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### MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES ON *Schizolobium parahyba* VAR. *Amazonicum* (HUBER EX DUCKE) BARNEBY PLANTS INTOXICATED BY GLYPHOSATE

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**ABSTRACT:** The objective of this study was to evaluate the morphological and physiological changes in paricá plants (*Schizolobium parahyba* var. *amazonicum*) intoxicated by glyphosate. The experiment was conducted in a protected environment using paricá plants during their planting stage, which were intoxicated with increasing doses of glyphosate: 0 (control); 43.2; 86.2; 129.6 and 172.8 g·ha<sup>-1</sup>. At 7 and 21 days after the application of the herbicide, the photosynthesis, transpiration, stomatal conductance and leaf temperature were measured. The visual intoxication degree and the growth of the shoot and the root of the plants were evaluated 21 days after the application. Paricá shows symptoms of visual intoxication characterized by chlorosis/winding, evolving to necrosis/abscission of the youngest leaflets. The growth of the stem and the roots of the intoxicated plants is preserved; however, an expressive leaf loss occurs, and paricá may have adaptation mechanisms to tolerate the action of the herbicide molecule. The photosynthesis decrease promoted by an indirect action of glyphosate represents the main reduction on the growth of plants. The decrease on the stomatal conductance, which was the most sensitive physiological variable to glyphosate, resulted in lower transpiration rates, which, consequently, caused increases on the leaf temperature.

### MODIFICAÇÕES MORFOLÓGICAS E FISIOLÓGICAS EM PLANTAS DE *Schizolobium parahyba* VAR. *amazonicum* (HUBER EX DUCKE) BARNEBY INTOXICADAS POR GLYPHOSATE

**RESUMO:** Objetivou-se avaliar as variações morfofisiológicas em plantas de paricá (*Schizolobium parahyba* var. *amazonicum*) intoxicadas por glyphosate. O experimento foi realizado em ambiente protegido utilizando plantas de paricá em idade de plantio, as quais foram intoxicadas com doses crescentes de glyphosate: 0 (testemunha); 43,2; 86,2; 129,6 e 172,8 g·ha<sup>-1</sup>. Aos 7 e 21 dias após a aplicação do herbicida, foram realizadas medições da fotossíntese líquida, transpiração, condutância estomática e temperatura da folha. O grau de intoxicação visual e crescimento da parte aérea e da raiz das plantas foram avaliados aos 21 dias após a aplicação. O paricá manifesta sintomas de intoxicação visual caracterizados por clorose/enrolamento e evolução para necrose/abscisão dos folíolos mais novos. O crescimento do caule e da raiz das plantas intoxicadas é conservado, contudo, há expressiva perda foliar, podendo o paricá possuir mecanismos de adaptação para tolerar a ação da molécula do herbicida. O decréscimo promovido na fotossíntese por ação indireta do glyphosate representa a principal causa da redução no crescimento das plantas. O decréscimo na condutância estomática, a qual demonstrou ser a variável fisiológica mais sensível ao glyphosate, resultou em menores taxas de transpiração, que, consequentemente, ocasionou elevações na temperatura da folha.

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## INTRODUCTION

In 2012, the Brazilian government presented the final version of the Low Carbon Agricultural Plan – ABC Plan, which, among other goals, established the commitment of expanding the planted forest areas to 9 million hectares until 2020 (MAPA/MDA, 2011). According to IBGE (2014), in 2014, the area with forest plantations had exceeded 9.3 million hectares, mostly occupied by eucalyptus and pine plantations. Among the native species of the Amazon, *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby (LEWIS, 2016), locally known as paricá, is currently one of the main species used for reforestation purposes, with over 89 thousand planted hectares (IBÁ, 2015).

The growing interest for paricá is due to its quick growth. It may reach a productivity of  $38 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$  (CARVALHO, 2007) even with no improvement degree; therefore, it is one of the few native species that may catch up with the productivity of eucalyptus. Another determining factor, according to Marques et al. (2006), is the increase on the demand for wood to produce laminates and plywood, a context in which paricá is inserted (ROSA, 2006a).

