

Response of trinexapac-ethyl-treated wheat to glyphosate drift

Respuesta del trigo tratado con trinexapac-etilo a la deriva de glifosato

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Abstract

The herbicide drift is problem in crops when it reaches non-target crops. The glyphosate drift after growth regulator application can influence in wheat response. This study assessed the effects of glyphosate drift after trinexapac-ethyl application on wheat. Two field experiments were conducted in winter season of 2018 and 2019. Cultivar TBIO Toruk and Sossego cultivars were used in 2018 and cultivar TBIO Audaz and Cultivar ORS Citrino in 2019. Wheat cultivars were treated with trinexapac-ethyl followed subdoses of glyphosate ranging from 0 to 72 g ae ha⁻¹. The phytotoxicity from glyphosate was less than 10% to para Cultivar TBIO Toruk and Sossego. Glyphosate symptoms were slightly higher in cultivar TBIO Audaz cultivar treated with trinexapac-ethyl. The isolated effects of Trinexapac-ethyl and glyphosate reduced plant height, but the interaction of trinexapac-ethyl and glyphosate factors had a greater reduction on plant height in 2019. The interaction between trinexapac-ethyl and glyphosate promoted more damage than they alone. Glyphosate reduced by up 11% cultivar TBIO Toruk yield but increase around 30% to cultivar Sossego yield. Cultivar TBIO Audaz was the most sensitive cultivar, with yield losses of up to 59% due to glyphosate drift. Overall, the effect of glyphosate on plant height was subdose dependent. Subsequent application of trinexapac-ethyl to plants exposed to the highest glyphosate subdoses resulted in decreased plant height and dry matter accumulation. Trinexapac-ethyl has no impact on wheat yield. The yield response was dependent of glyphosate subdose, cultivar and year.

Keywords: growth regulator, subdose, *Triticum aestivum*, yield.

Resumen

La deriva del herbicida es un problema en los cultivos cuando el cultivo no alcanza su objetivo. La deriva del glifosato después de la aplicación del regulador de crecimiento puede influir en la respuesta del trigo. Este estudio evaluó los efectos de la deriva del glifosato después de la aplicación de trinexapac-ethyl en el trigo. Se llevaron a cabo dos experimentos de campo en la temporada de invierno de 2018 y 2019. Se utilizaron los cultivares TBIO Toruk y Sossego en 2018 y el cultivar TBIO Audaz y el cultivar ORS Citrino en 2019. Los cultivares de trigo se trataron con trinexapac-ethyl seguido de subdosis de glifosato que variaron de 0 a 72 g ae ha⁻¹. La fitotoxicidad del glifosato fue inferior al 10% para los cultivares TBIO Toruk y Sossego. Los síntomas del glifosato fueron ligeramente mayores en el cultivar TBIO Audaz tratado con trinexapac-ethyl. Los efectos aislados de Trinexapac-ethyl y glifosato redujeron la altura de la planta, pero la interacción de los factores trinexapac-ethyl y glifosato tuvo una mayor reducción en la altura de la planta en 2019. La interacción entre trinexapac-ethyl y glifosato promovió más daño que ellos solos. El glifosato redujo hasta un 11% el rendimiento del cultivar TBIO Toruk, pero aumentó alrededor del 30% el rendimiento del cultivar Sossego. El cultivar TBIO Audaz fue el cultivar más sensible, con pérdidas de rendimiento de hasta el 59% debido a la deriva de glifosato. En general, el efecto del glifosato en la altura de la planta dependió de la subdosis. La aplicación posterior de trinexapac-ethyl a las plantas expuestas a las subdosis más altas de glifosato resultó en una disminución de la altura de la planta y la acumulación de materia seca. Trinexapac-ethyl no tiene impacto en el rendimiento del trigo. La respuesta del rendimiento dependió de la subdosis de glifosato, el cultivar y el año.

Palabras clave: rregulador de crecimiento, subdosis, *Triticum aestivum*, productividad.

Introduction

Glyphosate is one of the most widely used herbicides for weed management in various crops, a non-selective herbicide used to control annual and perennial weeds applied in desiccation management and postemergence of Glyphosate-resistant crops and widely used globally alone or in combination with others herbicides (Almeida *et al.*, 2015). Due to the use of glyphosate in several crops and many times of season crop, there is always a risk of a susceptible crop receiving glyphosate subdoses via drifting (Duke, 2015).

