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Effect of subdoses of sugarcane ripeners on lettuce physiology in a drift scenario

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Abstract

In order to understand the physiological effects of ripeners in sensitive crops, the objective of this work was to evaluate the effect of subdoses of the ripeners glyphosate, trinexapac-ethyl and sulfometuron methyl commonly used in sugarcane, in the growth of lettuce cultivar 'Lucy Brown' and 'Vanda'. To address the effects of the products in the lettuce physiology, analyses of fresh weight, dry weight, number of leaves, chlorophyll content, quantum efficiency of photosystem II, lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), glutathione reductase (GR), guaiacol peroxidase (GPOX) were performed. We observed that among the products tested, glyphosate had minor impact on plant growth, compared to trinexapac-ethyl and sulfometuron methyl. All products induced a decrease in chlorophyll content for both cultivars. Chlorophyll A fluorescence suffered a major reduction with trinexapac-ethyl and sulfometuron methyl in 'Vanda' and no differences were observed for 'Lucy Brown'. MDA content and enzyme quantification varied by cultivar and the sugarcane ripener tested. By disturbing chlorophyll content and quantum efficiency of photosystem II, through these sugarcane ripeners did not have direct mode of action affecting photosystem II, they can cause some level of damage and activate different mechanisms and at different times, in response to stress. In this sense, it is possible to observe that reduced doses of glyphosate, trinexapac ethyl, and sulfometuron methyl affect the development of lettuce at different levels and trigger an oxidative response that was cultivar dependent.

Keywords Plant growth regulators · Photosynthesis · Oxidative stress · Enzymes

Introduction

Sugarcane (*Saccharum officinarum*) is one of the most important sources of sugar and biofuel in many tropical countries (Anbanandan; Eswara, 2018; Antunes et al. 2019). In Brazil, this crop occupies around 5,686,000 ha, accounting for 55% of total Brazilian agriculture production (Nocelli et al. 2017; Camargo Filho and Camargo 2019). Brazil ranks first in world sugarcane production with over 670 million metric tons in 2017 (Antunes et al. 2019). Sugarcane is planted near-annual crops (Camargo Filho and Camargo 2019), such as lettuce (*Lactuca sativa* L.), an important vegetable due to the high content of vitamin C, polyphenol and fiber (Shatilov et al. 2019).

To regulate growth and increase the quantity of sugar per area, farmers apply ripeners such as ethephon, trinexapac-ethyl, sulfometuron-methyl, and glyphosate (Leite et al. 2011). The flowering process of sugarcane causes morphological and physiological changes in the plant. As a way of making the largest accumulation of sucrose feasible, ripeners are applied. Ripeners in general are plant growth regulators, capable of altering the morphology and physiology of plants, resulting in qualitative and quantitative changes in production (Caputo et al. 2007). For sugarcane, ripeners are used to delay or inhibit vegetative development, allowing the accumulation of sucrose in the stalk, promoting the maturation of the crop, and increasing productivity.

Trinexapac-ethyl (ethyl 4-(cyclopropyl(hydroxy)methylene)-3,5-dioxocyclohexanecarboxylate) inhibits synthesis of active forms of gibberellic acid, a growth regulator that

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acts on cell division and plant growth. There is an accumulation of the inactive form while the suppression of the active form of gibberellin results in the inhibition of vegetative growth, favoring the accumulation of sucrose in the sugarcane stalk (Van Heerden et al. 2015).

The group of sulfonylureas, which includes sulfometuron methyl (methyl 2-(4,6-dimethylpyrimidin-2-ylcarbamoylsulfamoyl) benzoate), acts by inhibiting the enzyme acetolactate synthase (ALS). ALS is the first enzyme in the branched-chain amino acid synthesis route for valine, isoleucine, and leucine. After absorption, they are quickly translocated to regions of active growth, interfering in the synthesis of the afore mentioned amino acids (Oliveira et al. 2011). The halt in the development of the apical meristem causes a shortening of the internode formed at the time of application, leading to a sucrose storage in the stalk.

Glyphosate (N-(phosphonomethyl) glycine), belonging to the group of EPSP-inhibiting herbicides (5-enolpyruvylshikimate-3-phosphate (EPSP) synthase), acts by inhibiting the synthesis of aromatic amino acids tryptophan, tyrosine, and phenylalanine. This inhibition occurs due to the accumulation of shikimate in the plant and, consequently, inhibition in the production of aromatic amino acids. Its absorption occurs slowly, via symplast, and is translocated to meristematic regions, together with photoassimilates (Duke and Powles 2008). As it is a systemic herbicide, it helps in the maturation of the sugarcane, by causing the death of the apical bud, when applied at low dosage.

The application of these plant-growth regulators is by airplane, putting in risk non-target crops in the surrounding areas because of the high probability of drift (Gandolfo et al. 2013, Streibig and Green 2017). Drift is defined as the movement of drops of phytosanitary products beyond the target area, which may occur at the moment of application or immediately after (USEPA 2014). As a result of these drift scenarios, many areas and crops can be contaminated (Duke et al. 2017) For lettuce, injury can negatively impact grower income by causing reductions in yield and quality.

As a result of plant chemical toxicity, crops can undergo a great overproduction of reactive oxygen species (ROS) which can cause oxidative damages to lipids (in the cell membranes), proteins, and DNA. This can result in a set of changes in the membrane fluidity, decreasing chlorophyll content, proteins, and photosynthetic rate that can lead to cell death and subsequently, plant death in extreme cases (Gomes et al. 2016, Sharma et al. 2017).

