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Article

CHLOROPHYLL FLUORESCENCE IN *Brachiaria decumbens* AND *Brachiaria ruziziensis* SUBMITTED TO HERBICIDES

*Fluorescência da Clorofila em **Brachiaria decumbens** e **Brachiaria ruziziensis** Submetidas a Herbicidas*

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ABSTRACT - Different doses of herbicides can promote a differentiated physiological response in grasses of the same genus. This study has been proposed in order to evaluate physiological responses of *B. ruziziensis* and *B. decumbens* submitted to glyphosate and fluazifop-p-butyl. Treatments were distributed in a 2 x 6 factorial design with five repetitions, being two grasses: *B. decumbens* cv. Basilisk and *B. ruziziensis* with six doses of herbicides: 0.00; 0.25; 0.50; 1.00; 1.5 and 2.00 times the commercial dose recommended by manufacturers. At 7, 15, 21, and 30 days after herbicide application (DAA) chlorophyll luminous energy uptake efficiency was evaluated. Plants subjected to glyphosate at 7 days after application (DAA) showed minimum fluorescence of 270 and 245 quantum^{-1} electrons to *B. ruziziensis* and *B. decumbens*, respectively, levels increased by 350% when compared to the control, and at 15 DAA the treated plants have completely dried up. At 15 DAA, both forage species submitted to the fluazifop-p-butyl showed a 25% reduction in F_v/F_m ratio compared with the control and there was no significant difference between the lowest and highest doses applied. *B. ruziziensis* and *B. decumbens* are more sensitive to glyphosate than fluazifop-p-butyl. Regarding fluazifop-p-butyl, *B. ruziziensis* was more sensitive than *B. decumbens* and at 45 days after cutting *B. decumbens* plants submitted to doses up to 100 g ha^{-1} were able to regenerate their photosynthetic apparatus.

Keywords: physiology, fluazifop-p-butyl, glyphosate, electron transport rate, *Urochloa.*

*RESUMO - A utilização de diferentes doses de herbicidas pode promover resposta fisiológica diferenciada em gramíneas do mesmo gênero. Este trabalho foi proposto com o objetivo de avaliar as respostas fisiológicas de **B. ruziziensis** e **B. decumbens** submetidas aos herbicidas glyphosate e fluazifop-p-butyl. Os tratamentos foram arranjados no esquema fatorial 2 x 6, com cinco repetições, sendo duas gramíneas (**B. decumbens** cv. Basilisk e **B. ruziziensis**) e seis doses dos herbicidas: 0,00, 0,25, 0,50, 1,00, 1,5 e 2,00 vezes a dose comercial indicada pelos fabricantes. Aos 7, 15, 21 e 30 dias após aplicação dos herbicidas (DAA), foi avaliada a eficiência de captação de energia luminosa da clorofila. Para as plantas submetidas ao glyphosate, aos 7 DAA, foi observada fluorescência mínima de 270 e 245 elétrons quantum^{-1} para **B. ruziziensis** e **B. decumbens**, respectivamente, valores aumentados em mais de 350% em comparação com a testemunha, e, aos 15 DAA, as plantas tratadas senesceram completamente. Aos 15 DAA, ambas as espécies forrageiras submetidas ao fluazifop-p-butyl apresentaram redução de 25% na relação Fv/Fm, em comparação com a testemunha, e não foi observada diferença significativa entre a menor e a maior dose aplicada. **B. ruziziensis** e **B. decumbens** são mais*

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sensíveis ao glyphosate que ao fluazifop-p-butyl. Com relação ao fluazifop-p-butyl, ***B. ruziziensis*** *foi mais sensível que* ***B. decumbens****, e, aos 45 dias após o corte,* ***B. decumbens*** *submetida a doses de até 100 g ha^{-1} foi capaz de regenerar seu aparato fotossintético.*

Palavras-chave: fisiologia, fluazifop-p-butyl, glyphosate, taxa de transporte de elétrons, *Urochloa*.

INTRODUCTION

The species *B. decumbens* Stapf was introduced in Brazil at the end of the 1960s and, due to the high adaptation to edaphoclimatic conditions, rusticity and competitive capacity in relation to other species, it quickly expanded throughout the national territory, being considered, besides forage, a weed of areas of agricultural crops and/or even pasture. In sites where *B. decumbens* was introduced, its replacement by another crop is not an easy practice, both in intercropped systems and for renewal of monoculture pastures. In addition, there are no graminicidal herbicides that are selective for fodder species and that control monocotyledonous weeds.