Corn crops in southern Brazil can be planted from July when it can be synchronized with wheat crop. Therefore, the use of glyphosate for weed management in maize may cause drift and affect wheat crops (Duke, 2015). Drift is the movement of herbicides to a non-target area and occurs in a combination of factors at the time of application such as wind speed and small droplet spectrum (Perkins *et al.*, 2022). The production of smaller particles by inadequate tips favors the drift and volatility of pesticides (Gandolfo *et al.*, 2014).

Various studies have reported the damaging effects of glyphosate drift on the development and yield of rice (McCoy *et al.*, 2021), maize (Brown *et al.*, 2009), cotton (Pio de Oliveira *et al.*, 2021), and wheat (Davis *et al.*, 2013; Deeds *et al.*, 2006; Wiersma & Durgan, 2017). However, the damages caused by glyphosate depend on the doses and growth stage of crop.

Simulated glyphosate drift presented severe yield loss in wheat when applied at the node emission stage compared to the flowering stage from 84 g ae ha⁻¹ (Deeds *et al.*, 2006). Similarly, Davis *et al.* (2013) verified a 25% decrease in crop yield when simulating a glyphosate drift (87 g ae ha⁻¹) at the wheat booting stage. In contrast, glyphosate subdoses up to 10 g ae ha⁻¹ may increase the yield of barley and white oat by up to 12% and 30%, respectively (Belz & Duke, 2014; Silva *et al.*, 2020).

Most published studies involving herbicide subdoses by studying drift or tank contamination and their effects on crops typically test herbicide application alone. However, under field conditions, these herbicides may interact with other agrochemicals applied to the crops or even the spray tank and alter the capacity of absorption, translocation, and metabolization of herbicides in crops. For Instance, Brown *et al.* (2009) and Kelley *et al.* (2005) verified that applying selective herbicides in post emergence might aggravate the symptoms caused by the herbicide drift.

In wheat crop, growth regulators are a management option to reduce plant height to prevent lodging (Shah *et al.*, 2016). Other benefits are related to using growth regulators to improve solar radiation capture by altering leaf architecture, obtain more considerable root growth, and reduce respiration (Chavarria *et al.*, 2015). Also, regulators may be used as stress-tolerating agents, as in the case of sugarcane, in which trinexapac-ethyl increases the reactivity of the antioxidant

enzyme system, which can minimize the effects of stress (Almeida *et al.*, 2020).

Likewise, the mixture of trinexapac-ethyl with fungicides may reduce the plant height and impact wheat lodging (Kleczewski & Whaley, 2018). There are no studies in the literature indicating the effects of the growth regulators application followed by glyphosate drift event in wheat. We hypothesized that: (1) the injury caused by low doses of glyphosate in wheat is lower in plants treated previously with trinexapac-ethyl; (2) low doses of glyphosate that do not show visual phytotoxicity increase yield of trinexapac-ethyl treated wheat. Hence, this study aimed to assess the effects of glyphosate drift after trinexapac-ethyl application on wheat cultivars.

Material and methods

Two field experiments were conducted in the winter seasons of 2018 (Experiment I) and 2019 (Experiment II) in soil classified as typical Dystrophic Red Oxisol with the following physicochemical characteristics in the 0 cm to 20 cm layer: 56% clay, pH = 5,9; SMP (H₂O) = 6,5; OM = 4,0%; P = 4,5 mg dm⁻³; K = 332,7 mg dm⁻³; Ca = 7,44 mg dm⁻³; Mg = 3,51 mg dm⁻³; CEC = 14,3 cmolc dm⁻³; SB = 82,4 cmolc dm⁻³; clay = 56,1%. The monthly average temperature and rainfall data observed during the experiments are shown in Figure 1.