Although some silvicultural aspects related to the culture of paricá have already been relatively widely studied, there are still gaps of information, such as in relation to the management of weeds. Rosa (2006b) mentions the procedure used by the company Pampa, Vigia – PA, as an example of weed management in paricá plantations in the Amazon, which uses mechanical hoeing and chemical control of weeds with glyphosate. According to the monitoring data of the company, the elimination of weeds resulted in a reduction from 25 to 6% of paricá mortality during the first year in the field.

Therefore, weed control is necessary with the purpose of minimizing the potential interferences on the culture of paricá that may, somehow, reduce the productivity of the species. The use of herbicides is an attractive option, since it reduces costs and increases the operational yield (SILVA et al., 2007a).

It is important to point out that there are no herbicides to be used on paricá plantations registered at the Ministry of Agriculture, Livestock and Supply, nor there are studies that evaluate the effects of herbicides on the species. It is necessary to determine the levels and types of damages that may be caused to paricá in case of herbicide intoxication, or even whether selectivity to the applied product occurs.

Studies conducted with eucalyptus under the effect of glyphosate drift found significant reductions

on the plant development and physiological changes (TUFFI SANTOS et al., 2007b; MACHADO et al., 2010). In practice, according to Tuffi Santos et al. (2007a), the damages caused by the glyphosate drift, or by some other herbicide, distributed at different levels on the plantation, would increase the heterogeneity of the plot, since the intraspecific competition is favorable to the less affected individuals. The commercial plantations of paricá are still constituted by seminal plantings, therefore, the drift effect would be added to the genetic variability of the plants, making the heterogeneity of the population more accentuated.

Considering the implications of the drift on the development of the forest plantation, our objective was to evaluate the morphological and physiological changes on young paricá plants (*Schizolobium parahyba* var. *amazonicum*) intoxicated by glyphosate.

## MATERIAL AND METHODS

The study was conducted in a protected environment with 50% shading and lateral openings for air circulation. The three-month old paricá plantings were planted on pots with capacity for 7 L, containing a substrate constituted by soil and nutshells at a 2:1 proportion (v/v). After planting, the seedlings went through an acclimatization period of 20 days, during which two fertilizations were conducted with 10 g of NPK per pot at a 10-28-20 formulation (CARVALHO, 2005; LOPES et al., 2015).

At the end of the acclimatization period, a drift simulation was conducted, applying five treatments related to reduced doses of glyphosate: 0 (control); 43.2; 86.2; 129.6 and  $172.8 \text{ g} \cdot \text{ha}^{-1}$  of active ingredient on acid equivalent, according to the sub-doses used by Machado et al. (2010) on a study with eucalyptus. The herbicide was applied by a  $\text{CO}_2$  pressurized sprayer, constituted by a 1m-wide bar equipped with two TT11002 spraying nozzles, previously calibrated for a spray volume of  $200 \text{ L} \cdot \text{ha}^{-1}$ . The sprayed solution was directly applied on the plant leaves.

On the day of the application, the plants were distributed so that the treatments had equal height means, which was equivalent to a general mean of 41.34 cm. The experiment was established on random blocks, with five replications, and an experimental unit was constituted by one pot with one plant.

The physiological evaluations were conducted 7 and 21 days after the application of glyphosate, between 9a.m. and 12p.m., every day, using an infrared gas analyzer (model LI-6400XT from LI-COR). The physiological

parameters analyzed were photosynthesis (A), stomatal conductance ( $g_s$ ), transpiration (E) and leaf temperature ( $T_l$ ), at constant light ( $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and  $\text{CO}_2$  ( $400 \mu\text{mol}$ ) levels. For the readings, were selected leaves located on the intermediate region of the plants.