In order to counteract the negative effect of the overproduction of ROS, plants use a complex antioxidant system that is split into two groups: enzymatic and non-enzymatic. The enzymatic group formed by superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (GPOX, EC 1.11.1.9), glutathione reductase (GR, EC 1.8.1.7), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), ascorbate peroxidase (APX, EC 1.11.1.11) and dehydroascorbate reductase (DHAR, EC 1.8.5.1) (Acosta-Motos et al. 2017). In contrast, the non-enzymatic group is mainly formed by glutathione (GSH), vitamin E (tocopherols and tocotrienols), ascorbate (vitamin C), and carotenoids (provitamin A) (Asensi-Fabado and Munné-Bosch 2010; Pintó-Marijuan and Munné-Bosch 2013; Kapoor et al. 2019).

The oxidative response of the plant cells can provide important information about plant tolerance to the application of agriculture chemistries such as sugarcane ripeners. Plant cell response will vary according to the species as well as the product mode of action (Caverzan et al. 2016). Research has addressed the effects of chemical ripeners, like glyphosate, on plant cell antioxidant response (Gomes et al. 2016, Gomes and Juneau 2016). However, there is little information about the effect of ripeners drift on the growth and physiology of lettuce.

Considering the economic importance of lettuce to growers its consumer demand, and its proximity to sugarcane production, which is one of the main users of ripeners, research was conducted to quantify the effects of reduced rates of glyphosate, sulfometuron-methyl and trinexapac-ethyl on the physiology of lettuce. The cultivars 'Lucy Brown' (Seminis®) and 'Vanda' (Sakata®) were evaluated in potential drift scenarios.

Material and methods

Study site and plant material

The experiment was conducted in from August to November of 2019 outdoors in an open field environment at Sao Paulo State University (21°14'05" south latitude and 48°17'09" west longitude), in Sao Paulo (SP), Brazil. The climate of the region is defined as Cwa, subtropical, according to the Koeppen classification (Chen and Chen 2013). During the experiment, the average air temperature was 24 °C and relative humidity was of 72% by Agroclimatological Station operated by Sao Paulo State University, Jaboticabal, SP (Rolim 2019).

Lettuce seedlings (average size approximately 4 leaves) of 'Lucy Brown' and 'Vanda' cultivars were purchased from Multitec®, a local vegetable plant supplier. Both cultivars are commonly planted in this region of the state of Sao Paulo and most of them are planted close to cane fields. Plants were acclimated, keeping them under shade with interception of 30% of sunlight for 2 days. After acclimatization, seedlings were transplanted with one plant per pot, considered an experimental unit. Five 5 L pots were filled to capacity with a mixture of horticultural substrate of BioPlant® in combination with soil, dark red latosol, in a 2:1 (v: v) ratio and five reps per treatment.

Crop management

Individual pots were fertilized at 10, 20 and 30 days after transplantation (DAT) with 60 kg ha^{-1} of nitrogen, applied to the side of each plant (Trani et al. 1997). During the experiment, pots were irrigated daily when necessary to maintain optimum growing conditions. Weeds were manually removed upon emergence.

Agricultural chemical treatments

There were 12 treatments, formed by 4 reduced rates of ripeners. The ripeners included sulfometuron methyl at 0.45, 0.90, 1.35, and 1.80 g a.i. ha^{-1} plus mineral oil (0.1% v;v), trinexapac-ethyl at 6, 12 18, and 24 g. a.i. ha^{-1}, and glyphosate at 6.5, 13.0, 19.4, and 25.9 g e.a. ha^{-1}. A non-treated (NTC) was included for all ripener (0 g ha^{-1}) dose scenarios, to make equal comparisons for each test group. Treatments represented the equivalent of 0, 3, 6, 9 and 12% of the recommended dose of Curavial® (15 g a. i. ha^{-1}, DuPont®), Moddus® (200 g a.i. ha^{-1}, Syngenta®), and Roundup Original® (216 g a.e. ha^{-1}; 360 g/L acid equivalent of glyphosate and 480 g/L of glyphosate, Monsanto®.), respectively. The products were purchased from COPLANA®, a local agricultural supplier from Jaboticabal, SP. Each treatment consisted of five replications. Ripeners were applied to 'Lucy Brown' at the beginning of head formation, and the 6 to 7 leaf growth stage for 'Vanda', according to the BBCH scale. The application of the products was carried out in a closed spray room. A backpack sprayer at constant pressure (CO_2), coupled to a bar containing four flat fan spray tips (XR11002) with a distance between nozzles of fifty centimeters, was used to apply a spray volume of 200 L ha^{-1}. At the time of application, the average air temperature was 20.7°C with 60% relative humidity.

Fresh and dry biomass, and number of leaves

At 20 days after application (DAA), all lettuce plants were cut at the soil level. Plants were then, defoliated separating the commercial and non-commercial leaves, counted, fresh biomass weighed (g), and then packed in paper bags. These were then placed in oven with forced air circulation (60 °C) until constant mass was achieved. Samples were then weighed to determine dry biomass (g).