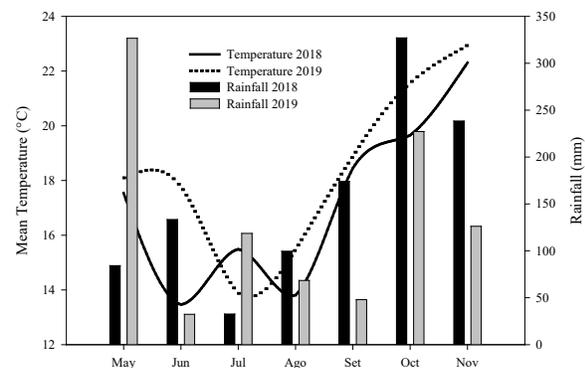


Figure 1. Mean temperature and rainfall data during experiments in 2018 and 2019

The experimental design used was a randomized complete block in a split plot factorial arrangement with four replications. The main plots were composed of wheat cultivars Cultivar TBIO Toruk (low height) and TBIO Sossego (medium height) (Exp. I), and TBIO Audaz (low height) and ORS Citrino (medium height) (Exp. II); Subplots comprised trinexapac-ethyl (TE) growth regulator (0 and 125 g ai ha⁻¹), and glyphosate subdoses: 0; 3,6; 7,2; 18 and 36 g ae ha⁻¹ (Exp. I), and 0; 9; 18; 36 and 72 g ae ha⁻¹ were allocated in sub subplot. The sub subplot size was 1,36 by 5 m.

The experiments were installed in succession with

soybean, carrying out weed control with glyphosate (1080 g ae ha⁻¹) 27 days before sowing (DBS), followed by the application of glufosinate (400 g ai ha⁻¹) at planting date (Exp. I), e glyphosate + metsulfuron-ethyl (1200 g ae ha⁻¹ + 4,2 g ai ha⁻¹) being carried out later at 18 days before sowing (DBS), followed by the application of paraquat (400 g ai ha⁻¹) at 3 DBS (Exp. II). The wheat cultivars were sowed with a 0,17 m spacing between rows, establishing a final population of 350 and 242 plants per square meter for (Exp. I) and (Exp. II) respectively. The base fertilization was 35, 104, 52, 13 and 10 kg ha⁻¹ of N, P₂O₅, K₂O, CaO and SO₄, respectively, and the nitrogen topdressing was carried out at the GS 14 and GS32 phenological stage according to the Zadoks scale with 32 and 45 kg Na ha⁻¹ in the form of urea, respectively. Pyroxsulam (18 g ai ha⁻¹) was used for weed control in Exp. I, and clodinafop (60 g ai ha⁻¹ + 0,5% v/v mineral oil) following the metsulfuron (3,2 g ai ha⁻¹) in Exp. II. The herbicides applications were performed in tillering stage of wheat (GS 14).

The treatments were applied sequentially, with a one-hour interval between the application of trinexapac-ethyl and glyphosate at the GS32 growth stage according to the Zadoks scale (second node detectable and first node perceptible). All treatments were carried out with a backpack sprayer pressurized with CO₂ and equipped with three 11002 fan spray tips distanced 0,50 m apart, with an application volume of 150 L ha⁻¹. In 2018, the applications were carried out at 8 am with temperature of 23 and 17,9 °C, relative humidity of 61 and 76% and wind velocity of 1,1 and 0,9 m/s in 2018 and 2019, respectively.

The variables assessed were crop injury at 28 days after the application (DAA) using a scale of 0 to 100%, where 0 represents no visible symptoms of treatments and 100% indicating plant death (SBCPD 1995). Plant height was measured at 28 DAA and pre-harvest (112 days after emergency) by taking five random plants and measuring the distance of the plant of the last leaf to the ground. The wheat aboveground dry matter was determined at 28 DAA by collecting plants within a 0,5 m section of two rows of each subplot. Subsequently, the plants were bagged and dried at 60 °C for seven days.

The wheat parcels were harvested manually in five rows by three meters in length. After, the samples were threshed and weighed to determine grain productivity, adjusted to 13% moisture.

The normality of the data was verified using the Shapiro-Wilk test and residue analysis. The analysis was performed by year separately. The data were submitted to an analysis of variance using $F \leq 0,05$. The cultivar and regulator factors were compared using Tukey's test ($p \leq 0,05$), and, the effect of glyphosate subdoses was fitted by linear, four-parameter Lorentzian, and three-parameter sigmoid regressions. The statistical analysis and charts were carried out with the assistance of the software RBio (Bhering, 2017) and SigmaPlot version 10.0.

