MEFENPYR-DIETHYL ACTION ON FENOXAPROP-*P*-ETHYL DETOXIFICATION IN WHEAT VARIETIES[1]

Ação de Mefenpyr-Diethyl sobre a Desintoxicação de Fenoxaprop-P-Ethyl em Variedades de Trigo

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ABSTRACT - Safeners protect crops against herbicide injury. The aim of this study was to examine the differential susceptibility of five wheat (Triticum aestivum) varieties to the herbicide fenoxaprop-p-ethyl, as well as the performance of mefenpyr-diethyl on minimizing herbicide injury and on lipid contents. Varieties BRS 49, CD 104, CEP 24, IAPAR 78 and Rubi were sprayed with fenoxaprop-p-ethyl (69 g ha^{1}), fenoxaprop-p-ethyl + mefenpyr-diethyl (69 g + 18.75 g ha^{-1}), or mefenpyr-diethyl (18.75 g ha^{-1}). Plants were evaluated visually for injury at 7 and 14 days after treatment (DAT). Glutathione S-transferase (GST) activity was assayed in aerial parts at 7 DAT, and lipid content was measured at 14 DAT. Varieties CEP 24, IAPAR 78 and Rubi were more tolerant to fenoxaprop-p-ethyl than BRS 49, and CD 104 rapidly recovering from the slight phytotoxicity symptoms produced by the herbicide. Mefenpyr-diethyl prevented crop injury associated with the herbicide. GST activity did not correlate directly with fenoxaprop-p-ethyl detoxification. However, lipid content was related to the susceptibility of wheat to fenoxaprop-p-ethyl treatment.

Keywords: safener, *Triticum aestivum*, selectivity, glutathione S-transferase, lipid content.

RESUMO - Protetores ou safeners protegem culturas contra a injúria de herbicidas. O propósito deste estudo foi investigar a suscetibilidade diferencial de cinco variedades de trigo (Triticum aestivum) ao herbicida fenoxaprop-p-ethyl e o efeito do protetor mefenpyr-diethyl na minimização da injúria do herbicida e sobre o conteúdo de lipídios. As variedades BRS 49, CD 104, CEP 24, IAPAR 78 e Rubi foram pulverizadas com fenoxaprop-p-ethyl (69 g ha^{-1}), fenoxaprop-p-ethyl + mefenpyr-diethyl (69 g + 18,75 g ha^{1}) ou mefenpyr-diethyl (18,75 g ha^{1}). A injúria das plantas foi avaliada visualmente aos 7 e 14 dias após o tratamento (DAT). A atividade da glutationa S-transferase (GST) foi analisada na parte aérea aos 7 DAT, e o conteúdo de lipídios, aos 14 DAT. As variedades CEP 24, IAPAR 78 e Rubi foram mais tolerantes ao fenoxaprop-p-ethyl do que a BRS 49, e a variedade CD 104 rapidamente se recuperou dos sintomas de intoxicação produzidos pelo herbicida. Mefenpyr-diethyl preveniu a injúria da cultura pelo herbicida. A atividade da GST não foi correlacionada diretamente com a desintoxicação do fenoxaprop-p-ethyl. Contudo, o conteúdo de lipídios esteve relacionado à suscetibilidade de trigo ao tratamento de fenoxaprop-p-ethyl.

Palavras-chave: *safener*, *Triticum aestivum*, seletividade, glutationa S-transferase, conteúdo de lipídios.

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INTRODUCTION

Herbicide fenoxaprop-*p*-ethyl, D - (+) - 2 – 4 – (6-chloro – 1, 3 – benzoxazol – 2pyloxy) phenoxypropionacid is a member of the aryloxyphenoxy propionic acid chemical family. The target site of this herbicide is acetyl coenzyme A carboxylase, which catalyzes the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. This reaction is the first step in the *de novo* biosynthesis of fatty acids in plants (Gronwald, 1991).

Some Poaceae crops may be protected from herbicide injury by chemicals called safeners, which have little intrinsic biological activity. Safeners, also known as antidotes, belong to well-defined chemical classes and are applied either as seed treatment or directly as tank mixes with the herbicides (Abu-Qare & Duncan, 2002). Thus, safeners reduce herbicide phytotoxicity to crops by a physiological or molecular mechanism, without compromising weed control efficacy (Hatzios & Burgos, 2004).

Herbicide safeners are used to protect several monocotyledonous crop species from herbicide injury, because they caused alterations on herbicide metabolism. Safeners might directly alter herbicide metabolism by chemically activating particular functional group(s) or by affecting enzymes involved in its metabolism (Fuerst et al., 1995). These compounds usually increase the activity of specific herbicide-metabolizing enzymes, such as glutathione S-transferase (GST, EC 2.5.1.18), P450-dependent monooxygenases, and glucosyltransferases, which lead to higher herbicide metabolism rate (Kreuz et al., 1996; Cummins et al., 1997; Abu-Qare & Duncan, 2002; Brazier-Hicks et al., 2008; Liu et al., 2009).

