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Physiological changes and in the carbohydrate content of sunflower plants submitted to sub-doses of glyphosate and trinexapac-ethyl

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ABSTRACT: The maturing of drift used in the culture of sugar cane can have harmful effects on other crops grown in the vicinity of sugar cane plantations. Among these, sunflower grown in the off-season can have its growth and productivity affected by drift. The objective of this research was to evaluate whether the drift of trinexapac-ethyl and glyphosate promotes changes in the photosynthetic metabolism of sunflower plants. Two trials were carried out to evaluate the effects of these products on gas exchange, chlorophyll fluorescence, chloroplastid pigments, membrane permeability, sugar content, as well as shikimic acid and malondialdehyde concentration in the treated plants. In the first experiment, we tested glyphosate in doses of 0 (control); 3.6; 7.2; 14.4; 28.8; and 86.4 g a.e.·ha^{-1} and in the second, trinexapac-ethyl at doses of 0 (control) 3.12; 6.25; 12.50; 25, and 75 g a.i.·ha^{-1}. The growth regulator trinexapac-ethyl did not change the photosynthetic metabolism of plants. However, glyphosate caused damage to the photosynthetic apparatus and a reduction in the carbohydrate concentration and chloroplastid pigments, with casual damage to cell membranes; these effect were more intense at increased doses. The effects of glyphosate were evidenced by the increased concentration of shikimic acid, derived from its mechanism of action. Concludes that, the photosynthetic metabolism of sunflower plants is not affected by the growth regulator trinexapac-ethyl, unlike to the evident effects after application of glyphosate.

Key words: gibberellin, EPSPs, photosystem II, photochemical efficiency.

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INTRODUCTION

The cultivation of energy crops has been expanded significantly in Brazil in recent years. Between 2005 and 2015, approximately 2.98 million hectares of sugar cane were added, increasing the cultivated area to 9.07 million hectares (Conab 2015). The high productivity and the increase in the area under cultivation of this crop have been highlighted as the main source of renewable energy in Brazil (Brasil 2013), in addition to the potential for use in the production of fuel and sugars.

The increased sugar cane productivity is due to, among other factors, the application of growth regulators (Fioreze and Rodrigues 2014), which are commonly applied via aerial spraying. This can lead to changes in the development of non-target plants grown in parallel. Herbicide drift and the application of growth regulators in many cultures have been a growing scientific concern (Bassa et al. 2011). However, there is little research about the effects of growth regulators on non-target crops, because of the breakthrough in the sugar-ethanol sector, are still incipient. The detection of changes in the development of adjacent plants, depending on the product derives can serve as a warning to assumptions errors in application technology.

Among the growth regulators frequently applied in sugar cane cultivation, the plant regulator trinexapac-ethyl and the herbicide glyphosate are of special importance. The mechanism of is based on inhibition of the enzyme 5-enolpyruvylshikimate-3 phosphate synthase (EPSPs), preventing the synthesis of three essential aromatic amino acids: phenylalanine, tryptophan and tyrosine (Gomes 2011). The inhibition of EPSPs enzyme affects the metabolic route of shikimic acid, responsible for the formation of phenolic compounds, which can represent up to 35% of the biomass plant (Gomes 2011). Trinexapac-ethyl promotes the sharp reduction of the stalk and, consequently, plant height through inhibition of gibberellin biosynthesis (Rajala 2003). This regulator acts as a GA_{12} aldehyde, inhibiting from this synthesis of gibberellins of biological efficiency, as: GA_1 and GA_3. This reduction in the level of gibberellic acid or in its sensitivity, with concomitant reduction in plant height, is important for the induction of tolerance to stresses in cereals (Korol and Klein 2002).

Among the methods currently available to quickly and accurately measure the harmful effects of driftnets of agrochemicals on plants, the use of morphological and physiological variables plays a crucial role. Fluorescence emission measurements of chlorophyll-*a* (Cl-*a*) and estimated chlorophyll concentration have been used to obtain qualitative and quantitative information about the photosynthetic process (Corrêa and Alves 2010). In regards to the application of growth regulators, the use of physiological and biochemical variables becomes extremely important in commercial species cultivated in areas close to sugar cane plantations.

In the Savannah "Cerrado" region, sunflowers are frequently planted in the vicinity of sugar cane crops treated with growth regulators. Sunflower crops are mainly cultivated for the production of biodiesel (Del Gatto et al. 2015), but they are also used for various other purposes, such as edible oil or animal feed. Furthermore, they can be easily cultivated in different conditions and soils (López-Valdez et al. 2011) by phenotypic plasticity, primarily because of their high water deficiency tolerance.

We hypothesized that sunflower plants are sensitive to sub-doses of the growth regulators trinexapac-ethyl and glyphosate used in sugar cane cultivation and that sensitivity can be measured through evaluations of gas exchange, chlorophyll fluorescence a, chloroplastid pigments, carbohydrates, and damage to cell membranes. Thus, the objective of this study was to evaluate the changes in the photosynthetic metabolism of sunflower plants subjected to simulated drift of the growth regulators trinexapac-ethyl and glyphosate.

MATERIAL AND METHODS

Plant material and experimental conditions

The experiments were conducted in an acclimatized greenhouse from October 2013 to January 2014. The humidity in the greenhouse ranged from 60 to 75% and the temperature, from 22 to 29 °C. Sunflower plants were grown in pots containing 8 kg of polyethylene substrate obtained from mixing one part sand and two parts of dystrophic Red Latosol. Chemical analysis revealed the following characteristics of the substrate: pH in H_2O equal to 4.7; 2.6 mg $P \cdot dm^{-3}$; 14 mg $K \cdot dm^{-3}$; 0.75 cmolc $Ca \cdot dm^{-3}$ Ca; 0.22 cmolc$(H + Al) \cdot dm^{-3}$; 13 $g \cdot kg^{-1}$ of organic matter, and 24% saturation of bases. Correction and substrate fertilization were carried out according to soil analysis and nutritional requirements of the culture (Ribeiro et al. 1999). The cultures were frequently irrigated depending on their water requirements, since we used 2 plants per pot standardized in size and effect.

