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CROP PROTECTION

Antioxidant detoxification system of wheat and ryegrass plants subjected to various herbicides

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ABSTRACT: Herbicide promotes physiological and biochemical changes even in tolerant species. The objective of this study was to evaluate the interference of the herbicides clodinafop-propargyl and 2,4-D in the antioxidant system of wheat, and iodosulfuron-methyl in wheat and ryegrass. Two studies, divided into three experiments, were conducted in an entirely randomized design in a greenhouse and phytotron. The first study tested herbicides iodosulfuron-methyl, clodinafop-propargyl, 2,4-D, and a control without application in wheat. The second, divided into two experiments with wheat and ryegrass, tested iodosulfuron-methyl doses (0, 1.75, 3.5, and 7.0 g a.i. ha^{-1}). The variables analyzed were the concentrations of chlorophylls *a*, *b*, carotenoids, hydrogen peroxide (H_2O_2), lipid peroxidation (TBARS), electrolyte leakage, and antioxidant system activity. The herbicide treatments iodosulfuron-methyl, clodinafop-propargyl, and 2,4-D decreased the concentrations of chlorophylls and carotenoids in wheat plants. The herbicides promoted oxidative stress with increased H_2O_2 and TBARS concentrations. Increasing the dose of iodosulfuron-methyl provided a reduction in the activity of the enzymatic antioxidant system in wheat and ryegrass.
Key words: *Triticum aestivum* L., *Lolium perenne* L. var. *multiflorum* (Lam.), oxidative stress.

Sistema de desintoxicação antioxidante de plantas de trigo e azevém submetidas a diversos herbicidas

RESUMO: A aplicação de herbicidas promove alterações fisiológicas e bioquímicas mesmo nas espécies tolerantes. O objetivo do estudo foi avaliar a interferência dos herbicidas clodinafop-propargyl e 2,4-D no sistema antioxidante do trigo, e iodosulfuron-methyl em trigo e azevém. Dois estudos, divididos em três experimentos, foram conduzidos em delineamento inteiramente casualizado em casa de vegetação e fitotron. O primeiro estudo testou os herbicidas iodosulfuron-methyl, clodinafop-propargyl, 2,4-D, e testemunha sem aplicação em trigo. O segundo, dividido em dois experimentos com trigo e azevém, testou doses de iodosulfuron-methyl doses (0, 1,75, 3,5, and 7,0 g i.a. ha^{-1}). As variáveis analisadas foram as concentrações de clorofilas *a*, *b*, carotenoides, peróxido de hidrogênio (H_2O_2), peroxidação lipídica (TBARS), extravazamento de eletrólitos e atividade do sistema antioxidante. O uso dos herbicidas iodosulfuron-methyl, clodinafop-propagyl e 2,4-D diminuíram o teor de clorofilas e carotenoides nas plantas de trigo. Os herbicidas promovem estresse oxidativo com aumento no teor de H_2O_2, TBARS e extravasamento de eletrólitos. O aumento da dose do iodosulfuron-methyl proporciona redução da atividade do sistema antioxidante enzimático no trigo e azevém.
Palavras-chave: *Triticum aestivum* L., *Lolium perenne* L. var. *multiflorum* (Lam.), estresse oxidativo.

INTRODUCTION

Xenobiotics are compounds that induce various responses in plants, resulting in cell damage that can lead to death. Herbicides are examples of xenobiotics used in agriculture, whose purpose is to control sensitive weeds (RADCHENKO et al., 2021). The use of chemical control is the most widely used method for weed management, considering this feature due to its convenience and efficiency when compared to other methods (AGOSTINETTO et al., 2016).

The selectivity of herbicides for plants is a combination of the factors of crops, herbicides, and the environment. Selectivity of herbicides is caused in most herbicide classes by the higher detoxification capacity in crop plants compared to weeds (KRAEHMER et al., 2014). However, even if there is selectivity for a particular species, physiological and biochemical changes can occur as a side effect of herbicide application, causing biochemical and physiological disturbances in plant metabolism, increasing toxicity.

