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Original Paper

Physiological and anatomical responses of *Eugenia dysenterica* to glyphosate

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Abstract

Brazil is among the countries that most use pesticides in the world. These chemicals cause undesirable changes in ecosystems, particularly the contamination of non-target native forest species through drift. The nuisances caused by pesticides go beyond environmental damage and include public health problems. The objective of this work was to evaluate the effects of glyphosate on leaf gas exchange, photosynthetic pigments and morphoanatomy of seedlings of *Eugenia dysenterica*. The visual toxicity, physiological and morphoanatomical characteristics of *E. dysenterica*, when exposed to concentrations of 0, 550, 1110 and 2220 g a.e. ha^{-1} of glyphosate, were analyzed. The results indicate that the herbicide caused toxicity in the leaves in all treatments. Reductions in photosynthesis (*A*), stomatal conductance (*gs*), and transpiration (*E*) at 47 DAA, were also identified. Glyphosate caused damage to the anatomical structures of *E. dysenterica* leaves. From the data analyzed it is possible to affirm that plants of *E. dysenterica* are sensitive to the action of glyphosate. Visible symptoms such as chlorosis and necrosis in the leaf edge are indicators that can be used by rural communities as a warning of the risk of contamination.

Key words: biomonitoring, *Cagaita*, Cerrado, toxicity.

Resumo

O Brasil está entre os países que mais utiliza agrotóxicos no mundo. Essas substâncias químicas provocam modificações indesejáveis nos ecossistemas, destacando a contaminação de espécies florestais nativas não alvo, através da deriva. Os transtornos causados pelos agrotóxicos extrapolam os danos ambientais, sendo considerados problema de saúde pública. O objetivo deste trabalho foi avaliar os efeitos do glifosato nas trocas gasosas foliares, pigmentos fotossintéticos e morfoanatomia de plântulas de *Eugenia dysenterica*. Foram analisadas a toxicidade visual, as características fisiológicas e morfoanatômicas de *E. dysenterica*, expostas a concentrações de 0, 550, 1110 e 2220 g a.e. ha^{-1} do glifosato. Os resultados apontam que o herbicida causou toxicidade nas folhas em todos os tratamentos. Redução da fotossíntese (*A*), condutância estomática (*gs*) e da transpiração (*E*) aos 47 DAA, também foram identificados. O glifosato causou danos às estruturas anatômicas das folhas de *E. dysenterica*. Diante dos dados analisados é possível afirmar que plantas de *E. dysenterica* são sensíveis a ação do glifosato. Sintomas visíveis como clorose e necrose na borda foliar são indicadores que podem ser utilizados pelas comunidades rurais como alerta do risco de contaminação.

Palavras-chave: biomonitoramento; Cagaita; Cerrado; toxicidade.

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Introduction

Brazil is among the countries that most use pesticides in the world. It is estimated that 19% of the world production is consumed in the country and of these, 60% are herbicides (Salomão *et al*. 2020). The high consumption of these products is related to the expansion of the agricultural frontier, where native vegetation is suppressed to make way for large monocultures. The expansion of agribusiness and the standardization of crops, besides requiring extensive areas, makes the plantation more vulnerable to the appearance of pests and consequently, creating a greater need for pesticides (Londres 2011; Bombardi 2017; Pignati *et al*. 2017; Salomão *et al*. 2020). These chemicals cause serious impacts to the environment through the contamination of natural resources (Dutra & Souza 2017). After being applied, part of these substances is dispersed in the environment through drift, affecting non-target species, contaminating forest fragments and, potentially, impacting animals and humans (Pereira *et al*. 2015; Lucadamo *et al*. 2018).

According to Gavrilescu (2005), of the total pesticides applied, approximately 55% do not reach their target, dispersing through the biotic and abiotic components (water, soil, plants and atmosphere) of the ecosystem. There is no use of pesticides without contamination of adjacent areas, and consequently, without affecting the people who live or work in the surrounding areas (Londres 2011).

The damage caused by pesticides go beyond environmental damage, being considered a public health problem (Londres 2011; Rigotto *et al*. 2014; Paumgartten 2020). Due to the deleterious potential on humans and the environment, it is essential to seek mechanisms to monitor the impacts of these substances *in situ*. Among the various techniques employed is biomonitoring, which makes it possible to diagnose the health of the environment through biological changes that indicate the exposure of a given individual to xenobiotic agents (Kapusta 2008; Rigotto *et al*. 2014).

Glyphosate is the most widely used herbicide in agricultural crops in Brazil (IBAMA 2019) since its rapid efficiency promotes the indiscriminate use of this product, especially in transgenic crops. Its absorption occurs through the leaves and stems, through which it is transported to the entire plant. It acts by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), interrupting the biosynthesis of essential aromatic amino acids (tryptophan, phenylalanine, and tyrosine), causing the accumulation of shikimic acid, and causing disorder in the metabolic processes of the plant that, in some cases, can lead to death (Orcaray *et al*. 2012; Rabello *et al*. 2012; Haas *et al*. 2018). The harmful effects of this substance reach several non-target organisms; studies have proven the presence of residues of the herbicide in animals and foods used in the human diet (Lorenzatti *et al*. 2004). Such facts corroborate the need for studies on the ecotoxicological effects of glyphosate on the environment (Moraes & Rossi 2010; Haas *et al*. 2018).

