



Article

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SUNFLOWER PLANT RESPONSE TO SIMULATED DRIFT OF GLYPHOSATE AND TRINEXAPAC-ETHYL

Respostas de Plantas de Girassol à Deriva Simulada de Glyphosate e Trinexapac-Ethyl

ABSTRACT - Chemical ripeners are frequently used on sugarcane crops in order to increase profitability. However, the drift of these products to neighbouring fields can expose susceptible non-target plants, such as sunflower, to these agents causing indirect impacts on growth and development. Therefore, this study aimed to assess the toxicity characterize possible changes in the growth of sunflower plants exposed to simulated drift of the ripeners glyphosate and trinexapac-ethyl. For each ripener, the following doses were used: glyphosate (0 (control), 3.6, 7.2, 14.4, 28.8 and 86.4 g a.e ha⁻¹) and trinexapac-ethyl (0, 3.12, 6.25, 12.50, 25 and 75 g a.i ha⁻¹). The effects of these products on plant height, number of leaves, leaf area, stem diameter and dry matter (leaves, stem, flower, root, shoot and total) were evaluated. We also conducted a visual analysis of symptoms of phytotoxicity after exposure. The effects of the ripeners on sunflower plants varied depending on the type of agent used and the dose. Plants treated with glyphosate showed altered growth patterns, indicating high sensitivity of these plants to the herbicide. With increasing doses, glyphosate drift promoted lower growth and development of sunflower plants and negatively affected productivity. Nevertheless, trinexapac-ethyl drift, in the evaluated doses, did not affect sunflower plants.

Keywords: *Helianthus annuus*, phytotoxicity, herbicide, growth regulator.

RESUMO - Atualmente, na cultura da cana-de-açúcar, têm-se utilizado com frequência maturadores químicos, de forma a aumentar sua rentabilidade. No entanto, a deriva desses produtos pode expor plantas suscetíveis não alvo, como o girassol, impactando indiretamente o crescimento e o desenvolvimento da cultura. Portanto, objetivou-se neste estudo avaliar a intoxicação e caracterizar possíveis alterações no crescimento de plantas de girassol expostas à deriva simulada de glyphosate e trinexapac-ethyl. Para cada maturador, foram utilizadas as seguintes doses: glyphosate (0 – controle; 3,6; 7,2; 14,4; 28,8; e 86,4 g e.a ha⁻¹); e trinexapac-ethyl: (0 – controle; 3,12; 6,25; 12,50; 25; e 75 g i.a ha⁻¹). Foram avaliados os efeitos desses produtos sobre a altura das plantas, número de folhas, área foliar, diâmetro de caule, matéria seca (folhas, caule, flores, raiz, parte aérea e total), além de análises visuais de sintomas de fitotoxicidade. Plantas de girassol apresentaram efeitos distintos em relação aos maturadores e doses; plantas tratadas com glyphosate apresentaram seu padrão de crescimento alterado, indicando sua alta sensibilidade ao herbicida. Com o aumento das doses, os resultados demonstraram que a deriva do glyphosate promoveu menor crescimento e desenvolvimento das plantas, podendo afetar negativamente sua produtividade. Contudo, a deriva do trinexapac-ethyl, nas doses avaliadas, não afetou as plantas de girassol.

Palavras-chave: *Helianthus annuus*, fitotoxicidade, herbicida, regulador de crescimento.

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INTRODUCTION

The sunflower (*Helianthus annuus*) is native to North America but, given that its productivity is only minimally influenced by conditions such as latitude, altitude and photoperiod, this plant is also cultivated in many regions around the world (Souza et al., 2010). The sunflower plant is considered of great commercial importance, mainly for oil production (Elezovic et al., 2012). In Brazil, its cultivation covers an area of approximately 119,400 hectares, with production estimated at 183,000 tons for the 2014/2015 harvest (Conab, 2015).

The sunflower is presented as a “Safrinha” (off-season) cultivation option for the Brazilian Midwest, since this crop has increased tolerance to drought stress. It is also a popular choice for oil production because it reduces idleness of beneficiary industries, optimizes the use of land, machinery and manpower, generating income and jobs (Capone et al., 2011). However, sunflower production areas may be located near areas of sugarcane cultivation where the intensive use of herbicides for weed control (Lourencetti et al., 2008) and artificial ripeners to obtain higher yields (Toppa et al., 2014) can affect the development of neighbouring plants. The transportation of molecules of these products to the adjacent areas may occur either through drift, after application using the sprayers attached to tractors or aircraft, or via leaching, runoff, volatilization or erosion/entrainment (Nunes et al., 2009).

The use of ripeners in the culture of sugar cane, promotes morphological and physiological changes in the plant (Meschede et al., 2012), which may result in a reduction in growth, enabling increments in sucrose content, precocity of maturation and an increase in productivity (Siqueira, 2014). Two of the most commonly used ripeners are glyphosate and trinexapac-ethyl. Trinexapac-ethyl operates in the synthesis of gibberellins (GA), after training of GA₁₂ aldehyde, inhibiting the 3-β-hydroxylation, preventing the synthesis of GAs of high biological activity, especially the GA₁, one of the most efficient in promoting cell elongation. After absorption, its translocation is quick, and plants can exhibit symptoms of growth inhibition within 48 hours after application (Rajala, 2003). Moreover, glyphosate slows the development of the plant by inhibiting the synthesis of 3-indole acetic acid, which is synthesized in the meristematic tissues. The plant then undergoes stress and begins to synthesize the ethylene and consequently speeds up the process of maturation by increasing the content of sucrose in the culm more consistently (Meschede et al., 2012).

The application of ripeners to sugarcane crops occurs mainly from October to December and from January to April, which represent the periods of highest risk for transporting fruit offsite (Masters et al., 2013). Moreover the period from January to April coincides with the cultivation of sunflower crops, whose proximity enhances the drift of ripeners, causing significant losses in the vegetative cultivation and reproductive stages of sunflower. Studies on the effects of ripeners on the morphological characteristics of sunflower plants still are incipient. Therefore, in this study we evaluated the hypothesis that *Helianthus annuus* plants subjected to simulated drift of trinexapac-ethyl and glyphosate ripeners will present morphological changes, which may result in lower productivity. This experiment aimed to evaluate the toxicity and characterize possible changes in the growth of sunflower plants exposed to simulated drift of glyphosate and trinexapac-ethyl.