Results and discussion

The phytotoxicity caused by subdoses of glyphosate was less than 10% for 36 g ae ha⁻¹ in exp. I (2018) (Supplementary material). In the exp. II (2019), there was a cultivar by trinexapac-ethyl by glyphosate subdoses interaction for phytotoxicity, which increased linearly for both cultivars, and the cultivar Citrino was slightly more tolerant to glyphosate when compared to cultivar TBIO Audaz (Figure 2). Trinexapac-ethyl potentialize the phytotoxicity symptoms of glyphosate for cultivar TBIO Audaz in 10% at 72 g ae ha⁻¹ of glyphosate, and whereas cultivar ORS Citrino was not influenced by trinexapac-ethyl. One of the possible explanations for the highest toxicity presented in the cultivar TBIO Audaz cultivar comes from the cultivar owner itself, which recommends avoiding pesticides mixtures because this cultivar is more sensitive than others and may present phytotoxicity symptoms (Biotrigo, 2023).

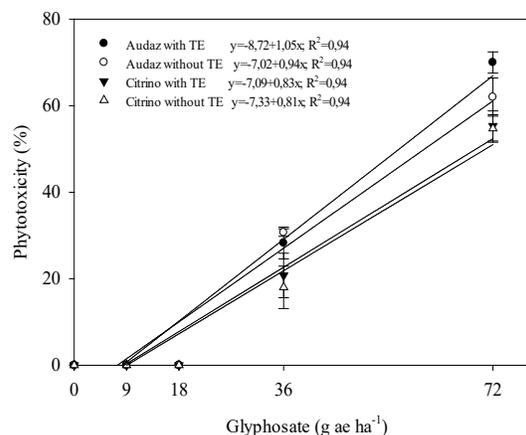


Figure 2. Phytotoxicity of wheat cultivar (TBIO Audaz and ORS Citrino) in response to trinexapac-ethyl and subdoses of glyphosate at 28 days after application in winter season of 2019. Bars represent 95% confidence interval

Cultivar sensitivity may also be related to an increase in glyphosate absorption and translocation in association with trinexapac-ethyl. McCullough & Hart (2010) verified greater absorption of bispiribac in *Poa annua*, *Lolium perenne* and *Agrostis stolonifera* when trinexapac-ethyl was early applied or in tank mixture with the herbicide. According to the authors, the increase in herbicide absorption provided by trinexapac-ethyl may be due to adjuvants in the formulation of the growth regulator. Similarly, trinexapac-ethyl in a tank mix with glyphosate increases the control of *P. annua* compared to the glyphosate alone (Baldwin *et al.*, 2015). Instead, Bearss *et al.* (2021) reported that trinexapac-ethyl in tank mixture

with fenoxaprop, quinclorac or mesotrione had no benefits or antagonism in controlling *Digitaria ischaemum*.

Cultivar by trinexapac-ethyl interaction and glyphosate subdoses effect were detected for plant height at 28 DAA and pre-harvest in winter season 2018 (Exp. I) (Table 1 and Figure 3). The cultivar Sossego was higher than Cultivar TBIO Toruk in treated or non-treated with trinexapac-ethyl at pre-harvest. However, the percentage of reduction in Sossego treated with trinexapac was twice as high compared than cultivar TBIO Toruk in pre-harvest plant height. The plant height was linearly reduced by glyphosate subdoses at 28 DAA and pre-harvest in 2018, with the reduction being more significant in the 36 g ae ha⁻¹, around 7,3 and 5,1% at 28 DAA and pre-harvest, respectively (Figure 3A).

Table 1. Plant height (cm) in response to trinexapac-ethyl and wheat cultivars at pre-harvest in winter season of 2018

Wheat cultivars	Trinexapac-ethyl	
	With	Without
TBIO Sossego	69,5 Ba	77,8 Aa
TBIO Toruk	63,6 B b	67,3 A b

Plant height results were averaged over glyphosate subdoses (0 to 36 g ae ha⁻¹). Identical upper- and lower-case letters in the row and column, respectively, do not differ by Tukey's test ($p \leq 0,05$). DAA= days after trinexapac-ethyl and glyphosate subdose application.

