

Photosynthetic Performance of Guaranazeiro Plants as Affected by Glyphosate Application

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Abstract

The unsuitable use of herbicides damages many cultures. In cases of high infestations and presence of aggressive weed species in guarana (*Paullinia cupana*) culture, glyphosate application is advisable, but its impact on guarana physiology is unknown. Therefore, leaf photosynthetic characteristics were measured with the aim of identifying if the photosynthetic performance of guaranazeiro plants is affected in response to glyphosate application. Three glyphosate doses (0 (control); 324 and 432 g a.i. ha⁻¹) were applied to two guaranazeiro cultivars (BRS-Andirá and BRS-Maués) selected on the basis of productive performance. An analysis was made of the effects of these doses on characteristics that represent the photosynthetic process: gas exchange, maximum quantum efficiency of PSII, performance index and chlorophyll content. The application of glyphosate did not affect the short-term responses relative chlorophyll content (SPAD index) and light use (chlorophyll *a* fluorescence). After 168 h, there were changes only in gas exchange variables. The effects of glyphosate doses on gas exchange was different between guaranazeiro cultivars. The photosynthetic performance of the guaranazeiro seems to be tolerant to the effects of short-term of glyphosate application.

Keywords: herbicide, photosynthesis, fluorescence, chlorophyll, *Paullinia cupana*

1. Introduction

Guaranazeiro is a very important crop for Brazil, which is the only commercial producer of guarana. Production is mostly concentrated in the States of Bahia and Amazonas, and the produce is mostly absorbed by the beverage industry in these regions (Companhia Nacional de Abastecimento [CONAB], 2019). In addition to their energetic properties, guaranazeiro seeds have more than 80 pharmacological properties that are beneficial to human well-being. For this reason, they are interesting for the pharmaceutical industry (Marques, Ferreira, Paula, Kleinc, & Mello, 2019). Over time, the culture has been undergoing a modernization process in its production system, as well as species improvement and phytosanitary management to increase production per hectare (Tricaudi, Piton, & Pereira, 2016). Among phytosanitary factors, weed control has great relevance in the production of guarana. The research of Soares, Albertino, Souza, Santos, and Silva (2019) found that in areas without weed control, production may be reduced by up to 50%. Although some studies have found evidence that the use of herbicides is efficient and cheap for weed control in guaranazeiro crops, there are no registered products in Brazil (Fontes & Nascimento Filho, 2007). For guaranazeiro (*Paullinia cupana*) crops, glyphosate is recommended against high infestations and presence of aggressive weed species, as it is an efficient method for this purpose (Pereira, 2005).

Herbicides are efficient in weed control; consequently, they increase crop productivity, but these products can affect the physiology and consequently the growth of non-target organisms through drift (Silva et al., 2016). The use of non-selective herbicides requires best practices to avoid unwanted deposition in the culture (Santos, Faria, Barros, Reis, & Tuffi-Santos, 2016). The use of non-selective herbicides, such as glyphosate, is increasing and required in crops, because it is easy to use and has efficient action (Moraes & Rossi, 2010). Glyphosate is one of

the most widely used post-emerging herbicides in the world (Companhia de Tecnologia Ambiental do Estado de São Paulo [CETESB], 2018). When deposited in plants, it is absorbed quickly, translocated by the phloem to all plants and accumulated in growth areas. Its effect, however, depends on factors such as species, age, environmental conditions, application mode (Yamada & Castro, 2007) and dose and frequency of application, which must be taken into account to avoid damage, even in tolerant cultivars (Albrecht et al., 2018). Glyphosate acts on the enzyme EPSPS synthase, directly affecting the synthesis of aromatic amino acids. However, indirect effects need to be considered. Stress caused by the herbicide glyphosate increases the production of reactive oxygen species, and these compounds affect photosynthetic processes (Gomes et al., 2014).