MATERIALS AND METHODS

The experiments were conducted in the Federal Institute of Goiás (IF Goiano) Campus Rio Verde - Goiás State, Brazil, under controlled conditions, in an acclimatized greenhouse, during the months of October 2013 to January 2014. The temperature in the acclimatized greenhouse ranged between 22 to 29 °C and the relative humidity between 60 and 75%.

Plant material and experimental conditions

Sunflower plants (*Helianthus annuus*) were cultivated in polyethylene pots containing 8 kg of substrate prepared with soil (dystrophic red oxisol) and sand (2:1). According to the chemical analysis of the soil, the substrate had the following characteristics: pH (H₂O) 4.7; 2.6 mg dm⁻³ P; 14 mg dm⁻³ K; 0.75 cmol_c dm⁻³ Ca; 0.22 cmol_c dm⁻³ H⁺Al; 13 g kg⁻¹ of organic matter and 24.4%

base saturation. The correction and fertilization of the substrate were carried out according to soil analysis and the nutritional requirements of culture (Ribeiro et al., 1999). Two plants, standardized in size and vigour, were grown per pot and irrigated daily.

Treatment imposition

Two independent experiments were conducted to evaluate the effects of glyphosate (480 g L⁻¹ acid equivalent) and trinexapac-ethyl (250 g L⁻¹ of active ingredient) on sunflower plants. The following concentrations were used, with the aim of achieving the simulated drift of each product: 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), corresponding to 0%; 1.25%; 2.5%; 5%; 10% and 30% of the dose recommended in the field. The applications were performed 30 days after the emergency, using a costal sprayer (Herbicat® Catanduva, Brazil) with constant pressure maintained by compressed CO₂, equipped with a bar with four spray tips and nozzles (Teejet), fan type model XR 110 02. The operating pressure used was 5 kgf cm⁻², providing a spray volume of 180 L ha⁻¹.

Morphological evaluation

Growth analyses were conducted for the following characteristics: plant height (cm), number of leaves, leaf area index (cm²), stem diameter (mm) and number of nodes. For both glyphosate, and trinexapac-ethyl, the evaluations were performed at 7, 14, 21 and 28 days after application (DAA). A graduated rule was used to determine the height of sunflower plants. The stem diameter was measured using a caliper rule and the leaf area was measured from the leaf blade across the width of all leaves of the plant and adjusted according to the regression model $AF = 1,7582L^{1,7067}$ (Maldane et al., 2009).

Visual evaluations and phytotoxicity

For visual evaluations, plants were photographed using a digital camera (Finepix SL 300), 30x optical zoom, 14 megapixels, high resolution (LCD) at 7 and 28 DAA. The phytotoxicity evaluations were performed at 7, 14, 21 and 28 DAA using the methodology proposed by ALAM (1974). This assessment is a percentage rating scale ranging from 0 to 100, in which 0 implies the absence of any injuries and 100 indicates the death of the plant.

Biomass evaluations

At 28 days after the chemical ripening application (DA), the plants were collected to obtain the following measurements: dry matter of leaves (DML), stem (DMS), flowers (DMFL), root (DMR), shoot (DMSH) and total dry matter (TDM). The different parts of the plants were separated into paper bags and placed in an oven with forced ventilation at 65 °C for 72 hours to obtain a constant dry weight. The dried material was then weighed and the result expressed in grams per plant.

Statistical analysis

The experiments were conducted in a randomized block design, in a split plot arrangement, with each plot allocated a different dose of glyphosate or trinexapac-ethyl and the subplots harvested for different times of assessments. Five replicates (n = 5) were conducted for each condition. The data obtained were submitted to analysis of variance (ANOVA) and adjusted for logistic regression models. Statistical analyses were performed by means of the *software* SISVAR version 5.3.

RESULTS AND DISCUSSION

After analyzing the effects of two different ripeners on sunflower plants, we observed that treatments with the growth regulator trinexapac-ethyl did not interfere in the morphological characteristics of plants. However, glyphosate negatively influenced the growth pattern of

sunflower plants, both in terms of doses as a function of time after application (Figure 1A, C and Figure 2A, B), indicating higher sensitivity of sunflower plants to glyphosate.

The study of morphological characteristics has great importance as it directly influences the plant architecture (Silva et al., 2010). Thus, plant height, number of leaves, leaf area, stem diameter, number of nodes and dry mass of the plant can directly influence the patterns of culture and the productivity of sunflower plants. These features enable not only assess plant conditions as a function of time but also what are the influences exerted by the stress factor (Cruz et al., 2010).

Sunflower plants treated with glyphosate showed the most prominent reductions at the dose of 86.4 g a.e ha⁻¹, 28 (DAA). Under these conditions, there was a 55% decrease in plant height (Figure 1A), 50.47% decrease in the number of leaves per plant (Figure 1B), 90% decrease in the leaf area (Figure 1C), 26.92% decrease in stem diameter (Figure 2A) and 47.66% decrease in the number of nodes (Figure 2B).

In Tables 1 and 2, the regression equations are presented adjusted to morphological characteristics, depending on the ripeners doses of glyphosate and days after application. These characteristics, linear and quadratic models were adjusted in the days after application, with reductions due to the increase of doses.