B. ruziziensis, comparing with *B. decumbens*, presents a higher requirement for soil fertility, lower rusticity and competitive capacity. This species is mainly used in intercropping with agricultural crops in direct planting systems, as it has good soil cover, weed suppression and ease of desiccation (Borges et al., 2014).

In the desiccation of grasses in the planting system, herbicidal molecules are used for plant control and stover formation. Among herbicides, glyphosate and fluazifop-p-butyl stand out. Glyphosate is a non-selective, broad-spectrum herbicide that controls monocotyledonous and dicotyledonous plants through the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Steinrücken and Amrhein, 1980). As for fluazifop-p-butyl, it is a selective herbicide that controls monocotyledonous, inhibiting the enzyme acetyl-CoA carboxylase (ACC) (Rendina and Felts, 1988). The use of different doses of these herbicides can promote a differentiated physiological response in the grasses, making it possible to devise strategies for renewing pastures cultivated with species of the same genus, controlling the desired species.

The evaluation of chlorophyll fluorescence is a nondestructive technique that has been used to elucidate the mechanisms of photosynthesis and changes caused by biotic or abiotic stresses in plants (Ferreira et al., 2015). During photosynthesis, light is absorbed by pigments of the antenna complex, which excite the electrons and transfer energy to the reaction centers of photosystems II and I. This capacity can be observed even in intact leaves, through chlorophyll fluorescence (Maxwell and Johnson, 2000).

There are studies that report the use of herbicides in the control of forage grasses of different genera (Santos et al., 2012). However, there are few studies in the literature with the use of herbicides evaluating the physiological responses of forage species of the same taxonomic genus, such as *Brachiaria*. In view of the above, the present study was proposed with the objective of evaluating the physiological responses of *B. ruziziensis* and *B. decumbens* (Syn. *Urochloa*) submitted to herbicides glyphosate (Roundup) and fluazifop-p-butyl (Fusilade).

MATERIAL AND METHODS

The trials were conducted in a greenhouse between February and August 2013. The soil was collected in a pasture area in the Brazilian city of Couto de Magalhães de Minas, MG, with 18º 4' of south latitude, 43° 28' of west longitude and 733 meters altitude. The results of soil chemical analysis (Red-Yellow Latosol) in the 0-20 cm depth layer are shown in Table 1. After the analysis, at each 100 kg of soil, 120 g of dolomitic limestone, 500 g of single superphosphate, 30 g of ammonium sulphate and 20 g of potassium chloride were added (Cantarutti et al., 2007).

The experimental design was the completely randomized with five replications. The treatments were arranged in a 2 x 6 factorial design, being two forage grasses: *Brachiaria decumbens* cv. Basilisk (signalgrass) and *B. ruziziensis* (congograss) (Syn. *Urochloa decumbens*

Table 1 - Soil chemical characteristics in the 0-20 cm depth layer. Couto de Magalhães de Minas, MG

pH (H_2O)	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	OM	Sand	Clay
	(mg dm^{-3})		($cmol_c$ dm^{-3})					(dag kg^{-1})	(%)	
5.12	1.02	25.3	0.40	0.20	0.76	5.20	0.66	0.70	47	53

SB = sum of the bases; OM = organic matter.

and *Urochloa ruziziensis*) and six doses of herbicides: 0.00, 0.25, 0.50, 1.00, 1.5 and 2.00 times the commercial dose indicated by the manufacturers. Two experiments were carried out simultaneously. In one trial glyphosate was used and in another, fluazifop-p-butyl. The doses of herbicides applied were: 0, 90, 180, 360, 540, and 720 g a.e. ha^{-1} of glyphosate and 0, 50, 100, 200, 300, and 400 g a.i. ha^{-1} of fluazifop-p-butyl.

Each experimental unit consisted of a pot containing 7 dm^3 of sieved soil. Signalgrass and Congo grass were sown in trays at depth of 1 cm, being transplanted 20 days after sowing. In each vessel, two signalgrass plants were kept interspersed with two Congo grass plants. The other plant species that occurred in the pots were manually eliminated. Irrigation was done daily and every 14 days cover fertilizations were carried out, with 2.5 g per pot of the formula $N\text{-}P_2O_5\text{-}K_2O$ (20-5-20) previously diluted in water.

The herbicides were applied at 30 days after transplanting the seedlings to the pots. The application was carried out with a knapsack sprayer with a flat jet nozzle (XR 11002) at a constant pressure of 210 kPa and spray mix volume equivalent to 200 L ha^{-1}. At the time of herbicide application, the plants were in an initial stage of development, containing three to four tillers formed and an average of 32 cm in height, simulating plant conditions in pasture area being established.