Ryegrass [*Lolium perenne* L. var. *multiflorum* (Lam.)] is one of the main weeds in wheat cultivation, and management methods for this weed should be adopted between 11 and 21 days after

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crop emergence to avoid yield loss due to competition (GALON et al., 2019). To avoid these losses, herbicides with different mechanisms of action are used to control ryegrass and other weeds, including acetyl-CoA carboxylase (ACCase) inhibitors, auxin mimics, and acetolactate synthase enzyme (ALS) inhibitors applied pre and post-emergence (OLIVEIRA et al., 2021). The herbicides iodosulfuron-methyl (ALS) and 2,4-D (auxin mimics) were shown to be selective to wheat in an evaluation performed 21 days after application, with visual phytotoxicity of less than 5% (AGOSTINETTO et al., 2016). However, little is known about the physiological effects and the ability to cause oxidative stress of these herbicides.

In plants, the exposure to certain xenobiotics often triggers oxidative stress as a primary response. This is characterized by the rapid generation of reactive oxygen species (ROS), with the most pronounced effect occurring shortly after herbicide exposure (SANTOS & SILVA, 2015). The ROS as superoxide anion ($O_2•^-$), hydroxyl radicals (OH•), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) are important ROS species causing damage in plant metabolism. The accentuated generation of ROS and oxidative stress are factors that can lead to increased phytotoxicity of herbicides to crops, characterized by the displacement of the balance between formation and elimination by antioxidant systems. It is known that 2,4-D mode of action is related to ROS production, since OH• is essential in the development of epinasty triggered by herbicide treatment (PAZMIÑO et al., 2014).

Essentially, herbicides induce the formation of these reactive oxygen species, which in turn leads to oxidative stress. As a part of the detoxification mechanism, the increased activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) plays a crucial role. This heightened enzymatic activity is a result of the detoxification process and serves to reduce lipid peroxidation (SANTOS & SILVA, 2015).

Herbicides can reduced chlorophylls and carotenoids negatively affecting the production of enzymes related to pigments, with this the chlorophyll concentration decreases making the photosynthetic process less stable, resulting in degradation of photosynthetic pigments and leads to cellular dysfunction (DIAS et al., 2014). Carotenoids, in addition to their role as pigments, serve as formidable antioxidants in combating the detrimental effects of reactive oxygen species.

These ROS are effectively countered by various components within the cell's antioxidant defense system, together, these components work harmoniously to uphold cellular homeostasis. The enzymatic antioxidant system operates in a carefully orchestrated sequence, which has been shown, particularly under stress conditions, to significantly enhance the activity of SOD. This heightened SOD activity catalyzes the dismutation of superoxide radicals into H_2O_2 and O_2. Notably, SOD plays a essential role in eliminating superoxide radicals from chloroplasts, subsequently converting them into H_2O_2. Following this conversion, several other antioxidant enzymes, such as CAT and APX, come into action, effectively neutralizing H_2O_2 and removing it from the cellular environment (SANTOS & SILVA, 2015; CANAKCI GULENGUL & KARABULUT, 2021).

In summary, carotenoids, along with this intricate network of enzymatic antioxidants, collectively contribute to safeguarding cells against oxidative damage and maintaining their vital balance. Thus, detecting how herbicide action occurs in signaling and inducing oxidative stress in wheat is a key element in determining the tolerance capacity of plants. This characterization will also be of interest for understanding the evolutionary dynamics of stress adaptation and the mechanisms of adaptation to novel stressors. The hypothesis of this study is that wheat and ryegrass plants are able to mitigate the adverse effects induced by the herbicides. Because of this, the objective of the study was to evaluate the interference of the herbicides clodinafop-propargyl, and 2,4-D on the antioxidant systems in wheat, and iodosulfuron-methyl in wheat and ryegrass.

MATERIALS AND METHODS

Two studies, divided into three experiments, were conducted in Brazil and the United States. The studies are described below.

Study 1 - Oxidative stress markers of wheat plants exposed to herbicides

This experiment was conducted in an entirely randomized design with six repetitions in a greenhouse. The determinations were performed in the laboratory at the Faculdade de Agronomia Eliseu Maciel (FAEM) from the Universidade Federal de Pelotas (UFPel), in Capão do Leão - RS. The experimental units consisted container of 8 liter, 23-cm diameter pots filled with Red-Yellow Argisol with sandy loam texture. The wheat cultivar used was 'Fundacep Horizonte' (precoce cycle takes about 133 days to reach harvest to a maturation, is resistant to leaf rust, has higher productive potencial and bread-making quality), and the wheat population per experimental unit was 15 plants.

