

Response of Eucalyptus (*Eucalyptus urograndis*) Plants at Different Doses of Glyphosate

Fernanda Campos Mastrotti Pereira¹, Mariluce Pascoalina Nepomuceno¹, Rodrigo Neto Pires¹, Mariana Casari Parreira¹ & Pedro Luis da Costa Aguiar Alves¹

¹ Universidade Estadual Paulista "Julio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias - Campus de Jaboticabal, Brazil

Correspondence: Fernanda Campos Mastrotti Pereira, Universidade Estadual Paulista "Julio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias-Campus de Jaboticabal, Brazil. E-mail: fernandamastrotti@hotmail.com

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Abstract

The aim of this study was to evaluate the response of eucalyptus plants submitted to the application of glyphosate increasing rates. The doses 0.0, 3.6, 7.2, 18, 36, 72 and 180 g ha⁻¹ of acid equivalent (ae) of glyphosate were applied on plants. At 7, 15, 22, 29, 36 and 42 days after application (DAA) was determined leaves relative chlorophyll content and quantum efficiency of photosystem II. At 22 and 42 DAA plant height and stem diameter were measured. At 42 DAA were determined the leaves dry mass, stem dry mass and foliar area. The height, diameter, leaves and stem dry masses and foliar area were converted to percentages, whereas the controls as 100%. These variables were fitted to the model described by Gauss equation. The data chlorophyll content and quantum efficiency of photosystem II were submitted to mean comparison, and when they differed, we performed the Tukey test at 5% probability. All the features presented here showed some hormetic effect promoted by reduced rates of glyphosate and the increments observed in these characteristics (when compared to control) ranged between 5.92 and 33.18% at 42 DAA. From average curve with height, diameter, leaves dry mass, stem dry mass and foliar area can be concluded that the increase averaged 12% on application of 16 g a.e. of glyphosate ha⁻¹. For leaves chlorophyll content significant differences were observed only at 29 DAA, while for quantum efficiency of photosystem II significant differences were found only at 7 DAA.

Keywords: chlorophyll, hormesis, photosystem II quantum efficiency, subdoses

1. Introduction

In Brazil, eucalyptus crop is rapidly expanding and each year the weighted average yield has increased, emerging as the main species for wood and cellulose production, also in the international market (ABRAF, 2011). Also according to this association were grown in 2010 about 4.7 million hectares, which produce 14.1 million tons of cellulose and 5.8 million tons of charcoal.

However, the interference exerted by weeds is one of the factors that can reduce the growth of this culture, compromising their productivity. Glyphosate is an herbicide widely used to weed control in eucalyptus, applied in full area for desiccation before planting, to control regrowth and to control weeds between plant rows. This herbicide acts on the enzyme 5-enolpyruvyl-shikimate-3-phosphate-synthase (EPSPs), blocking the aromatic amino acids synthesis and secondary amino acids (Jaworski, 1972). Inhibition of EPSPs also leads to the accumulation of shikimate in plant tissues, allowing the use of glyphosate as a sensitivity indicator.

However, the use of this herbicide in inadequate conditions can lead to drift, compromising weed control, leading to increase doses and, consequently, increasing spending and still can cause damage to non-target species and environment (Hemphill & Montgomery, 1981).

“Hormesis” (word derived from “hormaein”, which in Greek means “to excite”) was originally defined as a behavior in which a biological trait is stimulated by low doses of a compound and inhibited by high doses of the same compound (Bukowski & Lewis, 2000; Calabrese & Baldwin, 2001; Calabrese & Baldwin, 2002). This effect has been observed in bacteria, fungi, higher plants and animals (Calabrese, 2005).

Increments resulting from the effect of low doses of glyphosate have been observed in corn (Wagner, Kogan, & Parada, 2003), soybean (Godoy, 2007), coffee, pinus, citrus and *Commelina benghalensis* (Velini, Guerrini, Castro, Meschede, Trindade, Mori, Godoy, & Cataneo, 2006). These authors concluded that was positive effect on growth, increased biomass, shoot growth and root system of plants studied, as well as increases in absorption and accumulation of phosphorus in the leaves.