At 21 days after the application, the visual toxicity degree was verified according to grade scales from 0 to 100% of intoxication (FRANS, 1972), the stem base diameter and height were measured, and the root, stem and leaves of all plants were collected. After collecting the material, the leaf area was measured using a leaf area analyzer (model LAI 3100C). Finally, all of the collected material was subjected to the drying process on a force-air-circulation oven at  $50^\circ\text{C}$  for 72 hours, posteriorly weighting the dry mass of the root, stem and leaves.

The morphology and physiology data were subjected to the normality analysis by the Kolmogorov-Smirnov test at 5% of significance, homogeneity analysis through the Levene test at 5%, and homogenization of the treatments based on the standard deviation. A regression analysis was conducted with the use of models that showed an adequate adjustment. The comparisons with the control plants were conducted through the Dunnett test at 1 and 5% of significance.

## RESULTS AND DISCUSSION

The visual toxicity symptoms on paricá were clearly manifested on plants that received  $172.8 \text{ g}\cdot\text{ha}^{-1}$  of glyphosate. They were initially characterized by chlorosis and winding of the leaflets before evolving to necrosis and leaf abscission. The symptoms started to manifest between the fifth and the seventh day after application, always from the youngest leaflets to the oldest ones, from the top of the leaf blade toward the basis.

Since glyphosate is a molecule that is translocated through the phloem, a higher concentration of it and an effect on parts of the plant with greater carbohydrate discharge is common, such as on growth points and strong drains (HETHERINGTON et al., 1999; MONQUERO et al., 2004). Although no records of the effect of glyphosate on paricá were found, the symptoms observed on this study are similar to the ones observed on eucalyptus by Tuffi Santos et al. (2009), with the difference that the symptoms on paricá were more evident for the dose of  $172.8 \text{ g}\cdot\text{ha}^{-1}$  of glyphosate.

The visual intoxication observed 21 days after the application of glyphosate reached its maximum degree at a dose of  $172.8 \text{ g}\cdot\text{ha}^{-1}$  of a.i. on acid equivalent, causing 43% of leaf damages (Figure 1A). From the dose of  $86.2 \text{ g}\cdot\text{ha}^{-1}$ , significant damages occurred in relation to the control

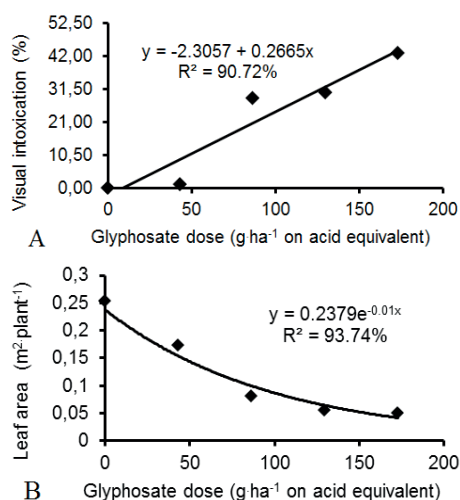
plant (Table 1). Although paricá has shown relatively reasonable resistance when intoxication symptoms are considered, it was observed that a reduction occurred on the leaf area from the dose of  $43.2 \text{ g}\cdot\text{ha}^{-1}$  of glyphosate in relation to the control. The mean leaf area of the plants sprayed with  $172.8 \text{ g}\cdot\text{ha}^{-1}$  was  $507.57 \text{ cm}^2$  (Table 1), which is equivalent to approximately 20% of the mean on the control (Figure 1B).

Evaluating the effect of glyphosate on different eucalyptus clones, Machado et al. (2010) verified, 21 days after the application, that the  $172.8 \text{ g}\cdot\text{ha}^{-1}$  dose of a.i. on acid equivalent caused from 70 to 86% of leaf damages. The same dose was tested on five eucalyptus species by Tuffi Santos et al. (2006), and a mean of 47.5% of intoxication 15 days after the application was observed; only *Eucalyptus resinifera* and *E. grandis* showed damages below 50%.