Treatment impositions

We conducted 2 independent experiments. The first one evaluated effects of glyphosate (480 g·L^{-1} of acid equivalent) and the second one the effects of trinexapac-ethyl (250 g·L^{-1} of active ingredient). We used the following doses for the treatments: glyphosate 0 (control); 3.6; 7.2; 14.4; 28.8 and 86.4 g a.e.·ha^{-1} and trinexapac-ethyl 0 (control); 3.12; 6.25; 12.50; 25 and 75 g a.i.·ha^{-1}. The applications were held to 30 days after the emergency, using a sprayer knapsack (Herbicat® Catanduva, Brazil) with constant pressure maintained by compressed CO_2 with a bar with four spray tips and nozzle (Teejet), such as the XR110 02 model range. The working pressure used was of 5 kgf·cm^{-2}, providing a volume of 180 L·ha^{-1}.

Physiological evaluation

Evaluations of gas exchange and chlorophyll fluorescence were carried out 1; 7; 14; 21 and 28 days after application (DAA) of growth regulators, using a fully expanded leaf. In addition, evaluation of carbohydrates, chloroplastid pigments, malondialdehyde content (MDA), electrolyte release rate (ERR), and shikimic acid were carried out 28 days after the application growth regulators.

Evaluations of gas exchange

Gas exchange measurements were carried out during the period from 7h00 to 10h00 in the morning to record photosynthetic rate (A, μmol·m^{-2}·s^{-1}), transpiration (E, mmol·m^{-2}·s^{-1}), stomatal conductance (g_s, mol H_2O·m^{-2}·s^{-1}), and the relationship between internal and external CO_2 concentration (C_i/C_a). We used the infrared gas analyzer (IRGA), model LI-6400XTR (Licor®/Nebraska, United States), coupled to an artificial light source focusing the photon flux density equal to 1,000 μmol·m^{-2}·s^{-1}.

Measurement of chlorophyll fluorescence a

Chlorophyll fluorescence measurement was conducted using a portable fluorometer modulated Model MINI-PAM (Walz®, Effeltrich, Germany) equipped with a special leaf clip model 2030-B (Bilger et al. 1995). The potential quantum yield of photosystem II (F_v/F_m) was calculated after 30 min of dark adaptation. The effective quantum efficiency of photosystem II (PSII) ($\Delta F/F_m'$) was determined by saturation pulse previously adapted to overlapping leaves to ambient light (Genty et al. 1989). The $\Delta F/F_m'$ was used to estimate the apparent electron transport rate (ETR) (Bilger et al. 1995), and the extinction coefficient non-photochemical quenching (NPQ) was calculated according Bilger and Bjorkman (1990).

Measurement of carbohydrates

Extraction method

We determined reducing sugars, non-reducing sugars, total soluble sugars, and starch. Samples of 1.0 g of fresh leaves were transferred to amber jars with 25 mL volume and completely covered with 80% ethanol previously heated to a temperature of 65 – 70 °C. After 30 min at room temperature, the jars were transferred to the refrigerator. Sugar extraction was performed through the maceration of the samples in 80% ethanol, followed by 3 successive filtering steps. The filtrate obtained was completed with 80% ethanol and the residue after drying was used for the measurement of concentration of starch. Determination of reducing sugars, total soluble sugars, and starch was performed using a UV-VIS spectrophotometer extracts in Evolution model 60S UV – VIS model Evolution 60S (Thermo Fischer Scientific*, Madison, United States).

Determination of reducing, total soluble, non-reducing sugars, and starch

The reducing sugars were determined using the dinitrosalicylic acid according to Miller (1959). We used a wavelength of 540 nm and a standard curve of glucose (1%) ranging from 0 to 40 μg. For the determination of total soluble sugars, we used the phenol-sulfuric method (Dubois et al. 1956) at a wavelength of 490 nm, using a standard curve of sucrose (1%) of 0 the interval 50 μg. Based on the results of the reducing and total sugars, we estimated the concentration of non-reducing sugars. Starch concentration was performed according to McCready et al. (1950) at a wavelength of 490 nm, using a standard curve of sucrose (1%) in the interval from 0 to 50 μg.

All analyses were performed in triplicate, and, from the values, calculations and the results expressed in percentages were made (%) using the equation proposed by Somogy (1945).

Concentration of Chloroplastid pigments

Chloroplastid pigments were determined through extraction with dimethylsulfoxide (DMSO) saturated with $CaCO_3$, using the adjusted methodology described by Silva et al. (2014). Three leaf disks of known fresh weight and 5 mm in diameter were incubated in DMSO in sealed amber jars and wrapped with aluminum foil for a period of 24 h at 65 °C. Subsequently, the absorbance of the extract was determined using the spectrophotometer UV - VIS Evolution model 60S (Thermo Fischer Scientific®, Madison, United States). The wavelengths and the equations to calculate the concentrations of Cl-*a*, chlorophyll-*b* (Cl-*b*) and carotenoids, total chlorophyll, and the ratio of Cl-*a*/Cl-*b* were based on Wellburn (1994).

Concentration of malondialdehyde and electrolyte release rate

Samples of 0.15 g of leaves were crushed in liquid nitrogen and homogenized in 2.0 mL of trichloroacetic acid (TCA) 0.1% (m/v), followed by filtering through 4 layers of gauze and centrifugation at 10,000 g for 15 min at 4 °C. For the reaction, 0.5 mL aliquot of the supernatant was added to 1.5 mL of thiobarbituric acid (TBA) 0.5% (w/v) TCA 20% (w/v). The tubes were closed and incubated in a water bath at 95 °C for 30 min. The reaction was stopped on ice for 1 min and centrifuged at 9.000 × *g* for 4 min at 25 °C. Absorbance was measured at 600 and 440 nm. The concentration of malonic aldehyde-TBA complex was obtained through subtraction of the absorbance and the use of the molar absorption coefficient of 155 $mM^{-1} \cdot cm^{-1}$ (Hodges et al.1999), and the results were expressed as $mmol \cdot g^{-1}$ of fresh weight. Membrane permeability was evaluated by the RRE of 15 leaf discs immersed in 30 mL of deionized water in amber jars, according to the methodology described by Pimentel et al. (2002).