Physiological evaluations were performed at 7, 15, 21 and 30 days after application of the herbicide (DAA). At 30 DAA, plants were harvested at ground level. Subsequently, new physiological evaluations were performed at 30 and 45 days after plant regrowth.

The photochemical efficiency of photosystem II (PS II) was analyzed at night, after 30 min of adaptation, with the use of a fluorometer (Junior-Pam, Walz, Germany) and device clamps placed in the middle third of the first fully expanded leaf of the most developed basal tiller. Minimum fluorescence (F_0) was obtained with modulated light intensity less than 0.1 ìmol m^{-2} s^{-1}. Maximum fluorescence (F_m) was determined by the light saturation pulse with wavelength of 450 nm, reproduced in 600 Hz of frequency per 0.3 second and maximum luminous intensity of 10,000 ìmol m^{-2} s^{-1}. The difference between F_0 and F_m is the variable fluorescence (F_v). With this, the photochemical efficiency was also determined, which is the ratio between variable fluorescence and maximum fluorescence (F_v/F_m) and the electron transport rate (ETR ìmols electrons m^{-2} s^{-1}).

The results were submitted to analysis of variance and the means were compared by the t test, adopting 5% as the critical probability level for the type I error, using the statistical program GENES.

RESULTS AND DISCUSSION

There was an effect of the commercial dose of the herbicides on the minimum fluorescence (F_0) of chlorophyll. A similar pattern of response to herbicides was observed in both forage species. For the plants submitted to glyphosate, at 7 DAA, minimum fluorescence of 270 and 245 $quantum^{-1}$ electrons was observed for *B. ruziziensis* and *B. decumbens*, respectively, values increased in over 350% comparing with the control, and at 15 DAA the plants treated were completely weakened.

For the commercial dose of fluazifop-p-butyl, average values of F_0 of 100 and 150 $quantum^{-1}$ electrons were observed for *B. ruziziensis* and *B. decumbens*, respectively. Comparing with the control, both species treated with herbicide showed high average values of F_0 (Figures 1A and B).