The improper use of glyphosate can alter numerous physiological functions of non-target plants directly and indirectly. Mechanisms such as gas exchange (Concenço et al., 2014), quantum performance functions of photosystem II and chlorophyll content (Correa & Alves, 2010) can be affected. These changes have been confirmed by several research studies on glyphosate drift (Torres et al., 2012; Ferreira et al., 2015; Silva et al., 2016). The use of low doses of simulated glyphosate drift has been widely researched in order to provide further insights into their effects on the sensitivity of crops (Langaro, Nohatto, Perboni, Tarauco & Agostinetto, 2014).

Although the use of the herbicide glyphosate occurs in big cultivation areas, there is limited knowledge about the effects of low doses of glyphosate on guaranazeiro. Thus, the objective of the present research was to investigate if the photosynthetic performance of guaranazeiro plants is affected in response to the application of glyphosate.

2. Method

2.1 Plant Material and Growing Conditions

The guaraná (*P. cupana*) seeds of BRS-Andirá and BRS-Maués cultivars were obtained from the Jayoro farm, located in the municipality of Presidente Figueiredo, (01°96'04" S and 60°14'37" W) 120 km from the city of Manaus, in the State of Amazonas. One-year old seedlings were transferred to 3 kg pots, filled with substrate, whose composition and fertilization followed the method described by Pereira (2005). The experiment was conducted in a nursery, with irradiation reduced by 50% and intermittent mist fogging, controlled by evaporation equilibrium to avoid tissue dehydration. Seedling irrigation was performed on alternate days, with 50 ml of water per pot, on average, based on field capacity. The seedlings that were treated and evaluated were selected and standardized to avoid interference.

2.2 Treatments and Experimental Design

The treatments consisted of the application of three doses of the herbicide glyphosate (0 (control); 324 and 432 g a.i. ha⁻¹) directly on two cultivars of guaranazeiro (BRS-Maués and BRS-Andirá) and observation at three times after application (0, 48h and 168h). The experiment was conducted in a completely randomized design with a 3 x 2 factorial scheme (doses and cultivars) at three times. The experimental unit consisted of nine plants, with four replicates.

2.3 Herbicide and Application Method

We used a solution of glyphosate at 0 (control); 324 and 432 g a.i. ha⁻¹ (0; 22.5 and 30% of active ingredient Original Roundup, 360L⁻¹ g a.i. SC, Monsanto Company®, USA) recommended for cleaning 1ha of tillage. The herbicide was applied using a backup sprayer with a 110.02 fan nozzle at 147.1 kPa pressure (YAMAHO FT-16), in the morning. The group of seedlings from each treatment was placed in separate locations for the application of the herbicide, and for four hour's time, the seedlings did not receive nebulization and were kept separate. This measure was taken to avoid the possible influence between the doses as well as interference from nebulization in the effect of the herbicide. After this period, the seedlings returned to the countertops for evaluation.

2.4 Guaranazeiro Cultivars

The cultivars BRS-Maués and BRS-Andirá were selected for their productive and agronomic characteristics. They both have high yield per area and high caffeine content; also, they are tolerant to major diseases, and well adapted to the production areas (Nascimento Filho et al., 1999; Nascimento Filho, Atroch, Pereira, & Araújo, 2007). The BRS-Maués cultivar is used in almost all planting areas in the State of Amazonas and BRS-Andirá is well accepted among producers.

2.5 Leaf Photosynthesis-Related Measurements

All experiments were evaluated at 0, 48 and 168 h after herbicide application. The measurements were made during the morning (from 08:00 to 10:00 a.m.), on the second pair of fully expanded leaves with good sanitary aspect.

The gas exchange variables measured were: net photosynthesis, transpiration, stomatal conductance and dark respiration. The measurements were performed with an infrared gas analyzer (LI-6400, LiCor, Lincoln, NE, USA) adjusted to work with $400 \mu\text{mol s}^{-1}$ flux, and leaf chamber for CO_2 concentration, temperature and H_2O steam around $380 \pm 4 \mu\text{mol mol}^{-1}$, $31 \pm 1 \text{ }^\circ\text{C}$ and $21 \pm 1 \text{ mmol mol}^{-1}$, respectively, with a saturating light pulse of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, with specific measures, on one leaf per plant.