The treatment factor was composed of different herbicides: iodosulfuron-methyl (Hussar®; Bayer S.A.), clodinafop-propargyl (Topik®; Syngenta Proteção de Cultivos Ltda.), 2,4-D (U 46 D-FLUID®; Sumitomo Chemical Brasil Indústria Química S.A.), and a control without application (untreated). The doses used were recommended, respectively, 3.5, 60, and 1290 g a.i. ha^{-1} for the herbicides listed above. Hoefix® adhesive spreader was added to the iodosulfuron-methyl spray at a dose of 0.3 % v/v, and Assist® adhesive spreader was added to the clodinafop-propargyl at a dose of 0.5 % v/v. The herbicides were applied 15 days after emergence (DAE) when the plants were at the five-leaf stage, using a CO_2 pressurized backpack sprayer equipped with 110.02 fan spray tips, calibrated to apply 150 L ha^{-1} of herbicide solution.

Study 2 - Doses of iodosulfuron-methyl on wheat and ryegrass enzymatic activity

Two experiments were conducted at Texas A&M University, College Station, TX, US. The experiments were conducted in an entirely randomized design with four replications. For one experiment, seeds of the wheat cultivar 'TAM 304' were used, and for the other experiment, the ryegrass biotype 'Elbon Rye' was used. The species were sown in one-liter capacity pots filled with commercial Fafard 2 MIX® substrate. The population of wheat and ryegrass per experimental unit was 10 plants total.

The experiments were conducted in a phytotron growth chamber, where the plants were maintained at a temperature of 22/18 °C and photoperiod of 12h/12h day and night, respectively, throughout the experimental period, with a relative humidity of 75%. The determinations were performed in the laboratory at Texas A&M University, College Station, TX, US. For both experiments, the treatment factor consisted of doses of the herbicide iodosulfuron-methyl. The doses used were 0, 1.75, 3.5, and 7.0 g a.i. ha^{-1}, equivalent to 0, 50, 100, and 200% of the recommended rate. The herbicides were applied at 10 DAE with the aid of a CO_2 pressurized spray chamber equipped with a 110.02 fan spray tip calibrated to apply 200 L ha^{-1} of herbicide solution.

Variables analyzed

For Study 1, samples were taken from the aerial parts of the wheat plants at different times, namely 0, 12, 24, 72, 96, and 120 hours after application (HAP). The samples were collected when the plants were at the five-leaf stage and were collected from the fully expanded leaves. A sample of each repetition was made with all the plants of the experimental unit (15 plants), which were immediately taken to the laboratory and stored at –80 °C until the moment of the evaluations.

The variables analyzed were chlorophylls *a*, *b*, carotenoids, hydrogen peroxide (H_2O_2), lipid peroxidation in terms of thiobarbituric acid reactive species (TBARS) and electrolyte leakage. The concentrations of chlorophylls and carotenoids were determined following the ARNON (1979). The concentrations were calculated by LICHTENTHALER (1987) from the absorbance of the supernatant solution was measured in a spectrophotometer at 663, 645, and 470 nm for determining chlorophyll *a*, chlorophyll *b*, and carotenoids, respectively, with the results expressed in mg g^{-1} of fresh mass (MF). The analyzed variables for cell damage in tissues, determined by H_2O_2 concentration, as described by LORETO & VELIKOVA (2001); TBARS were determined by malondialdehyde (MDA) accumulation, proposed by HODGES et al. (1999); and electrolyte leakage, as described by TARHANEN et al. (1999).

For Study 2, plant collections were made at 0, 1, 2, 3, 24, 48, and 72 HAP, from leaves that were fully expanded. A composite sample was taken from each repetition with all the plants of the experimental unit, which were stored at –80 °C until the quantification of the enzymatic activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). To determine enzymatic activity, the samples were extracted according to the methodology proposed by ZHU et al. (2004), and the supernatant was used to assess enzymatic activity and protein content (BRADFORD, 1976). The SOD activity was determined according to the methodology of GIANNOPOLITIS & RIES (1977). CAT was determined by the methodology of AZEVEDO et al. (1998) and APX by of NAKANO & ASADA (1981). Enzymatic activities of the total extract were determined by calculating the amount of extract that reduced the absorbance reading in an active unit (AU) and expressed in AU mg^{-1} protein min^{-1}.