**TABLE 1** Morphological variables of *S. parahyba* var. *amazonicum* 21 days after the application of glyphosate.

Variables	Glyphosate dose ( $\text{g}\cdot\text{ha}^{-1}$ on acid equivalent)				
	0	43,2	86,2	129,6	172,8
Visual intoxication	0.001	1.33ns	28.71*	30.50**	43.00**
Leaf area	2,527.82	1,725.27*	806.64**	548.32**	507.57**
Stem Diameter	9.25	9.14ns	8.73ns	7.57*	8.22ns
Height	57.08	51.90ns	52.08ns	46.28**	48.58*
Leaf dry mass	10.82	8.32ns	3.94**	2.63**	2.33**
Stem dry mass	9.35	8.23ns	6.89ns	5.51*	6.39ns
Root dry mass	2.24	2.48ns	1.65ns	1.42ns	1.57ns
Total dry mass	22.42	19.03ns	12.48**	9.56**	10.29**

<sup>1</sup>Comparison with dose 0 (control) on the row: ns – non-significant; \* and \*\* significant difference according to the Dunnett test at 5 and 1%, respectively.



**FIGURE 1** Visual intoxication degree and leaf area of *S. parahyba* var. *amazonicum* 21 days after the application of glyphosate.

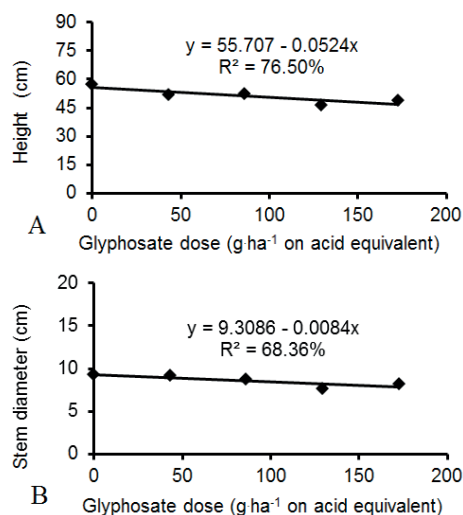
On a study conducted by Wagner Júnior et al. (2008) with passion fruit (*Passiflora edulis*), the intoxication was below 10% up to 28 days after the application of 172.8 g·ha<sup>-1</sup> of glyphosate, similarly to what Yamashita et al. (2006) observed on *Parkia multijuga* plants. The drift effect on *Jatropha curcas* evaluated on the study by Costa et al. (2009) reached 38.7% of intoxication 21 days after the application of 180 g·ha<sup>-1</sup>.

Costa et al. (2012) observed a more mild leaf loss tendency on *Eucalyptus grandis*, reaching a reduction of 57.6% with 120 g·ha<sup>-1</sup> of glyphosate applied only on the leaves. Magalhães et al. (2001a) and Magalhães et al. (2001b) did not observe a significant variation on the leaf area of maize and sorghum, respectively, using doses of up to 172.8 g·ha<sup>-1</sup> of glyphosate.

The leaf abscission observed must have occurred probably due to the increase on the ethylene production through an indirect action of glyphosate (FUCHS et al., 2002; GRAVENA, 2006). According to Yamada and Castro (2007), ethylene increases the cellulase activity, which makes the leaves more susceptible to abscission, in addition to promoting the loss of chlorophyll; therefore, it is one of the factors responsible for the chlorosis. Ethylene derives from the amino acid methionine (SCHALLER; KIEBER, 2002), which is, in turn, produced at a greater amount when the molecule of the herbicide inhibits the route that originates the aromatic amino acids phenylalanine, tryptophan and tyrosine (YAMADA; CASTRO, 2007).