Plants are sensitive to environmental disturbances and, when exposed to a stressor, react in predictable and measurable ways through changes in their vital functions or chemical composition. Before the visible symptoms appear, biochemical, morphological, physiological and anatomical changes occur in plants, which serve as a warning about the implicit consequences of these pollutants for the environment (Lima 2001; De Figueiredo Aquino *et al*. 2011; Rai 2016; Prestes & Vincenci 2019). Biochemical, physiological, anatomical and morphological parameters of higher plants are highly effective bioindicators and can be employed in short- or long-term monitoring with different concentrations of pollutants (Lima 2001; Savóia *et al*. 2009). Physiological indicators of glyphosate toxicity such as decreases in photosynthetic rate, in chlorophylls, and in the efficiency of photosystem II were identified in species native from the Brazilian Cerrado (Rezende-Silva *et al*. 2022).

The presence of visible anatomical and ultrastructural damages suggests that plants has remarkable potential as a bioindicator of glyphosate presence in the environment. In addiction, an accumulation of shikimic acid in the leaves and decrease in leaf gas exchange and chlorophyll fluorescence variables have been observed in plants, after exposure to glyphosate (Freitas-Silva *et al*. 2020).

Among the various fruit species of the Cerrado, *Eugenia dysenterica* (Mart.) DC. (Myrtacea), popularly known as *cagaita*, stands out for its multiple uses by local communities. *Cagaita* has long been exploited for medicinal purposes, which is why it is considered a species of great relevance to regional populations. This species is commonly found in areas near monocultures, in remnants of native cerrado vegetation (Lima *et al*. 2010; Scariot & Ribeiro 2015; Gonçalves *et al*. 2015).

This study evaluated the hypothesis that *E. dysenterica* is sensitive to the action of glyphosate and that physiological and morphoanatomical changes may contribute to the diagnosis of the presence of this herbicide in this species. As it is a widely used species of Cerrado, studies that seek to evaluate the effects of the presence of glyphosate are important tools for the biomonitoring and allow indicating whether there is a risk of contamination to the health of the populations that uses. In this sense, decisions in the political and health spheres may be made, helping to define strategies for the prevention and monitoring of environmental and human health (Prestes & Vincenci 2019). The objective of this work was to evaluate the effects of glyphosate on leaf gas exchange, photosynthetic pigments and morphoanatomy of seedlings of *E. dysenterica*.

Materials and Methods

Plant material and experimental conditions

Seedlings of *Eugenia dysenterica* were adquired from the Sempre Viva nursery (10°09'13.64"S, 48°20'26.90"W), located in Palmas, Tocantins, Brazil, at an age of ten months. The substrate used was composed of a mixture of Cerrado Red Latosol, topsoil and organic compost (rice straw, bovine manure and foliage) at a ratio of 3:1:1, plus NPK fertilizer 4:14:8 and dolomitic limestone. In addition to daily irrigation, the seedlings received once a week an application of organic foliar fertilizer at a proportion of 50 ml of fertilizer diluted in 500 ml ofwater; the fertilizer was composed of castor bean meal, cotton meal and bovine manure. It was used to supply the main macronutrients for the plants (nitrogen, phosphorus, and potassium).

Of the 60 seedlings acquired, sixteen healthy individuals of standardized height were selected and then brought to the greenhouse. The average height of the seedlings was 52 cm, were kept in de 18 × 25 cm pots (1,8 L).

The experiment was conducted in a greenhouse at the Federal University of Tocantins - UFT, Palmas campus, with geographical coordinates 10°10'35.8"S and 48°21'29.3"W, in the period between March and November 2020.

After the acclimatization period (115 days), glyphosate was applied, only once, outside the greenhouse in the morning at 8 am. The following atmospheric conditions were recorded at the time of application: 35 °C (mean temperature); 33.3% (relative humidity) and 3.9 km/h (wind speed), without shading.

For glyphosate application a handheld compression sprayer was used at 2.8 bar pressure, equipped with a boom containing a spike with a BX-AP/70 empty cone spray tip, with a 200 l/ha spray volume.

The pesticide used was Roundup Original® DI at the following concentrations: 0 (T0), 555 (T1), 1110 (T2) and 2220 (T3) g a.e. ha^{-1}, corresponding to 0, 25, 50 and 100% of the commercial dose, simulating possible concentration of the product to reach, through drift, adjacent non-target plants.

The experiment was conducted in a completely randomized design (CRD), composed of 4 treatments with 4 replicates, each individual being considered an experimental unit.

Visible symptoms were recorded using photographs taken with a 12-megapixel digital camera from the third day. The toxicity index considered the symptoms presented by the individuals at 60 days after application (DAA) and followed the EWRC scale (Frans 1972), with adjustments. Scores were assigned from 1 to 7, according to the toxicity symptoms described below: 1 - null; 2 - very mild; 3 - mild; 4 - medium; 5 - strong; 6 - very strong; 7 - severe.