Moreover, according to the results observed by Maciel, Velini, Santos and Viana (2007) in *Ananas erectifolius*, the application of reduced rates of glyphosate did not cause stimulation or increase in vegetative growth of plants.

The aim of this study was to evaluate the response of eucalyptus plants submitted to the application of glyphosate rates.

2. Material and Methods

This work was conducted under semi-controlled conditions (no restriction of water and light) in 2011, from July to October. Pre-selected seedlings from a hybrid of *Eucalyptus grandis* W. Hill ex. Maiden com *E. urophylla* S.T. Blake (*E. urograndis*) were transplanted in plastic pots (19 x 25 x 14) cm, of height and diameters. This commercial material was chosen because it is widely grown in the state of São Paulo - Brazil, has a fast growth, tolerance to drought periods and good wood quality.

The pots were filled with soil from the surface of a clayey red Latosol with pH 6.8. To mineral nutrition of the seedlings were performed fortnightly covering fertilization using 300 kg ha⁻¹ of the formula 4-14-8 (NPK formulation).

The doses 0.0; 3.6; 7.2; 18; 36; 72 e 180 g ha⁻¹ of acid equivalent (a.e.) of glyphosate were applied on the plants one month after planting, seeking simulate the effect of a possible drift in the field during the applications of glyphosate for weed control.

Upon application the plants were, on average, height of 35 cm and diameter stem of 4.5 cm. The herbicide used was Scout® (720 g a.e. kg⁻¹ of glyphosate), applied by a CO₂ constant pressure backpack sprayer provided with spray nozzles model XR 11002 calibrated of 3 bar pressure. The spray volume applied was 200 L ha⁻¹.

The experimental design was a randomized block with four replications. Each experimental unit consisted of one plant per pot.

At 7, 15, 22, 29, 36 and 42 days after application (DAA) was determined the relative chlorophyll content in the leaves, using a portable chlorophyll SPAD-502 (Minolta Camera Co. Ltd.). The procedure was performed between 8 and 10 AM, in the fifth leaf fully developed, performing four readings and considering the value obtained from their averages. On the same dates the quantum efficiency of photosystem II (PS II) was evaluated (the ratio between variable fluorescence and maximum fluorescence of chlorophyll a), using a fluorometer Handy PEA Chlorophyll Fluorometer (Hansatech Instruments Ltd.). The leaves were pre-adapted to the darkness, with use of forceps for 30 minutes before determination.

At 22 and 42 DAA plant height (measured from soil level to last leaf enter) and stem diameter (measured at 10 cm of soil) were measured. Even at 42 DAA were determined the foliar area (Li Cor, modelo LI 3000A), the dry mass of leaves and stem, then dried at about 70°C during 7 days.

The height, diameter and plant dry masses were transformed to percentage, considering the control (plants exempted from the glyphosate application) to 100%. These variables were fitted to the model described by Gauss equation:

$$y = y_0 + (A/W * \text{sqrt}(PI/2)) * \exp(-2 * ((x - x_c)/w)^2)$$

The chlorophyll content and the quantum efficiency of photosystem II were submitted to means comparison, and when it differed, was performed the Tukey test at 5% of probability.

3. Results and Discussion

Although not evaluated the phytotoxicity promoted by simulated glyphosate drift, visual symptoms were observed in plants, which were characterized by yellowing of the tip leaves from the dose of 36 g a.e. ha⁻¹ of glyphosate. The symptoms are intensified as the applied dose increased to 72 and 180 g a.e. ha⁻¹ of glyphosate. The yellowing of leaves was followed by the death of the tip and loss of apical dominance to the dose 180 g a.e. ha⁻¹ of glyphosate.

The yellowing of leaf tissue on the application of this herbicide was also certified by Salgado, Alves, Kuva, Takahashi, Dias and Lemes (2011) in *E. urograndis* for doses from 158.6 g a.e. ha⁻¹ of glyphosate; Tuffi Santos, Ferreira, Ferreira, Duarte, Tiburcio, and Sanyos (2006) in *E. grandis*, *E. urophylla*, *E. saligna*, *E. pellita* e *E. resinifera* after application of doses higher than 86.4 g a.e. ha⁻¹ of glyphosate; Yamashita and Guimarães (2005) in

cotton, after application of 270 g a.e. ha⁻¹ of glyphosate and Magalhães et al. (2001a), in maize and sorghum, for doses about 172 g a.e. ha⁻¹ of glyphosate. According to Campbell, Evans and Reed (1976), this fact may be related with the chloroplasts degradation, very common phenomenon in plants treated with glyphosate.