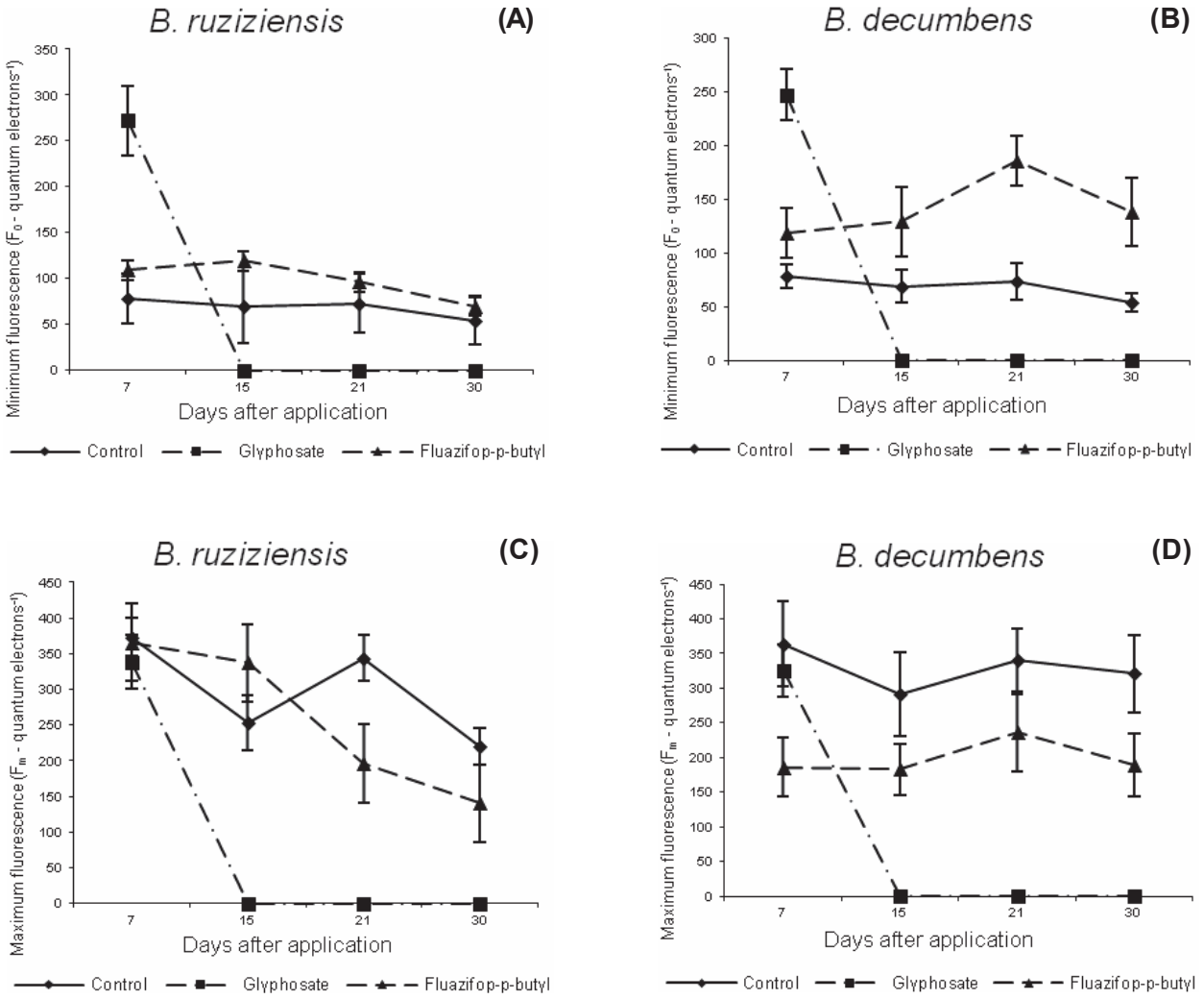

Figure 1 - Effect of the commercial dose of glyphosate (360 g ha^{-1}) and fluazifop-p-butyl (200 g ha^{-1}) on the minimum fluorescence (F_0, charts A and B) and maximum fluorescence of chlorophyll (F_m, charts C and D) due to the days after application.

F_0 represents the energy released by chlorophyll molecules before the electrons migrate to the reaction center. Any change in these values is indicative of possible damage to PS II (Mathis and Pallotini, 1981). According to Bolhàr-Nordenkampf et al. (1989), F_0 may increase or decrease if the PS II reaction centers are compromised or if the transfer of excitation energy from the antenna to the reaction centers is impaired. Increase in the values of F_0 may occur due to reduction in the transfer of excitation energy from the light collecting system to the reaction center (Baker and Rosenqvist, 2004).

At 7 DAA, the *B. ruziziensis* plants did not show difference for the maximum fluorescence values (F_m) of chlorophyll, with an average of 350 $quantum^{-1}$ electrons. However, at 15 DAA there was a decrease of the F_m values of the plants under influence of the herbicides comparing with the control. For glyphosate, at 15 DAA it was observed that both forage species were completely weakened, while for fluazifop-p-butyl a different response pattern was observed for each forage species. *B. ruziziensis* showed reduction in the F_m values, according to the evaluation days: at 30 DAA, F_m was of 150 $quantum^{-1}$ electrons and F_m values observed in plants without herbicide, on average, 250 $quantum^{-1}$ electrons (Figure 1C). *B. decumbens* under influence of fluazifop-p-butyl during all the evaluation periods showed F_m values between 150 and 200 $quantum^{-1}$ electrons. And in the control the F_m values remained between 300 and 350 $quantum^{-1}$ electrons (Figure 1D). Decreased F_m values is probably the result of decreased reoxidation of quinones of PS II, resulting in lower electron flow efficiency between photosystems II and I (De Las Rivas and Barber, 1997).

In the first week after application of the commercial dose of glyphosate, drastic reduction in the F_v/F_m ratio was observed in both forage species (Figures 2A and B). And in the second one a complete stoppage of PS II, which resulted in plant death. A similar result was reported by Campos and Ronchi (2015) when assessing *B. decumbens* treated with glyphosate. These authors have observed a decrease in the capacity of use of the photons for assimilation of CO_2 with the increase of the dose applied. It is known that glyphosate works by inhibiting the key enzyme in the biosynthesis of aromatic amino acids, enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. Although it is considered a reversible competitive inhibition because it is a non-covalent bond with the active site of the enzyme, the inactivity of this key enzyme interrupts innumerable essential reactions in the plant metabolism, resulting in the paralysis of development and accumulation of some compounds that accelerate plant death (Taiz and Zeiger, 2013).