Chlorophyll content and wuantum efficiency of photosystem II

At 5, 10, 15 and 20 days after application (DAA), quantification of the relative content of total chlorophyll using a chlorophyll meter (ClorofiLog, Falker, Brazil) was conducted. Three readings per leaf (FALKER index) and determinations of the quantum efficiency of photosystem II (Fv / Fm) by means of a fluorimeter (Mini PAM, WALZ, Germany) in which clips were placed on the sheet to be measured, remaining in the dark for about 15 min before reading, were performed. All measurements were performed on the fourth basal leaf of each plant.

Lipid peroxidation

At the 12% ripener treatment applications, sampling of the fourth basal expanded leaf was taken at 24, 48 and 72 h. These leaves were detached and placed in liquid nitrogen, and kept frozen at −90 °C until biochemical analysis in the Laboratory of Plant Physiology at Sao Paulo State University, Jaboticabal, SP.

Lipid peroxidation was analyzed by determining the content of MDA (malondialdehyde) and it is highly correlated to damage of cell membrane, by reactive oxygen species. In this experiment, the content of MDA was measured according to Shimizu et al. (2006). For analysis, leaves were taken from the freezer, then macerated in trichloroacetic acid (TCA, 0.1%) and centrifuged at 1500 × *g* for 15 min at 4 °C. After that, the supernatant was added to 1.5 ml of 0.5% of thiobarbituric acid (TBA). This mixture was agitated and incubated at 95 °C for 30 min. Sample absorbance was then recorded at 532 nm and 600 nm using a lambda spectrophotometer (PerkinElmer, USA).

Hydrogen peroxide (H_2O_2)

In order to quantify the content of H_2O_2, the leaves were macerated (0.1% TCA) and centrifuged (1500 g by 15 min at 4 C). In the tube of the supernatant was added 200 µl of 100 mM potassium phosphate buffer (pH 7.5) and 800 µl of a 1 M KI solution. The new solution was placed on ice within 1 h. After that, we measured the absorbance (390 nm) with PerkinElmer – Lambda spectrophotometer (Alexieva et al. 2001).

Enzymes

Glutathione Reductase activity (GR, EC 1.8.1.7)

To quantify the activity of this enzyme, 100 mM phosphate buffer pH 7.5, 500 µl of 5′5′-dithio-bis (2-nitrobenzoic) acid (DTNB) in a water bath (30 °C) were mixed. After the water bath, 1 mM of oxidized glutathione, 0.1 mM NADPH, and 50 µl of extract were added and finally read with PerkinElmer – Lambda spectrophotometer at the wavelength of 412 nm (Cakmak and Horst 1991; Azevedo et al. 1998).

Guaiacol Peroxidase activity (GPOX 1.11.1.7)

The activity of this enzyme is determined according to the methodology proposed by Gomes-Junior et al. (2007). Two hundred and fifty µL of the phosphate-citrate buffer (sodium phosphate dibasic 0.2 M: citric acid 0.1 M) pH 5.0 is mixed, also 25 ul of 0.5% guaiacol and the plant extract were added. The mixture was incubated in a vortex at 30 °C for 15 min. Then, the mixture was placed on ice and 25 ul of 2% sodium metabisulphite solution was added. The reaction mixture was held for 10 min, and the GPOx activity was evaluated by monitoring the absorbance at 450 nm.

Experimental analysis and statistical analysis

The experimental design was a randomized block in a 3 × 5 factorial for each cultivar, with five replications. The first factor corresponded to chemical ripeners (3) and the second to rates (5). Chlorophyll content, quantum efficiency of photosystem II, number of leaves, fresh and dry biomass were analyzed considering the 3 × 5 factorial. Lipid peroxidation, hydrogen peroxide, and enzymes were analyzed as a 3 × 3 factorial. The first factor corresponded to the chemical ripeners (3) and the second to the periods of collection (24, 48, 72 h) after treatment application, at the 12% dose of the ripeners.

Data were subjected to analysis of variance (F test), with the means compared by Tukey's test at 5% ($p < 0.05$) probability in the AgroEstat® program. H_2O_2 contents, lipid peroxidation (MDA), antioxidant response, chlorophyll content, quantum efficiency of photosystem II, fresh biomass (g), number of leaves and dry mass (g) were submitted to regression analysis in Graphpad Prism 7 (GraphPad Software Inc.; La Jolla, CA, USA), when significant at 5%.

Results

Fresh biomass, dry biomass and number of leaves

In the 'Lucy Brown', the difference between ripeners and subdoses was evident. All products reduced the fresh biomass of lettuce collected to 20 DAA ($p < 0.01$). When estimating the lettuce biomass, it was possible to observe that for each 1% of subdose applied, there was a 2.92 g decrease for glyphosate ($p < 0.01$), 7.70 g for trinexapac-ethyl ($p < 0.01$) and 7.37 g for sulfometuron methyl ($p < 0.05$). At 12% dose levels, lettuce biomass reductions culminated in up to 15% for glyphosate, and 39% for trinexapac-ethyl and sulfometuron methyl, respectively, compared to the reduced rate corresponding to the non-treated control (subdose 0) (Fig. 1a). No differences were observed for cultivar 'Vanda', regarding fresh biomass.