In 2019, glyphosate subdoses effect was detected for plant height at 28 DAA, and glyphosate at 72 g ae ha⁻¹ reduced 22% the wheat plant height in average over trinexapac-ethyl and cultivars treatments (Figure 3B). Plant height at pre-harvest was influenced by cultivar by glyphosate subdoses and trinexapac-ethyl by glyphosate subdoses interactions (Figure 3C and 3D). The application of glyphosate at 72 g ae ha⁻¹ reduced cultivar TBIO Audaz plant height in 24%, while cultivar ORS Citrino plant height was reduced by 16% at the highest tested subdose averaged over trinexapac-ethyl treatments (Figure 3C). The trinexapac-ethyl associated with glyphosate had impact on plant height reductions from 11,6% to 15,2% from 9 to 72 g ae ha⁻¹ of glyphosate compared plants treated with glyphosate alone (Figure 3D).

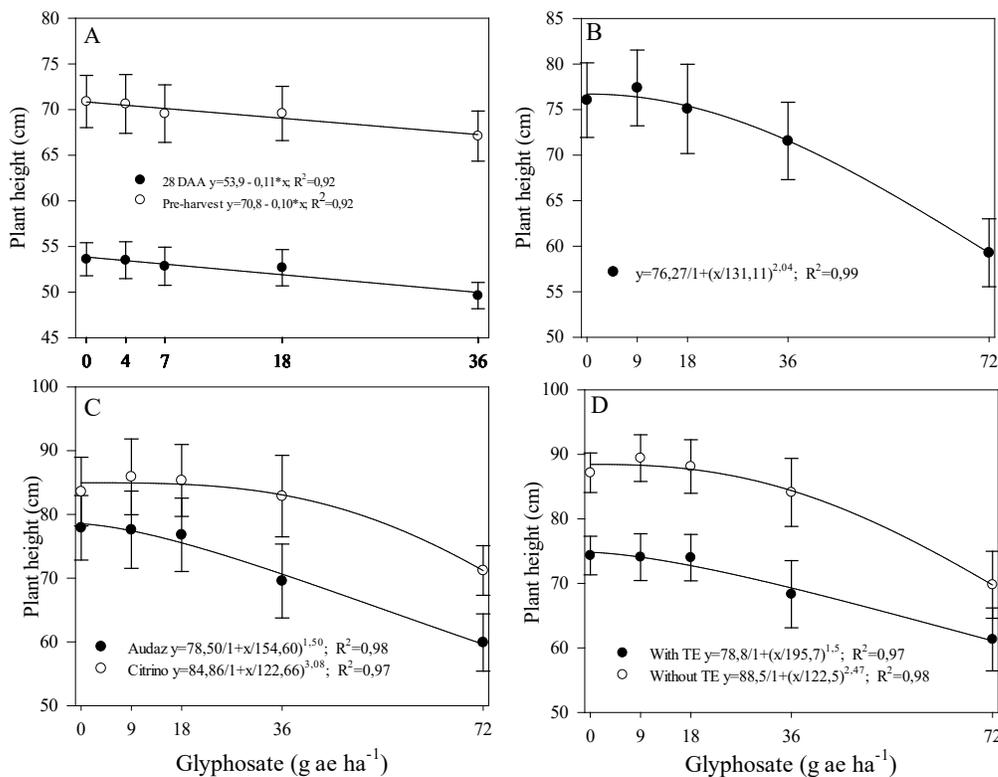


Figure 3. Plant height in response to glyphosate subdoses at in winter season of 2018 (A), and at 28 DAA (B) in winter season of 2019. Plant height at pre-harvest in response to glyphosate subdoses and wheat cultivars (C) and in response to glyphosate subdoses and trinexapac-ethyl (D) in winter season of 2019. Bars represent 95% confidence interval.

DAA= days after trinexapac-ethyl and glyphosate subdose application, DAE= days after emergence

According to Lassiter *et al.* (2007), among the visible lesions caused by herbicides is the delay in plant development, and the plant height reduction is directly related to the action mechanism of glyphosate, which acts by inhibiting the activity of EPSPs enzyme. As a result, the shikimate pathway is interrupted and the synthesis of proteins in the apical meristems, resulting in the interruption of development. Associated with this is the effect of trinexapac-ethyl, which acts in reducing the active gibberellic acid levels, by inhibits the β -hydroxylase enzyme (Zagonel & Fernandes, 2007). Hence, the decrease in the gibberellin levels in the plant results in less significant growth, given that it is responsible for cell division and elongation (Taiz & Zeiger, 2013).