For the measurements of chlorophyll *a* fluorescence, we submitted two leaves of each plant to dark adaptation for 30 minutes, using appropriate clips. Chlorophyll *a* fluorescence was recorded with a portable fluorometer (PEA, MK2-9600-Hansatech, Norfolk, UK), with $3000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and wavelength 650 nm for 1 s, with evaluation of maximum quantum efficiency of PSII (*Fv/Fm*) and performance index (PI abs).

Chlorophyll content was estimated by the SPAD Index with a portable chlorophyll meter (model SPAD-502 Plus), on two leaves per plant.

2.6 Statistical Analysis

All data were submitted to the variance test and the Skott-knot test with 5% probability ($p < 0.05$). The tests were performed for multiple comparison of means and submitted to the Shapiro-Wilk normality using Assisat software (Version 1.0, Brazil).

3. Results

The action of glyphosate doses over guaranazeiro cultivars did not have any significant effect on the chlorophyll content variable, whose averages ranged between 35 and 54 (Figure 1).

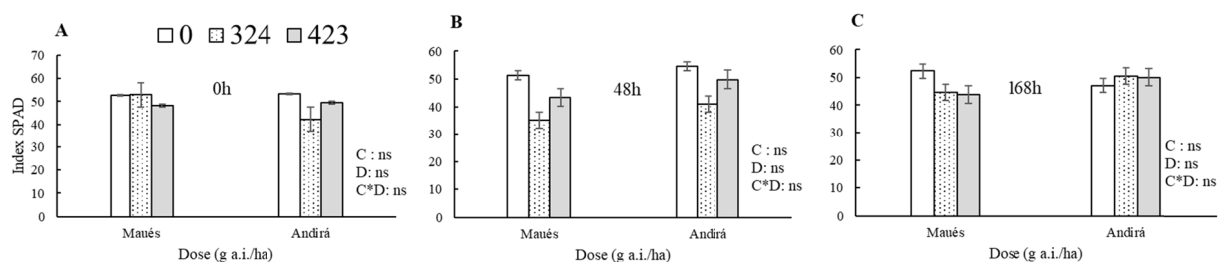


Figure 1. Chlorophyll content values in two guaranazeiro crops, submitted to three herbicide doses and its interaction, in three periods. The values without any letter are significantly equal at $P < 0.05$. C: cultivar; D: dose; C*D: interaction between cultivar and dose

There was no interaction between the doses and the cultivars for the chlorophyll *a* fluorescence variable. The average values of maximum quantum efficiency of PSII (*Fv/Fm*) ranged between 0.760 and 0.830, regardless of doses and evaluation times (Figures 2A, 2B and 2C). The average performance index (PI abs) values ranged between 5.44 and 12.16 (Figures 2D, 2E and 2F).

At 168 h, performance of BRS-Maués was reduced as a result of the doses applied (Figure 2F).