Shikimic acid

Extraction method

Eight leaf/foliar disks were collected from six mm (± 25 mg) of the second leaf beneath the apical meristem of each plant. The samples were frozen and stored in liquid nitrogen until extraction. Extraction of the crude extract was performed according to the method described by Singh and Shaner (1998), with modifications. The frozen samples were macerated in microtubes containing HCl buffer (0.25 N) in relation 1:10 [fabric weight (g) / volume of HCl 0.25 N (mL)]. The extract was centrifuged at 15,000g speed at 4 °C for 25 min. The supernatant was then collected and analyzed for shikimic acid.

Determination of shikimic acid

Shikimic acid was determined according to the methodology proposed by Perez-Jones et al. (2005), with modifications. We collected 30 µL aliquots of the supernatant transferred to microtubes and added 500 µL of a 1% solution of periodic acid. The microtubes were closed and incubated in a water bath at 37 °C for 45 min. We then added 500 µL of sodium hydroxide (1 N) and 300 µL of glycine (0.1 M). Readings were performed at 380 nm in a spectrophotometer UV - VIS Evolution model 60S (Thermo Fischer Scientific®, Madison, USA). The concentration of shikimic acid was obtained by using the molar absorptivity coefficient 4.76×10^4 $mM^{-1} \cdot cm^{-1}$ (Gaitonde and Gordon 1958), and results were expressed as $mg \cdot g^{-1}$ fresh weight. All analyses were performed in triplicate.

Statistical analysis

The experiments were conducted in a randomized block design in a subdivided plot arrangement; in the plots, we allocated the doses of glyphosate or trinexapac-ethyl and, in subplots, the evaluation periods with 5 replicates. The data were subjected to analysis of variance (ANOVA) and adjusted to the regression models. Statistical analyses were performed using SISVAR software version 5.3.

RESULTS AND DISCUSSION

Growth regulators increase the concentration of sucrose in the stalks of sugar cane plants, promoting lower development and higher productivity. However, the drift of these products can have significant impacts on susceptible non-target plants (Martins and Tomquelski 2015). In this study, the photosynthetic variables in non-target plants, namely sunflowers, responded in a contrasting way to the treatments, and for the growth regulator trinexapac-ethyl, we observed no significant effects between the doses used and →

the time after application. The evaluated variables showed the following averages over a period of 28 days: A ($\hat{Y} = \bar{Y} = 20.3990$ µmol·m^{-2}·s^{-1}), g_s ($\hat{Y} = \bar{Y} = 0.5662$ mol H_2O·m^{-2}·s^{-1}), E ($\hat{Y} = \bar{Y} = 1.5664$ mmol·m^{-2}·s^{-1}), and C_i/C_a ($\hat{Y} = \bar{Y} = 0.6379$). However, for glyphosate, there were significant differences between doses as well as between DAA. As the interactions for these factors were significant, gas exchange rates are shown to function at doses of DAA (Figure 1a – d). Photosynthetic rate, stomatal conductance, and transpiration decreased with increasing doses and over the following days, but the C_i/C_a ratio increased due to the increase in doses and periods after application. Fioreze and Rodrigues (2014), studying the effect of trinexapac-ethyl on wheat plants, observed a higher concentration of carbohydrates as a result of increased carbon assimilation in treated plants. The application of growth regulators, such as trinexapac-ethyl, can result in changes in developing stomata in quantity or size or might even control the opening and closing of stomata, resulting in higher values of transpiration and absorption of CO_2 (Fioreze and Rodrigues 2014). However, this was not observed in our study.

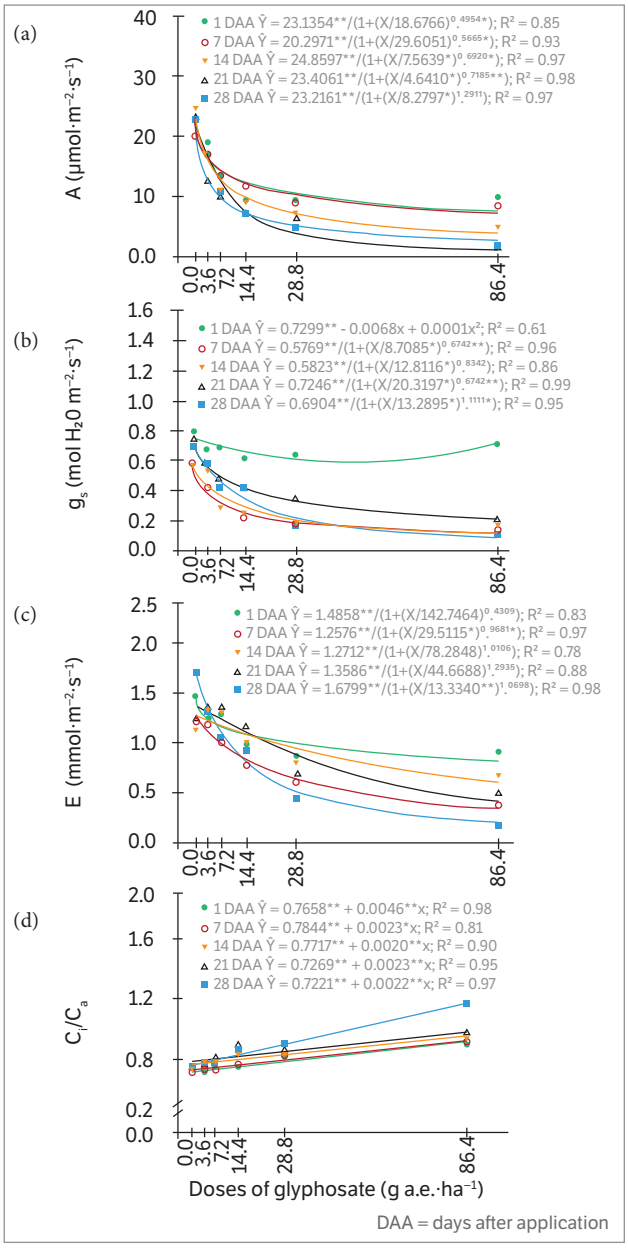

Figure 1. Photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E) and relation between internal and external CO_2 concentration (C_i/C_a) of sunflower plants treated with increasing doses of glyphosate (a) – (d) and evaluated at different times. Values are averages (n = 5). Significance: **p < 0.01; *p < 0.05.