For the commercial dose of fluazifop-p-butyl, a similar behavior was observed in both forage species. From 15 DAA, the F_v/F_m ratio was kept below the range considered ideal, but PS II activity was not completely stopped, as observed for glyphosate (Figures 2A and B). The action of the fluazifop-p-butyl molecule occurs through inhibition of the first stage specific to the synthesis of fatty acids that are involved in several essential metabolic processes, such as photosynthesis, biosynthesis of carotenoids and amino acids, as well as membrane polarization (Rendina and Felts, 1988). The commercial dose of fluazifop-p-butyl decreased 20% the quantum yield of PS II for *B. ruziziensis* and 30% for *B. decumbens*. It is important to note that the quantum yield of

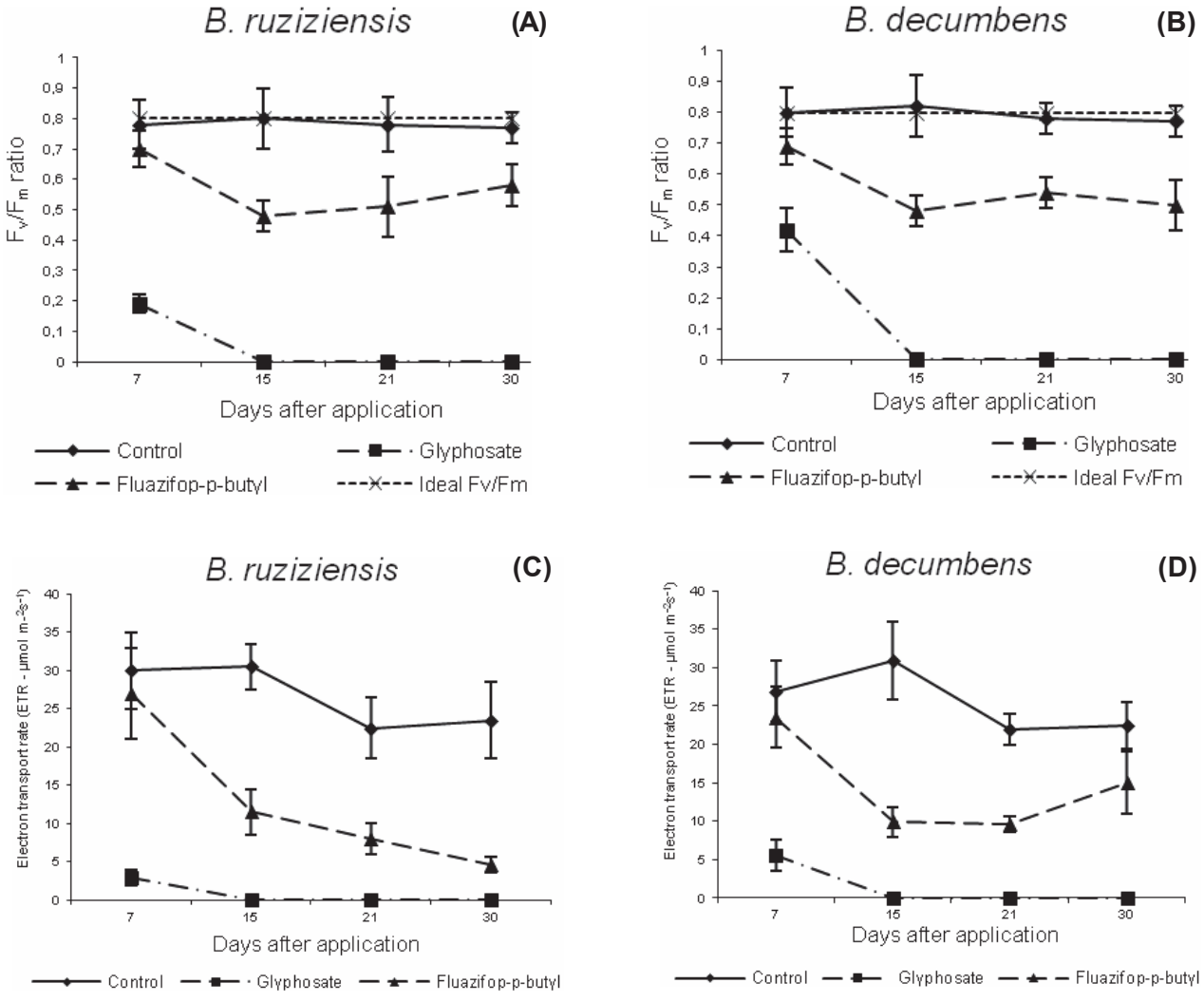

Figure 2 - Effect of the commercial dose of glyphosate (360 g ha^{-1}) and fluazifop-p-butyl (200 g ha^{-1}) on the variable fluorescence/maximum fluorescence ratio of chlorophyll (F_v/F_m, charts A and B) and electron transport rate (ETR, charts C and D) due to the days after application.

