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BASIC AREAS
Article

Enzymatic antioxidant defense system and ALA-D enzyme activity in soybean Enlist™ line

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ABSTRACT: Enlist™ technology confers resistance to 2,4-D herbicides, glyphosate, and ammonium glufosinate for soybeans. However, the application of herbicides to plants can generate stress, even in resistant crops. Thus, here we evaluated whether the application of herbicides in transgenic soybean farms, resistant to these herbicides, adversely affects the activity of antioxidant enzymes and porphobilinogen formation by delta-aminolevulinate dehydratase (ALA-D) activity. At seven days after herbicide application, the aerial part of the plants was collected and used to determine the activity of antioxidant enzymes as catalase, ascorbate peroxidase, and guaiacol peroxidase, such as lipid peroxidation levels and the activity of the ALA-D enzyme. At seven days after herbicide application, the aerial part of plants was collect and used to determine the activity of antioxidant enzymes, such as catalase, ascorbate peroxidase and guaiacol peroxidase, as well as the levels of lipid peroxidation and the activity of the ALA-D enzyme. The activity of important antioxidant enzymes involved in the scavenging of reactive oxygen species (ROS) was increased in the soybean cultivar with the Enlist™ technology, while no severe damage to lipids was detected. However, the activity of ALA-D was inhibited, which could potentially impair the formation of porphobilinogen and decrease photosynthetic efficiency. Thus, these results indicate that herbicides can affect the activity of ROS-scavenging enzymes even in transgenic, herbicide-resistant plants.

Key words: *Glycine max* L., oxidative stress, herbicide resistance, resistant crops, antioxidant enzymes.

INTRODUCTION

New genetically modified cultivars are emerging, such as Cortex Agro Sciences™ Enlist™ that confers resistance to 2,4-dichlorophenol (2,4-D) herbicides in a variety of crops, including soybeans, corn, and cotton. This resistance to 2,4-D herbicides comes from the insertion of a transgene that encodes a bacterial isoenzyme specific for aryloxyalkanoate dioxygenase (AAD), which cleaves 2,4-D into a metabolite that does not contain herbicidal action (Wright et al. 2010). Enlist™ soy, in turn, harbors three genes: aryloxyalkanoate dioxygenase-12 (AAD-12), originally isolated from the bacterium *Delftia acidovorans*, which is responsible for 2,4-D resistance; the 5-enolpyruvylchikimate-3-phosphate synthase (EPSPS); and phosphinothricin acetyltransferase (*pat*), which confers resistance to two other herbicides, glyphosate, and ammonium glufosinate, respectively (Gazola et al. 2021).

In susceptible plants, glyphosate acts on the shikimic acid pathway and specifically inhibits the enzyme EPSPS, preventing the biosynthesis of aromatic amino acids such as phenylalanine, tryptophan, and tyrosine (Brito et al. 2018). The 2,4-D is an auxinic herbicide, acting as auxin agonists, mimicking indole-3-acetic plant growth hormone (AIA). However, in high doses, it causes alterations on the normal growth and development of plants and even death (Senseman 2007). Ammonium

glufosinate inhibits the glutamine synthetase enzyme in non-resistant plants generating ammonium accumulation at toxic levels and inhibits photorespiration (Duke and Dayan 2011).

It is known that herbicides can trigger stress in plants, even in crops considered resistant, thereby compromising their productivity and growth (Gill and Tuteja 2010). For instance, herbicides are capable to induce the overproduction of reactive oxygen species (ROS). In excess, ROS are very reactive and toxic, with the ability to promote damage to proteins, lipids, carbohydrates, and DNA, resulting in oxidative stress (Kapoor et al. 2019; Singh and Tiwari 2020).