Leaf gas exchange

Leaf gas exchange was measured using a portable photosysnthesis system LI-6400 XT (LI-COR Biosciences Inc., Nebraska, USA) on fully expanded leaves located along the upper third of the plant with 3 replicates for each individual. Measurements of net photosynthetic rate (*A*, μmol CO_2 m^{-2} s^{-1}), leaf transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), stomatal conductance to water vapor (*gs*, mol H_2O m^{-2} s^{-1}) and internal to external CO_2 rate (*Ci*/*Ca*, μmol $μmol^{-1}$) occurred in the morning (8 to 9 am) and was performed on 47 DAA.

The leaf gas exchanges were measured at air temperature of 30 °C, at 9 am, relative humidity ranging from 50% a 60%, atmospheric CO_2 concentration between 380 to 400 μmol mol^{-1} and artificial saturating photon irradiance of 2000 μmol m^{-2} s^{-1}.

Leaf pigments

The concentration of chloroplast pigments and carotenoids was measured at 60 DAA of the herbicide in leaf sample and followed the methodology described by Ronen & Galun (1984), modified, with 3 repetitions for each individual, at 8 am. For pigment

extraction, chlorophylls *a* (*Chl a*) and *b* (*Chl b*), the solvent dimethyl sulfoxide (DMSO), previously saturated with calcium carbonate ($CaCO_3$), were used. The readings were performed in a UV-VIS spectrophotometer, Evolution 60S model (Thermo Fisher Scientific, Madison - USA). The leaves used to determine chloroplastic pigments were used to measure gas exchange.

The wavelengths, equations, and calculations to determine the content of chlorophylls *a* (480 nm), *b* (649 nm) and carotenoids (665 nm) followed the Wellburn methodology (1994). The quantities of total chlorophyll were calculated by chlorophylls a + chlorophylls b concentrations. Chlorophyll degradation was evaluated by spectrophotometry, adopting the pheophytinization index (PI = A435/A415). This index corresponds to the transformation of chlorophylls in pheophytin, when disorganization in membranes occurs under acidic pH (Wellburn 1994).

Leaf anatomy

On 60 DAA of glyphosate application, fully expanded leaves were collected from each individual for anatomical analyses. A section was cut in the median region, closest to the right margin, avoiding the vein, with 3 repetitions for each individual. The samples were fixed in FAA (50) solution and stored in 70% ethyl alcohol. For the dehydration process, the samples were submitted to ethyl and butyl series (80, 90 and 100%, ethyl butyl (3:1), ethyl butyl (1:1), ethyl butyl (1:3) and pure butyl), followed by embedding in paraffin + 8% beeswax (Johansen 1940).

Transverse sections were obtained using a semi-motorized rotary microtome (RM2245-Leica, Germany) at 12 µm thickness, which were adhered to the slide with Haupt's adhesive (Haupt 1930) and subsequently stained in 1% safranin and astra blue for 20 min (Gerlach 1984).

The slides were washed in distilled water, dehydrated in an increasing ethyl series (30% to 100%) and submitted to a xylol series, with subsequent mounting of slides and coverslips with Canada balsam. For morphometric analyses, three sections were randomly selected on each slide. Images were captured by a Leica DM 500 optical microscope with a Leica ICC50 HD camera attached. The morphometry of leaf tissues (adaxial and abaxial epidermis, palisade, and spongy parenchyma) was performed with the aid of the image analysis software ANATI QUANTI, version 2.0 for Windows® (Aguiar *et al*. 2007).

Statistical analyses

The data were submitted to analysis of variance (ANOVA), with means compared by the Scott-Knott test at 5% probability, with the aid of SISVAR software version 5.7 (Ferreira 2018) and of R software version 4.2.

Results

Toxicity

Plants of *Eugenia dysenterica* submitted to glyphosate treatments showed toxicity symptoms (Fig. 1). Leaf damage such as chlorosis and necrosis was observed in all treatments whith glyphosate, which varied according to the dose. At a dose of 550 g a.e. ha^{-1}, the first symptoms became visible at 25 DAA, with the appearance of small discolorations (Fig. 1c). Plants that received the dose of 1110 g.e.a.ha^{-1} showed the first chlorotic spots at 11 DAA (Fig. 1e). At a dose of 2220 g a.e. ha^{-1}, the first symptoms became visible 3 DAA, with the appearance of necrotic spots distributed on the leaf blade (Fig. 1g).

Regarding of the level of intoxication, it was found that the higher the dose of glyphosate, the more visible were the symptoms and the higher the toxicity index.

At the dose of 555 g a.e. ha^{-1}, 75% of the individuals had a low level of intoxication, only minor discolorations on the leaf blade (note 2) (Fig. 1d), while at the dose of 1110 g a.e. ha^{-1}, 50% of the plants had strong discoloration with chlorosis on the leaf blade (note 4) (Fig. 1f). All individuals exposed to a dose of 2220 g a.e. ha^{-1} (100%) showed symptoms of necrosis and chlorosis in their leaves (note 5) (Fig. 1h).

Leaf gas exchange

In plants exposed to glyphosate, significant effects were observed at 47 DAA, with reductions in (*A*), (*gs*) and (*E*). Thus, it is possible to state of exposure glyphosate affected the leaf gas exchange of *E. dysenterica*.