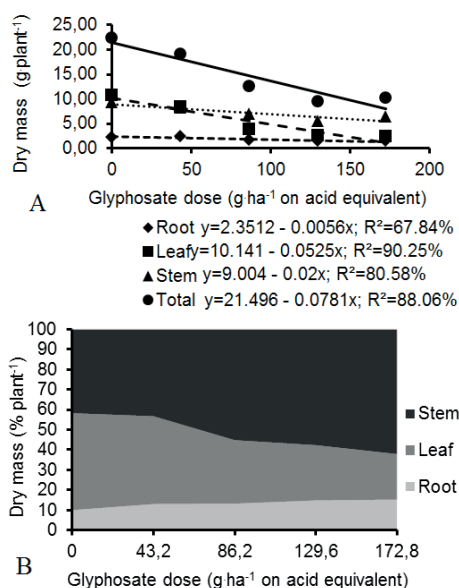
The height of the plants varied significantly from the dose of 129.6 g·ha<sup>-1</sup> (Table I), and a reduction occurred on approximately 15% with the dose of 172.8 g·ha<sup>-1</sup> in relation to the control (Figure 2A). There was a smaller variation on the diameter, and a decrease slightly above 11% occurred at the highest dose in comparison to the control (Figure 2B).

Using clones of hybrids of *Eucalyptus grandis* x *E. urophylla*, Santos Junior et al. (2005) evaluated the total height and diameter (measured at 1.3 m) 30 days after the application of 1,080 g·ha<sup>-1</sup> of glyphosate on 100% of the basal branches and observed reductions of 33.19 and 31.44%, respectively. Costa et al. (2012) verified a reduction of 37.5% on the stem diameter 28 days after the application of 120 g·ha<sup>-1</sup> of glyphosate. Tuffi Santos et al. (2006) did not observe variations on the height and diameter of *E. urophylla* 45 days after the application of up to 172.8 g·ha<sup>-1</sup> of glyphosate, although it has also been compared together with other molecules and combinations.



**FIGURE 2** Height and stem diameter of *S. parahyba* var. *amazonicum* 21 days after the application of glyphosate.

The total dry mass was reduced in approximately 55% with the application of 172.8 g·ha<sup>-1</sup> (Figure 3A), observing a significant variation from the 86.2 g·ha<sup>-1</sup> dose in relation to the control mean (Table I). Both the stem dry mass and the root dry mass suffered a reduction of approximately 30% on the most intoxicated plants when compared to the control plants. Although the decrease on the total dry mass was expressive, this is mostly due to the loss of leaf dry mass (Figure 3A and B), with a reduction of almost 80% upon the application of the highest dose. Only the mean of the lowest dose was equal to the control (Table I).



**FIGURE 3** Dry mass of the root, leaf, stem, total dry mass (A) and participation of assimilates on the total dry mass (B) of *S. parahyba* var. *amazonicum* 21 days after the application of glyphosate.



In order to reduce the dry mass of the root, leaves and stem of paricá in 50%, doses of 219.86; 90.10 and 216.35 g ha<sup>-1</sup>, respectively, are necessary, while the total dry mass is reduced with 131.73 g ha<sup>-1</sup>.

In opposition to the reduction on the leaf area and the leaf dry mass, the growth of the diameter and the root was relatively not much affected; this could be the effect of some self-preservation mechanism, such as the change on the translocation of carbohydrates in order to limit the action of the herbicide molecule (SILVA et al., 2007b).

According to Salgado et al. (2011), the dry mass of the leaves and stem of hybrids of *Eucalyptus grandis* x *E. urophylla* is reduced in half with the application of 143.3 to 277.4 g ha<sup>-1</sup> of glyphosate. In the case of the study conducted by Silva et al. (2015), the mass of the shoot components of *Eucalyptus* "urograndis" was reduced in 50% with doses of 72 g ha<sup>-1</sup> (stem) and 122 g ha<sup>-1</sup> (leaf) of glyphosate, while, for *Pinus taeda*, the same effect was promoted by doses of 120 g ha<sup>-1</sup> (stem) and 44 g ha<sup>-1</sup> (leaf) of glyphosate.

