al., 2001), and the final evaluation point (Cedergreen et al., 2009), that is, the period after exposure to the product that it will be evaluated. Thus, more studies must be developed in this sense in order to clarify the possible variations that may occur in field situations.

4. Conclusion

We concluded that the subdoses of 3.78 to 270 g a.e. ha^{-1} of glyphosate promoted an increase in growth and in the physiological parameters of *Panicum maximum* cv. Mombaça. The application of glyphosate in doses of 540 g a.e. ha^{-1} or higher, caused a deleterious effect on plant growth.

Authors' contributions

PLCAA, and ARSJ: conceptualization, methodology development and project administration. GJTG: conducted the experiment. ALB, WCC, and GJTG: performed formal analyses and wrote the original manuscript. All authors collaborated in the process of revising and editing the final version of this manuscript.

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