Data analysis

The data obtained were analyzed for normality (Shapiro-Wilks test) and then submitted to analysis of variance ($P \leq 0.05$). In the case of significance, the means of the treatments were compared within each evaluation season by Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Antioxidant system and oxidative stress markers of wheat plants exposed to herbicides

The herbicides showed a reduction of chlorophyll a, in general, especially in the last evaluation that showed a reduction of 43% (120 HAP, Figure 1a). When comparing herbicides, clodinafop-propargyl treated plants showed lower chlorophyll *a* concentration at 24 and 72 HAP than the others (Figure 1a). At 96 HAP, iodosulfuron-methyl-treated plants were the ones that had the lowest concentration of chlorophyll *a*, differing from the control without application and from clodinafop-propargyl-treated plants, but not from 2,4-D-treated plants. In the last evaluation at 120 HAP, the herbicides did not differ, showing lower chlorophyll concentrations than the control treatment (Figure 1a).

According to the results, it can be inferred that chlorophyll *a* concentrations was sensitive to the use of herbicides, especially clodinafop-propargyl, which reduced the value of chlorophyll *a* and could compromise the photosynthetic activity of wheat plants. In a similar study, it was observed that the application of iodosulfuron-methyl and 2,4-D and 120 HAP reduced the concentration of chlorophyll *a*, *b* and *a*+*b* compared to the control, coinciding with the reduction of photosynthetic and transpiration rates at 120 HAP (AGOSTINETTO et al., 2016).

For the chlorophyll *b*, in general, the herbicide treatments differed from the control, except at 0 and 120 HAP (Figure 1b). At 12 HAP, the herbicides iodosulfuron-methyl and clodinafop-propargyl caused higher concentration of chlorophyll *b* compared to the control (Figure 1b). Comparing the herbicides, iodosulfuron-methyl and 2,4-D caused a greater reduction in chlorophyll *b* compared to clodinafop-propargyl at 12 HAP (Figure 1b), while at 24, 72 and 96 HAP 2,4-D caused an increase in chlorophyll *b*. Likely, the application of the herbicide treatments interfered in the chlorophyll *b* conversion process as a stabilizing factor in chlorophyll *a* concentrations