In *A*, doses of 1110 g a.e. ha^{-1} (T2) and 2220 g a.e ha^{-1} (T3), differed significantly from the control and the smallest dose 555 g a.e. h^{-1} (T1), with a decrease of 60.21% and 47.41%, respectively at 47 DAA (Fig. 2a).

For *gs* and *E*, the effects observed were similar to the photosynthetic rate. The doses of 555 g a.e. ha^{-1}, 1100 g a.e. ha^{-1}, and 2220 g a.e. ha^{-1} showed significant decreases when compared to the control (Fig. 2b-c).

In the internal and external carbon (*Ci/Ca*) ratio, the results were also due as a function of the longer time of exposure to glyphosate. The highest internal CO_2 concentration was observed at the dose of 2220 g a.e. ha^{-1} at 47 DAA and was characterized by a 41% increase in relation to the control (Fig. 2d).

Concentrations of leaf chloroplast pigments

Regarding the chloroplast pigments of the plants studied, it was observed that the higher the dose of glyphosate, the greater the degradation of the photosynthesizing pigments. Individuals exposed to the dose of 2220 g a.e. ha^{-1} showed a greater reduction in the content of chlorophylls a (*Chl a*), b (*Chl b*) and total chlorophyll (*Chl a* + *Chl b*), by 41%, 23%, 39%, respectively, when compared to the control. Regarding carotenoids (*CaT*) and the pheophytinization index (*PI*), there were no significant differences among treatments (Tab. 1).

Leaf anatomy

The leaves of *Eugenia dysenterica* have a unistratified epidermis on both surfaces (adaxial and abaxial) and are covered by a thick cuticle. Stomates are present only on the abaxial side (hypostomatic leaf) (Fig. 3a). The dorsiventral mesophyll has one layer of palisade parenchyma and several layers (between 4 to 7) of spongy parenchyma, which presents few intercellular spaces. The presence of secretory cavities is visible throughout the leaf mesophyll (Fig. 3b). The chloroplasts show a lenticular shape. The central vein region is composed of an arch-shaped collateral vascular bundle, surrounded by pericyclic fibers. There are prismatic crystals along the mesophyll and associated with the vascular bundles (Fig. 3c).

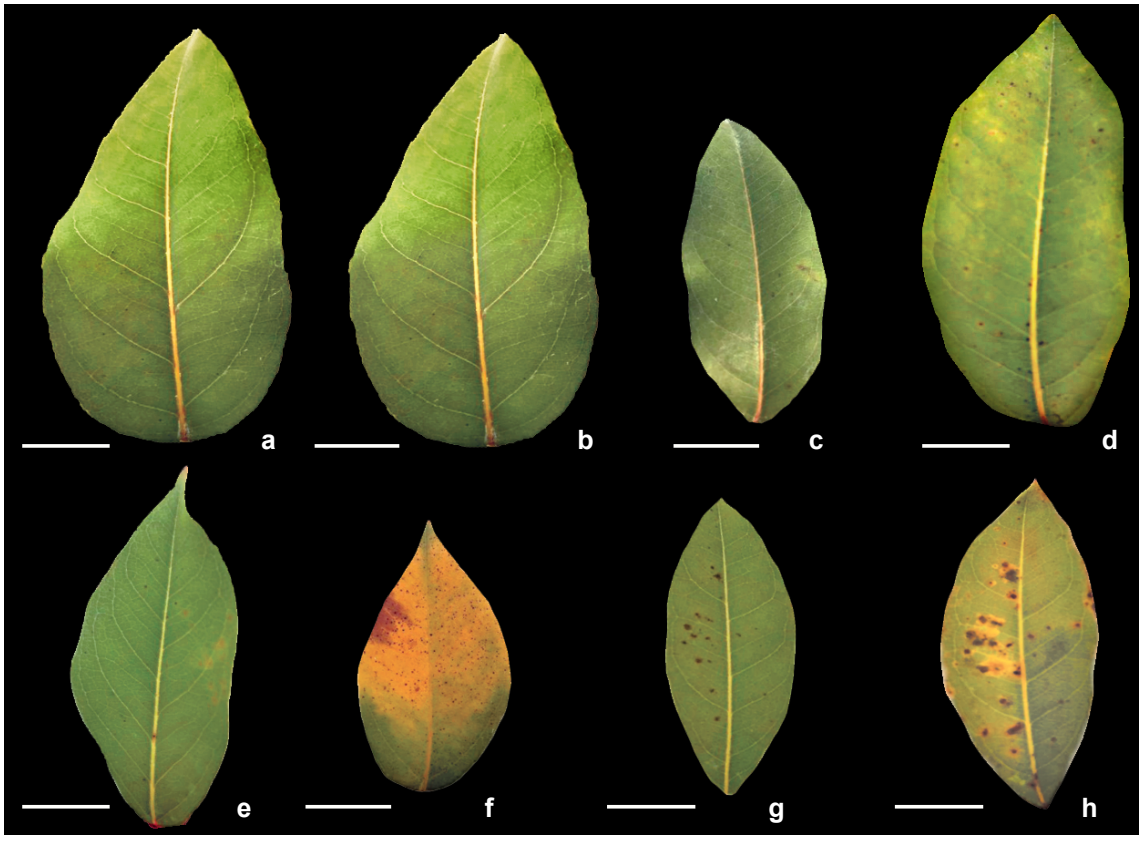

Figure 1 – a-h. Visible toxicity symptoms in *Eugenia dysenterica* plants submitted to different doses of glyphosate – a-b. treatment with 0 g a.e. ha^{-1} (T0); c-d. treatment with 555 g a.e. ha^{-1} (T1); e-f. treatment with 1100 g a.e. ha^{-1} (T2); g-h. treatment with 2220 g a.e. ha^{-1} (T3). a, c at 25 DAA; b, d, f, g at 60 DAA; e at 11 DAA; g at 3 DAA. Scale bar = 2 cm. DAA = Days After Application.