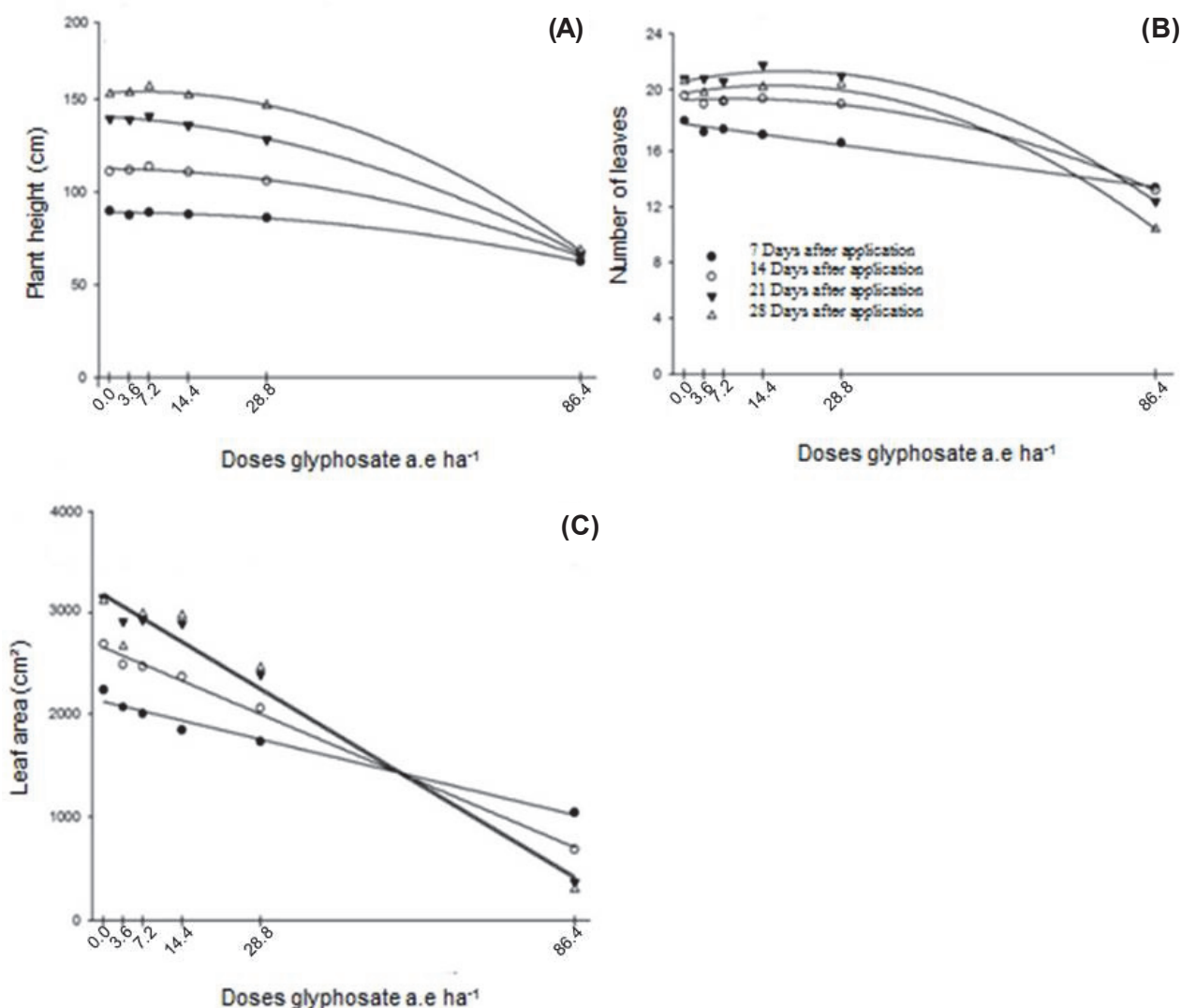


Figure 1 - Plant Height (cm) (A), the number of sheets (B), and leaf area (cm²) (C) sunflower plants subjected to increasing levels of glyphosate and evaluated at 7, 14, 21 and 28 days after application DAA.

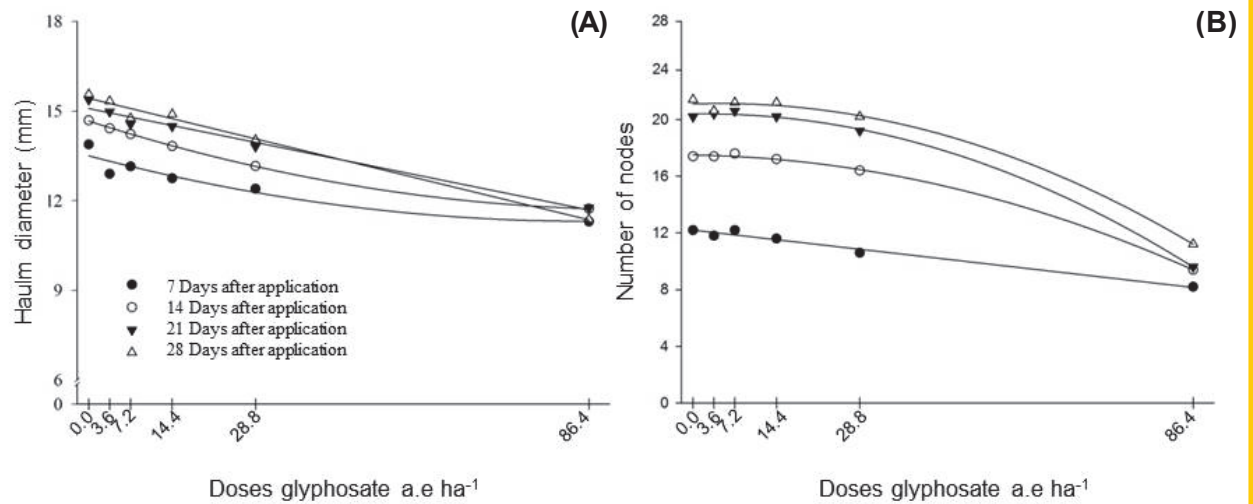


Figure 2 - Haulm diameter (A) and number of nodes (B) of sunflower plants exposed to increasing doses of glyphosate and evaluated at 7, 14, 21 and 28 days after application DAA.

Table 1 - Regression equations adjusted to plant height, leaf number and leaf area of sunflower plants exposed to increasing doses of glyphosate and evaluated at 7, 14, 21 and 28 days after application, referring to Figure 1

| Characteristic | Unit | Adjusted equations | R ² |
|------------------------------|--------|--|----------------|
| Plant height (cm) | 7 DAA | $\hat{Y} = 88.7277 + 0.0356x - 0.0039x^2$ | 0.99** |
| | 14 DAA | $\hat{Y} = 112.3866 - 0.0302x - 0.0059x^2$ | 0.99** |
| | 21 DAA | $\hat{Y} = 140.4787 - 0.1838x - 0.0077x^2$ | 0.99** |
| | 28 DAA | $\hat{Y} = 153.6205 + 0.1433x - 0.0131x^2$ | 0.99** |
| Number of leaves | 7 DAA | $\hat{Y} = 17,9584 - 0.0523x$ | 0.98** |
| | 14 DAA | $\hat{Y} = 19.6596 + 0.0205x - 0.0011x^2$ | 0.99** |
| | 21 DAA | $\hat{Y} = 21.0233 + 0.0766x - 0.0020x^2$ | 0.99** |
| | 28 DAA | $\hat{Y} = 20.1613 + 0.0677x - 0.0021x^2$ | 0.97** |
| Leaf area (cm ²) | 7 DAA | $\hat{Y} = 2115.8309 - 12.6607x$ | 0.97** |
| | 14 DAA | $\hat{Y} = 2640.4476 - 22.3565x$ | 0.99** |
| | 21 DAA | $\hat{Y} = 3160.5660 - 31.5752x$ | 0.98** |
| | 28 DAA | $\hat{Y} = 3137.2785 - 31.5999x$ | 0.95** |