PS II (F_v/F_m) may range from 0.75 to 0.85 under ideal growth conditions and the reduction of this ratio is an excellent indicator of plants submitted to stress (Bolhàr-Nordenkampf et al., 1989).

In fact, the activity of chlorophyll has been analyzed to understand the changes in photosynthesis caused by biotic or abiotic stresses, such as temperature (Oliveira et al., 2002), radiation (Mazza et al., 2000), water deficiency (Silva et al., 2006), salinity (Belkhodja et al., 1994), presence of insects (Bown et al., 2002) and herbicides (Ferreira et al., 2015). According to Baker and Rosenqvist (2004), under the effect of herbicide the reduction of chlorophyll activity occurs due to the reduction in the capture efficiency of the excitation energy by the reaction centers opened by PS II, which generates reduction in the quantum yield of the electron transport.

For both species and herbicides, the most pronounced effect on chlorophyll activity was observed at 15 DAA. According to Araldi et al. (2011), herbicides glyphosate and fluazifop-p-butyl affect the photosynthesis of plants in an indirect and slower way when compared to herbicides inhibiting PS II, such as, for example, atrazine.

Regarding the electron transport rate (ETR), the plant response pattern to herbicides was similar to that observed for the F_v/F_m ratio. For glyphosate, at 7 DAA reduction of 90% and 81% was observed in ETR, for *B. ruziziensis* and *B. decumbens*, respectively (Figures 2C and D). In fluazifop-p-butyl the effect was observed at 15 DAA, with reduction of 67% in ETR of both forage species (Figures 2C and D). As for the F_v/F_m ratio, ETR presented the most evident response from 15 DAA. These results confirm the statement by Araldi et al. (2011), because these herbicides act by indirectly ceasing electron transport, resulting in slow inhibition in the ETR of the plants