Glyphosate caused damage to the anatomical structures of *E. dysenterica* leaves at all doses tested. Changes in the volume and shape of the mesophyll cells were observed. In the palisade parenchyma cells, protoplast shrinkage was observed (Fig. 3d). Large intercellular spaces were identified in spongy parenchyma and changes in chloroplast shape, which was spherical (Fig. 3e-g).

The interior of the fibers that contour the vascular bundles presented accumulation of pigmentation, differing from the control (Fig. 3c), which fibers did not show this pigmentation (Fig. 3f). In the secretory cavities, it was observed that the secretion presented a granular aspect with reddish coloration, different from the control, whose secretion was translucent (Fig. 3h).

Regarding the morphometry of leaf tissues, individuals exposed to glyphosate showed a significant reduction in abaxial epidermal thickness at doses of 1110 g a.e. ha^{-1} (T2) and 2220 g a.e. ha^{-1} (T3), at values of 22.48% and 19.79%, respectively, statistically different from the control. The other tissues showed no significant difference when compared to the control (Tab. 2).

Discussion

Leaf damage such as chlorosis and necrosis was observed in all treatments whith glyphosate. *Eugenia dysenterica* plants exposed to higher doses of glyphosate showed more pronounced toxicity symptoms. When exposed to glyphosate, the characteristic visual symptoms of the action of this herbicide are the appearance of chlorosis followed by necrosis of the leaf tissue, which starts from the leaf tissue, which from the edge towards the center of the leaves (Da Silva Borges *et al*. 2021).