Table 2 - Regression equations adjusted for stem diameter and number of nodes of sunflower plants exposed to increasing doses of glyphosate and evaluated at 7, 14, 21 and 28 days after application, referring to Figure 2

| Characteristic | Unit | Adjusted equations | R ² |
|---------------------|--------|---|----------------|
| Diameter haulm (mm) | 7 DAA | $\hat{Y} = 13.5005 - 0.0511x + 0.0003x^2$ | 0.90** |
| | 14 DAA | $\hat{Y} = 14.6630 - 0.0619x + 0.0003x^2$ | 0.99** |
| | 21 DAA | $\hat{Y} = 15.0819 - 0.0392x$ | 0.97** |
| | 28 DAA | $\hat{Y} = 15.4252 - 0.0471x$ | 0.98** |
| Number of Nodes | 7 DAA | $\hat{Y} = 12.1958 - 0.0468x$ | 0.97** |
| | 14 DAA | $\hat{Y} = 17.4959 - 0.0067x - 0.0010x^2$ | 0.99** |
| | 21 DAA | $\hat{Y} = 20.3897 - 0.0062x - 0.0015x^2$ | 0.99** |
| | 28 DAA | $\hat{Y} = 1.0985 + 0.0138x - 0.00148x^2$ | 0.99** |

The reduction in the plant height is directly related to the mechanism of action of glyphosate, which after being absorbed acts by inhibiting the activity of the enzyme 5-enolpiruvilchiquimato-3-phosphate synthase (EPSPs). This enzyme, which catalyzes the reaction in which the shikimate-3-phosphate (S3P) reacts with the phosphoenolpyruvate (PEP) forming the 5-enolpiruvilchiquimato-

3-phosphate (EPSP) and inorganic phosphate (P_i) (França, 2009). As a result the pathway of shikimate is interrupted thereby inhibiting the synthesis of tryptophan, phenylalanine and tyrosine, in addition to essential amino acids the protein synthesis in the apical meristems, resulting in the interruption of development (Silva et al., 2012). Yamashita et al. (2009), for example, observed a strong reduction in plant height and symptoms of toxicity in the apical meristem of *Schizolobium amazonicum* and *Ceiba petandra* after exposure to simulated drift of glyphosate.

In addition to the effects of glyphosate on the height of sunflower plants, the reduction in the number of leaves and leaf area directly influences the production of achenes, mainly to maintain a direct relation with the nutritional status of the plant and the production of assimilates (Zobiolo et al., 2010). The leaf is the primary photosynthetic active body, accumulating nutrients and organic compounds which are then translocated to the reproductive organs and grains. Thus, changes in leaf structure can affect both the vegetative and reproductive of state the plant (Castro and Farias, 2005).

Another important characteristic for sunflower cultivation is the stem diameter, being your good development allows occurs less lodging, facilitating its handling, treatment and harvesting (Alves et al., 2010). In this study, even though a noticeable reduction in stem diameter was observed in plants treated with glyphosate, the values, which ranged from 10 to 80 mm, were found to be within normal ranges (Castro and Farias, 2005). According to Naves (1993), the stem diameter has a closer relationship with photosynthesis than the growth in height, as is directly dependent on the accumulation of carbohydrates and favourable balance between net photosynthesis and respiration. During the vegetative stage, the accumulation of photoassimilates in the stem allows better allocation of carbohydrates to reproductive organs, in the case of sunflower, for greater production of achenes.

Contrary to the differences observed in sunflower plants treated with glyphosate, there was no interaction between the dose of the ripener trinexapac-ethyl and the exposure time of the plants. Being significant only in function of time demonstrating the growth of the plant in the vegetative cycle relation (Figure 3A, B and C). According to the time, we observed a 78% increase in plant height (Figure 3A), a 13% increase in the number of leaves (Figure 3B), 56% increase in leaf area (Figure 3C), 19% increase in stem diameter (Figure 4A) and 72% increase in the number of nodes (Figure 2B).

Zagonel and Ferreira (2013) demonstrated a similar absence of response of morphological characteristics to trinexapac-ethyl in certain corn hybrids. Also according to the author, the response absence occurred because the product did not substantially affect the height of plants and other morphological characteristics of the plant. Also, Alvarez et al. (2007) found no changes in growth characteristics in some cultivars of rice subjected to various doses of plant growth regulators. These data reinforce the idea that the effect of trinexapac-ethyl is dependent on the timing and application of doses (Dunand, 2003) and grow crops (Rajala and Peltonen-Sainio, 2001).

The phytotoxicity caused by glyphosate in sunflower plants included noticeable symptoms of poisoning beginning at 7 DAA (Figure 5), which severely damaged the plants. Higher percentages of intoxication were observed at a dose of 86.4 g a.e ha⁻¹ at all evaluated times. However, particularly at 14 DAA, at doses up to 28.8 g a.e ha⁻¹, reductions in intoxication were observed (Figure 5), only this possible recovery was not enough to prevent visual symptoms as yellowing and chlorosis progressing to necrosis (Figure 6). In addition, the reduction in plant height, the formations of the chapter and death of the shoot apical meristem (Figure 7).

After studying the effect of application of glyphosate on coffee plants, França (2009) observed a reduction in leaf nitrogen, phosphorus and potash, which are all essential nutrients for plant development. As the roots of sensitive plants exposed to this herbicide becomes functionless possibly greatly influence the absorption of water and nutrients. Reductions in the concentration of nutrients such as nitrogen induce susceptibility of plants to oxidative stress. This is mainly due to the malformation of chlorophyll, which requires nitrogen and magnesium for its proper formation. In their absence, the reduction in chlorophyll leads to chlorosis and necrosis, especially sheets expanding, as evidenced in this study.