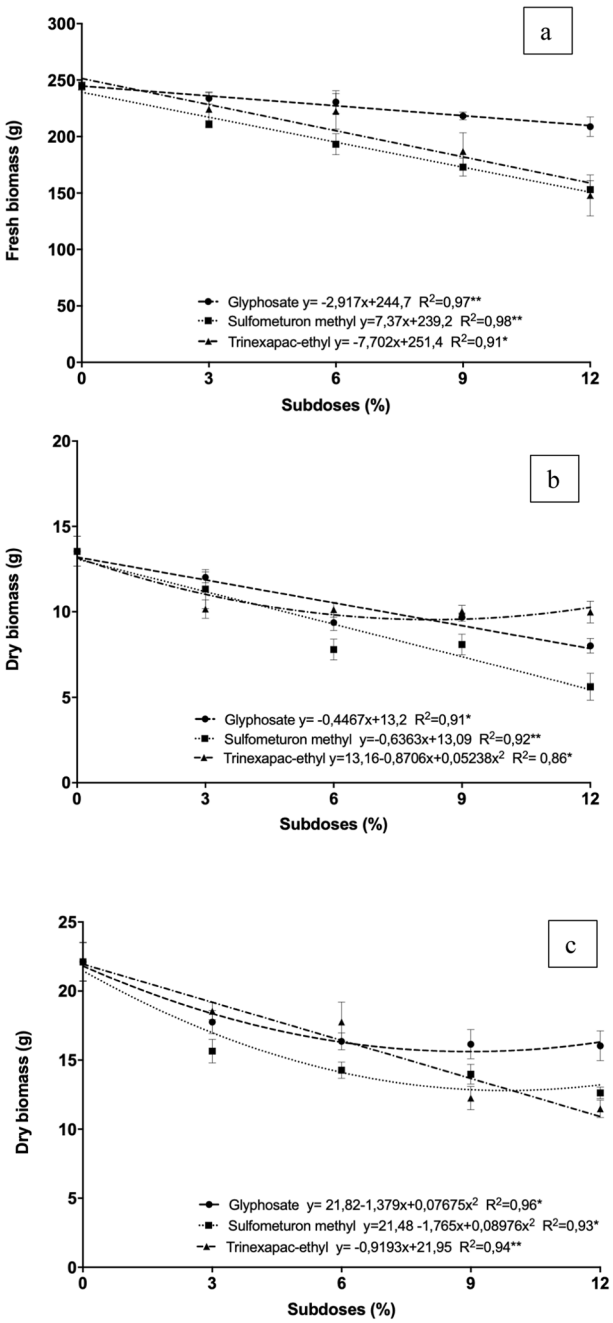

Fig. 1 Fresh and dry biomass of 'Lucy Brown' (**a** and **b**) and dry biomass of 'Vanda' (**c**) at 20 DAA depending on the subdoses of the ripeners, being * significant at 5% and ** significant at 1% by the F test

For dry biomass, a significant interaction was also observed for the factors tested ($p < 0.05$) in 'Lucy Brown' and 'Vanda' (Fig. 1b and 1c). For 'Lucy Brown' (Fig. 1b) it was noted that for each 1% of reduced rate, there is a decrease of 0.45 and 0.63 g with the use of glyphosate ($p < 0.05$) and methyl sulfometuron ($p < 0.01$), respectively, (linear regression model). However, trinexapac-ethyl ($p < 0.05$), adjusted to the quadratic model and the major

reduction observed was in the rate starting from 8%, equivalent to 16 g. i, a. ha^{-1}, which corresponds to a decrease of up to 29.5% in biomass (Fig. 1b). At 12% of the dose, reductions in dry biomass reached approximately 41% for glyphosate and methyl sulfometuron, and 58.5% for trinexapac-ethyl, when compared to the non-treated control (subdose 0).

For 'Vanda' (Fig. 1c), glyphosate ($p < 0.05$), in the estimated 8.9% subdose, provided the greatest reduction in dry biomass, with a decrease of up to 29% per plant. For methyl sulfometuron ($p < 0.05$), this reduction was even greater, at 41% in the estimated 9.8% subdose. Trinexapac ethyl ($p < 0.01$), caused a greater reduction at 12%, with a decrease of 48% in dry biomass (Fig. 1).

For number of leaves, there was no interaction between ripeners and subdoses ($p > 0.05$). Thus, the number of leaves counted at 20 DAA did not differ among the ripeners used for both cultivars tested.

Chlorophyll content and Quantum efficiency of photosystem II

In the evaluation of the total chlorophyll content, in the 'Lucy Brown' cultivar we noticed that only at 15 DAA ($p < 0.01$) (Fig. 2), all ripeners, subdoses and interactions were statistically different (Fig. 2). However, in the 'Vanda' at 15 DAA ($p < 0.01$) and 20 DAA ($p < 0.05$), the interaction was statistically significant (Fig. 3).

In the case of 'Lucy Brown', data indicated that the estimated subdose of 8% for glyphosate ($p < 0.05$) and 10% for trinexapac-ethyl ($p < 0.01$) provided a greater reduction in the total chlorophyll content, representing a decrease of 28% and 20% respectively. As for methyl sulfometuron ($p < 0.05$), 12% provided the greatest reduction in the reading of chlorophyll contents, culminating in 30%.

Analyzing the total chlorophyll content for 'Vanda' at 15 DAA, it was possible to observe that for glyphosate ($p < 0.01$), the estimated subdose of 9% was responsible for reducing chlorophyll contents by up to 30% when compared to the non-treated control. For sulfometuron methyl ($p < 0.05$) the estimated 10% resulted in a decrease of approximately 33% and, finally, trinexapac ethyl ($p < 0.05$), provided the largest reduction in 9%, which was approximately 27.5%. At 20 DAA, the reductions were even greater. Glyphosate ($p < 0.05$), at 8% reduced the chlorophyll present by approximately 51%, with the greatest decrease observed. Sulfometuron methyl ($p < 0.05$), in the estimated 10% rate, reduced by up to 37.5% and trinexapac ethyl ($p < 0.05$), in the 9%, by up to 46% in chlorophyll contents.