Tuffi Santos et al. (2006) also showed the losses of glyphosate drift on the eucalyptus growth, reporting reductions in height stem diameter and plant dry mass at 45 days after herbicide application. The authors also warned of the need for care and use of technology for the application of herbicides in eucalyptus.

For plant height (Figure 1) was observed that the fit by Gauss model was significant, indicating that doses up to 123 and 94 g a.e. ha⁻¹ of glyphosate provided higher values than the controls (considered 100%) at 22 and 42 days after application (DAA).

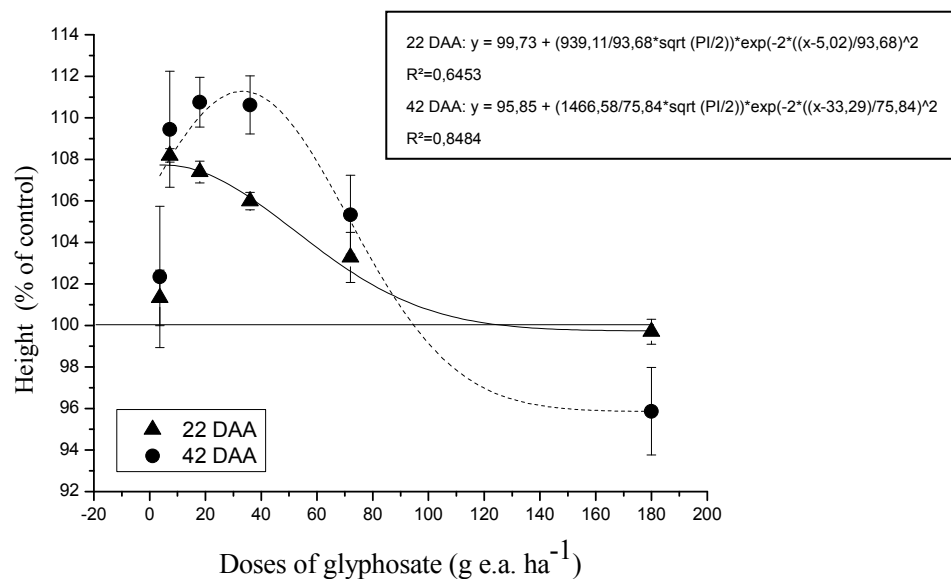


Figure 1. Height of eucalyptus plants subjected to glyphosate rates, expressed as a percentage, assessed at 22 and 42 days after application (straight line represent height at 22 DAA and dashed line represent height at 42 DAA)

At 22 DAA, this increase was up to 7.70% when compared to control. Already at 42 DAA, even for doses of 3.6 and 93.7 g a.e. ha⁻¹ of glyphosate, the increases in plant height reached up to 11%. Since doses from 130 and 100 g a.e. ha⁻¹ of glyphosate was responsible for the plants intoxication (Figure 1) at 22 and 42 DAA.

The stimulatory effect of low doses of many herbicides, including glyphosate, was observed and related (Cedergreen, Streibig, Kudsk, Mathiassen, & Duke, 2007; Velini et al., 2008; Wagner, Kogan, & Parada, 2003). Research studying this phenomenon caused by herbicides on crops and aquatic plants indicated that may pass increments up to 100% in the evaluated parameters. However, the observed average stimulus varies between 20 and 30% compared to the control (Cedergreen, Ritz and Streibig, 2005). Duke, Cedergreen, Velini and Belz (2006) related that, in general, the hormesis response was more pronounced in woody genera, as in *Eucalyptus* spp.

The coefficient of determination (R^2) ranged between 0.8484 and 0.9851, respectively, demonstrating good fit for height, diameter, leaf dry mass, stem dry mass and foliar area to the chosen model. There were exceptions to the height of the plants at 22 DAA, which showed R^2 of 0.6453, which reflects the closeness of height values observed for doses up to 20 g a.e. ha⁻¹ of glyphosate.