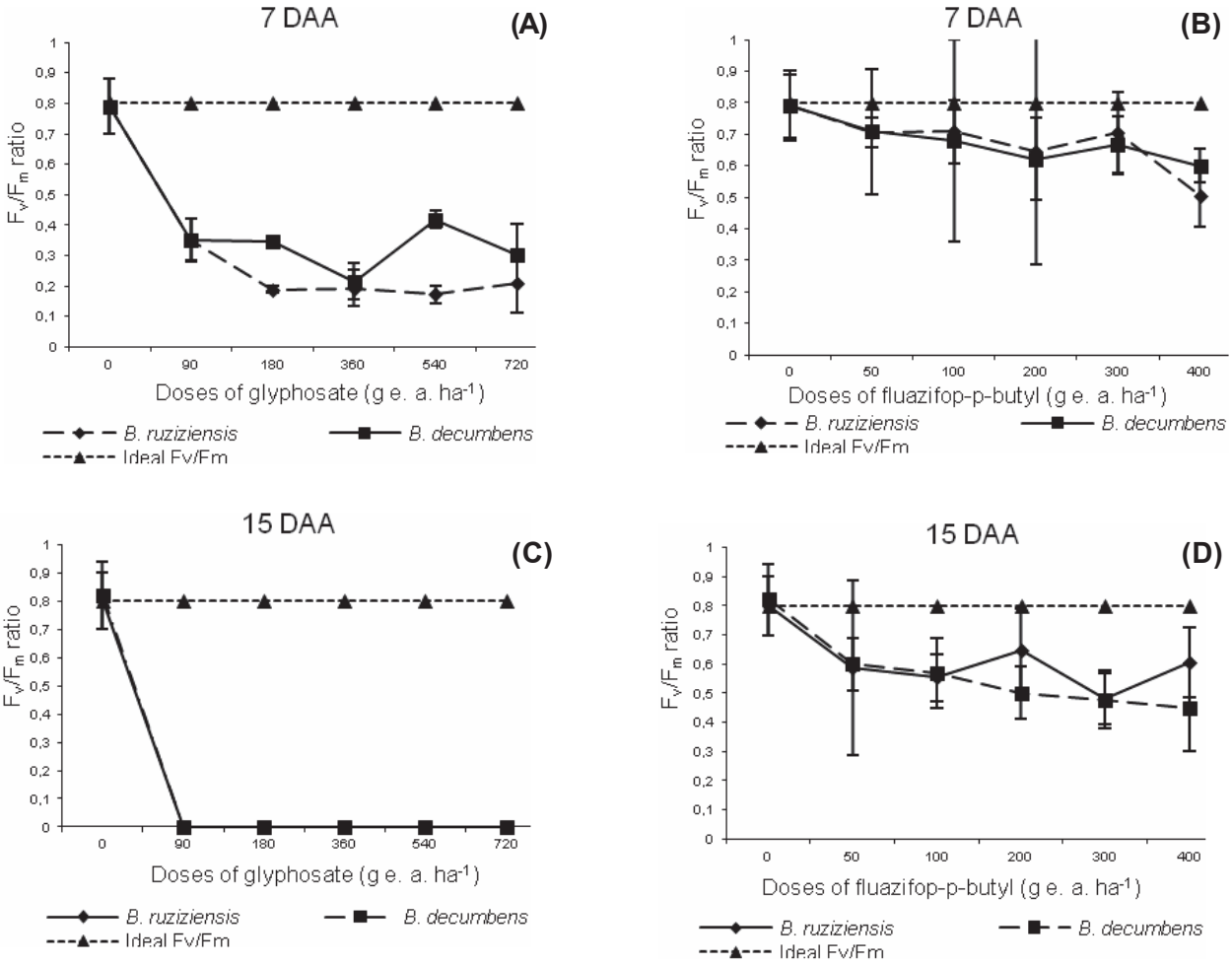

Figure 3 - Effect of herbicides on the variable fluorescence/maximum fluorescence ratio of chlorophyll (F_v/F_m) at 7 (charts A and B) and at 15 (charts C and D) days after the application of the herbicides (DAA) for each forage species due to the doses applied.

treated. These authors, working with *B. decumbens* and different doses of glyphosate, have observed increasing reduction in ETR with the passage of hours after application of the herbicide, with reduction of 40%, 66%, 85% and 100% at 24, 72, 120 and 168 hours after application of the herbicide. However, in a study developed by Dayan et al. (2009), the ETR of *Digitaria sanguinalis* and *Abutilon theophrasti* submitted to the application of amicarbazone and atrazine was completely inhibited eight hours after the application of the herbicides, in both species. In addition, monitoring ETR is important, since it is correlated with the gross photosynthetic rate (Mohammed et al., 1995).

Effect of doses of herbicides in the F_v/F_m ratio was observed at 7 and 15 DAA. The F_v/F_m ratio at 15 DAA did not differ from the 21 and 30 DAA, and this is reason why only the charts related to 7 and 15 DAA were presented (Figure 3). For both forage species submitted to glyphosate at 7 DAA a 44% reduction was observed in the F_v/F_m ratio comparing with the control. But the response was similar between the lower and higher doses used (Figure 3A). For fluazifop-p-butyl, at 7 DAA significant reduction was observed only with the higher dose of the herbicide. Doses lower than 400 g ha^{-1} resulted in values of the F_v/F_m ratio within the range considered ideal (Figure 3B). At 15 DAA, the forage species submitted to glyphosate, regardless of the dose used, resulted in a null F_v/F_m ratio due to the plants complete weakening (Figure 3C). Although variable, at 15 DAA both forage species submitted to fluazifop-p-butyl showed a 25% reduction in the $F_v/F_{m,}$ ratio comparing with the control. And no significant difference was observed between the lower and higher doses applied (Figure 3D). From these results it is possible to infer that both species were similarly affected by glyphosate and fluazifop-p-butyl in relation to the quantum yield of PS II. In the doses used, glyphosate was the most powerful herbicide in completely inhibiting PS II.