In the evaluation of the quantum efficiency of photosystem II for 'Lucy Brown', the interaction between ripeners and reduced rates was not significant ($p < 0.05$) in any of the evaluations performed. However, in 'Vanda', the analysis of variance showed significance for ripeners and subdoses, which was significant in the evaluation of 5 DAA ($p < 0.05$) and 10 DAA ($p < 0.01$) (Fig. 4).

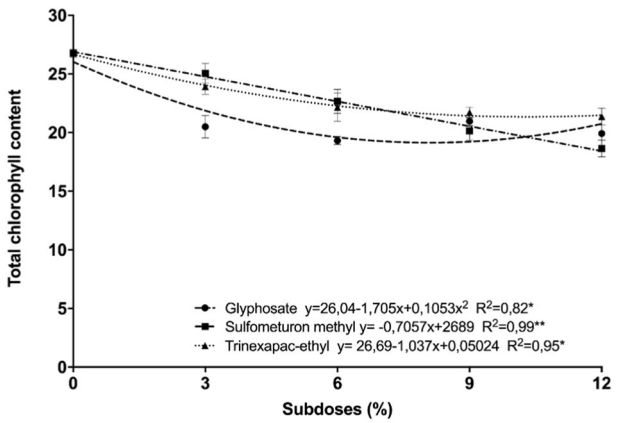

Fig. 2 Total chlorophyll content in lettuce leaves cultivar 'Lucy Brown' at 15 DAA according to the subdoses of the ripeners, being * significant at 5% and ** significant at 1% by the F test

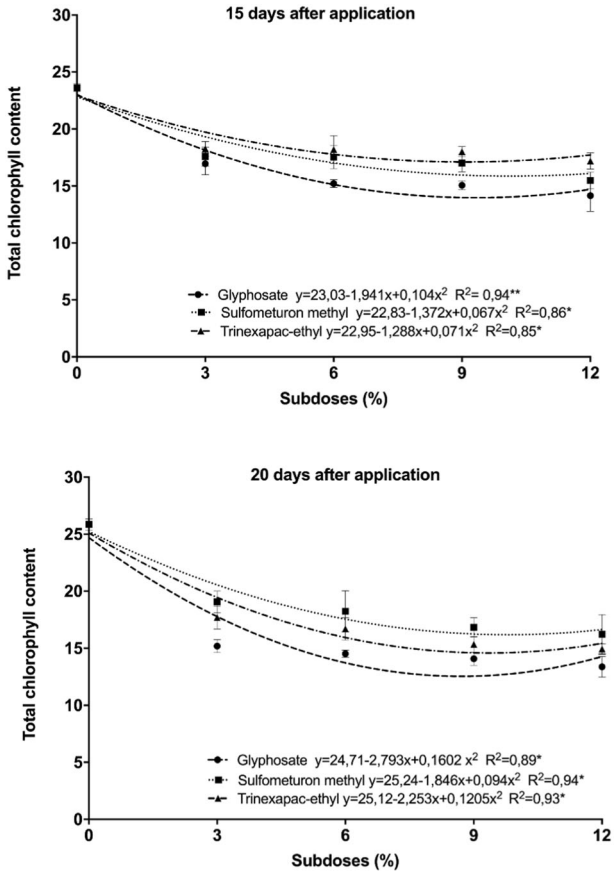

Fig. 3 Total chlorophyll content in lettuce leaves cultivar 'Vanda' at 15 and 20 DAA according to the reduced rates of the ripeners, being * significant at 5% and ** significant at 1% by the F test

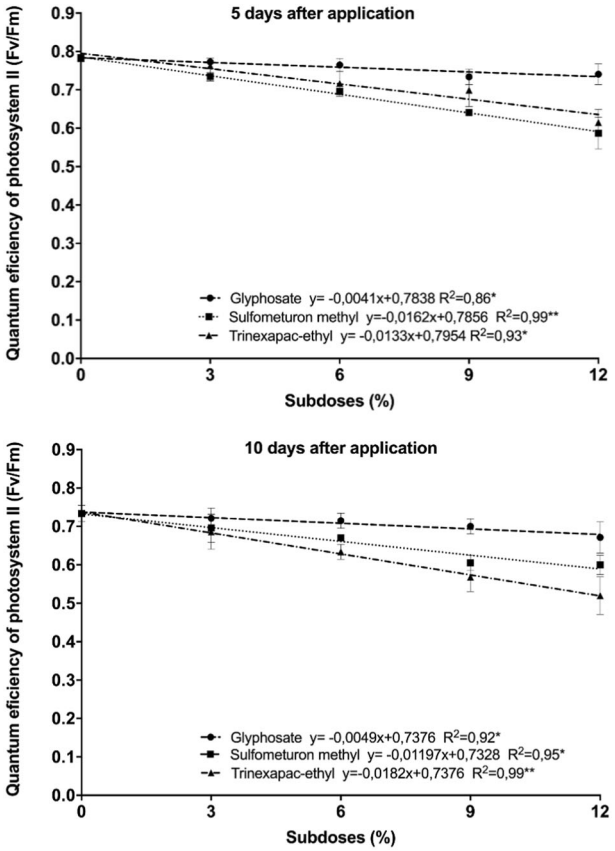

Fig. 4 Quantum efficiency of photosystem II as measured in 'Vanda' leaves at 5 and 10 DAA depending on the subdoses of the ripeners, being * significant at 5% and ** significant at 1% by the F test