The results of this study suggest that glyphosate has deleterious effects on non-target species such as sunflowers, mainly by changing the photosynthetic metabolism, which can be seen in the reduction in A, g_s, and E, and an increase in the ratio C_i/C_a. Under conditions of reduced stomata opening and greater concentration of intercellular carbon, reductions are observed in the Calvin cycle efficiency (Silva 2015). The efficiency in CO_2 fixation can be affected by the energy supply in the form of ATP and NADPH and by inhibition of enzymes of the Calvin cycle itself. Cedergreen and Olesen (2010), for example, reported that in barley plants treated with glyphosate, the reduction in stomatal conductance was a result of cessation of CO_2 fixation based on a decreased Rubisco regeneration process instead of a direct effect on stomatal conductance. This fact could be observed in this study, since the ratio C_i/C_a increased from 1 DAA and the g_s, to 7 DAA.

In plants treated with trinexapac-ethyl, we did not observe changes in the soluble total sugars characteristics, reducing sugars, non-reducing sugars, and starch (Figure 2). In contrast, in plants treated with glyphosate, the concentration of sugars and starch were significantly lower, with a reduction at the highest dose of 72% for soluble sugars, 71% for reducing sugars, 73% for non-reducing sugars, and 92% for starch (Figure 3a – d) when compared with the control treatment. Changes in the photosynthetic machinery directly reflect the concentration of carbohydrates generated by photosynthesis. These compounds are essential sources of carbon and energy for the growth and development of plants (Wen-Wen et al. 2015). In this research, it was possible to verify that in plants treated with glyphosate, carbohydrate reserves are drastically reduced.

For fluorescence characteristics of chlorophyll *a*, the growth regulator trinexapac-ethyl showed no significant effects between the doses used and the time after application. The evaluated variables showed the following averages over a period of 28 days: F_v/F_m ($\hat{Y} = \bar{Y} = 0.8535$), $\Delta F/F_m'$ ($\hat{Y} = \bar{Y} = 0.3720$), ETR ($\hat{Y} = \bar{Y} = 106.7434$), and NPQ ($\hat{Y} = \bar{Y} = 0.64786$). In plants subjected to glyphosate, we noted reductions in maximum quantum efficiency of PSII (F_v/F_m), effective quantum efficiency of PSII ($\Delta F/F_m'$), and the apparent rate of electron transport (ETR) (Figure 4a – d). More pronounced reductions occurred at 21 and 28 DAA for the ratio F_v/F_m, and from 7 DAA for $\Delta F/F_m'$ and ETR. These changes resulted in an increase in the flow of energy dissipated as heat (non-photochemical), as noted by the most pronounced values of NPQ from 14 DAA.

ATP and NADPH consumption rates are the main factors that determine operational efficiency of PSII in many stressful situations. Changes in carboxylation efficiency, the →

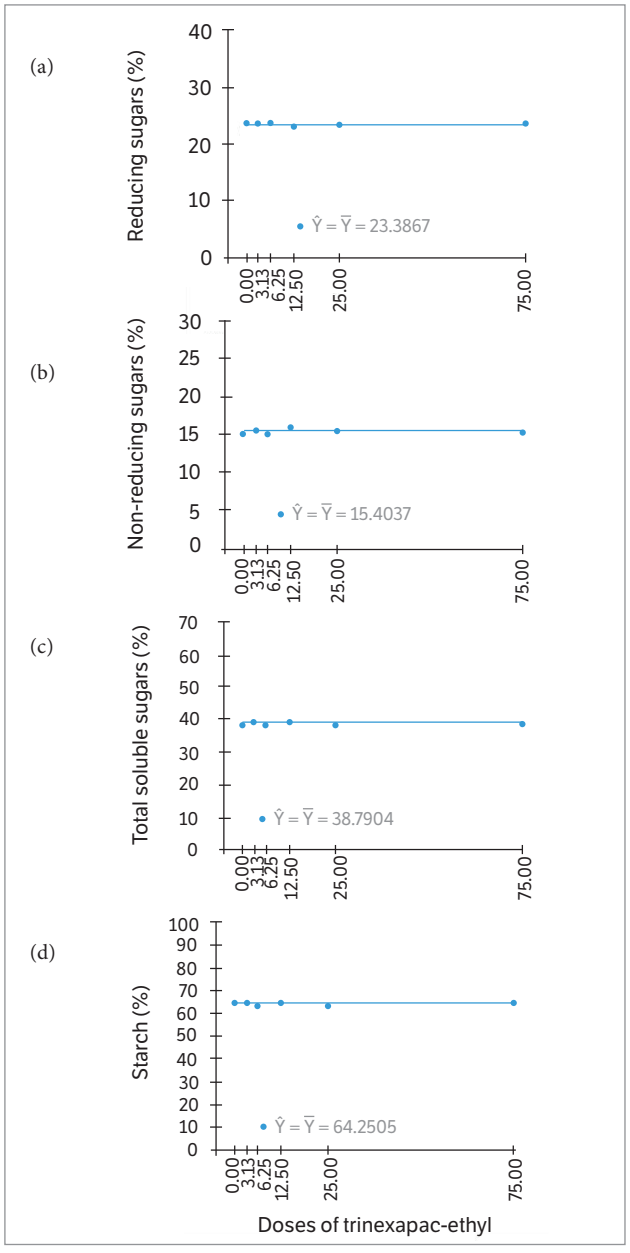

Figure 2. Total soluble sugars (a), non-reducing sugars (b), reducing sugars (c) and starch (d) of sunflower plants treated with increasing doses of trinexapac-ethyl at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01, *p < 0.05.