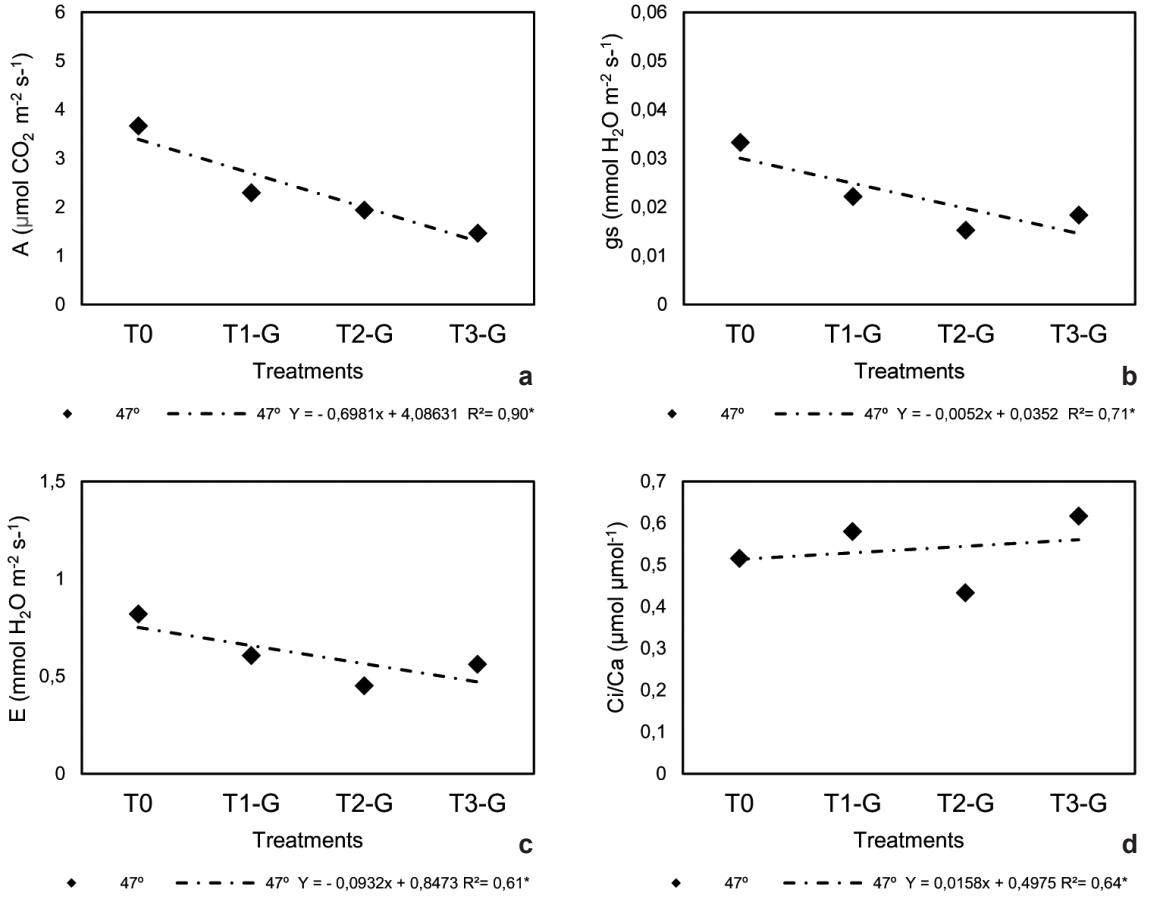

Figure 2 – a. Net photosynthetic rate (*A*); b. stomatal conductance to water vapor (*gs*); c. leaf transpiration rate (*E*); d. internal to external CO_2 ratio (*Ci/Ca*) of *Eugenia dysenterica* plants submitted to glyphosate application at doses of 0 (T0), 555 g a.e. h^{-1} (T1), 1110 g a.e. ha^{-1} (T2) and 2220 g a.e. ha^{-1} (T3) at 47 DAA. (n = 4) Significant by factorial analysis (*$p \leq 0.05$).

Table 1 – Chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll (*Chl a + Chl b*), carotenoids (*CaT*) and pheophytinization index (*PI*) of *Eugenia dysenterica* plants submitted to glyphosate application at doses of 0 (T0), 555 g a.e. h^{-1} (T1), 1110 g a.e. ha^{-1} (T2) and 2220 g a.e. ha^{-1} (T3) at 60 DAA. MF (fresh past); CV = coefficient of variation. Means followed by the same letter do not differ by the Scott-Knott test at 5% probability. (n = 4)

Chloroplast pigments	Treatment				CV
	T0	T1	T2	T3	
Chl a (mg g^{-1} MF)	0.86a	0.61ab	0.64ab	0.44b	24.05%
Chl b (mg g^{-1} MF)	0.52a	0.43b	0.48ab	0.4b	16.73%
Chl a+b	1.38a	1.05ab	1.11ab	0.84b	20.57%
CaT	4.57a	3.86a	4.82a	4.56a	23.42%
PI	0.9a	0.88a	0.85a	0.78a	4.05%

In function of the visual damage observed in leaves of *E. dysenterica*, it is possible to infer that the species studied is sensitive to the action of glyphosate, and has the capacity to show response, making possible its use in biomonitoring studies of areas exposed to the action of this herbicide. Other native species of cerrado have been identified in the literature as sensitive to glyphosate. Rezende-Silva *et al*. (2019), found similar results for *Pouteria torta*, where visual symptoms of intoxication caused by glyphosate were observed, showing the potential for use of this species to monitor the effects of this herbicide in Cerrado vegetation. Cruz *et al*. (2021), identified visible symptoms in *Eugenia uniflora* only 3 DAA of glyphosate, presenting itself as a promising species for biomonitoring in native vegetation.

The concentrations of glyphosate affected the exchange of gases in seedling *E. dysenterica*, and these results confirm the sensitivity of *E. dysenterica* to the herbicide glyphosate. The effects of glyphosate on plants indicates that photosynthesis is not the primary mechanism of action of the herbicide, which acts gradually and indirectly on photosynthetic processes (Sprankle *et al*. 1975; Wagner & Merotto Junior 2014). It acts on the guard cells, promoting the closure of the stomata, making guard cells one of the most sensitive systems to disruption of cellular metabolism caused by glyphosate (Yamada & Castro 2007). Photosynthesis is intrinsically related to stomatal conductance. Closing of stomata limit the exchange of gases, compromise the assimilation of CO_2, and cause a reduction in carboxylation efficiency, with significant effects on the metabolic activities of plants (Machado *et al*. 2010; Dem & Ka 2016). Besides that, glyphosate interferes in the action of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, with the potential to cause a collapse in the metabolic pathway of shikimic acid, and as a consequence, the carbon accumulates, making it unavailable for the synthesis of photo-assimilates necessary for the development, protection, and growth of plants (Stephenson *et al*. 2006; Duke & Powles 2008). Additional species, such as *Caryocar brasiliense* (Silva *et al*. 2016), *Pouteria torta* (Batista *et al*. 2018), *Cenostigma macrophyllum* (De Sousa Santos *et al*. 2020), and *Eugenia uniflora* (Cruz *et al*. 2021), have also shown similar results after exposure to glyphosate.

The *Ci/Ca* ratio refers to the amount of CO_2 present in the intercellular substomatal chamber (*Ci*) and in the atmosphere (*Ca*), changes in these concentrations are related to limitations in stomatal conductance and/or involved in the photochemical and biochemical photosynthetic process of plants, inhibiting the efficiency of rubisco (carboxylase or oxygenase), and reducing its ability to assimilate CO_2 and O_2 present in the stroma (Farquhar & Sharkey 1982; Ding *et al*. 2011). It is possible to afirm that glyphosate affected Rubisco activity or Ribulose 1,5-bisphosphate regeneration in *E. dysenterica* seedlings, increasing the CO_2 concentration in the substomatal region. According to the data presented, there is a stomatal limitation impacting photosynthesis and the Ci/Ca ratio. The results of this study corroborate those found by Yanniccari *et al*. 2012, De Sousa Santos *et al*. (2020), and Cruz *et al*. (2021).