Among the main ROS generated in the plant cells, there are the superoxide radical ($O_2 \cdot^-$), hydroxyl radical (OH·), hydrogen peroxide (H_2O_2), and singlet oxygen ($1O_2$) (Gill and Tuteja 2010). To mitigate the consequences of oxidative stress, plants have developed enzymatic and non-enzymatic defense mechanisms that play an important role in controlling the accumulation of ROS (Kapoor et al. 2019). The enzyme system is formed by superoxide dismutase (SOD, EC 1.15.1.1) that converts $O_2 \cdot^-$ to H_2O_2, which is also toxic to cells. H_2O_2 is rapidly degraded to H_2O and O_2 by peroxidases such ascorbate peroxidase (APX, EC 1.11.1.11), catalase (CAT, 1.11.1.6), and guaiacol peroxidase (GPX, EC 1.11.1.7) (Sharma et al. 2019, Zaid and Wani 2019).

In addition, there are reports that herbicide exposure may affect the activity of the enzyme δ-aminolevulinate dehydratase (ALA-D, EC4.2.1.24), which is a key enzyme in plants responsible for catalyzing the condensation of two molecules of delta-aminolevulinic acid (δ-ALA), with the formation of the porphobilinogen monopyrrole compound (PBG), an essential molecule on respiration and photosynthesis (Gashi et al. 2020).

Considering the importance of the plant's ability to reverse stress situations through the antioxidant defense and knowing that herbicides can generate stress conditions in plants, even in resistant crops, the present study aimed to evaluate whether the application of herbicides negatively affects the activity of antioxidant enzymes and the ALA-D in transgenic soybean cultivars that are resistant to glyphosate, 2,4-D, and ammonium-glufosinate herbicides.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse with a transgenic soybean cultivar with resistance to glyphosate, ammonium-glufosinate, and 2,4-D (Enlist™ - DAS 44406-6). Plants were grown in 0.5-L pots containing soil and substrate in a 1:1 ratio. The design was completely randomized, with four replications, and two plants per replicate. The treatments were composed of control (without application) and single or combined herbicides applied following the commercial dose recommended for weed control (Table 1).

Table 1. Treatments and doses of herbicides applied to Enlist™ soybean (DAS 44406-6). Universidade Federal da Fronteira Sul, Erechim/RS, Brazil, 2018.

Treatments	Dose (g·ha^{-1} i.a or e.a)*	Commercial name	Commercial dose (L·ha^{-1})
Control without herbicides	---	---	---
Glyphosate	1,080	Roundup Original®	3
2,4-D	670	DMA 806 BR®	1
Amonio-glufosinate	400	Finale®	2
Glyphosate + 2,4-D	1,080 + 670	Roundup Original® + DMA 806 BR®	3 + 1
Glyphosate + amonio-glufosinate	1,080 + 400	Roundup Original® + Finale®	3 + 2
Amonio-glufosinate + 2,4-D	400 + 670	Finale® + DMA 806 BR®	2 + 1
Glyphosate + amonio-glufosinate + 2,4-D	1,080 + 400 + 670	Roundup + Finale® + DMA	3 + 2 + 1

*Doses recommended by the manufacturer for weed control.

The application of treatments was carried out 22 days after the emergence of soybean plants, which coincided with the V1 stage (with the first visible node). Plants were sprayed with a CO_2 pressurized costal sprayer, coupled with a four-point spray bar of type DG 110.02, which sprayed at a 150 $L \cdot ha^{-1}$ flow rate. Seven days after the treatment application, whole plants were collected at a distance of 1 cm from the soil with the aid of scissors. Collected plant samples were immediately frozen in liquid nitrogen to avoid degradation. Then, the samples were macerated in liquid nitrogen and kept in an ultra-freezer at -80°C until the biochemical analyses were performed.

Determination of the antioxidant system enzymes

Extract preparation for enzymatic antioxidant system

The enzymatic extract used for the determination of catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) activity was obtained by homogenizing 0.3 g of plant tissue in polyvinylpolypyrrolidone (PVPP 10%) and 1.5 mL extraction buffer containing 200 mM potassium, with bovine serum albumin used as a standard.