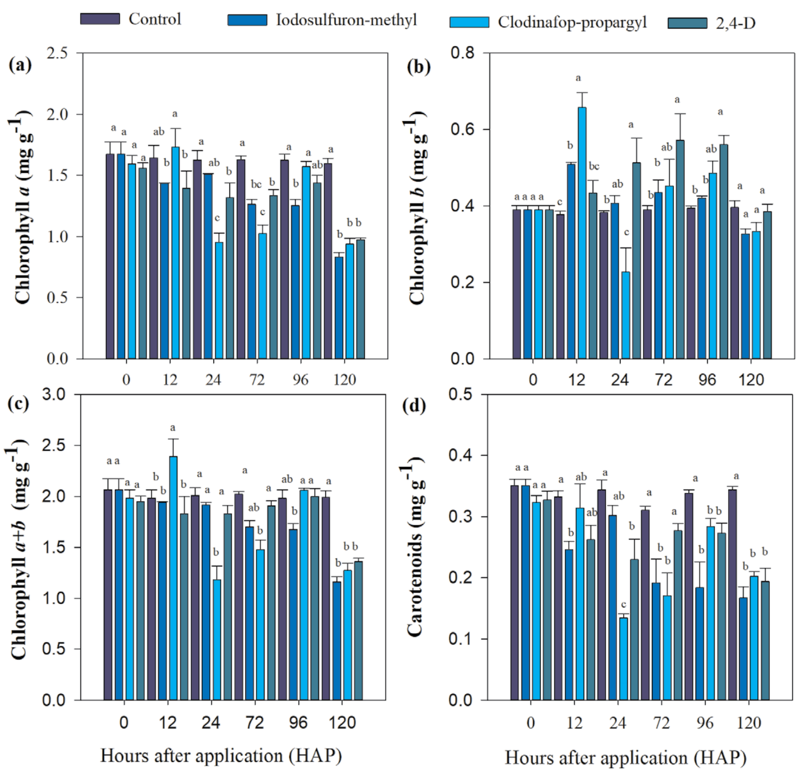

Figure 1 - Chlorophyll *a* (a), chlorophyll *b* (b), chlorophyll *a*+*b* (c), and carotenoids (d) concentrations of wheat plants in response to the use of different herbicides evaluated at 0, 12, 24, 72, 96, and 120 hours after application (HAP). Means followed by the same lower case letter in the bars do not differ within each evaluation season by Tukey's test ($P \leq 0.05$).

(ASHRAF & HARRIS, 2013), evidenced by the low values of the former in the control. Consequently, this characteristic may have interfered with the chlorophyll *a* concentration observed and, consequently, with the photosynthetic efficiency of the plants.

The chlorophyll *a*+*b* showed differences between the herbicide treatments and control, except for 0 HAP (Figure 1c). When comparing herbicides, iodosulfuron-methyl-treated plants showed a reduction in chlorophyll *a*+*b* at 12 and 96 HAP (Figure 1c). As for clodinafop-propargyl-treated plants, the reduction occurred at 24 and 72 HAP, while 2,4-D-treated plants, in general, did not change relative to the others. At 120 HAP, all herbicides showed a difference relative to the control treatment (Figure 1c), relating this effect to the reduction in chlorophyll *a* concentration (Figure 1a).

The decrease in chlorophyll content in wheat seedlings serves as evidence of these plants incapacity to synthesize all the essential photo-assimilates required for their sustenance. This phenomenon was observed in three distinct wheat cultivars exposed to concentrations of 2,4-D, resulting in the degradation of these vital pigments (CANAKCI GULENGUL & KARABULUT, 2021).

For carotenoids, in general, all herbicides showed a reduction of the variable relative to the control as of 24 HAP, and at 96 and 120 HAP, all showed a reduction compared to the control (Figure 1d). Carotenoids are antioxidants that perform multiple functions in plant metabolism, including prevent damage to the photosynthetic apparatus by oxidative stress by acting in the removal of ROS. The decrease in photosynthetic pigments is related to reduction in photosynthesis (SHARMA et al., 2018).

Under stress conditions, the concentrations of chlorophylls and carotenoids can have significant alterations. These changes can serve as indicators of herbicide-induced stress within chloroplasts, resulting in an increase in reactive oxygen species. Consequently, this can lead to the degradation of chlorophyll, thereby impairing the functionality of photosynthetic machinery (SANTOS & SILVA, 2015).

In general, all herbicides induced the production of H_2O_2 from 12 HAP, where herbicide 2,4-D-treated plants showed higher levels of H_2O_2 compared to the control in all HAP (Figure 2a). Clodinafop-propargyl-treated plants evidenced higher H_2O_2 production between 24 and 96 HAP (Figure 2a). In maize, it was also observed that the application of 2,4-D resulted in a rise in H_2O_2 and MDA accumulation in the leaves, when compared to the control group (ABOU-ZEID et al., 2020).

In the case of TBARS, it was evident that all herbicides, across all evaluation time of evaluation, led to an elevation in MDA levels when compared to the control group (Figure 2b). These results are probably because the increase of H_2O_2 contributed to the higher values of MDA detected. Among herbicides, there was a difference only at 120 HAP, where clodinafop-propargyl-treated plants obtained a higher TBARS concentrations than the others did. Substantial rises in the concentration of MDA, an essential parameter signifying the intensity of oxidative stress, are observed. MDA serves as the characteristic product of lipid peroxidation within cellular membranes. The elevation in MDA levels indicates an augmentation in oxidative stress-induced lipid peroxidation within plant biomembranes (WANG et al., 2015).