At 22 DAA were observed increases in stem diameter of the plants (Figure 2) varying between 1.15% and 4.20% for doses 3.6 and 18 g a.e. ha⁻¹ of glyphosate, respectively. In this evaluation, the maximum increment in stem diameter was 3.93%, found with application of 27.73 g a.e. ha⁻¹ of glyphosate. Even at 22 DAA, doses from 62.50 g a.e. ha⁻¹ of glyphosate decreased stem diameter, which was up 3.92% lower than observed in control with the application of 180 g a.e. ha⁻¹ of glyphosate.

At 22 DAA, doses up to 41.95 g a.e. ha⁻¹ of glyphosate led to increases in the diameter of eucalyptus plants, which exceeded the control by up to 5.92% with the application of 18 g a.e. ha⁻¹ of glyphosate (Figure 2). The dose of 21.40 g a.e. ha⁻¹ of glyphosate was responsible for the maximum diameter increase at 42 DAA. Doses of 41.95 g a.e. ha⁻¹ of glyphosate restrict diameter plants, which was about 2% smaller than control.

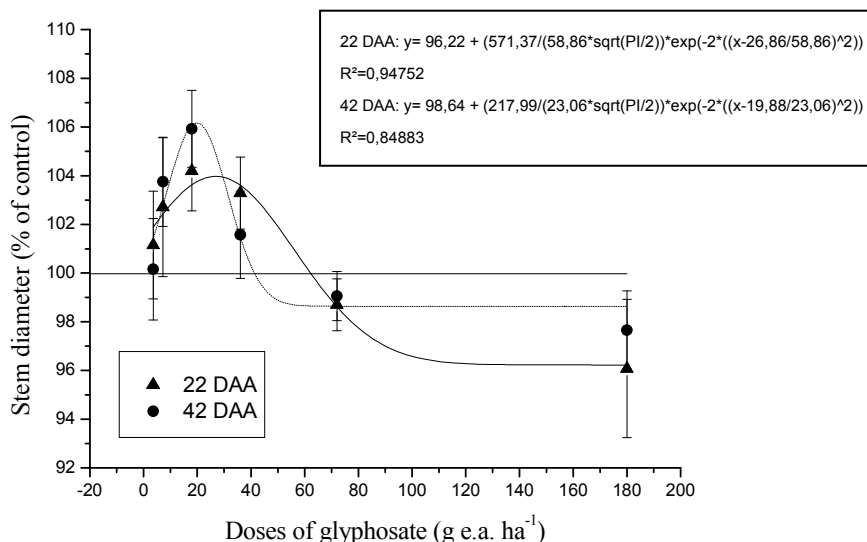


Figure 2. Stem diameter of eucalyptus plants subjected to glyphosate rates, expressed as a percentage, measured at 22 and 42 days after application (straight line represent stem diameter at 22 DAA and dashed line represent stem diameter at 42 DAA)

For stem dry mass, at 42 DAA, relative values greater than control were observed using doses between 4 and 39 g a.e. ha⁻¹ of glyphosate (figure 3). In this evaluation, the largest increase was 22.8% compared to control, with application of 19.8 g a.e. ha⁻¹ of glyphosate. The accumulation of stem dry mass is restricted by the application of doses higher than 39 g a.e. ha⁻¹ of glyphosate. For doses above 70 g a.e. ha⁻¹ of glyphosate, the restriction was 26.52% compared to control.

Even at 42 DAA, doses up to 50 g a.e. ha⁻¹ of glyphosate increased the leaves dry mass (Figure 3), and the maximum accumulation was 9%, observed to dose 15.60 g a.e. ha⁻¹ of glyphosate. Doses up to 50 g a.e. ha⁻¹ of glyphosate restrict the leaves dry mass, and the highest dose administered (180 g a.e. ha⁻¹ of glyphosate) caused reduction of 40% in leaves dry mass. According Salgado et al. (2011), doses of 158.6 and 195.1 g a.e. ha⁻¹ of glyphosate were sufficient to reduce 50% of stems and leaves dry mass accumulation.