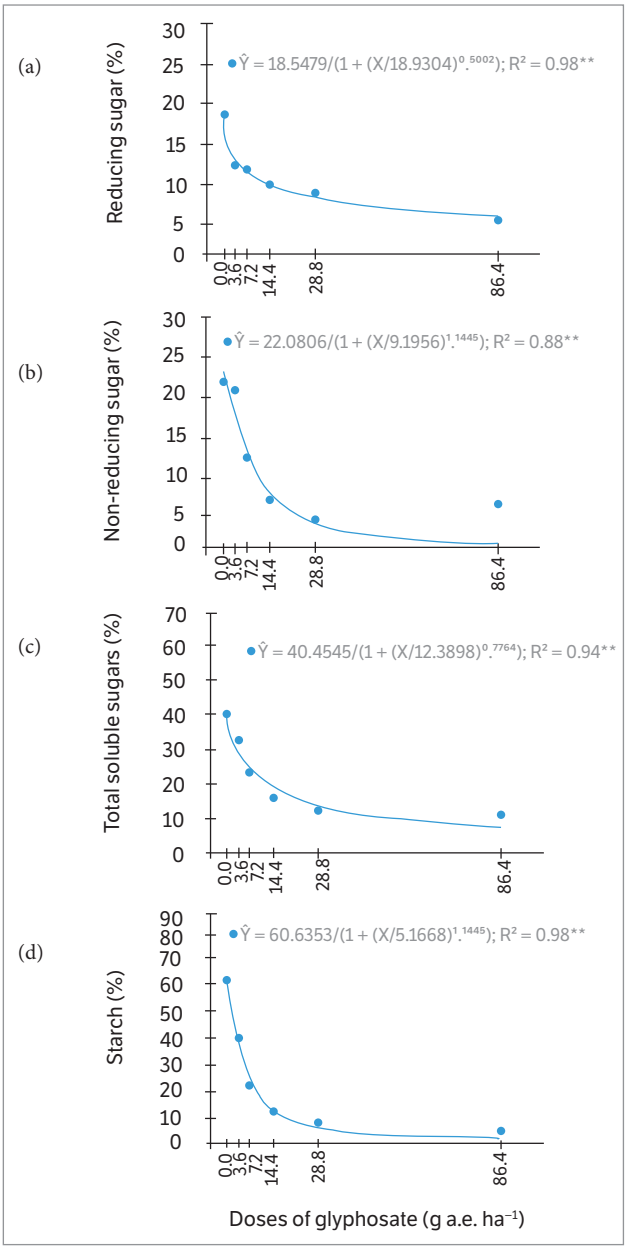

Figure 3. Total soluble sugars (a), non-reducing sugars (b), reducing sugars (c) and starch (d) of sunflower plants treated with increasing doses of glyphosate at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01; *p < 0.05.

supply of CO_2, and carbohydrate transport rate out of the cell can influence ATP and NADPH consumption rates and, consequently, result in reduced PSII rates. Several studies report that glyphosate harms the FSII (Yanniccari et al. 2012). In this study, low values of F_v/F_m, $\Delta F/F_m$', and ETR are indicative of damage to the photosynthetic apparatus. The F_v/F_m ratio represents the maximum efficiency in which light is absorbed by PSII used for the reduction of Q_A, optimal

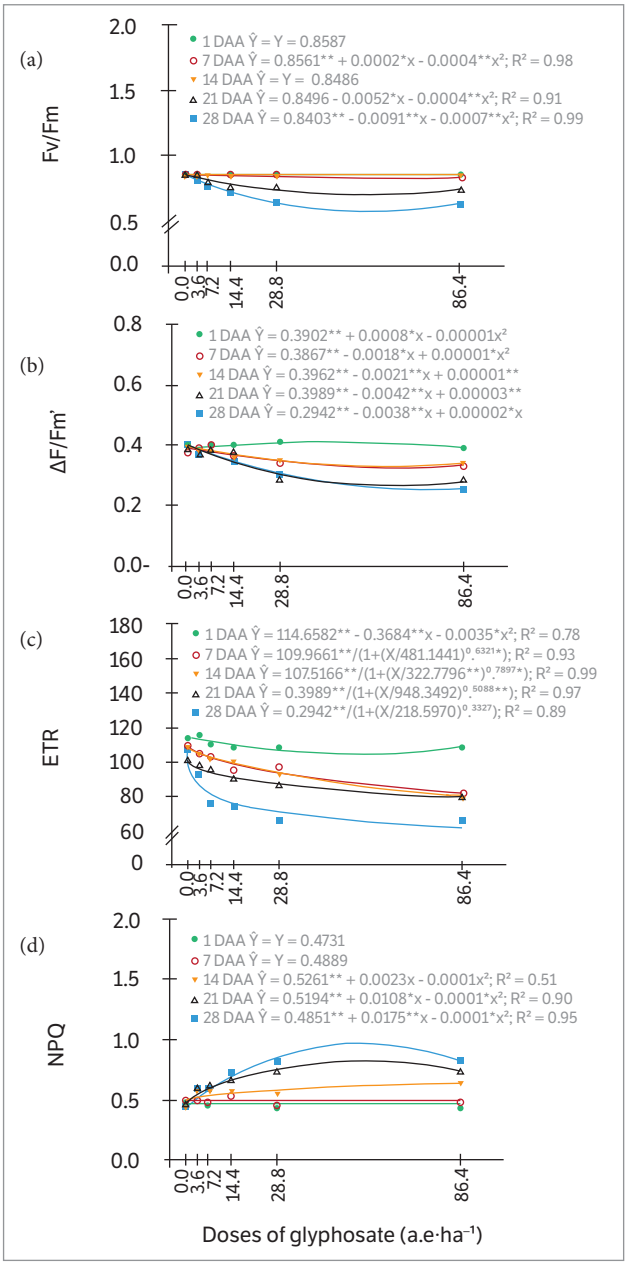

Figure 4. Maximum quantum yield (F_v/F_m) and effective quantum yield of PSII ($\Delta F/F_m$'), electron transport rate (ETR) and non-photochemical quenching (NPQ) of sunflower plants treated with increasing doses of glyphosate (a) – (d) and evaluated at different times. Values are averages (n = 5). Significance: **p < 0.01; *p < 0.05.

values are around 0.75 to 0.85 for most species (Baker 2008). However, decreases in these values are indicative of photo-inhibition or photo-oxidation, as evidenced in this research.