For the electrolyte leakage variable, the 24, 72, and 96 HAP herbicide applications showed an increase in the variable compared to the control, while the 120 HAP obtained, in general, lower values (Figure 2c). Comparing herbicides, there was a difference only at 120 HAP, where clodinafop-propargyl-treated plants showed lower values compared to iodosulfuron-methyl and 2,4-D-treated plants. These results possibly occurred due to the decrease in chlorophylls obtained in this study, and consequently, there was an increase in H_2O_2 and TBARS causing damage to the plasma membrane, leading to extravasation of cell contents. Thus, according to this study, the application of herbicides in wheat, altered the production of ROS and consequently caused oxidative stress, increasing the concentration of metabolites such as H_2O_2 and TBARS (Figure 2), with negative interference in photosynthesizing pigments (Figure 1).

Doses of iodosulfuron-methyl on enzyme activity of wheat and ryegrass

For wheat, SOD activity decreased at all doses of the herbicide iodosulfuron-methyl used compared to the control, except for 0 HAP (Figure 3a). In the comparison between doses, in general, the dose of 7.0 g i.a. ha^{-1} caused lower activity of the enzyme. For ryegrass, similarly to the wheat, the increase of the doses reduced the variable compared to the control without application, except for 0 and 1 HAP, and in the comparison between doses; in general, the highest dose reduced the enzyme activity (Figure 3b).

For CAT and APX activity for both crop and ryegrass, in general, the result was similar to that for SOD, the enzyme value decreased with increasing doses compared to the control (Figure 3c–f). Between doses, in general, the highest doses of 3.5 and 7.0 g i.a ha^{-1} caused the lowest enzyme activities. According

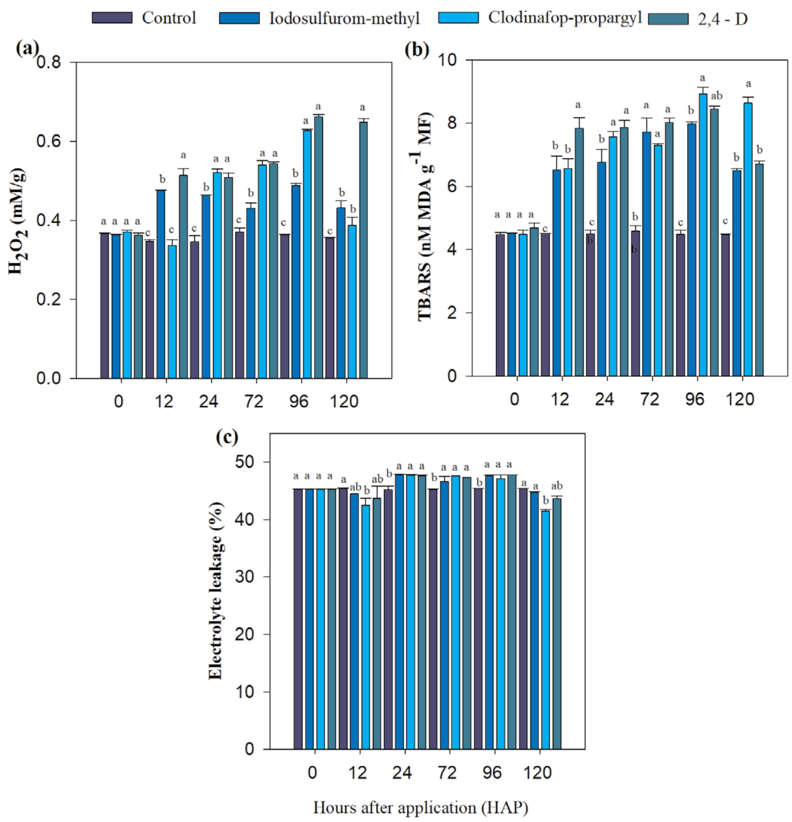

Figure 2 - Hydrogen peroxide (H_2O_2) concentration (a), thiobarbituric acid reactive species (TBARS) (b), and cellular electrolyte leakage (c) of wheat plants in response to the use of different herbicides evaluated at 0, 12, 24, 72, 96, and 120 hours after application (HAP). Means followed by the same lower case letter do not differ within each evaluation season by Tukey's test ($P \leq 0.05$).

to the results, it can be observed that the increase in the dose of the herbicide iodosulfuron-methyl caused the decrease in the activity of the enzymes SOD, CAT, and APX in all HAP. This may have occurred because the higher doses and the exposure time to the herbicide reduced the antioxidant protection system of the plants, trying to eliminate the excess H_2O_2 that may have increased due to the action of the herbicide, thus reducing the activity of the enzymes studied. In ryegrass genotypes exposed to haloxyfop, glyphosate, and iodosulfuron also exhibited oxidative stress, primarily attributed to a downregulated decrease in SOD activity and an increase in lipid peroxidation (TAMAGNO et al., 2022).