For foliar area the highest value was observed in the application of 18.77 g a.e. ha⁻¹ of glyphosate, which exceeded the control in 33.18% (Figure 3). However, doses up to 55 g a.e. ha⁻¹ of glyphosate caused an increase in foliar area. Doses up to 56 g a.e. ha⁻¹ of glyphosate caused decreases in foliar area, and the maximum reduction in this variable was approximately 37%, observed in the application of 120g a.e. ha⁻¹ of glyphosate.

The average equation still allowed to infer that doses above 110 g a.e. ha⁻¹ of glyphosate reduced about 20% of the values of height, diameter, leaves dry mass, stem dry mass and foliar area.

From height, diameter, stem and leaves dry mass and foliar area (only at 42 DAA evaluation) data was made an average equation (Figure 4), which indicated that doses close to 16 g a.e. ha⁻¹ of glyphosate led to gains of up to 12% in the characteristics mentioned above, evaluated at 42 DAA. Doses above 58 g a.e. ha⁻¹ of glyphosate caused reductions in these characteristics, which showed lower relative values in comparison with control. The average equation still allowed inferring that doses above 110 g a.e. ha⁻¹ of glyphosate reduced about 2% of height, diameter, stem and leaves dry mass and leaf area values.

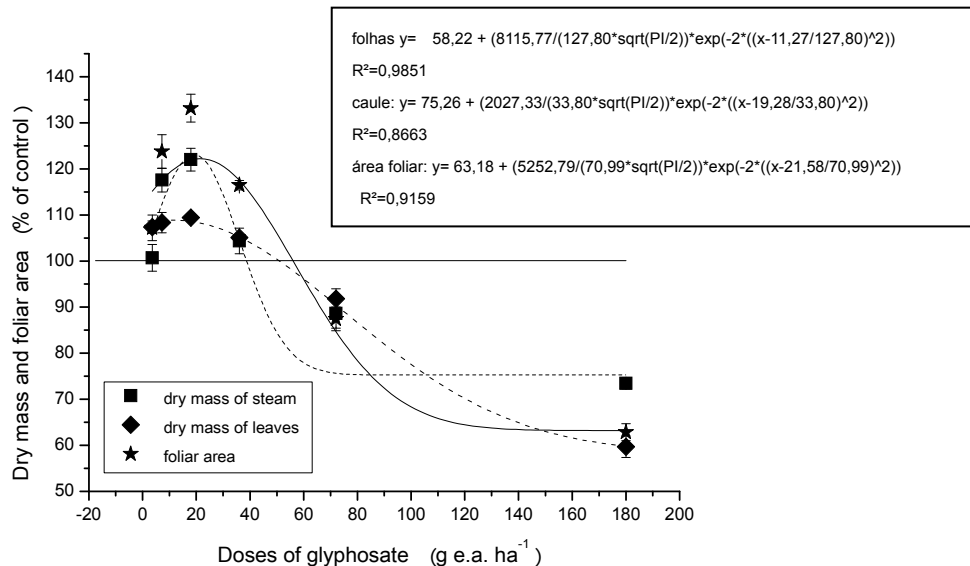


Figure 3. Leaves dry mass, stem dry mass and foliar area of eucalyptus plants subjected to glyphosate rates, expressed as a percentage, measured at 42 days after application (straight line represent foliar area, dashed line represent dry mass of stem and dashed dot line represent dry mass of leaves)