Catalase, ascorbate peroxidase, guaiacol peroxidase, thiobarbituric acid and ALA-D determination

The extract preparation followed the procedure described before. The CAT activity was carried out using the method described by Havir and McHale (1987), and the spectrophotometric reading as described by Anderson et al. (1995), expressed in Units.mg protein.min. APX activity was assessed using the method of Nakano and Asada (1981), and expressed as μmol $OA \cdot min^{-1} \cdot$mg protein. GPX activity was determined according to Zeraik et al. (2008), and expressed as $U \cdot$mg $protein^{-1}$.

The malondialdehyde (MDA) contents were estimated according to the methodology proposed by Hodges et al. (1999) by quantifying substances that react with thiobarbituric acid (TBARS), and expressed as nmol of $MDA \cdot$mL leaf $tissue^{-1}$.

δ-Aminolevulinate dehydratase (δ ALA-D)

The plant tissue was homogenized in 10 mM Tris-HCl buffer, pH 7.4, in a ratio of 1:1 (w/v). The homogenate was centrifuged at 6,000 rpm at 4°C for 10 min. The supernatant was pretreated with 1% Triton X-100 and 0.5 mM dithiothreitol (DTT). The activity of ALA-D was determined as described by Morsch et al. (2002). The reaction product was determined with the Erlich reagent at 555 nm using a molar absorption coefficient of $6.1 \times 10\text{-}4$ $mol^{-1}cm^{-1}$ (Sassa 1982) for Erlich-porphobilinogen. The results were expressed in nmol of $PBG \cdot h^{-1} \cdot mL^{-1}$.

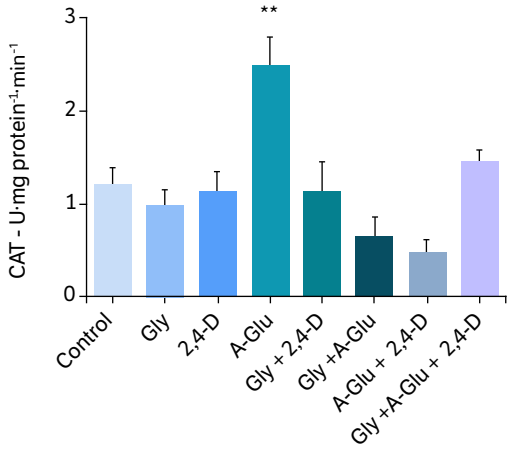
Statistical analysis

All data were submitted to analysis of variance (ANOVA) using GraphPad Prism 7.0 software, followed by a post-hoc Dunnet's test at $p < 0.05$.

RESULTS

Catalase

The activity of the CAT enzyme (Fig. 1) was increased by 157.80 and 173.39% compared to the control when the plants were treated with single haloxyfop-R and the ammonium-glufosinate + haloxyfop-R mixture, respectively. The other treatments did not significantly alter the activity of this enzyme compared to the control.

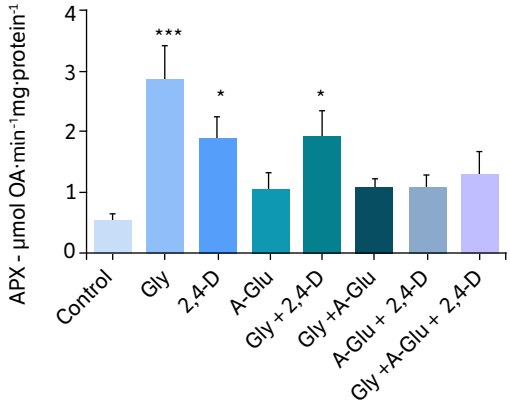


*p < 0.05; **p < 0.005; ***p < 0.001.

Figure 1. Effect on catalase activity (CAT) of herbicides glyphosate (Gly), ammonium-glufosinate (A-Glu), 2-4-D, and its mixtures at seven days after application on soybean Enlist. Data are expressed in mean ± standard error range of four replicates and analyzed using post hoc Dunnet's test compared to the control (without herbicide).