However, for wheat, rye, and maize crops subjected to different doses of iodosulfuron-methyl, it was observed that the activity of CAT and APX enzymes increased with increasing doses of the herbicides (LUKATKIN et al., 2013). The changes in the activity of antioxidant enzymes through different mechanisms of action of herbicides and doses used aim to overcome oxidative stress by eliminating the ROS produced during the stress process. As observed in this study, the differences in the activity of these enzymes may be related to the herbicide, the dose, and the species. However, the non-enzymatic antioxidant system plays an important role in overcoming oxidative stress in wheat plants, as shown in Experiment 1. Further studies should be conducted to better understand these effects.

CONCLUSION

The use of the herbicides iodosulfuron-methyl, clodinafop-propargyl, and 2,4-D decreased the concentrations of chlorophylls and carotenoids in wheat plants. In addition, they promote oxidative stress with increased H_2O_2, TBARS, and electrolyte leakage.

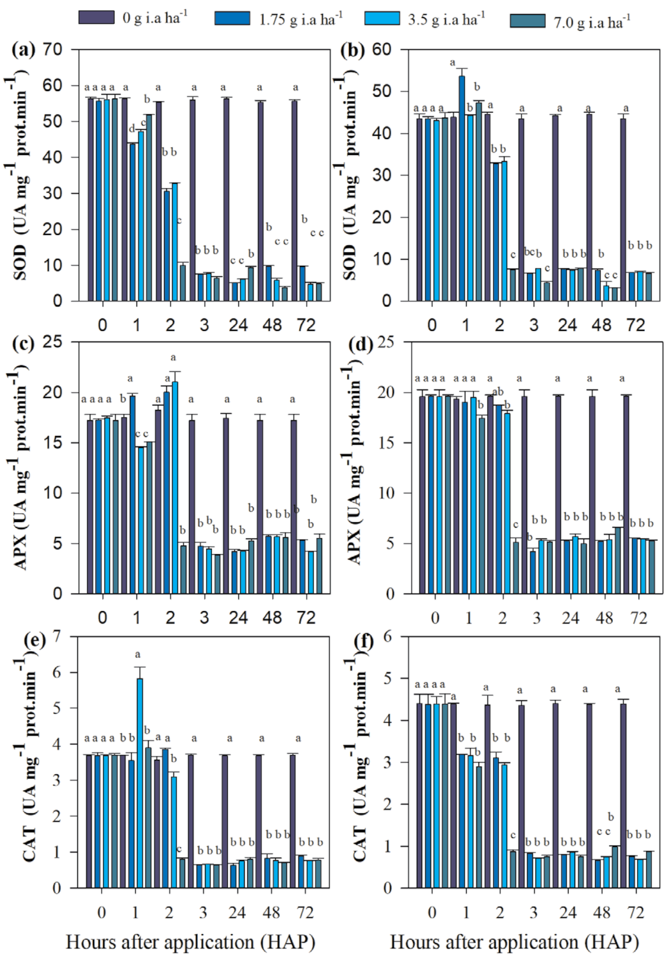

Figure 3 - Activity of SOD (a, b), APX (c, d), and CAT (e, f) of wheat (a, c, e) and ryegrass (b, d, f) plants in response to the use of doses of iodosulfuron-methyl sodium evaluated at 0, 1, 2, 3, 24, 48, and 72 hours after application (HAP). Means followed by the same lower case letter in the bars do not differ within each evaluation season by Tukey's test ($P \leq 0.05$).

Increasing the dose of iodosulfuron-methyl from 1.75 to 7.0 g i.a. ha^{-1} reduced the activity of SOD, CAT, and APX enzymes in wheat and ryegrass plants.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare there is no conflict of interests in carrying the research and publishing the manuscript.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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