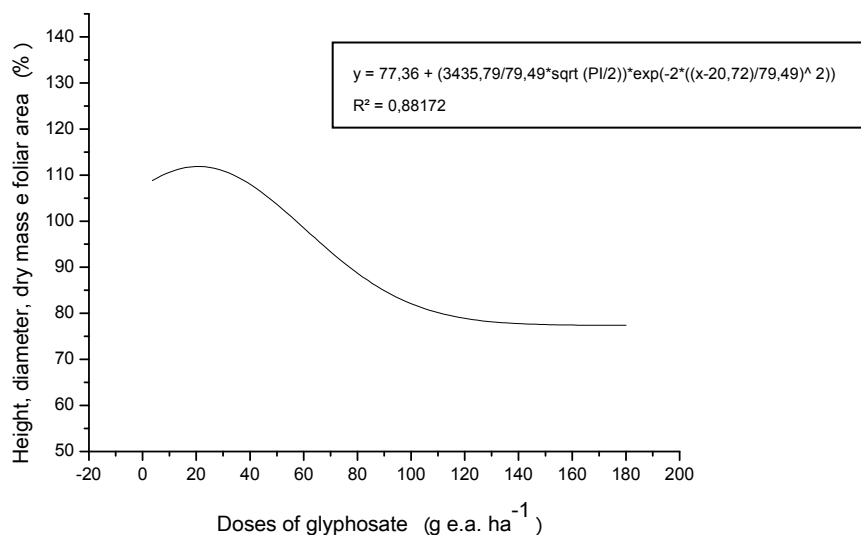


Figure 4. Average equation constructed from height, diameter, leaves dry mass, stem dry mass and foliar area data, measured at 42 DAA

The results of this study corroborate with those obtained by Velini et al. (2008) and Cedergreen (2008), who found hormesis to glyphosate rates below 36 g a.e. ha⁻¹ of glyphosate.

Velini et al. (2008) noted that when glyphosate was applied at doses between 1.8 and 36.0 g a.e. ha⁻¹ were stimuli from 50 to 100% increase in leaves, stem and root for *Commelina benghalensis*, *E. grandis* e *Pinnus caribea*. The authors also found hormetic effects on corn and soybeans. In this cases, the ground dry mass increases of 27.81% and 25.46% for doses of 14.2 and 22.6 g e.a. ha⁻¹ of glyphosate, respectively.

Cedergreen, Streibig, Kudsk, Mathiassen, and Duke (2007), observed 687 dose-response curves for organisms to herbicide sublethal (separated in three species of plants or algae and 10 types of herbicides) concluded that the treatment were between 25 and 76% higher than controls, depending on species.

Cedergreen (2008), evaluating the effect of glyphosate and seven others herbicides applied through the solution growth and spray in barley plants grown in hydroponics, concluded that glyphosate induced a real stimulation in dry mass accumulation on the application corresponding to doses of 5 and 10% of the recommended for field application. Also according to the author, the growth stimuli were relatively small (increase of 25% in plants dry mass) and very dependent on plant growth variations. The hormetic effect is related to the glyphosate's sites action, since this effect was not observed in plants resistant to this herbicide (Velini et al., 2008).

For leaves chlorophyll content (Table 1), significant differences were observed only at 29 DAA. The chlorophyll content was statistically lower than the levels observed in treatments 0, 3.6, 7.2 and 36 g a.e. ha⁻¹ of glyphosate, and superior to the others before the application of 180 g a.e. ha⁻¹ de glyphosate.

Possibly, the glyphosate affected the chlorophyll, since this molecule is part of same sites of action. In chloroplasts, glyphosate joins to the EPSP-S3P enzyme complex, inhibiting the essential amino acidssynthesis (Vidal, 1997). The glyphosate may also affect the secondary metabolites synthesis, due to blockage of shikimic acid pathway (Lydon & Duke, 1988).

The differences only at 29 DAA can be justified by some stress suffered by the plant, including considering the manifestation of a stress presented one month after glyphosate application. However, further studies need to confirm this result, as well as because of its occurrence.

Table 1. Total chlorophyll content in eucalyptus leaves in function of glyphosate rates applied

Glyphosate doses (g a.e. ha ⁻¹)	Evaluation time (days after application)					
	7	15	22	29	36	42
0	49,1A	40,4A	44,7A	42,1B	46,6A	42,0A
3,6	46,8A	42,3A	43,0A	38,9B	40,8A	39,5A
7,2	47,4A	43,1A	44,2A	41,9B	41,3A	40,4A
18	49,1A	42,8A	45,7A	44,3AB	42,2A	42,2A
36	47,7A	40,5A	44,5A	42,1B	42,1A	40,0A
72	54,6A	41,2A	48,4A	43,7AB	45,2A	44,8A
180	46,1A	45,3A	50,8A	55,4A	49,2A	46,0A
F	0,685ns	0,185ns	0,260ns	0,017*	0,221ns	0,346ns
VC (%)	7,34	5,80	9,35	11,55	10,71	10,08
SMD	10,05	6,00	11,29	12,41	11,55	10,63

Means followed by same letter in column do not differ significantly by Tukey test at 5% probability. * Significant at 5% probability. ** Significant at 1% probability. ns- nosignificant.