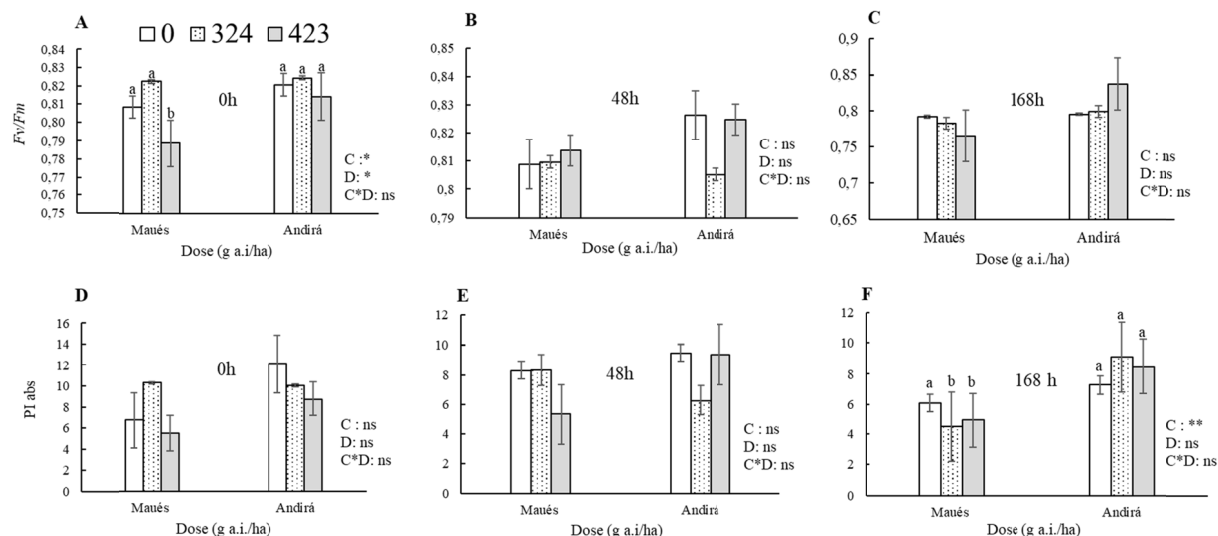


Figure 2. Maximum quantum efficiency of PSII (F_v/F_m) and performance index (PI abs) in two guaranazeiro crops, submitted to three glyphosate doses and their interaction, in three periods. According to the Skott-Knott Test, C, cultivar; D dose; C*D, interaction between cultivar and dose; distinct letters are significantly different at $P < 0.05$ (*) or $P < 0.01$ (**)

Just 168 h after glyphosate application, there was an interaction between doses and cultivars. In this period, all of the investigated variables were changed. The dose of 423 g a.i. ha^{-1} did not affect gas exchanges of guaranazeiro cultivars in any period. However, the dose of 324 g a.i. ha^{-1} promoted variable increment for BRS-Maués and reduction for BRS-Andirá (Figures 3C; 3F; 3I and 3L).