Symptoms and reduction in plant height from glyphosate subdoses vary according to cultivars, growth stages and environmental conditions. The results found in this study are similar to those found by Roider *et al.* (2007), which symptoms by glyphosate in wheat ranged from 49% to 70 g ae ha⁻¹ and 80% to 84 g ae ha⁻¹ when applied in the jointing growth stage, and reduction of up to 26% in plant height at 70 g ae ha⁻¹. Nevertheless, symptoms caused by subdoses of glyphosate up to 84 g ae ha⁻¹ are minimal when applied at the panicle initiation growth stage (Davis *et al.*, 2013).

Wheat aboveground dry weight response was influenced by cultivar by trinexapac-ethyl by glyphosate subdoses interactions in 2019 (Figure 4B). Trinexapac-ethyl reduced cultivar TBIO Audaz aboveground dry weight by about 22% in nontreated plants with subdoses of glyphosate; however, trinexapac-ethyl did not alter Cultivar ORS Citrino aboveground dry weight. According to Hawerroth *et al.* (2015) trinexapac-ethyl can decrease vegetative growth, consequently reducing the production of straw. Contrary,

isolated trinexapac-ethyl does not interfere in the Cultivar ORS Citrino aboveground dry weight, however trinexapac-ethyl and glyphosate interaction were more harmful, and in the absence of trinexapac-ethyl, an increase of 16% aboveground dry weight was estimated at 20 g ae ha⁻¹ of glyphosate.

Changes in the carbon distribution shoots root ratio is a response to the use of trinexapac-ethyl. Chavarria *et al.* (2015) reported a change in carbon accumulation between shoot and root, with an increase of up to 44% in plant root mass. The increase in Cultivar ORS Citrino aboveground dry weight under low doses of glyphosate may be described as a hormesis effect. Similar results were observed by Silva *et al.* (2020) in white oat, for which the authors verified an increase of up to 43% in dry matter at 14,9 g ae ha⁻¹ of glyphosate. Other studies reported in the literature review by Belz and Duke (2014), evinced increases in dry matter of crops such as soybean, maize, barley, and eucalyptus with low doses varying from 10 g ae ha⁻¹ to 25 g ae ha⁻¹ of glyphosate.

Wheat yield was affected by cultivar by subdose of glyphosate interaction, therefore, wheat yield was not affected by the main effects of trinexapac-ethyl (Figure 4A and C). In 2018, cultivar TBIO Toruk yield of nontreated plants with glyphosate was 9% higher than Sossego. However, the application of glyphosate at 36 g ae ha⁻¹ reduced cultivar TBIO Toruk yield by 11%. The Sossego yield was increased by up to 30% by glyphosate subdoses ranging between 3,6 to 18 g ae ha⁻¹ compared to untreated plants, and wheat yield from plants treated with glyphosate applied at 36 g ae ha⁻¹ was similar to the nontreated plants. Several studies have shown that low-dose glyphosate exposure is not without effects on individual species, but can lead to changes in plant growth, such as hormesis (Brito *et al.*, 2018).