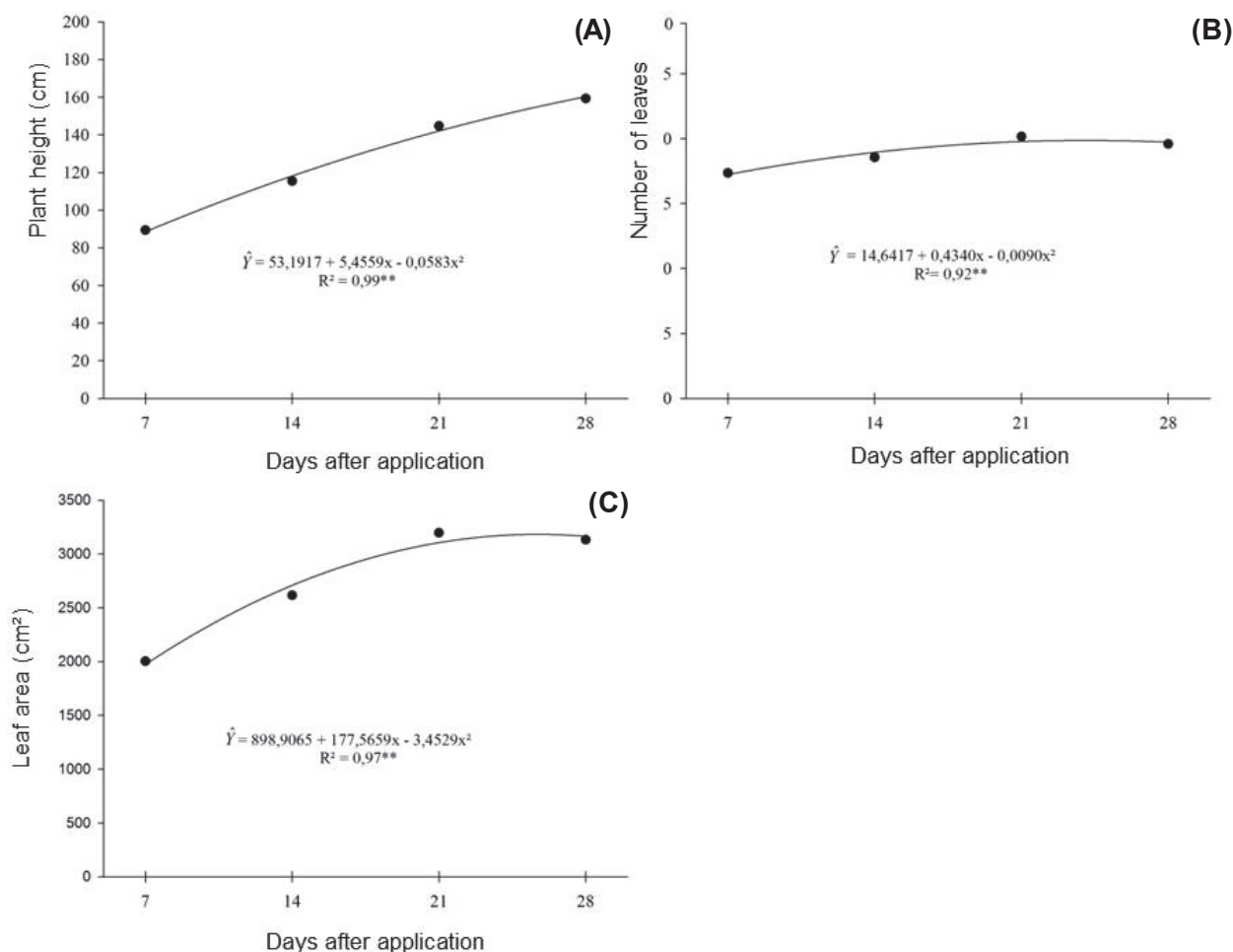


Figure 3 - Plant height (cm) (A), the number of sheets (B), and leaf area (cm²) (C) sunflower plants evaluated at 7, 14, 21 and 28 days after application DAA of trinexapac-ethyl.

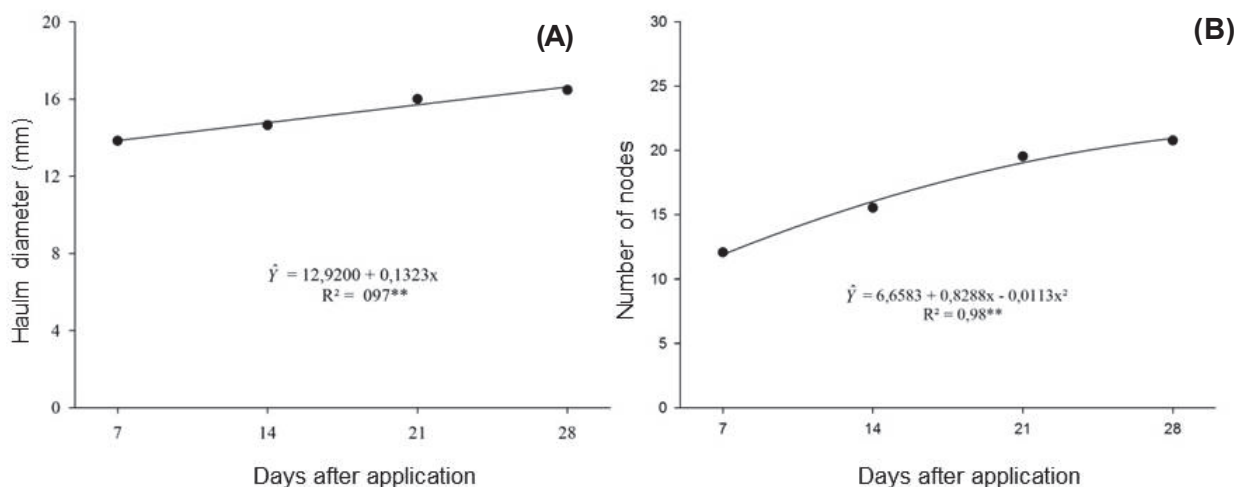


Figure 4 - Diameter of the haulm (A) and the number of nodes (B) of sunflower plants evaluated at 7, 14, 21 and 28 days after application DAA of trinexapac-ethyl.

Consistent with the data obtained in this work, yellowing symptoms, followed by chlorosis and necrosis, have also been demonstrated in cotton (Yamashita and Guimaraes, 2006), with poisoning occurring faster in younger plants and at the highest dose of glyphosate, and in some cases resulting in the death of the plant. In addition to the morphological results, Figures 8 and