Ascorbate peroxidase

The ammonium-glufosinate and haloxyfop-R herbicides induced the activity of ascorbate peroxidase by 183.92 and 127.33%, respectively (Fig. 2). Among the herbicide combinations, mixtures of glyphosate + 2,4-D (173.52%), glyphosate + haloxyfop-R (111.18%), 2,4-D + ammonium glufosinate (317.62%), ammonium glufosinate + haloxyfop-R (189.98%), and glyphosate + ammonium-glufosinate + haloxyfop-R (145.18%) (Fig. 3) were those showing an increase in enzyme activity when compared with the control, suggesting that ROS were generated after exposure to these herbicides.



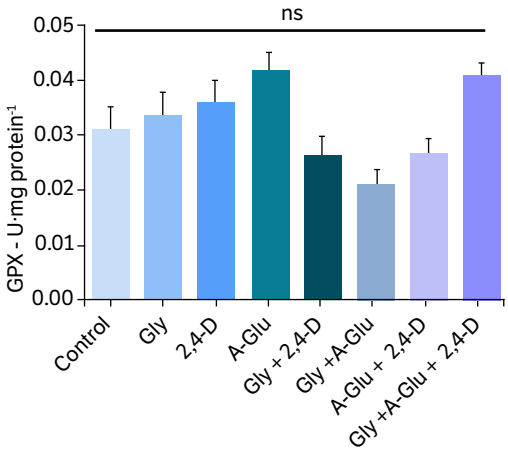
*p < 0.05; **p < 0.005; ***p < 0.001.

Figure 2. Effect on ascorbate peroxidase activity (APX) of herbicides glyphosate (Gly), ammonium-glufosinate (A-Glu), 2-4-D, and its mixtures at seven days after application on soybean Enlist. Data are expressed in mean ± standard error range of four replicates and analyzed using post hoc Dunnet's compared to the control (without herbicide).

Guaiacol peroxidase

Another enzyme that was affected by the treatments was GPX, which was inhibited by some mixtures, but not impaired single herbicide treatments. The glyphosate + ammonium-glufosinate and 2,4-D + haloxyfop-R mixtures reduced 48.57 and 50.86% GPX activity compared to the control, respectively.

The lowest GPX activity occurred when mixing glyphosate + 2,4-D + ammonium glufosinate (75.38%), glyphosate + 2,4-D + haloxyfop-R (92.51%), glyphosate + ammonium glufosinate + haloxyfop-R (75.59%), 2,4-D + ammonium glufosinate + haloxyfop-R (87.93%), and glyphosate + 2,4-D + ammonium glufosinate + haloxyfop-R (91. 92%) compared to the control (Fig. 3). These results suggest that mixtures involving the association of a greater number of mechanisms of action were more damaging.

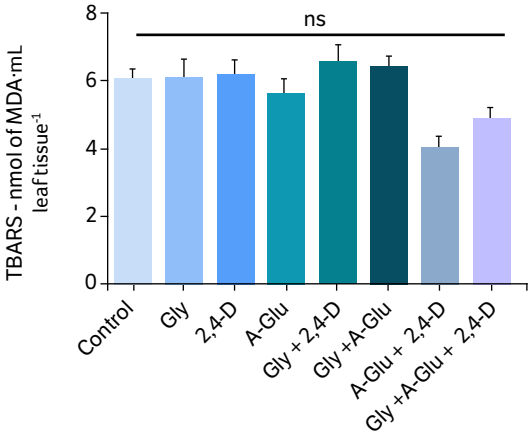


ns: non-significant.

Figure 3. Effect on guaiacol peroxidase activity (GPX) of herbicides glyphosate (Gly), ammonium-glufosinate (A-Glu), 2-4-D, and its mixtures at seven days after application on soybean Enlist. Data are expressed in mean ± standard error range of four replicates and analyzed using post hoc Dunnet's compared to the control (without herbicide), $p < 0.05$.