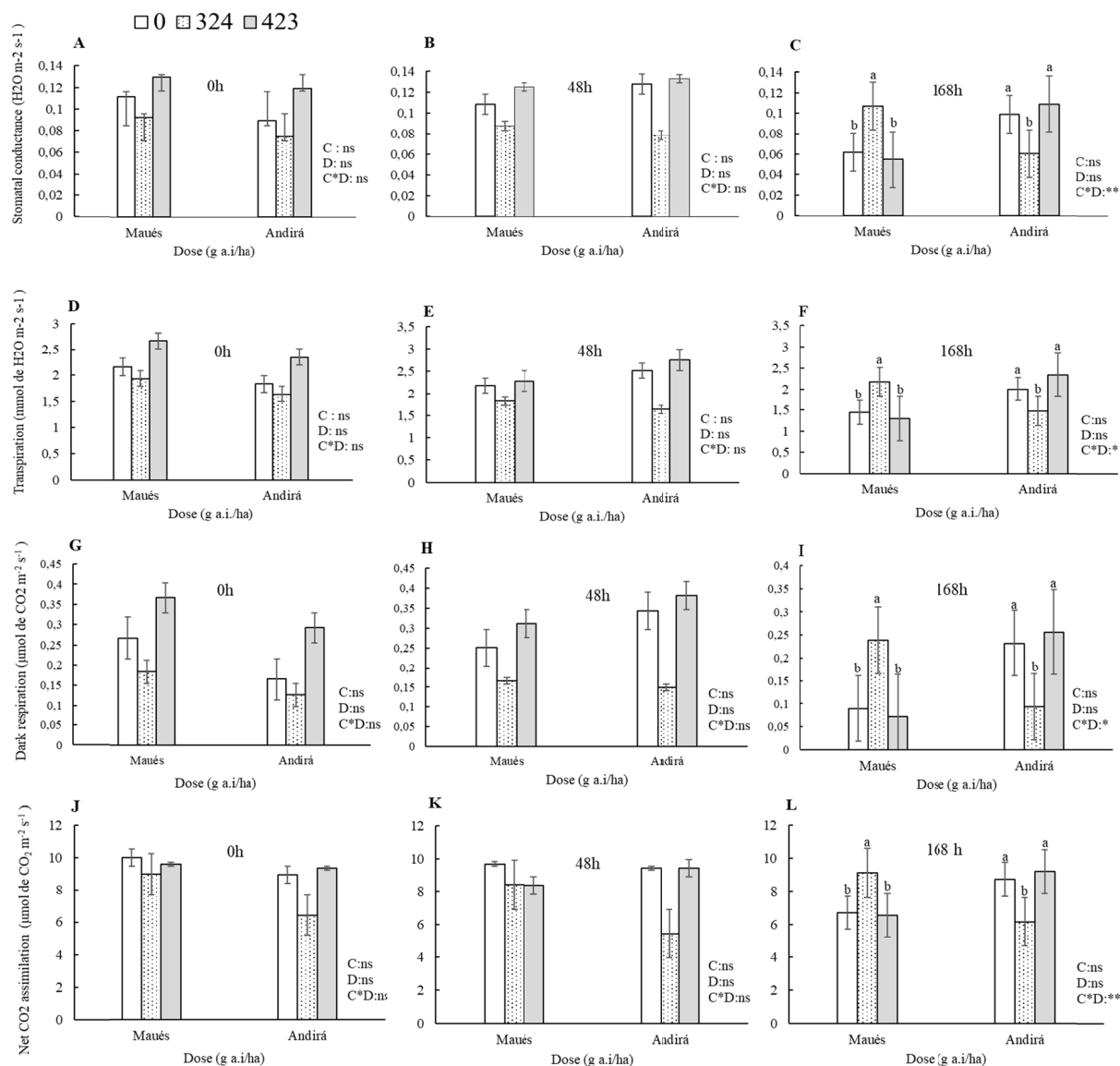


Figure 3. Values of stomatal conductance, transpiration, dark respiration and liquid assimilation of CO₂ in two guaranazeiro cultivars submitted to three glyphosate doses and their interaction, in three periods. According to the Skott-Knott Test, C, cultivar; D dose; C*D, interaction between cultivar and dose; distinct letters are significantly different at P < 0.05(*) or < 0.01 (**)

4. Discussion

In this study, the low doses of glyphosate applied to guaranazeiro seedlings altered gas exchanges without affecting the parameters chlorophyll *a* fluorescence and chlorophyll content. There was a difference between the doses applied and the crops. Herbicidal action depends on many factors, such as age, species, environmental conditions and doses applied (Torres et al., 2012). Olesen and Cedergreen (2010) found that the action of glyphosate on *Hordeum vulgare* affected gas exchanges, without affecting chlorophyll *a* fluorescence; therefore, glyphosate-induced chemical stress breaks the interconnection between CO₂ assimilation and electron transport, similarly to the effects of abiotic stresses, e.g., the cold. It is likely that the guaranazeiro seedlings also disassociated this interconnection, which indicates tolerance levels of the species to glyphosate, because the action of low glyphosate doses alters maximum quantum efficiency of PSII (F_v/F_m) in susceptible species (Campos & Ronchi, 2015).

Importantly, the crops of this research showed F_v/F_m values that can be considered as normal according to the studies of Björkman and Demmig (1987), with 44 species, with diverse ways of life and growths; of Li, Niu,

Jiang, and Liu (2004) with 99 species, and also of Gonçalves, Silva, Guimarães & Bernardes (2010) with species from the Amazon region, in natural conditions. Another important variable that detects stress is PI abs, which is even more sensitive than the *Fv/Fm* ratio (Christen, Schönmann, Jermini, Strasser, & Défago, 2007). In our research, we found that glyphosate doses reduced PI abs only for the BRS-Maués cultivar.