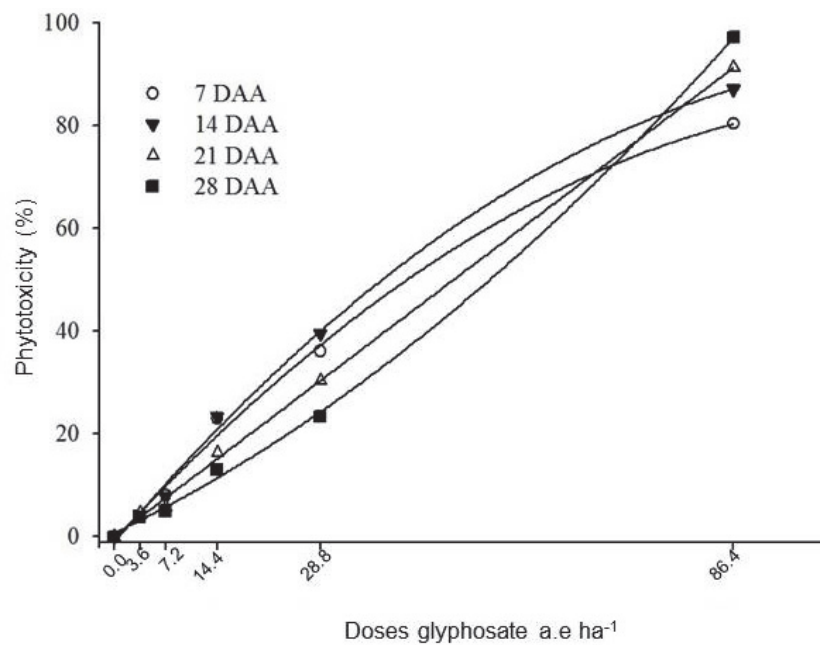


Figure 5 - Phytotoxicity (%) in sunflower plants exposed to increasing doses of glyphosate and evaluated at five different times.



Figure 6 - Visual symptoms of the aerial part of sunflower plants subject to different doses of the herbicide glyphosate 7 days after treatment application.

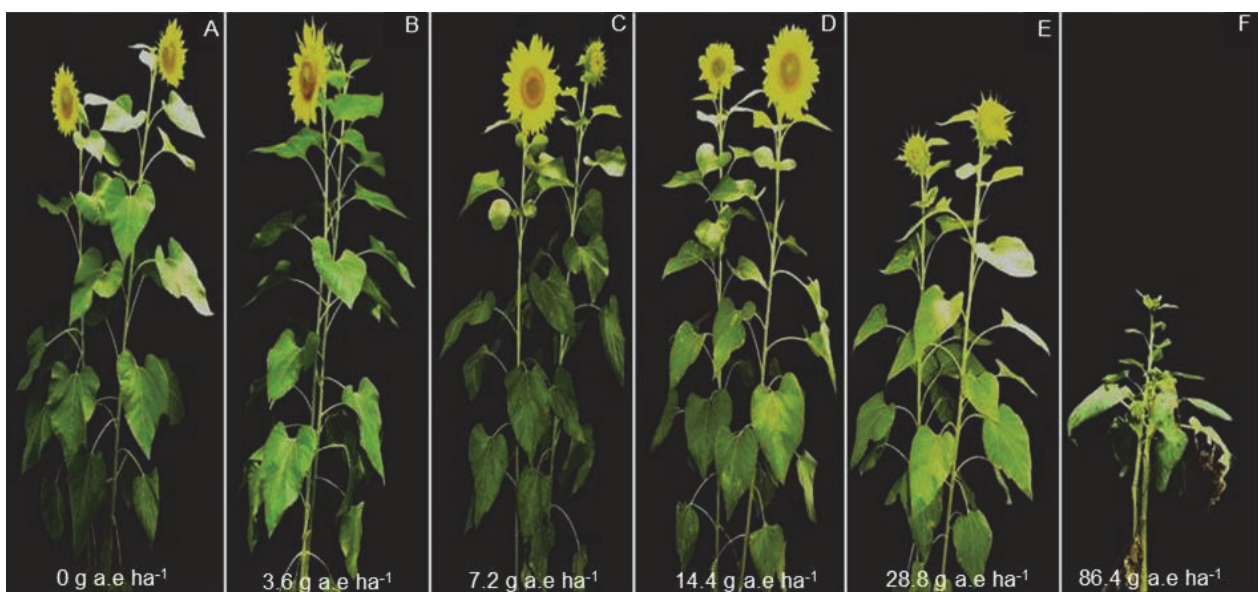


Figure 7 - Visual symptoms of sunflower plants exposed to different doses of the herbicide glyphosate 28 days after treatment application.

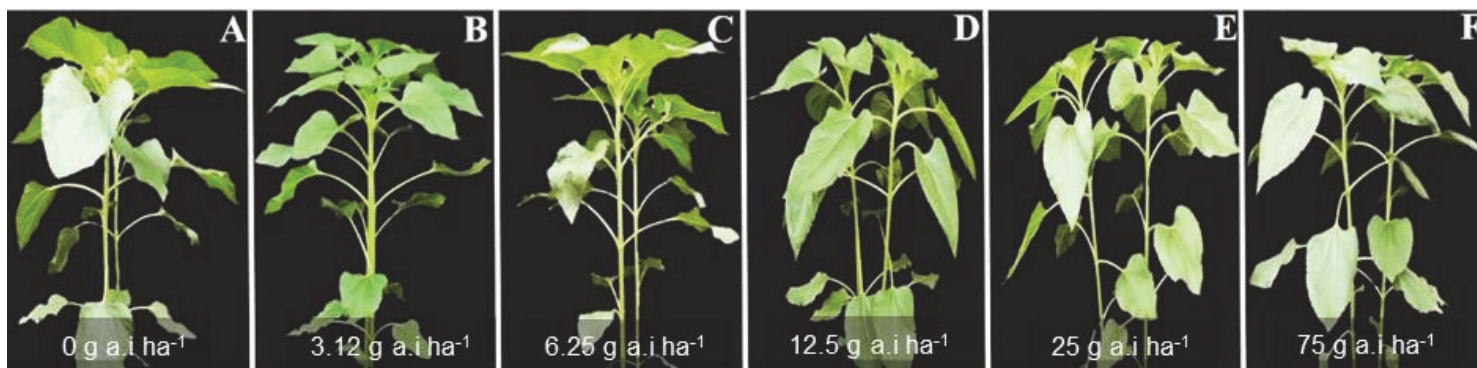


Figure 8 - Visual symptoms of sunflower plants exposed to different doses of the herbicide trinexapac-ethyl at 7 days after treatment application.