Many studies have suggested that safeners can induce the activity of mixed-function oxidase enzymes. This induction may enhance herbicide oxidation and hydroxylation (Jablonkai & Hatzios, 1994), but there is no conclusive evidence regarding the induction of these enzymes by any of the currently available safeners. Herbicide conjugation with reduced glutathione (GSH) is a common metabolic pathway, and the conjugation rate is related to plant tolerance to herbicides (Cataneo et al., 2002, 2003; Andrews et al., 2005). Previous reports have demonstrated that safeners induce GST activity in plants (Andrews et al., 2005; Cummins et al., 2006; DeRidder & Gouldsbrough, 2006). Thus, an increase not only in GSH content but also in the activity of GST, the enzyme that catalyzes herbicide conjugation to GSH, may lead to a higher rate of herbicide conversion into non-toxic metabolites. Therefore, conclusive elucidation of the mechanisms underlying safener action still remains a challenge.

The aims of this study were to: (a) examine lipid levels and GST activity in five wheat (*Triticum aestivum*) varieties having different tolerance levels to fenoxaprop-*p*-ethyl; (b) determine a possible relationship of lipid levels and/or GST activity and the tolerance of these varieties to fenoxaprop-*p*-ethyl; and (c) evaluate the effect of mefenpyr-diethyl safener on lipid levels and/or GST-mediated fenoxaprop-*p*-ethyl detoxification.

MATERIAL AND METHODS

Plant material and growth conditions

The experiment was carried out in a greenhouse at 30±3/20±3 °C day/night temperature with a photoperiod of about 14 h. Seeds of BRS 49, CD 104, CEP 24, IAPAR 78 and Rubi wheat varieties were used. Twenty-five seeds of each variety were sown at 2 cm depth in 700 mL plastic pots filled with a soil mixture (75% soil and 25% sand v/v). Pots were kept in greenhouse at 25 °C, and plants were watered when necessary. Plants were thinned to the most uniform fifteen seedlings per pot at 6 days after germination.

Experimental design and treatments

Experimental design was completely randomized in a 5 x 4 factorial arrangement, i.e., five wheat varieties and four treatments as fenoxaprop-p-ethyl plus mefenpyr-diethyl, fenoxaprop-p-ethyl, mefenpyr-diethyl and water control, with six replicates.

Commercial formulations including herbicide fenoxaprop-*p*-ethyl with safener (Puma S: fenoxaprop-*p*-ethyl 69 g a.i. L^{-1} plus mefenpyr-diethyl 18.75 g a.i. L^{-1}), with no

safener (Whip S: fenoxaprop-*p*-ethyl 69 g a.i. L^{-1}), or the safener alone (mefenpyr-diethyl 18.75 g a.i. L^{-1}) were applied using a motorized knapsack mist blower equipped with four flat-fan nozzles (XR 110.02) spaced 50 cm apart, and calibrated to deliver 200 L ha^{-1} at 310.27 kPa. Treatments were applied at sixteen days after planting.

Visual evaluation of injury

Injury to shoots (fifteen seedlings per pot) was visually assessed at 7 and 14 days after treatments (DAT). These evaluations were based on a percentage scale, in which zero and one hundred indicate no injury and death of all seedlings, respectively. The numbers between zero and one hundred represented different degrees of injury (such as chlorosis) and were given based on this scale 0: no damage; 20: 20% of seedlings with chlorosis; 40: 40% of seedlings with chlorosis; 60: 60% of seedlings with chlorosis; 80: 80% of seedlings with chlorosis; and 100: complete death of seedlings.

GST extraction and assay

Shoot tissue samples (1.0 g) of both treated and control seedlings were harvested at 7 DAT, frozen in liquid nitrogen and stored at -80 °C before analysis. Samples were washed with distilled water and superficially dried with filter paper. GST extraction was carried out according to the procedure of Knörzer et al. (1996). Shoot segments (1.0 g fresh matter) were powdered using a pestle and a cold mortar containing a small amount of quartz sand and 5 mL of 50 mM Tris-HCl buffer, pH 7.0, containing 20% (v v^{-1}) glycerol, 1 mM ascorbic acid, 1 mM dithiothreitol, 1 mM EDTA, 1 mM GSH and 5 mM $MgCl_2$, including 1% (w v^{-1}) polyvinylpyrrolidone. After two centrifugation steps (6 min at 12.000 x *g*, and 16 min at 26.000 x *g*) at 4 °C, the supernatant was collected and used as crude extract to assess GST activity according to Wu et al. (1996) using 1 chloro-2,4-dinitrobenzene (CDNB) as substrate. Each reaction (3 mL) contained 30 µL enzyme extract, 2 mL 100 mM potassium phosphate buffer pH 6.9, 0.9 mL 3.3 mM GSH (0.9 mL), and 100 µL 30 mM CDNB in 96% ethanol. The mixture was incubated at 25 °C for 60 min. The reaction started after the addition of CDNB, and the change in absorbance due to the formation of glutathione-CDNB conjugate over time was measured spectrophotometrically at 340 nm. The rate of change in absorbance obtained was then used to quantify the enzyme activity in the mixture using the molar extinction coefficient of glutathione-CDNB conjugate (9.6 mM cm^{-1}). The enzyme activity was corrected using the nonenzymatic conjugation, which was determined through the same reaction without the crude plant extract. Specific GST activity was expressed as mmol min^{-1} mg^{-1} protein. Protein concentrations in the shoot extracts were determined according to Lowry et al. (1951) using bovine serum albumin as standard.