The same was observed in quantum yield data of PSII ($\Delta F/F_m$') and the apparent ETR, indicating that there was a reduction in the amount of energy used by the plant to perform photochemical processes such CO_2 fixation and reduction of NADPH. When the energy from light is not efficiently used in the photochemical step, reactive oxygen species (ROS) that are responsible for the loss of activity of PSII and the degradation of D_1 protein might be produced. Moreover, according Vivancos et al. (2011), glyphosate can inhibit protein synthesis D_1 hurting the repair of PSII. There are 2 possible paths to this end: the first is that the herbicide, after inhibiting $EPSP_S$, reduces the synthesis of aromatic amino acids, thus harming the amino acid pool. This effect subsequently disrupts normal levels of synthesis of a new protein D_1. Another possible mechanism is the increased production of ROS, which also inhibits the synthesis of new proteins D_1 (Takahashi and Murata 2008).

Additionally, according to Maxwell and Johnson (2000), decrease in photosynthetic efficiency is most often accompanied by increasing the NPQ during periods of stress, as observed in this study. The answer to high voltage light in reaction centers is provided by the non-photochemical dissipation. Thus, while minimizing further damage to the centers reactions, part of the absorbed energy can be dissipated in the form of photochemical processes, including thermal dissipation, measured by the NPQ. In this study, the decline in the effective quantum yield of PSII and the apparent rate of electron transport, combined with the increase in NPQ, suggest that most of the excitation energy was dissipated in non-photochemical processes, and this mechanism protects the foil against damage induced by light.

Changes in gas exchange and chlorophyll fluorescence a in this study are in accordance with those found in the concentration of chloroplastid pigments, in that the glyphosate promoted reduction in Cl-*a*, Cl-*b*, and carotenoids. For glyphosate, we observed reductions in the concentration of Cl-*a* (Figure 5a), Cl-*b* (Figure 5b), total chlorophyll (Figure 5c), and carotenoids (Figure 6a). For example, at the highest dose and 86.4 g a.e.·ha^{-1}, the concentration of Cl-*a* decreased by about 45%, Cl-*b* by 38%, total chlorophyll by 43, and carotenoids by 63% compared to the control. For the reason Cl-*a*/Cl-*b* (Figure 6b) significant effects were

not observed. Trinexapac-ethyl did not cause significant decreases in the concentration of chloroplastid pigments (Figure 7a – c and Figure 8a,b). Reduction of pigments is related to the reduction in photosynthesis occasionally by direct of glyphosate in chlorophyll (Zobiole et al. 2010). This is primarily due to the immobilization of Mg and Mn necessary for chlorophyll formation. As this herbicide is a phosphonic acid, it acts as a chelating agent, forming stable complexes with divalent cations and trivalent metals (Zobiole et al. 2010). This chelating effect immobilizes essential micronutrients such as magnesium and manganese, which are necessary cofactors and regulators of physiological functions in plants (Zobiole et al. 2011).

Stress caused by glyphosate resulted in increased MDA concentrations and electrolyte release rates (ERRs). ROS in high concentrations damage cellular components, and MDA is a stable compound formed by lipid peroxidation. In this study, plants subjected to trinexapac-ethyl had increased MDA values (Figure 9b). Therefore, glyphosate has a significant effect on the MDA concentration of sunflower plants (Figure 9), and we observed an increase of 180% at the highest dose compared with the control plants. Similar to MDA, there was no increase in the ERR in plants treated with trinexapac-ethyl (Figure 10b). However, the change in the concentration of MDA in plants treated with glyphosate resulted in an increase in TLE (Figure 10a). MDA is one of the end products of lipid peroxidation. The severity of damage can occur at various levels, ranging from localized reductions in membrane fluidity to total rupture (Halliwell and Gutteridge 2005). Thus, a direct consequence of damage to cell membranes by lipid peroxidation is the extravasation of cellular contents to the center which is wound around the damaged tissue when the damage is enough for both (Kruse et al. 2006) as observed in this study.

The leaf is the main organ involved in the penetration of herbicides, and this statement was verified by the reduction of the characteristics evaluated, especially for glyphosate. To confirm the effect of glyphosate on sunflower

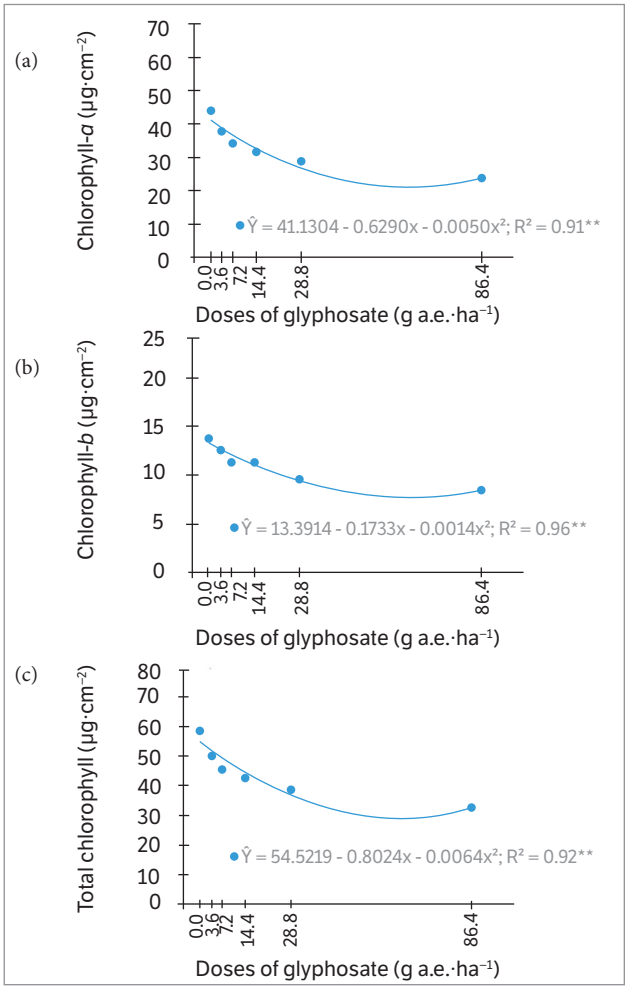

Figure 5. Concentration of chlorophyll-*a* (a), chlorophyll-*b* (b) and total chlorophyll (c) of sunflower plants, treated with increasing doses of glyphosate and evaluated at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01; *p < 0.05.