Lipid peroxidation

The peroxidation of lipids produces MDA, in addition to other products, and it is through this compound that it is possible to estimate the lipid peroxidation state of plant cell membranes. In the present work, an increase in lipid peroxidation levels (Fig. 4) was observed in the EnlistTM soybean seven days after the application of treatments. The use of glyphosate alone induced 41.37% of lipid peroxidation. Increased lipid peroxidation was also detected when the following mixtures were applied: glyphosate + 2,4-D (50.61%), 2,4-D + ammonium glufosinate (45.83%), 2-glyphosate + 2,4-D + ammonium glufosinate (51.61%), glyphosate + 2-glyphosate + 4-D + haloxyfop-R (36.46%), ammonium glufosinate + haloxyfop (46.68%), and glyphosate + ammonium-glufosinate + haloxyfop-R (48.60%). Thus, these plants exhibited signs of oxidative stress (Fig. 4).

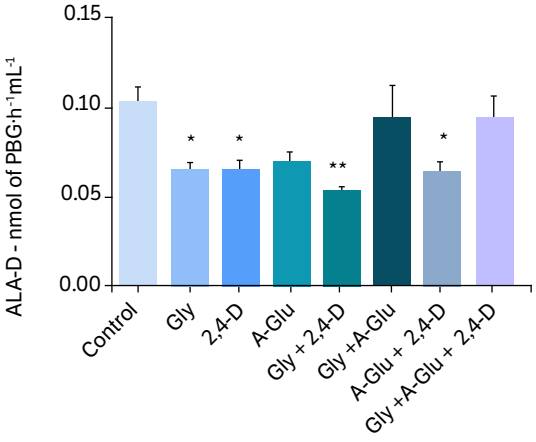


*p < 0.05; **p < 0.005; ***p < 0.001; ns: non-significant.

Figure 4. Lipid peroxidation (TBARS) of herbicides glyphosate (Gly), ammonium-glufosinate (A-Glu), 2-4-D, and its mixtures at seven days after application on soybean Enlist. Data are expressed in mean ± standard error range of four replicates and analyzed using post hoc Dunnet's compared to the control (without herbicide)

δ-aminolevulinate dehydratase (ALA-D)

The activity of ALA-D (Fig. 5) was reduced by using the herbicide treatments on EnlistTM soyben compared to the control without herbicide. Regarding herbicides applied alone, glyphosate, 2,4-D, ammonium glufosinate, and haloxyfop-R inhibited the enzyme by 52.7, 59.82, 67.63, and 66.52%, respectively.



*p < 0.05; **p < 0.005; ***p < 0.001.

Figure 5. Effect of δacid aminolevulinate dehydratase (ALA-D) of herbicides glyphosate (Gly), ammonium-glufosinate (A-Glu), 2-4-D, and its mixtures at seven days after application on soybean Enlist. Data are expressed in mean ± standard error range of four replicates and analyzed using post hoc Dunnet's compared to the control (without herbicide).

Similar inhibition occurred in the mixtures of herbicides: glyphosate + 2,4-D (67.91%), glyphosate + ammonium glufosinate (60.66%), glyphosate + haloxyfop-R (64.84%), 4-D + haloxyfop-R (50.06%), ammonio-glufosinate + haloxyfop-R (66.33%), and glyphosate + 2,4-D + ammonium glufosinate (64.56%).

The other mixtures evaluated also caused some inhibition, but less dramatically: glyphosate + 2,4-D + haloxyfop-R (41.27%), glyphosate + ammonium glufosinate + haloxyfop-R (39.62%), 4-D + ammonium glufosinate + haloxyfop-R (41.55%), and glyphosate + 2,4-D + ammonium glufosinate + haloxyfop-R (41.40%).