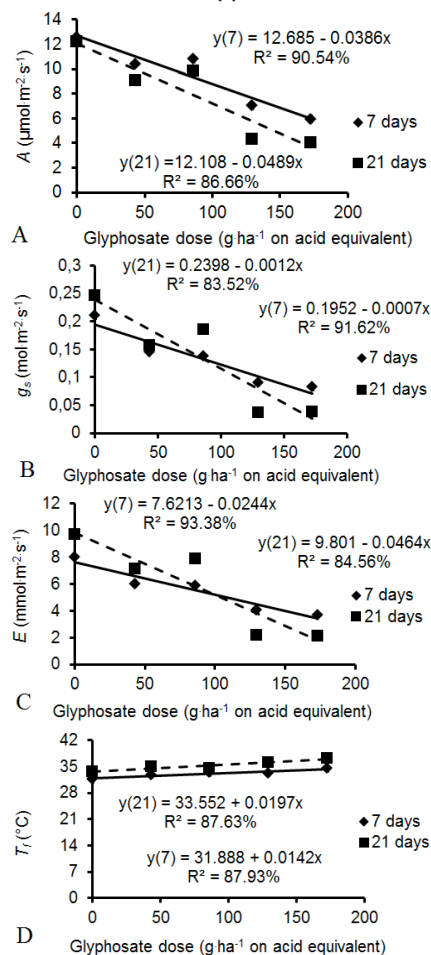
The growth inhibition due to the action of glyphosate, both on the shoot and on the root, occurs partially due to the reduction on the synthesis and the concentration of indoleacetic acid (IAA) (LEE; DUMAS, 1983; LEE; DUMAS, 1985), as well as due to the reduction on the translocation speed of the hormone (BAUR, 1979). Yamada and Castro (2007) explain that, by inhibiting the synthesis of phenolic compounds, glyphosate reduces the concentration of the IAA precursor (tryptophan) and increases the IAA-oxidase activity, which would be normally regulated by the phenolic compounds. With the increase on the activity of the IAA-oxidase enzyme, the metabolism of free IAA increases, especially on plants that are sensitive to the herbicide, as observed by Lee and Dumas (1985).

The effect of glyphosate on A for paricá led to a more accentuated reduction 21 days after the application (Figure 4A), and there was a significant difference in relation to the control from the 129.6 g ha<sup>-1</sup> dose (Table 2). Considering the data collected 21 days after the application, the dose of 124.7 g ha<sup>-1</sup> of active ingredient on acid equivalent of glyphosate is enough to cause a 50% reduction of A for paricá.

On a study with *Eucalyptus*, Machado et al. (2010) only observed a change on A 21 days after the intoxication by glyphosate. Carvalho et al. (2013) observed a decrease on the photosynthesis rate of coffee 15 days after the application of 60.8 g ha<sup>-1</sup> of glyphosate.

The reduction of A is related, among other factors, to an important secondary effect of glyphosate, which consists in deviating erythrose-4-phosphate, which would be used for the regeneration of ribulose biphosphate on the Calvin cycle, for the deregulated

route of shikimate, thus, reducing the carbon fixation capacity of the plant (YAMADA; CASTRO, 2007). Such phenomenon was shown by Servaites et al. (1987) on a study with common beat (*Beta vulgaris*), in which they observed a 50% reduction on the ribulose biphosphate activity 12 hours after the application of 17 mmol plant<sup>-1</sup>.



Air temperature: 32.39° C (7 days) and 33.87° C (21 days).

**FIGURE 4** Photosynthesis (A), stomatal conductance (gs), transpiration (E) and leaf temperature (Tf) of *S. parahyba* var. *amazonicum* 7 and 21 days after the application of glyphosate.

**TABLE 2** Physiological variables of *S. parahyba* var. *amazonicum* 7 and 21 days after the application of glyphosate.