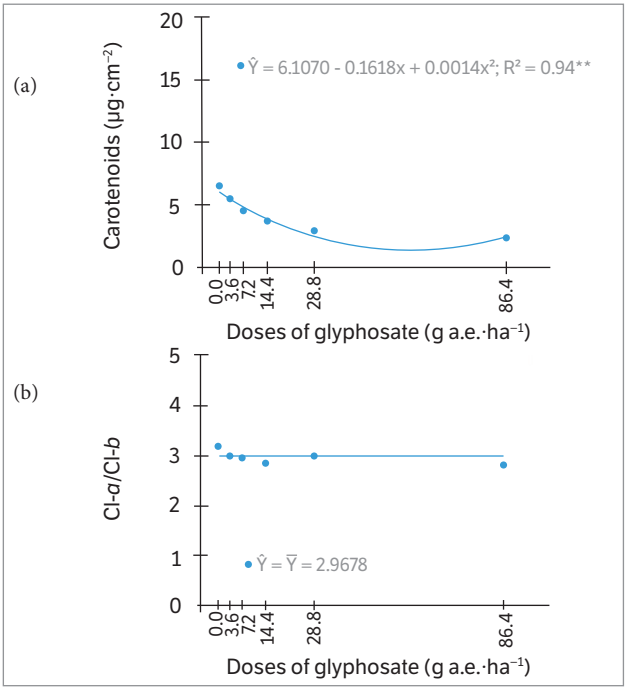

Figure 6. Concentration of carotenoids (a) and ratio Cl-*a*/Cl-*b* (b) of sunflower plants with increasing doses of glyphosate and evaluated at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01; *p < 0.05.

plants, we assessed the concentration of shikimic acid (Figure 11). Glyphosate inhibits the action of the enzyme EPSPs and carries the shikimic acid accumulation in plant tissues (Velini et al. 2009), which compromises the biosynthesis of the amino acids phenylalanine, tyrosine, and tryptophan. In this study, the concentration of shikimic acid, mainly in the dose of 86.4 g·ha^{-1}, increased by 169% compared with the control. Due to the decrease of the evaluated characteristics, we also visually observed injuries in the leaves, possibly due to the imbalance caused by the herbicide absorption cuticle sheet. The lack of effect of trinexapac-ethyl may be related to cuticular absorption and plant detoxification mechanisms. Future studies are needed to examine the mechanisms by which plants prevent being impacted by growth regulators.

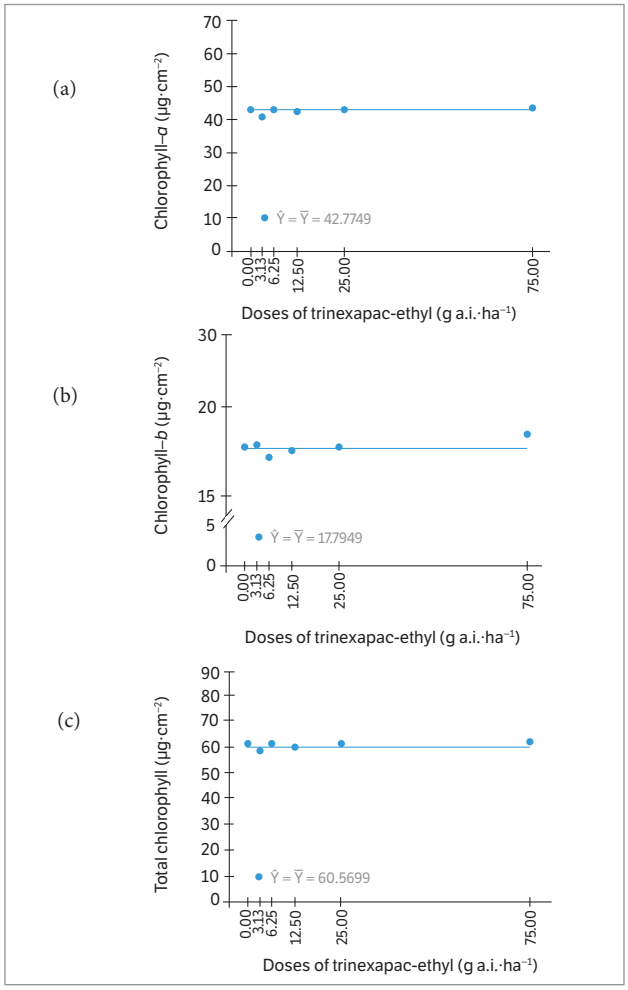

Figure 7. Concentration of chlorophyll-*a* (a), chlorophyll-*b* (b) and total chlorophyll (c) of sunflower plants, treated with increasing doses of trinexapac-ethyl and evaluated at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01; *p < 0.05.

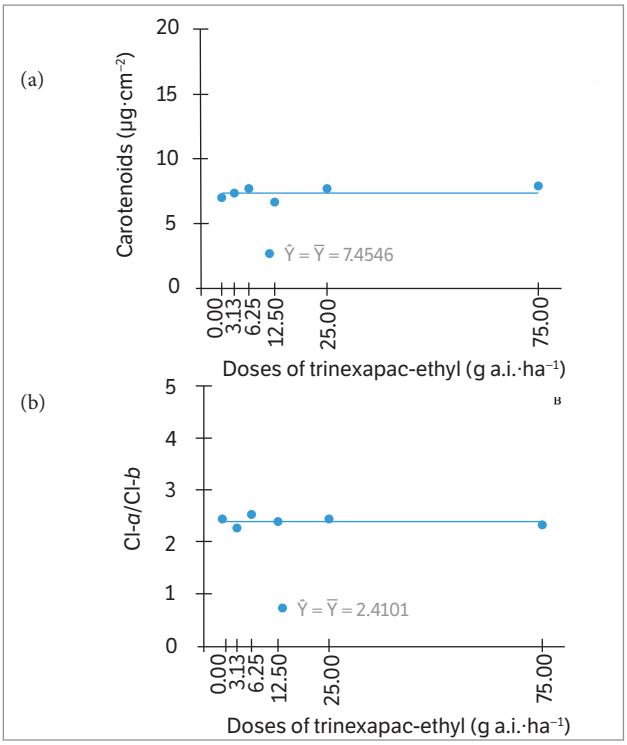

Figure 8. Concentration of carotenoids (a) and ratio Cl-*a*/Cl-*b* (b) of sunflower plants treated with increasing doses of trinexapac-ethyl and evaluated at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01; *p < 0.05.