The low doses of glyphosate may have unfavorable effects on gas exchanges of plant species. In a study with *Arachis hypogea*, even in low doses, glyphosate negatively affected gas exchanges for the species; owing to oxidative stress, there was an increase in antioxidant activity in the leaves, as a defense against glyphosate toxicity (Radwan & Faye, 2016). In *Glycine max* cultivars, with higher doses than those established in this study, glyphosate severely affected the gas exchange of species, especially in the initial stages of crop establishment, caused by the reduction in the capture and use of light energy (Zobiolo, Kremer, Oliveira Junior, & Constantin, 2010). However, these processes were not affected in our research.

The change in gas exchange in plants is also subject to the characteristics of each genotype; a study with *Eucalyptus × urograndis* found that the changes caused by glyphosate were partially due to the differential accumulation of shikimic acid, rather than to the morphological, anatomical and defense characteristics of the species (Carvalho, Duke, & Alves, 2018). However, a study with guaranazeiro showed that the differences in morphology and physiology of cultivars contribute to the photosynthetic performance of the species and their adaptability to adverse environmental conditions (Gonçalves et al., 2006). The morphological characteristics of each genotype of the same species alter the absorption of glyphosate by the plant, as in the study with *Eucalyptus grandis*, favoring the differential tolerance between the genotypes (Machado et al., 2009).

Despite the negative effects on gas exchange, low doses of glyphosate can also promote an increase. Carvalho, Alves, Silvano, and Prado (2012) found a stimulating effect of the application of glyphosate in young plants of *Coffea arabica*, although contradictory with the findings of other studies. The authors suggest that the processes behind this effect may be related to molecular characteristics, partial EPSPS inhibition or the use of CO₂ however, they concluded that the growth phase of the species is crucial for this result. Vercamp et al. (2016) suggested that the energy generated through photosynthesis in young plants is directed, in part, to defense mechanisms, helping to overcome chemical stress. As mentioned above, this oxidative increase in plants can promote changes in gas exchange and may be related mainly to the young stage of the species. Low doses of glyphosate also stimulated gas exchange in plants of *Hordeum vulgare*, after seven days of treatment, owing to the increased efficiency of CO₂ fixation. The authors concluded that the effect of glyphosate induces changes in the enzyme invertase, which may partially explain this stimulus (Cedergreen & Olesen, 2010).

Nascentes et al. (2017), explain that low doses of glyphosate can promote hormetic effects in plants, depending on the study species, the dose of the herbicide and the stage of plant development. In studies with *Saccharum officinarum* and *E. grandis*, sublethal doses promoted biomass gain and plant growth, consequently increasing photosynthesis. In *Phaseolus vulgaris* plants, the hormonal effect of low doses of glyphosate was dependent on the cultivar and the dose; of the three evaluated cultivars, one suffered significant yield losses, while the other had increased yield in the highest underdoses (Silva, Arf, Gerlach, Kuryiama, & Rodrigues, 2012).

There are countless processes that may account for the tolerance or intolerance of species to low doses of glyphosate, especially in terms of their effects on photosynthetic processes. Further research still needs to be carried out to clarify each of the mechanisms involved in the action of glyphosate, as the genetic characteristics of each species have their specificities, especially species from tropical environments, such as guaranazeiro. Another factor that needs to be taken into account is medium- and long-term responses, as they show other mechanisms and processes of adaptation to this stress.

5. Conclusion

The short-term responses relative to the photosynthetic performance of plants indicate the tolerance of cultivars BRS-Maués and BRS-Andirá to glyphosate application. However, we suggest conducting more comprehensive research with more doses for application to guaranazeiro.

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