DISCUSSION

As cell membranes are the primary targets of many plant stresses, maintaining their integrity and stability under stress conditions is an important component of herbicide resistance in plants. In this work, according to the evaluation of lipid peroxidation, the transgenic Enlist™ soybean did not present significant changes in any of the treatments when compared to the control at seven days after herbicide application (Fig. 1). This result may be related to the increase in antioxidant defense. However, this action cannot be attributed solely to the studied antioxidant enzymes, such as CAT and APX, which directly decompose H_2O_2. Since peroxide generally does not damage lipids, other antioxidant enzymes (SOD, glutathione reductase, among others) and non-enzymatic antioxidant system such as ascorbic acid (AsA), glutathione (GSH), and carotenoids could have also been involved (Alves et al. 2018). This antioxidant enzymatic system response has the potential to act in the prevention of oxidative peroxidation of biological membranes, which occurs when there is an imbalance between the production and elimination of these reactive species. To avoid oxidative damage, even at small H_2O_2 levels, there is an induction of CAT and APX enzymes that may be associated with the fact that these enzymes act in the presence of peroxides such as H_2O_2, thus controlling oxidative stress.

At seven days after the application of ammonium glufosinate, there was an increase in CAT activity in soybean (Fig. 2). This change in CAT activity is probably linked to the action of ammonium-glufosinate, even after its herbicidal

activity has been eliminated through the Pat gene; that is, CAT activation indicates the increased generation of ROS, especially H_2O_2, which is its substrate. However, when this herbicide was associated with others in the mixtures, it did not cause any increase in this enzyme compared to with the control.

However, in contrast to the CAT increase, ammonium glufosinate did not increase APX activity (Fig. 3). APX is one of the most important and fundamental enzymes of antioxidant metabolism in plants, since it catalyzes the decomposition of H2O2 into water, using ascorbate as an electron donor (Pandey et al. 2017). In this work, an increase in its activity in transgenic soybean was observed when glyphosate herbicide was applied (Fig. 3). The activity of the enzyme APX increased after the plants were exposed to glyphosate, 2,4-D, and in the mixture of the two herbicides.

Although the main mechanism of action of glyphosate does not exert oxidative stress, the increased activity of enzymes such as CAT, GPX, and APX indicates the generation of ROS and antioxidant responses after treatment with herbicides (Pandey et al. 2017). There are already indications in studies that glyphosate can affect other physiological processes of the plants beyond its main site of action (Gomes et al. 2017). The results from the present study reinforce this possibility. One hypothesis is that glyphosate may have affected the flow of electrons during plant cell respiration, thus causing ROS generation in the Enlist™ soybean.

A study that addressed the specific site of ROS induction by glyphosate was performed by Gomes et al. (2017) on lentil (*Lemna minor* L.). The authors noted that glyphosate can interfere with mitochondrial activity via the normal flow of electrons in the electron transport chain during the cellular respiration of plants, as it acts by inhibiting the activity of complex III and this may end up damaging the energy metabolism generating ROS, consequently promoting a reduction of productivity and plant growth.

The 2,4-D increased APX activity, suggesting that the herbicide also causes ROS generation. It is likely that with the introduction of the gene that enables 2,4-D resistance in soybeans; there is a cleavage of this herbicide into an action-free metabolite, 2,4-dichlorophenol (2,4-DCP). Therefore, it is suggested that the metabolism of this compound could generate the formation of ROS. In a study with *Skeletonema costatum*, an increase in the activity of APX and SOD enzymes was observed after 96-hour exposure to 2,4-DCP (Yang et al. 2002). In addition, the mixture of the two herbicides (glyphosate + 2,4-D) also promoted APX activation (Fig. 2), but it was lower than when applied in isolation.

The behavior seen between the two enzymes (CAT and APX) can be explained by their distinct affinities to the substrate. APX has a high affinity with H_2O_2 and acts when it is present in low concentrations; CAT, on the other hand, has the opposite behavior (Gill and Tuteja 2010). Therefore, the results may be related to the number of H_2O_2 formed due to exposure to each herbicide. A hypothesis would be that, when the transgenic soybean plants were submitted to glyphosate and 2,4-D herbicides, both in isolation and in the mixture, the amount of H_2O_2 produced was only sufficient to activate APX. This would explain the increase in APX activity to treatments with glyphosate, 2,4-D, and glyphosate + 2,4-D, whereas CAT was not activated in these treatments. CAT had its activity increased only after exposure to ammonium-glufosinate. It occurs because of the presence of H_2O_2 in the chloroplasts, which is eliminated by APX enzymes; in contrast, when it is produced in the peroxisomes/glyoxysomes, it is removed by CAT (Gupta et al. 2018).