Plants of *E. dysenterica* exposed to the action of the herbicide showed changes in chlorophylls a (*Chl a*), b (*Chl b*) and total chlorophyll (*Chl a + Chl b*). These pigments play an important role in

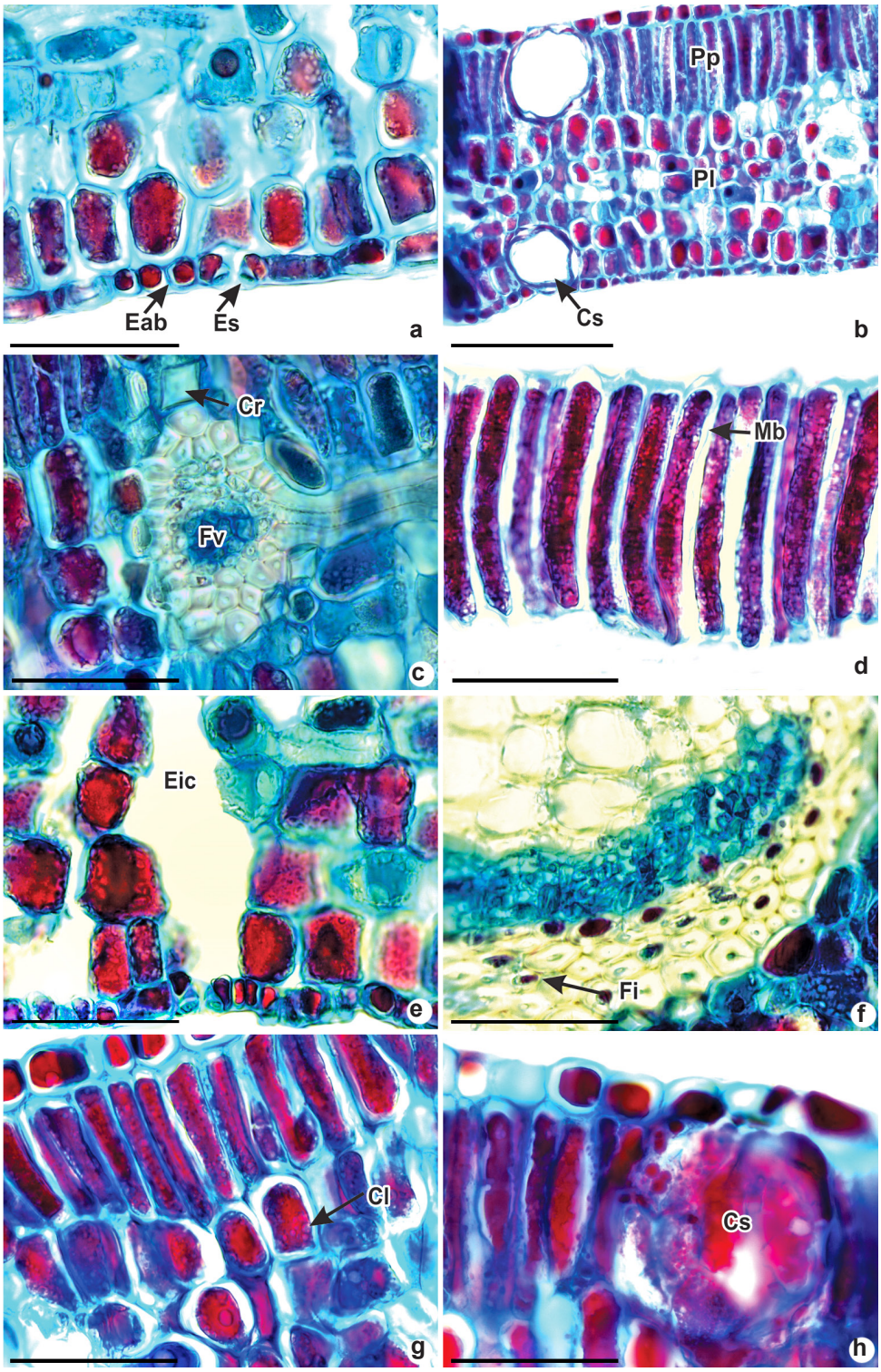

Figure 3 – a-h. Anatomical sections of *Eugenia dysenterica* leaves at 60 DAA of glyphosate. Control (a, b, c), when submitted to doses of 555 g a.e. h^{-1} (d), 1110 g a.e. ha^{-1} (e, f) and 2220 g a.e. ha^{-1} (g, h). a. abaxial epidermis (Eab) and stomata (Es); b. palisade parenchyma (Pp), spongy parenchyma (Pl) and secretory cavities (Cs); c. vascular bundle (Fv) and prismatic crystals (Cr); d. retraction of cell membranes (Mb) of palisade parenchyma; e. meatus (Eic); f. pigmented fibers (Fi). g. change in chloroplast (Cl); h. secretory cavities (Cs). Scale bar = 50 µm.