SMD-significant mean difference.

Evaluating the quantum efficiency of photosystem II, significant differences were found only at 7 DAA (Table 2). On this occasion, the lowest value (0.725) was observed at 18 g a.e. ha⁻¹ of glyphosate, and the highest value (0.806) at 180 g a.e. ha⁻¹ of glyphosate.

However, the observed values in all evaluations, regardless of glyphosate rate applied, ranging between 0.725 and 0.801. Such values are not indicative of stress, according to Maxwell and Johnson (2000). According to these authors, the value of quantum efficiency of photosystem II can vary, for most species and stress-free conditions, between 0.750 and 0.850. Only values below 0,750 indicate stress, resulting in a reduction in quantum efficiency of photosystem II, and consequently, in the photosynthetic plant potential.

The quantum efficiency of photosystem II indicates the efficiency of excitation energy capture by open reaction centers or may also indicate the existence of photo inhibitory effects (Correia & Alves, 2010).

Table 2. Quantum efficiency of photosystem II as a function of glyphosate rates applied

Glyphosate doses (g a.e. ha ⁻¹)	Evaluation time (days after application)					
	7	15	22	29	36	42
0	0,75A-D	0,758A	0,759 A	0,797A	0,755A	0,809A
3,6	0,780A-C	0,749A	0,797 A	0,768A	0,725A	0,801A
7,2	0,727CD	0,749A	0,778 A	0,758A	0,774A	0,781A
18	0,725D	0,763A	0,796 A	0,722A	0,704A	0,770A
36	0,745B-D	0,785A	0,791 A	0,754A	0,733A	0,791A
72	0,782AB	0,804A	0,772 A	0,775A	0,694A	0,796A
180	0,806A	0,645A	0,814 A	0,714A	0,766A	0,806A
F	0,000**	0,000**	0,279ns	0,731ns	0,688ns	0,294ns
VC (%)	2,96	3,67	3,59	9,09	10,66	2,94
SMD	0,06	0,09	0,06	0,16	0,19	0,05

Means followed by same letter in column do not differ significantly by Tukey test at 5% probability. * Significant at 5% probability. ** Significant at 1% probability. ns- no significant.

SMD - significant mean difference

For a long time, the hormetic effect has been observed in studies designed to investigate other problems, such as herbicides high doses effect (Streibig, 1980). Hormesis still confront major controversy within agronomy areas, environmental protection and medical toxicology (Calabrese, 2005; Thayer, Melnick, Burns, Davis, & Huff, 2005).

However, Calabrese and Baldwin (2001) stated that hormesis is the rule and not the exception, and that this phenomenon represents an evolutionary response to perturbations induced by the environment. Many herbicides were originally developed as plant growth regulators, thus proving the existence of hormesis. Glyphosate, for example, is the predecessor of a compound used as a growth regulator.

Currently, research have been conducted in order to study the hormetic effect in plants; however, few studies have investigated the mechanisms of growth stimulation, and when investigated, the results were inconclusive (Morre, 2000; Appleby, 2001), indicating the relevance of studies in the area.

Low doses of glyphosate are not recommended to stimulate the crops growth, since the hormetic effects depends on many factors, such as climate, variety and stage of plant development as well as the formulation of glyphosate applied (Velini, Trindade, Barberis, & Duke, 2010).

4. Conclusion

The plants intoxication visual symptoms were observed and characterized by yellowing leaves from the dose of 36 g ha⁻¹ glyphosate. The symptoms are intensified as the applied dose increased to 72 and 180 g ha⁻¹ glyphosate. All the features presented here showed some hermetic effect promoted by reduced rates of glyphosate and the increments observed in these characteristics (when compared to control ranged between 5.92 and 33.18% at 42 DAA. From average curve with height, diameter, leaves dry mass, stem dry mass and foliar area can be concluded that the increase averaged 12% on application of 16 g a.e. of glyphosate ha⁻¹. For leaves chlorophyll content significant differences were observed only at 29 DAA, while for quantum efficiency of photosystem II significant differences were found only at 7 DAA.

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