Additionally, as reported for CAT and APX, GPX is also specialized in the removal of H_2O_2 (Møller et al. 2007). However, in this study, GPX activity was not influenced by the performed treatments compared to the control group.

In general, ammonium-glufosinate-containing mixtures associated with other herbicides, such as glyphosate + ammonium-glufosinate, ammonium-glufosinate + 2,4-D, and glyphosate + ammonium-glufosinate + 2,4-D, did not cause a significant increase in activity of the antioxidant enzymes in the Enlist™ soybean cultivar (Figs. 2, 3, and 4). This can be explained by a possible antagonistic effect, which is common in herbicide mixtures that are not commercial and happens when the interaction represents a smaller effect than the sum of the effects of the products applied in isolation (Galon et al. 2021). This may have been caused by the mixture of ammonium glufosinate, glyphosate contact herbicide, and/or 2,4-D, which are systemic, and may have resulted in lower herbicidal activity. However, this small antagonistic effect turned out to be beneficial, not causing stress and managing to maintain a balance in the production of ROS.

Cases of antagonism of the blends described before are reported in a few studies in the literature. One of them reported an antagonistic effect for the mixture of glyphosate + ammonium-glufosinate in Indian goosegrass (*Eleusine indica* L.) (Chuah et al. 2008). This antagonism was hypothetically explained by the fact that ammonium glufosinate exhibits a rapid action,

which would prevent glyphosate; this is systemic and has a slower action, thus preventing its absorption and translocation (Chuah et al. 2008). Therefore, this would be a hypothesis for the results in this work; that is, ammonium-glufosinate would have acted faster against systemic herbicides by damaging its actions in soybean cultivation, even resulting in resistance. Similar antagonistic response was also reported when glyphosate was mixed with another contact herbicide such as diquat (Wehtje et al. 2008).

ALA-D, another enzyme considered to be an oxidative stress biomarker, but which has no antioxidant role and has been poorly studied in plants (Chai et al. 2017), was also evaluated in this study. The activity of the enzyme was inhibited seven days after the application of glyphosate in transgenic soybean (Fig. 5). Here, exposure to the herbicide resulted in a decrease in the activity of the ALA-D enzyme (Fig. 5) compared to the herbicide-free control in glyphosate (34%), 2,4-D (35%), glyphosate + 2,4-D (47%) and ammonio-glufosinate + 2,4-D (37%). The other treatments did not present significant differences when compared to the control.

It is known that a molecule that blocks the synthesis of ALA-D would have long-term effects on biochemical and physiological processes in plants because this enzyme is one of the most important in chlorophyll biosynthesis (Tanaka and Tanaka 2007). It catalyzes a reaction that is part of the biosynthetic pathway of tetrapyrrole compounds (corrins, bilins, chlorophylls, and heme), which play important roles in photosynthesis and plant respiration. Its inhibition, therefore, affects chlorophyll synthesis through inhibition of the porphobilinogen formation pathway. The inhibition of chlorophyll synthesis reduces the amount of light absorbed and energy generated. In this case, the change in chlorophyll content will affect the photosynthetic process, which is a determinant of agricultural productivity (Li et al. 2019). The sensitivity of chlorophyll to glyphosate is addressed in some studies, which show that there is a decrease in its content in plants when exposed to herbicides (Gomes et al. 2017), as well as in transgenic cultivars (Zobiole et al. 2010), as observed in this work.

Moreover, another hypothesis for detected inhibition of ALA-D would be that there was a possible inhibition of delta-aminolevulinic acid (δ ALA), a substrate of ALA-D, caused by glyphosate. In addition, inhibition of the formation of δ ALA by glyphosate has already been reported (Zobiole et al. 2010).