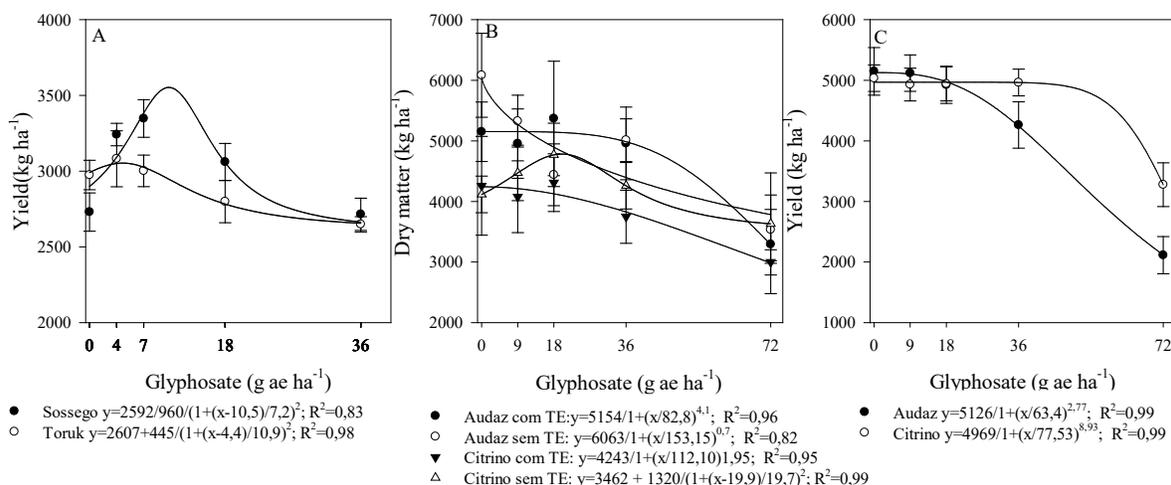


Figure 4. Effect of glyphosate subdoses, trinexapac-ethyl (TE) and wheat cultivars interactions on wheat yield (A and C) aboveground dry weight (B) in winter season in 2018 (A) and in 2019 (B e C). Bars represent 95% confidence interval

In 2019, subdoses of glyphosate from 9 to 18 g ae ha⁻¹ does not affect on wheat yield of cultivar TBIO Audaz, but yield was reduced 17 and 59%, respectively, when treated with 36 and 72 g ae ha⁻¹ of glyphosate (Figure 4C). The Cultivar ORS Citrino yield remained unchanged up to the subdose of 36 g ae ha⁻¹ of glyphosate, and with estimated yield loss of 34% at 72 g ae ha⁻¹. Although Cultivar ORS Citrino plants showed phytotoxicity symptoms of 22% in the subdose of 36 g ae ha⁻¹, this was not enough to reduce yield. There were no differences in yield between cultivars TBIO Audaz and ORS Citrino up to 18 g ae ha⁻¹ of glyphosate; however, the differences were estimated at 884 and 1158 kg ae ha⁻¹ for the subdoses of 36 and 72 g ae ha⁻¹, respectively. The ED50 parameter of the logistic equations may also be used to describe this higher tolerance of cultivar ORS Citrino than cultivar TBIO Audaz, given that 77,5 g ae ha⁻¹ of glyphosate is needed to reduce the yield by 50%, while this dose is 63,4 g ae ha⁻¹ for the cultivar TBIO Audaz.

Literature reports indicate that wheat yield response as a function of subdose of glyphosate is highly variable. Generally, glyphosate drift can have greater impacts on wheat yields when it occurs in the early growth stages (Davis *et al.*, 2013). Deeds *et al.* (2006) found a yield loss ranged from 0 to 80% when wheat were subjected to 25,5 g ae ha⁻¹ of glyphosate at the jointing and flowering growth stages. Results obtained by Roider *et al.*, (2007) indicate lower impacts of glyphosate on wheat yield, with a 29% reduction in the 70 g ae ha⁻¹.

The low herbicide doses in wheat seem response to others factors including genetic, environmental (temperature, soil fertility, precipitation), intraspecific competition, herbicide deposition (Belz & Sinkkonen, 2019; Brito *et al.*, 2018), since plant response between the two years. The long-term repeated glyphosate drift can evolve recurrent selection for herbicide in crops or weeds. In crops, the effect of recurrent of low doses herbicide is reported in barley, which generated progeny fast growing plants and more tolerant to glyphosate (Belz & Sinkkonen, 2021).

The wheat response to trinexapac-ethyl varies depending on the environment, management and genetics, and tall cultivars are more responsive to regulator growth. Similar results were also observed by Miziniak and Matysiak (2016) who verified the wheat response to trinexapac-ethyl dependent on a series of conditions that imply in cultivar genetics, season and growth regulator application rate, use of mixed agrochemicals and environmental conditions.

Conclusion

The cultivar differences play a significant role in wheat response to glyphosate drift after trinexapac-ethyl application. Trinexapac-ethyl did not consistently reduce glyphosate injury. Trinexapac-ethyl treatment reduced plant height, particularly in cultivar TBIO Audaz and at higher glyphosate doses. Cultivar ORS Citrino exhibited a higher overall tolerance to

glyphosate compared to cultivar TBIO Audaz, with minimal yield reductions even at moderate glyphosate subdoses. Low doses of glyphosate stimulus Sossego cultivar yield

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