Table 2 – Morphometry of leaf tissues of *Eugenia dysenterica* plants submitted to glyphosate application at doses of 0 (T0), 555 g a.e. h^{-1} (T1), 1110 g a.e. ha^{-1} (T2) and 2220 g a.e. ha^{-1} (T3) at 60 DAA. CV = coefficient of variation. Means followed by the same letter do not differ by the Scott-Knott test at 5% probability. (n = 4)

Leaf tissues	Treatment				CV
	T0	T1	T2	T3	
Adaxial Epidermis (µm)	16,89a	15,3a	14,38a	15,13a	12,36%
Palisade Parenchyma (µm)	112,27a	102,79a	111,53a	115,29a	8,40%
Spongy Parenchyma (µm)	61,58a	68,73a	65,1a	64,3a	14,18%
Abaxial Epidermis (µm)	9,65a	8,86a	7,48b	7,74b	11,84%
Mesophyll (µm)	173,86a	171,53a	176,63a	179,59a	8,57%
Leaf Blade (µm)	200,40a	195,70a	198,50a	202,48a	7,55%

the photosynthetic process (Taiz *et al*. 2017) and their reduction causes severe damage to plants. Glyphosate interferes with chlorophyll biosynthesis because it inhibits the enzymatic activities of EPSPS (Galli & Montezuma 2005; Kaspary *et al*. 2014) and the synthesis of aminolevulinic acid, decreasing the production of photosynthesizing pigments (Kitchen *et al*. 1981; Carvalho & Alves 2011). Furthermore, the chelating activity of glyphosate also causes iron deficiency by impairing the action of two enzymes (catalase and peroxidase), which are essential in chlorophyll biosynthesis (Zobiole *et al*. 2010). Such facts are in line with the results observed regarding the pigment content of the species studied. Silva *et al*. (2016), Batista *et al*. (2018), and Cruz *et al*. (2021), have also reported changes in photosynthesizing pigments in plants exposed to glyphosate.

The anatomical characteristics described, in the control plants, in the present study corroborate those reported by Palhares (2003) for the species *E. dysenterica*. Anatomical evaluations of leaves are important tools in the identification of environmental disturbances because they allow identifying the sensitivity of a given species to the stressor agent even before visible symptoms appear (Silva *et al*. 2006; Freitas-Silva *et al*. 2016). After application, glyphosate is rapidly absorbed by the plant through the cuticle, being translocated to leaves, roots and meristematic regions. Because it has a very stable formulation with prolonged residual power, its effects are irreversible (Gruys & Sikorski 1999). Visible symptoms of intoxication (chlorosis and necrosis) in plants exposed to the herbicide may be related to the degeneration of chloroplasts and the production of reactive oxygen species (ROS) (Duke & Powles 2008; Islam *et al*. 2016). Leaf damage such as chlorosis and necrosis were observed in all treatments in *E. dysenterica* in this study.

In morphometry of leaf tissues, a reduction of the abaxial epidermis was observed. Tissue reduction has been observed in the adaxial and abaxial epidermis and the palisade and spongy parenchyma in four species native to the cerrado (*Plathymenia reticulata*, *Bowdichia virgilioides*, *Kielmeyera lathrophyton*, and *Solanum lycocarpum*) after exposure to glyphosate (Machado *et al*. 2013). Damage to the epidermis it is common effect due to the interaction between the leaf surface and herbicide droplets (Freitas-Silva *et al*. 2020). As the leaf of *E. dysenterica* is hypostomatic, the effects of the herbicide in the abaxial epidermis also affected the stomata, corroborating with the results found regarding the interference of glyphosate on stomatal conductance and, consequently, on photosynthesis and transpiration. De Sousa Santos *et al*. (2020), observed a similar response, regarding the reduction of abaxial epidermis in *Cenostigma macrophyllum* submitted to different concentrations of the herbicide.

In the species studied, modifications in leaf structures, the appearance of meatuses, and changes in the volume and shape of the mesophyll cells and chloroplasts were observed. These changes compromise the functionality of the chlorophyllous parenchyma, directly interfering with the efficiency of photosynthesis, because they promote changes in gas diffusion inside the leaves (Heath 1994; Flexas *et al*. 2008; Freitas-Silva *et al*. 2020), thus corroborating the results found with the gas exchange analyses.

Changes in the synthesis the organic substances, in plants exposed to herbicides, have caused modifications in the chemical composition

of the secretion present in the leaves (Tuffi Santos *et al*. 2005; De Sousa Santos *et al*. 2020). *E. dysenterica* were also identified changes in the coloration of chemical compounds present in the secretory cavities. Glyphosate interferes in the metabolic pathways of shikimic and pyruvic acid, compromising the synthesis of the essential amino acids phenylalanine, tyrosine, and tryptophan (Galli & Montezuma 2005). These amino acids are indispensable for protein synthesis and serve as substrates for the production of various secondary compounds. Interference in glyphosate on shikimic acid synthesis can cause metabolic imbalance (Gruys & Sikorski 1999; Yamada & Castro 2007).

Eugenia dysenterica is sensitive to the action of glyphosate. Negative effects of this pesticide were identified in morphological, physiological and anatomical parameters of the plants studied. It is possible to infer that *E. dysenterica* has the potential to be used in programs for biomonitoring of environments exposed to glyphosate at doses above 550 g a.e. ha^{-1}. It would be interesting to carry out further studies with exposure of subdoses of glyphosate. Visible symptoms such as chlorosis and necrosis in the leaf edge are indicators that can be used by rural communities as a warning of the risk of contamination.

Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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