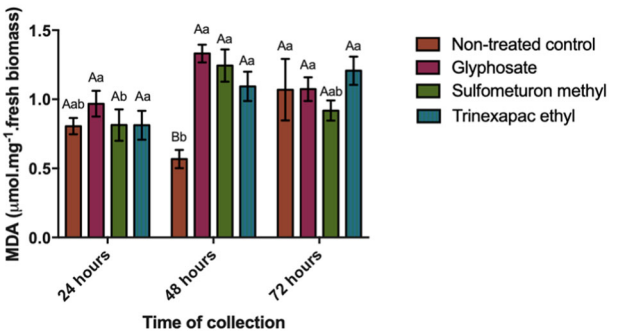

Fig. 5 Average MDA levels observed at 24, 48, 72 h after application (HAA) in 'Lucy Brown'. Averages followed by the same letter do not differ by Tukey's test at 5% probability, with lower case letters comparing the time of collection within each product and upper-case letters comparing products within the time of collection

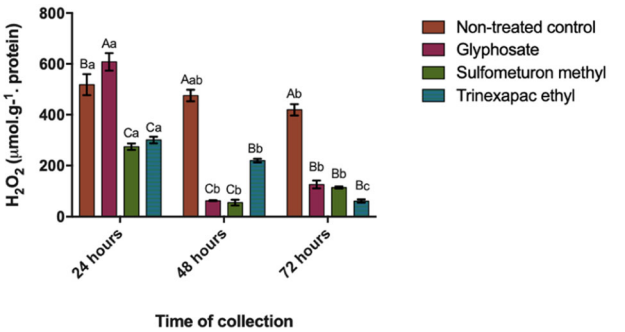

Fig. 6 Average levels of reactive oxygen species (ROS) observed at 24, 48, 72 h after application (HAA) in the cultivar 'Lucy Brown'. Averages followed by the same letter do not differ by Tukey's test at 5% probability, with lower case letters comparing the time of collection within each product and upper-case letters comparing products within the time of collection

At 5 DAA, when compared to the non-treated control and at 12% of the dose of the products, glyphosate ($p < 0.05$), was the one that provided the least reduction in the Fv/Fm readings, with a decrease of approximately 5%. In the evaluation of 10 DAA, a greater reduction of 8% was observed for the subdose corresponding to 12% ($p < 0.05$). For sulfometuron methyl ($p < 0.01$), at 5 DAA in the subdose for 12% of the recommended dose, there was a 25% reduction in Fv/Fm, this being 0.587. At 10 DAA, the reduction was smaller, with 18% and reading of 0.680 ($p < 0.05$). Considering all the subdoses tested, evidence of damage to the quantum yield of photosystem II can be seen from the 6% subdose at 5 DAA and 10 DAA.

Lipid peroxidation

MDA concentration in the leaves of the cultivar 'Lucy Brown' (Fig. 5) was significative in the 48 h evaluation ($p < 0.05$), when was possible to observe differences between the non-treated control and the treatments applied. In fact, the MDA content in the non-treated control is almost twice smaller than in plants with ripener application. For 'Vanda', there were no differences in the interactions between products and subdoses, which were not significant ($p > 0.05$).

Hydrogen peroxide (H_2O_2)

For 'Lucy Brown' (Fig. 6), it was observed that all the collection periods evaluated showed different behavior ($p < 0.05$). Only glyphosate at 24 h showed higher values than the non-treated control. In the other collection periods, at 48 and 72 h, all the products tested, presented amounts (almost 5 times) of H_2O_2 lower than the non-treated control.

For 'Vanda' (Fig. 7), it was observed that the H_2O_2 content varied depending on the tested products ($p < 0.01$). Thus, sulfometuron methyl, in the 24 h ($p < 0.01$), significantly increased the H_2O_2 content compared to the non-treated control. At 48 h ($p < 0.01$), in response to trinexapac ethyl, the leaves had a high content of H_2O_2. While in the 72 h, there was no difference regarding the oxygen peroxide content between the tested products ($p < 0.05$).

Enzymes

Glutathione reductase activity (GR, EC 1.8.1.7)

Glutathione reductase (GR) (Fig. 8) showed activity in at least two times of collection periods for 'Lucy Brown'. In fact, this enzyme was more active at 24 and 72 h ($p < 0.05$) in treatments with ripeners, although only it was statistically significant at 72 h. Whereas at 48 h, compared to the non-treated control, all products decreased the activity of this enzyme from 4 to 10 times.

Analyzing the interaction between the factors for 'Vanda', it was possible to observe GR enzyme activity in all collection periods ($p < 0.01$). In general, the ripeners used reduced the activity of this enzyme, but in the evaluation made at 72 h, glyphosate increased in 28% the activity of GR.