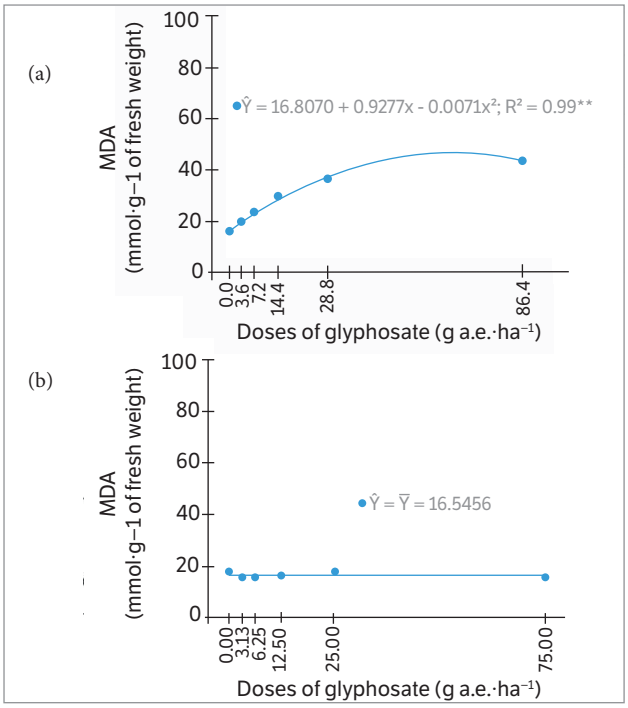

Figure 9. Concentration of malondialdehyde (MDA) in sunflower plants treated with increasing doses of glyphosate (a) and trinexapac-ethyl (b) and evaluated at 28 days after application. Values are averages (n = 30). **p < 0.01; *p < 0.05.

Additionally, the data from this study suggest that glyphosate should be used with utmost care, because, at low concentrations, it can cause changes in plant physiology. In addition, it has the potential to alter gene expression and metabolic pathways and damage proteins, plant development and, consequently, induce the decrease of antioxidant defenses or cause immediate oxidative damage to organisms (Valavanidis et al. 2006).

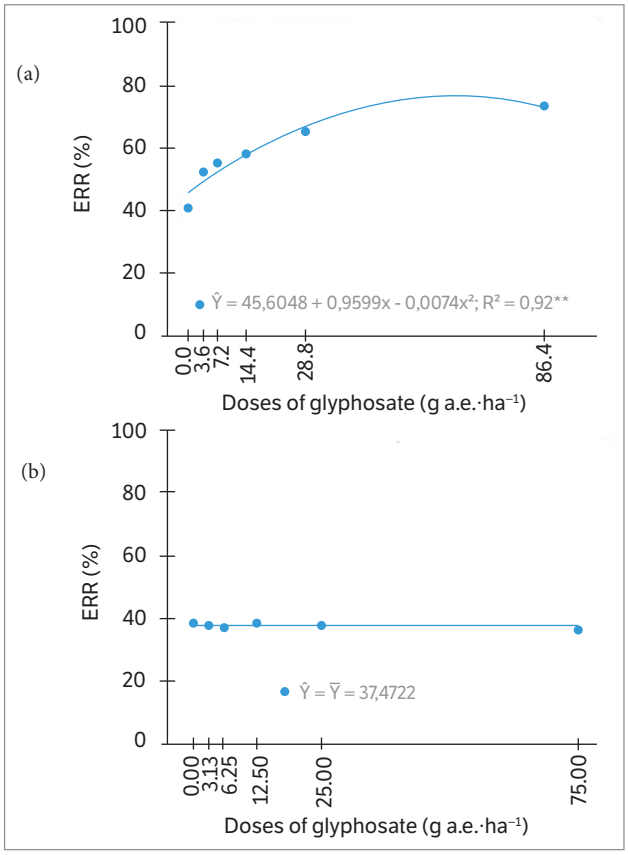

Figure 10. Electrolytes release rate (ERR) in sunflower plants treated with increasing doses of glyphosate (a) and trinexapac-ethyl (b) evaluated at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01; *p < 0.05.

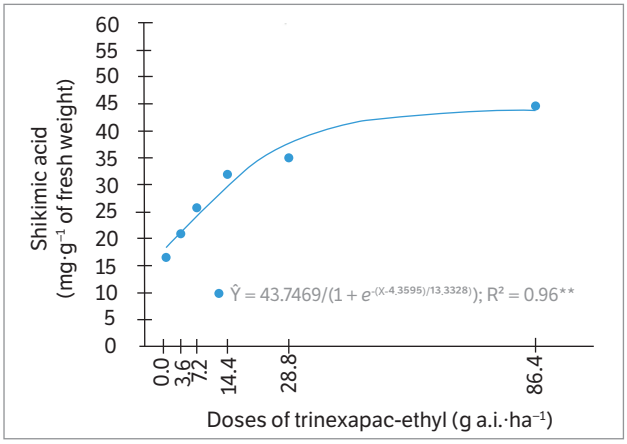

Figure 11. Concentration of shikimic acid in sunflower plants treated with increasing doses of glyphosate and evaluated at 28 days after application. Values are averages (n = 30). Significance: **p < 0.01; *p < 0.05.

CONCLUSION

The photosynthetic metabolism of sunflower plants is not affected by the growth regulator trinexapac-ethyl. However, with the application of glyphosate, plants suffered changes in the photosynthetic apparatus, with reduction in the concentration of carbohydrates and chloroplastid pigments and subsequent damage to cell membranes.

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