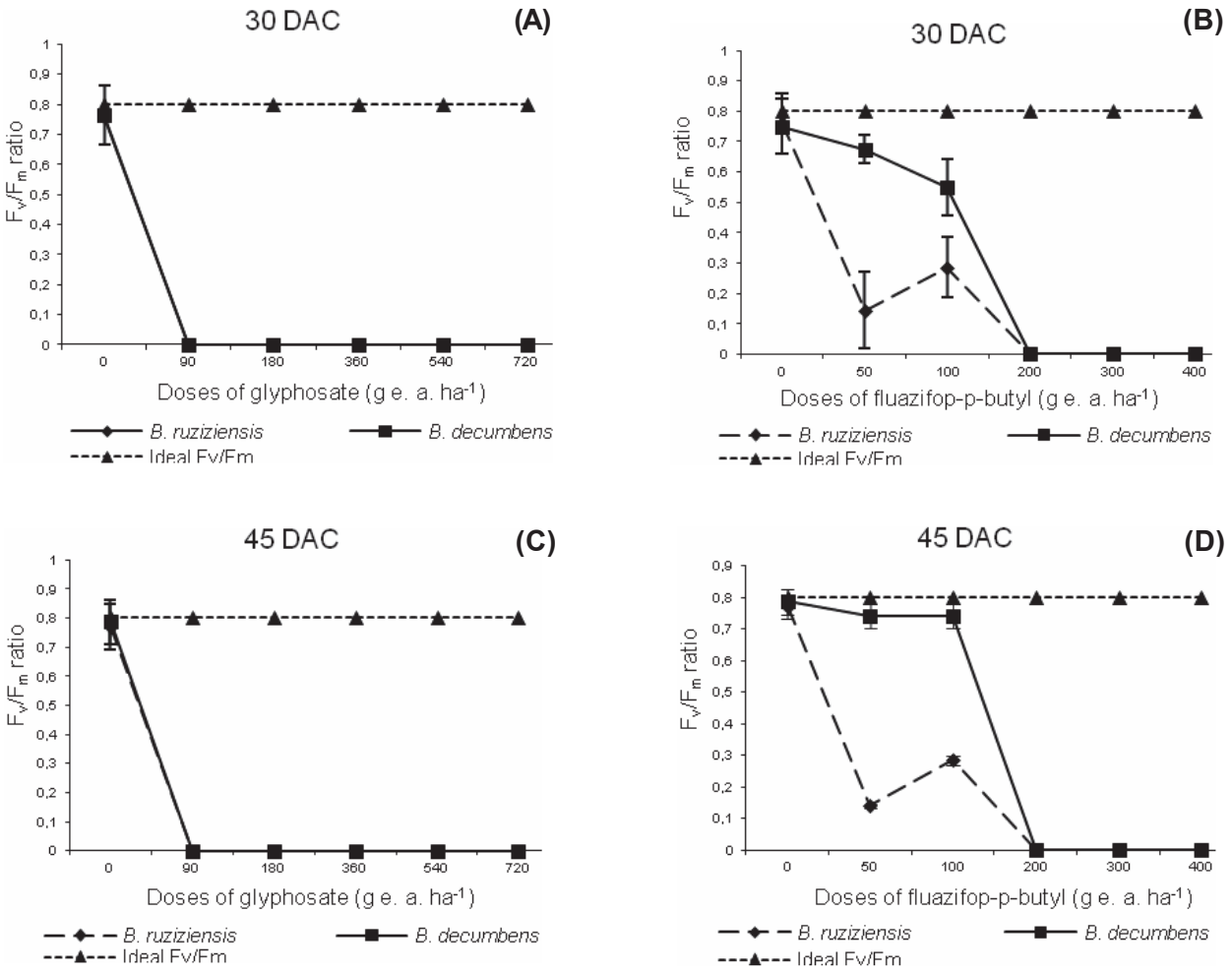

Figure 4 - Effect of herbicides on the variable fluorescence/maximum fluorescence ratio of chlorophyll (F_v/F_m) at 30 (charts A and B) and at 45 (charts C and D) days after cutting the plants (DAC) due to the doses applied.

It is important to highlight that at 30 DAA plant cutting was carried and both forage species, when submitted to doses higher than 200 g ha^{-1} of fluazifop-p-butyl and 90 g ha^{-1} of glyphosate did not show regrowth. Therefore the values were null for the F_v/F_m ratio (Figure 4). Higher sensitivity of *B. ruziziensis* submitted to doses of 50 and 100 g ha^{-1} of fluazifop-p-butyl was observed at 30 and 45 days after plant cutting (DAC) comparing with *B. decumbens*. For *B. ruziziensis* the response pattern was similar at 30 and 45 DAC. With the dose of 50 g ha^{-1} of fluazifop-p-butyl an 81% reduction was observed in the quantum yield of PS II. As for *B. decumbens*, it presented regeneration capacity of PS II, for at 30 DAC 13% and 31% reductions were observed for doses of 50 and 100 g ha^{-1} of fluazifop-p-butyl, respectively. However, at 45 DAC, *B. decumbens* showed a F_v/F_m ratio similar to the ratio considered ideal (Figures 4B and D). Photosynthetic capacity decreases in proportion to size and severity of the damage undergone by plants. However, depending on the type of damage, photosynthetic capacity may recover in a few hours or days or, on the contrary, result in tissue death (Bolhàr-Nordenkampf et al., 1989).

Taken as a whole, the results obtained in this study have shown that both species, *B. ruziziensis* and *B. decumbens*, are more sensitive to glyphosate than to fluazifop-p-butyl, where glyphosate caused plant death at the dose of 90 g ha^{-1}. Regarding fluazifop-p-butyl, *B. ruziziensis* proved to be more sensitive than *B. decumbens* and, at 45 DAC, *B. decumbens* submitted to doses of up to 100 g ha^{-1} could regenerate its photosynthetic apparatus.

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