Variables	Glyphosate dose (g ha <sup>-1</sup> on acid equivalent)				
	0	43,2	86,2	129,6	172,8
7 days after the application					
Photosynthesis	12.59 <sup>i</sup>	10.40ns	10.80ns	7.03ns	5.94*
Stomatal conductance	0.21	0.15ns	0.14ns	0.09*	0.08*
Transpiration	7.97	6.01ns	5.85ns	4.04*	3.68*
Leaf temperature	31.63	32.87ns	33.32ns	33.24ns	34.52*
21 days after the application					
Photosynthesis	12.200	9.071ns	9.821ns	4.302**	4.025**
Stomatal conductance	0.246	0.157ns	0.186ns	0.037**	0.039**
Transpiration	9.668	7.140ns	7.846ns	2.175**	2.124**
Leaf temperature	33.546	34.947ns	34.434ns	36.145**	37.206**

<sup>i</sup>Comparison with dose 0 (control) on the row: ns – non-significant; \* and \*\* significant difference according to the Dunnett test at 5 and 1%, respectively.

The  $g_s$  varied substantially from the 129.6 g ha<sup>-1</sup> dose of glyphosate (Table 2), both 7 and 21 days after the application, however, with a more accentuated difference between two higher doses and the other doses on the second evaluation. Among the analyzed physiological variables, the  $g_s$  response was the most expressive one due to the intoxication, reaching a reduction of almost 85% on the 172.8 g ha<sup>-1</sup> dose on the evaluation after 21 days (Figure 4B).

When working with common bean, Geiger et al. (1986) observed a considerable decrease on  $g_s$  approximately four hours after the application of 17 mmol plant<sup>-1</sup> of glyphosate with and without surfactant. Machado et al. (2010) reported an accentuated reduction on  $g_s$  of eucalyptus 21 days after the application of up to 172.8 g ha<sup>-1</sup> of glyphosate.

It is known that the stomatal opening is promoted by the blue light through a specific stimulation and through the activation of the photosynthesis process on the chloroplasts or guard cells (TAIZ; ZEIGER, 2002). Therefore, if glyphosate has a negative effect on the photosynthesis, then, the opening and  $g_s$  are partially reduced.

As a consequence of the reduction on  $g_s$ , there was a decrease on  $E$  for paricá as the glyphosate doses increased. On both evaluations, the 129.6 and 172.8 g ha<sup>-1</sup> doses differed from the control (Table 2), with a more expressive variation 21 days after the application (Figure 4C). As a response to the lower transpiration rate, an increase was observed on the  $T_f$  (Figure 4D) with a significant variation, in comparison to the control, at the dose of 172.8 g ha<sup>-1</sup> at 7 days, and from the 129.6 g ha<sup>-1</sup> at 21 days after the application (Table 2).

The rise on the  $T_f$  leads to an increase of the photorespiration and, concomitantly, to a decrease of the rubisco activity (ribulose-1,5-bisphosphate carboxylase/oxygenase) and of the quant yield of the photosynthesis on C<sub>3</sub> plants, which becomes lower than on C<sub>4</sub> plants from approximately 30° C on (TAIZ; ZEIGER, 2002). The increase on the photorespiration rate results on a linear decrease of the photosynthesis, that is, a lower production of photoassimilates.

## CONCLUSIONS

Morphological and physiological changes occur on paricá when it is intoxicated by glyphosate. Paricá is susceptible to glyphosate with intoxication symptoms from a dose of 172.8 g ha<sup>-1</sup> of active ingredient on acid equivalent. There are no relevant damages on the stem and root of intoxicated plants, however, severe leaf loss occurs. Increasing doses of glyphosate reduce the photosynthesis, stomatal conductance and transpiration. The leaf temperature rises as the glyphosate dose increases.

The application of 196.27 g ha<sup>-1</sup> of active ingredient on acid equivalent causes 50% of intoxication on paricá.

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