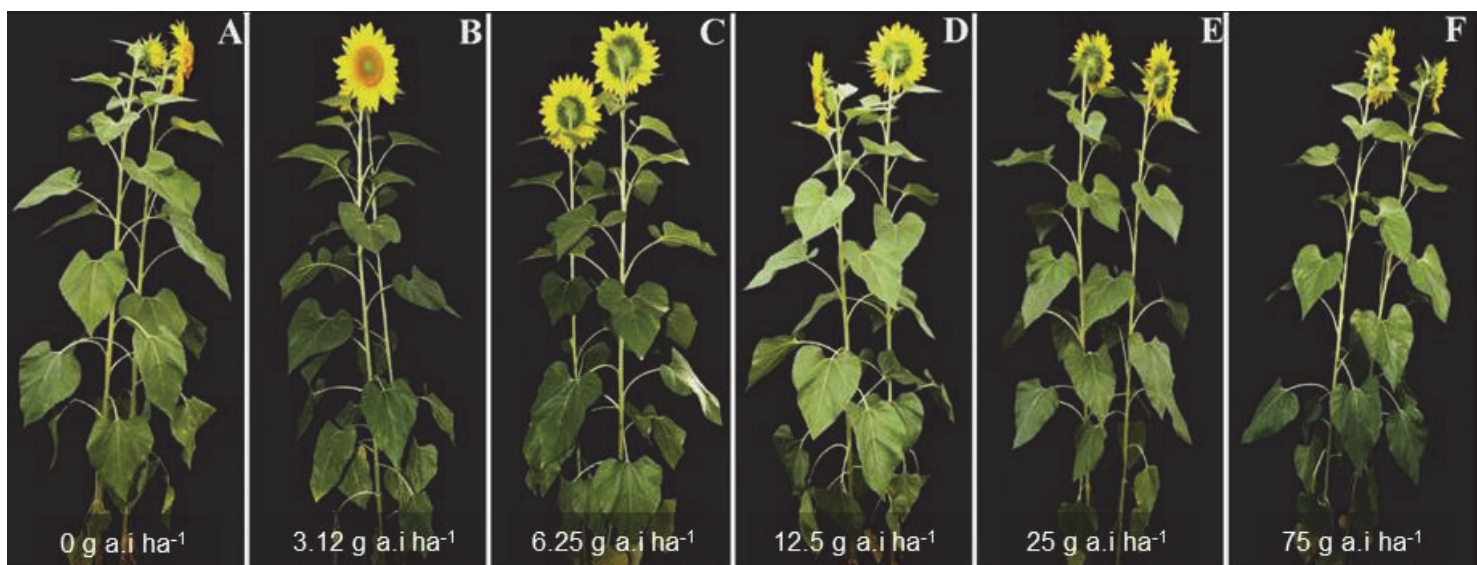


Figure 9 - Visual symptoms of sunflower plants exposed to different doses of trinexapac-ethyl herbicide at 28 days after treatment application.

Table 3 - Regression equations adjusted to phytotoxicity of sunflower plants subjected to increasing levels of glyphosate and evaluated in five different times, referring to Figure 5

| Characteristic | Unit | Adjusted equations | R ² |
|-------------------|--------|---|----------------|
| Phytotoxicity (%) | 7 DAA | $\hat{Y} = -0.6874 + 1.5115x - 0.0066x^2$ | 0.99** |
| | 14 DAA | $\hat{Y} = -1.0744 + 1.6286x - 0.0070x^2$ | 0.99** |
| | 21 DAA | $\hat{Y} = -0.0694 + 1.0571x$ | 0.99** |
| | 28 DAA | $\hat{Y} = 0.6692 + 0.6721x + 0.0051x^2$ | 0.99** |

9 indicate the absence of symptoms in sunflower plants subjected to trinexapac-ethyl, which may have occurred due to the low concentrations of the regulator applied. Maciel et al. (2010) reported that the application of trinexapac-ethyl on emerald grass at a dose of 250 g a.i ha⁻¹ promoted necrosis at the edges of the leaf blade, which was not observed in the control.

The lower growth and development of sunflower plants, in addition to the glyphosate phytotoxicity, most directly affect the formation of biomass. Decreases in the production of dry matter in leaves (Figure 10A), shoots (Figure 10B), stem (Figure 10C), roots (Figure 10D), flowers