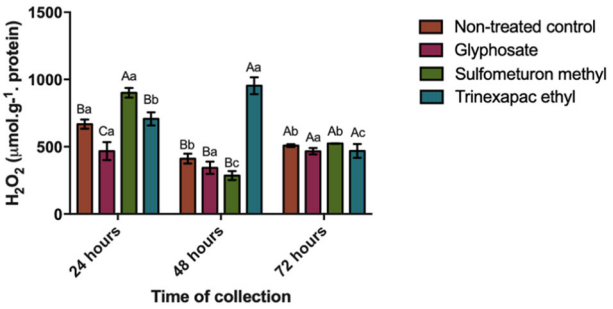

Fig. 7 Average levels of reactive oxygen species (ROS) observed 24, 48, 72 h after application (HAA) in the cultivar 'Vanda'. Averages followed by the same letter do not differ by Tukey's test at 5% probability, with lower case letters comparing the subdoses within each product and upper-case letters comparing products within the subdoses

Guaiacol Peroxidase activity (GPOX 1.11.1.7)

'Lucy Brown' (Fig. 9), GPOX showed high activity in the 24 h ($p < 0.05$), where treatment with glyphosate caused a significant increase at activity of this enzyme (97.6%), while treatments with sulfometuron methyl and trinexapac ethyl showed levels of activities of 45 and 41.6% respectively, compared to the non-treated control. At 48 h ($p < 0.05$), only methyl sulfometuron showed high activity with an increase of 26%, and at 72 h there was no difference between treatments ($p > 0.05$). For cultivar 'Vanda', no interaction was observed.

Discussion

The drift of the application of chemical ripeners in sugarcane is an important problem since it can cause a drop in the growth and yield of horticultural crops (El-Nahhal and Hamdona 2017). In fact, in this experiment it was noted that as the doses of ripeners increased, lettuce cultivars fresh and dry biomass decreased.

In addition, it was noted that for both cultivars, glyphosate had the least impact on their growth. Suggesting that both cultivars may have tolerance to glyphosate at low doses. Insensitive species to glyphosate, few doses sound capable of significantly reducing plant growth, for example, in sunflower, lower doses than those tested in this experiment caused an important drop in the growth of this species (Vital et al. 2017a). In peanut, low doses of glyphosate reduced yield and seed kernel mass, but germination was not affected (Grey and Prostko 2010). However, in maize, *Commelina benghalensis, Eucalyptus grandis*, and *Pinus caribea*, plant growth stimulation was observed at low

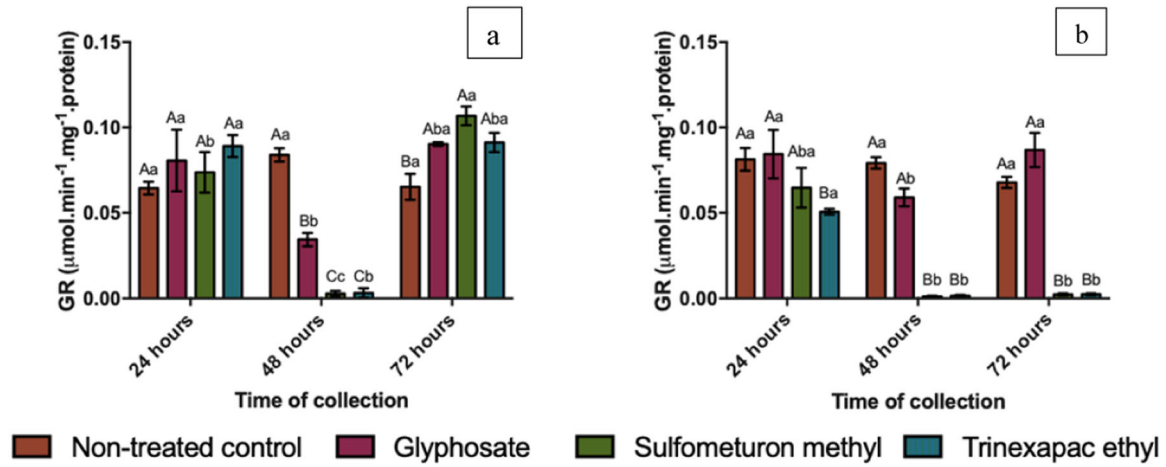

Fig. 8 Average GR enzyme contents observed at 24, 48, 72 h after application (HAA) in 'Lucy Brown' (**a**) and 'Vanda' (**b**). Averages followed by the same letter do not differ by Tukey's test at 5% probability, with lower case letters comparing the time of collection within each product and upper-case letters comparing products within the time of collection

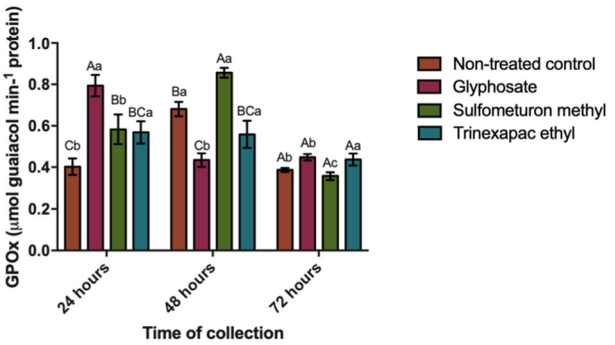

Fig. 9 Average levels of the GPx enzyme observed at 24, 48, 72 h after application (HAA) in 'Lucy Brown'. Averages followed by the same letter do not differ by Tukey's test at 5% probability, with lower case letters comparing the time of collection within each product and upper-case letters comparing products within the time of collection

doses (Velini et al. 2008). This effect is known as hormesis and according to Velini et al. (2008), plants that responded with an increase in growth showed some level of shikimic acid accumulation, and may be related to the EPSPS enzyme inhibition pathway. The mechanisms involved in the stimulation or tolerance of a plant expose to glyphosate at low doses are not clarified and will depends on species, plant stage during the exposure to glyphosate and rate applied (Velini et al. 2008).