Also, ALA-D was inhibited in transgenic soybean when exposed to 2,4-D (synthetic auxin) (Fig. 5), showing its potential to affect the formation of chlorophyll in the plant and to interfere with photosynthesis. In a study by Wu et al. (2010), the herbicide fluroxypyr of the same mechanism of action of 2,4-D decreased the chlorophyll formation in *Oryza sativa* L. plants.

The inhibition of ALA-D was also seen after exposure to the glyphosate and 2,4-D herbicide mixture (Fig. 5), which was slightly more pronounced than the one that occurred in comparison to exposure to the two individually; thus, its association can cause a sum of their effects and increase the damage to plants. The ammonium-glufosinate + 2,4-D mixture also promoted inhibition of the ALA-D enzyme, which has the potential to affect chlorophyll synthesis as a side effect, since the plant contains the protein resistance; this was also observed when the herbicide was applied in isolation, as it promoted inhibition in ALA-D, but it was not significant.

In addition, inhibition of this enzyme after the application of these herbicides may also be related to the generation of ROS observed by the activation of antioxidant enzymes in this work. The low activity of the enzyme ALA-D can be related to an increase in the level of ROS, especially H_2O_2, which shows that inhibition of the ALA-D enzyme induces plant stress, as observed by Chai et al. (2017) in the mutant plant of *Gossypium hirsutum*.

Treatments with ammonium glufosinate associated with other herbicides, such as glyphosate + ammonium glufosinate, ammonium glufosinate + 2,4-D, and glyphosate + ammonium glufosinate + 2,4-D, were the least toxic to soybean since the activities of enzymes that remove H_2O_2 were close to those of the control. Also, there was no lipid peroxidation, which shows that the herbicides maintained the levels of lipid damage close to those of the control by a possible antagonistic effect in the mixtures, which was positive in this case. In addition, these mixtures did not inhibit the activity of ALA-D, except for ammonium-glufosinate + 2,4-D. This makes the mixtures a favorable option for farmers to use.

Despite this, inhibition of the ALA-D enzyme occurred in the glyphosate and 2,4-D isolated treatments and the ammonium-glufosinate mixture with 2,4-D. Thus, together, the results suggest that the herbicides affect homeostasis in the plant, promoting an increase in the antioxidant enzymes APX and CAT. Nevertheless, the plant, through its defense mechanisms, managed to prevent lipid peroxidation, showing itself as a promising cultivar for agricultural crop insertion.

CONCLUSION

The Enlist™ soybean cultivar presented activation of the important antioxidant enzymes in the mechanism of elimination, such as CAT and APX, avoiding severe damage to plant lipids, as indicated by the MDA (TBARS). However, there was inhibition of the ALA-D enzyme, which is important in the formation of compounds like chlorophyll. Thus, the effects of herbicides on enzymes related to ROS provide evidence that they can affect plants that are genetically engineered to resist these herbicides.

AUTHORS' CONTRIBUTION

Conceptualization: Concato, A.C., Galon, L. and Kaizer, R.R.; **Data curation:** Concato, A.C., Tamagno, W.A. and Alves, C.; **Formal analysis:** Tamagno, W.A., Galon, L. and Vargas, L.; **Investigation:** Concato, A.C., Tamagno, W.A. and Kaizer, R.R.; **Methodology:** Kaizer, R.R., Concato, A.C. and Galon, L.; **Resources:** Kaizer, R.R, Vargas, L. and Galon, L.; **Software:** Tamagno, W.A., Alves, C. and Sutorillo, N.T.; **Supervision:** Concato, A.C., Vanin, A.P. and Kaizer, R.R.; **Visualization:** Galon, L., Vargas, L., Sutorillo, N.T., **Writing – original draft:** Concato, A.C., Tamagno, W.A. and Alves, C.; **Writing – review and editing:** Kaizer, R.R., Concato, A.C. and Galon, L.

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