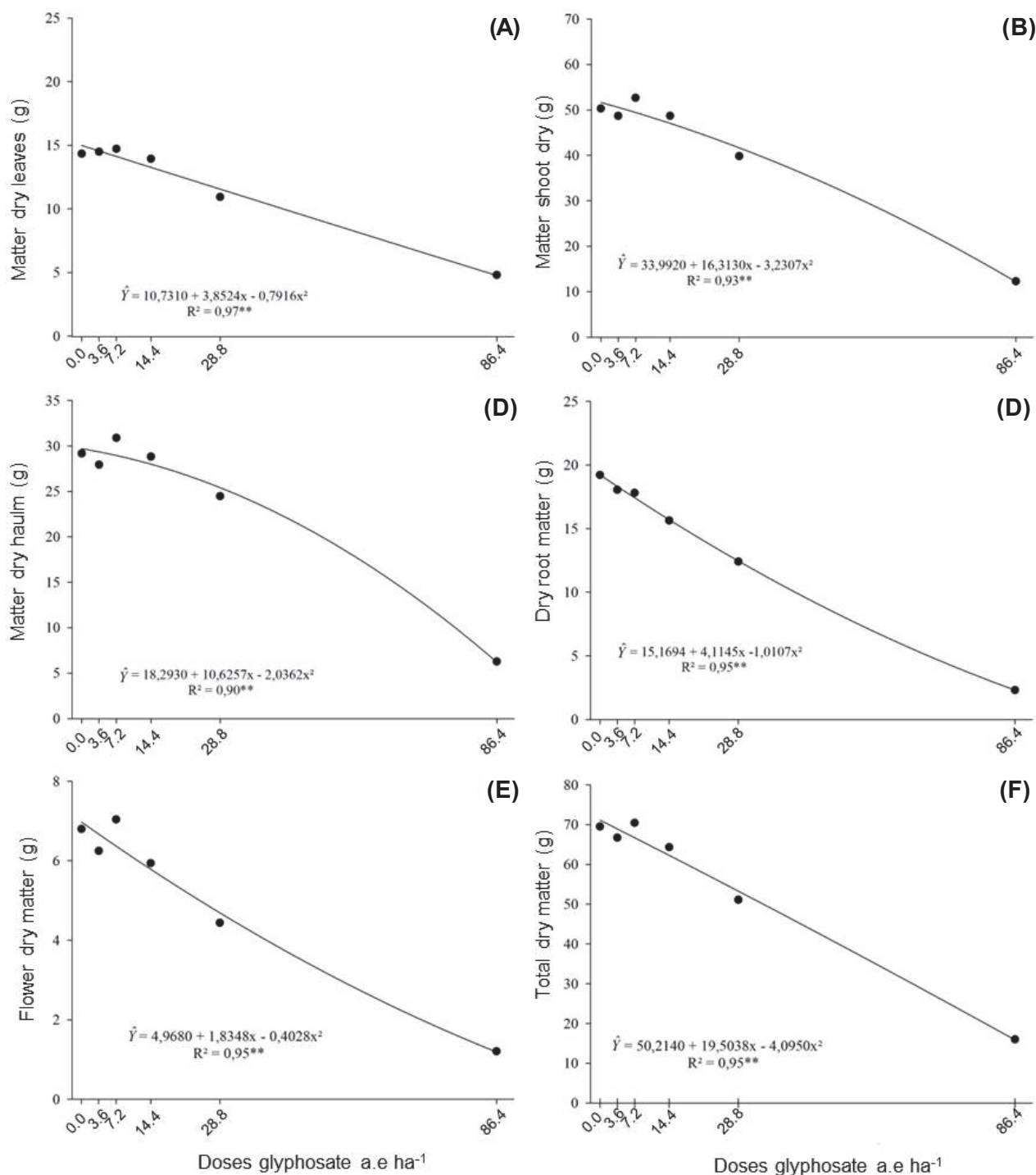


Figure 10 - Leaf dry matter (g) (A), aerial parts (g) (B), stem (g) (C), Root (g) (D), flowers (g) (E) and total dry matter (g) (F) of sunflower plants exposed to increasing doses of glyphosate 28 days after application.

(Figure 10E) and dry matter total (Figure 10F) were observed with increasing concentrations of glyphosate applied to sunflower plants evaluated at 28 DAA. These decreases were most pronounced at a dose of 86.4 g a.e ha⁻¹. Conversely, sunflower plants treated with trinexapac-ethyl did not show significant morphological differences, as compared to control, as shown in (Figure 11A, B, C, D, E and F).

This reduction in dry matter production may be due to the accelerated death of root meristems and stems caused by the toxicity of glyphosate. In addition, glyphosate is known to inhibit the shikimate pathway. Approximately 20% of the carbon fixed by green plants is present in this

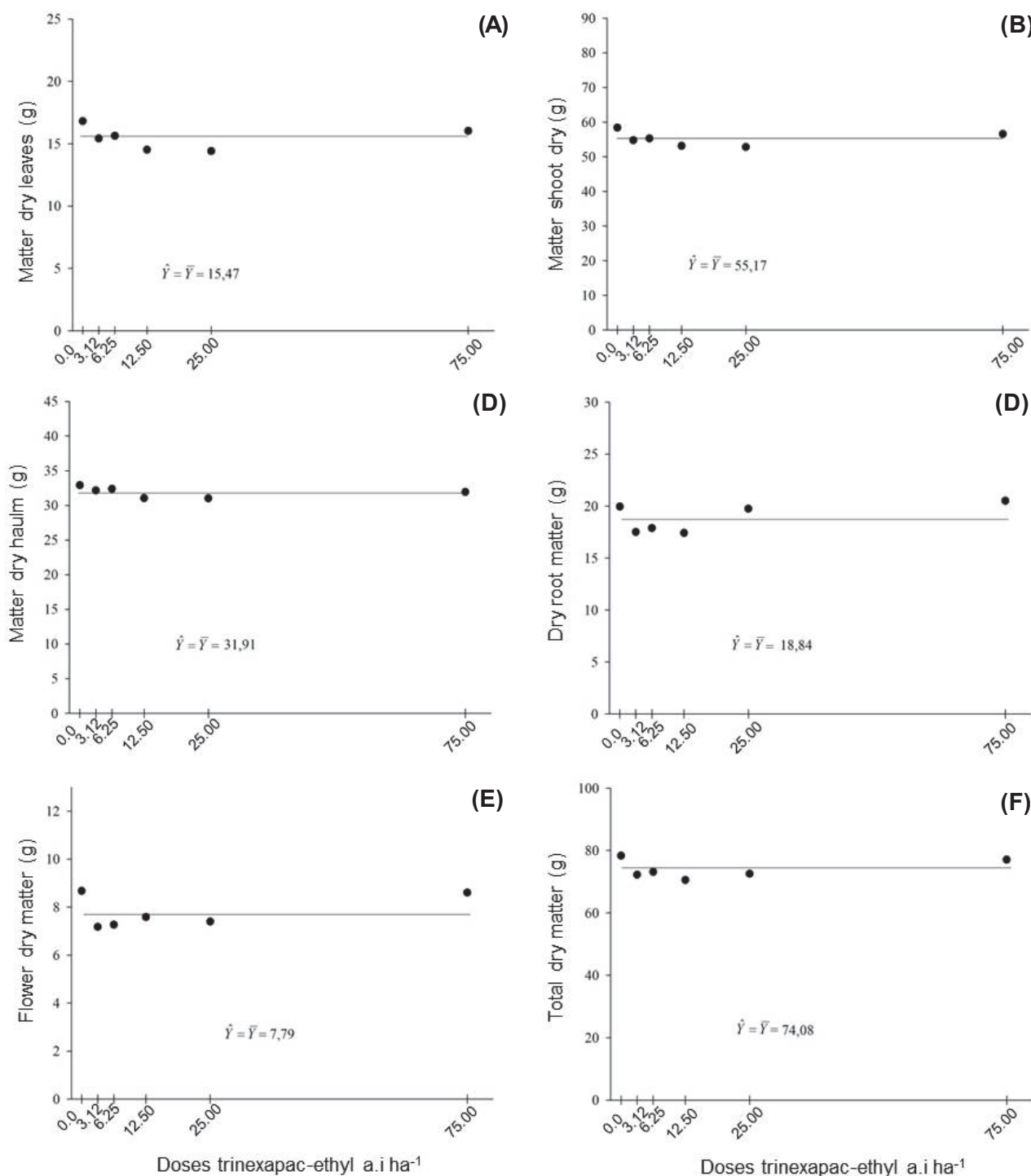


Figure 11 - Leaf dry matter (g) (A), aerial parts (g) (B), stem (g) (C), Root (g) (D), flowers (g) (E) and total dry matter (g) (F) of sunflower plants exposed to increasing doses of trinexapac-ethyl at 28 DAA.

metabolic pathway (Cedergreen and Olesen, 2010), with many end products, such as vitamins, lignans, alkaloids, flavonoids and auxin, altering the plant biomass.

Given the results described above, we conclude that sunflower plants have high sensitivity to glyphosate, as demonstrated by changes in the morphology and dry weight of sunflower plants and the induction of phytotoxicity visualized in the plants. Conversely, sunflower plants were not sensitive to trinexapac-ethyl, as plants exposed to this agent did not display morphological changes or signs of toxicity.

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