Trinexapac ethyl and sulfometuron methyl had a high impact on the decrease in fresh and dry biomass, especially at 12% and other researchers report that sulfometuron methyl can reduce the growth of the soybean area by almost 50% with increasing doses (Correia and Leite 2012). The decrease in lettuce biomass of 'Lucy Brown' and 'Vanda' when affected by the ripeners may be related to the reduction in chlorophyll content observed in both cultivars, especially at 12% of sub-dose levels. Chlorophylls play an important role in the amount of solar radiation that is absorbed by plants and, therefore, its leaf concentration is directly related to photosynthetic capacity (Croft et al. 2017).

It was reported that the application of glyphosate can significantly reduce the content of chloroplasts in annual species (Singh et al. 2017) as observed in our results in both lettuce cultivars. However, this response is affected by factors such as the nutritional status of the plants and the dose applied (Vital et al. 2017b; Bacha et al. 2018). In the case of trinexapac-ethyl, this ripener had an impact on the chlorophyll content in the cultivar 'Lucy Brown', while trinexapac ethyl and sulfometuron methyl had a strong impact on the chlorophyll levels in 'Vanda'. These results suggest that the detoxification system of these cultivars for the applied duration did not manage to protect the stability of the chlorophylls (Singh et al. 2017). Other studies report that sulfometuron methyl, although changes in the photosynthetic apparatus are not the main target of the sulfonylurea group, there are changes in the chlorophyll content (Riethmuller-Haage et al. 2006) as occurred in this experiment.

Chlorophyll A fluorescence is an important measurement of photosynthetic efficiency and plant productivity, which, according to Stirbet et al. (2018), can be used for a study of photosynthetic aspects, such as changes in the organization of the thylakoid membrane. The reference value for the parameter Fv/Fm, found for most vascular plants and when it has its photosynthetic apparatus intact, is 0.832 ± 0.004, according to Bjorkman and Demming (1987). However, some authors consider the range between 0.75 and 0.85 to be acceptable (Maxwell and Johnson 2000; Wagner and Merotto Junior 2014).

As previously mentioned, glyphosate did not show an important impact on growth reduction in both, although this ripener significantly reduced the chlorophyll content, which may be related to a high efficiency of photosystem II shown by the plants that grew under this treatment when compared to the treatments with trinexapac ethyl and sulfometuron methyl.

It is worth mentioning that for glyphosate in specific, Gomes et al. (2016) explain that its physiological effects have been recently studied, demonstrating that there are possible damages to photosystem II as well as influences on the electron transport chain. In the case of trinexapac ethyl and sulfometuron methyl, these directly affected the health of the FSII, endangering the electron transport chain in this work.

Reactive oxygen species (ROS) act as important signaling molecules in various biological processes in plants. When its accumulation occurs, phytotoxic effects can be observed, and at the cellular level, damage can occur in cellular components, such as DNA, lipids and proteins (Tripathi et al. 2020). In our experiment, it was found that only in 48 hr there was significant damage to the membranes by the ripeners used (high MDA content) to cultivar 'Lucy Brown'. On the same sampling date, a drop in H_2O_2 content was found, which may suggest that other ROS species are damaging membranes (Kapoor et al. 2019). Even so, the lack of could be related to a drop in the activities of antioxidant enzymes such as GR as noted in this experiment. In the case of cultivar 'Vanda', there were no differences between the treatments studied, suggesting that other cell structures are probably being damaged.

At 72 h no significant differences were found compared to the non-treated control in the cultivar 'Lucy Brown' and in 'Vanda'. In the case of the cultivar 'Lucy Brown', these results may indicate a recovery of the plants from the third day, which can be verified with a low amount of hydrogen peroxide found at that moment which may be related to a good activity of the antioxidant enzyme system such as GR and GPOx. Both GR and GPOx are related to abiotic stress (Harshavardhan et al. 2017; Kataria et al. 2019).

The effects of chemical ripener drift can vary significantly, even within the same crop species, as can be seen in this research. The results indicate that some level of reduction in the dry and fresh lettuce biomasses are observed, even in subdoses. This may be related to the levels of chlorophyll and quantum efficiency of photosystem II that have undergone some level of physiological changes, as observed in this research. This demonstrates that although the tested ripener products do not have a direct mode of action in these locations of the plant, other detrimental effects were observed. These include an increase in ROS activity for glyphosate at 24 h after collection in the 'Lucy Brown' and sulfometuron methyl and trinexapac-ethyl at 24 and 48 h, respectively, for 'Vanda'. GR showed activity for both cultivars, at 24 and 72 h. When evaluating GPOx activity, only for 'Lucy' at 24 and 48 h of collection, activity was observed. Due to the abiotic plant stresses, cultivars reacted in different ways, demonstrating that different mechanisms to overcome stress were being activated. Further studies should, therefore, investigate the different effects of trinexapac-ethyl, glyphosate, and sulfometuron methyl on plant physiology, more specifically in the photosynthesis pathway, and understand which mechanisms to overcome stress are related to the primary and secondary modes of action of the products.

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Compliance with ethical standards

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