Lipid content

Fifteen seedling shoots were harvested at 14 DAT for lipid percentage quantification in dry biomass according to the method proposed by the Association of Official Agricultural Chemists (AOAC), as described by Silva (1990).

Statistical analysis

Visual evaluation (percent injury) was statistically analyzed only for fenoxaprop-*p*-ethyl, because no injury was detected for any of the other treatments. GST activity and lipid percentage data were transformed in square root. ANOVA was performed to test the significance of the observed differences. Differences among varieties and chemical treatments were evaluated by the Duncan test, and p-values ≤ 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Wheat varieties differed in their susceptibility to fenoxaprop-*p*-ethyl according to visual evaluation of plant injury at 7 and 14 DAT (Table 1). Crop injury (expressed as chlorosis and sometimes death) increased in all wheat varieties from 7 to 14 DAT. Varieties CD 104 and BRS 49 were most susceptible to fenoxaprop-*p*-ethyl, whereas the variety Rubi was the most tolerant one. None of the wheat varieties showed symptoms of phytotoxicity at

Table 1 - Injury of shoots of *Triticum aestivum* varieties associated with the application of fenoxaprop-*p*-ethyl[1/]

Variety	Days after application	
	7[2/]	14
BRS 49	16.33 ± 1.97 B[3/]	31.67 ± 2.58 B
CD 104	27.67 ± 2.25 A	57.17 ± 2.48 A
CEP 24	9.17 ± 0.98 C	13.00 ± 2.45 C
IAPAR 78	8.00 ± 0.00 C	9.50 ± 0.55 D
Rubi	3.83 ± 0.98 D	6.33 ± 1.51 E

[1/] According to a visual evaluation using a scale from 0 (no damage) to 100 (complete death); [2/] Data represent the mean ± S.E. of six replicates; [3/] Means followed by the same letter in column did not differ significantly according to Duncan's test. Standard error = 1.7962; α = 0.05; CV = 9.83%; Degrees of freedom = 50.

7 and 14 DAT with fenoxaprop-*p*-ethyl plus mefenpyr-diethyl or the safener alone.

Wheat varieties differed in their natural GST activity in the absence of treatment (Table 2). Wheat plants from CD 104 and CEP 24 varieties treated only with fenoxaprop-*p*-ethyl had ~2-fold higher GST activity relative to the untreated controls. Compared with control plants, GST activity in the BRS 49, IAPAR 78, and Rubi varieties, increased by 72%, 50%, and 37%, respectively over control. For all tested varieties except BRS 49, the treatment involving mefenpyr-diethyl safener plus fenoxaprop-*p*-ethyl led to decreased GST activity in plants as compared with the treatment using fenoxaprop-*p*-ethyl only. The treatment with mefenpyr-diethyl safener only led to decreased GST activity in all varieties compared with those plants treated only with fenoxaprop-*p*-ethyl. The GST activity in the BRS 49, CD 104 and IAPAR 78 varieties treated with the safener alone decreased relative to the mefenpyr-diethyl plus fenoxaprop-*p*-ethyl treatment, whereas the activity in CEP 24 and Rubi increased.

Herbicide selectivity is frequently based on the ability of crops to detoxify these compounds (Cummins et al., 1997). The conjugation of herbicides with GSH is an important and irreversible mode of detoxification catalyzed by GSTs. However, the highest GST activities measured in the wheat varieties BRS 49 and CD 104 did not reveal more tolerance to fenoxaprop-*p*-ethyl, because visual evaluations indicated that these varieties had higher susceptibility to this herbicide (Table 1). These results are contradictory to those of Wu et al., (1996) who considered that an increase in GST activity in wheat is one of the mechanisms responsible for fenoxaprop-*p*-ethyl detoxification, improving tolerance to the herbicide. Furthermore, field populations of the black-grass weed (*Alopecurus myosuroides* Huds.), which are more resistant to fenoxaprop-*p*-ethyl, had higher GST activities than populations susceptible to this herbicide (Cocker et al., 1999).

Based on the relationship between high GST activity and wheat seedling tolerance to the herbicide dimethenamid, GST activity was used as a biochemical marker for such tolerance (Riechers et al., 1996). However, this is not true for fenoxaprop-*p*-ethyl, because no

Table 2 - GST activities[1/] of wheat cultivars shoots at 7 DAT

Variety	Treatments			
	Untreated/Control[2/]	Fenoxaprop-*p*-ethyl (69 g ha^{-1})	Fenoxaprop-*p*-ethyl + mefenpyr-diethyl (69 g + 18.75 g ha^{-1})	Mefenpyr-diethyl (18.75 g ha^{-1})
BRS 49	62.50 ± 4.77 A[3/]b	107.66 ± 12.89 B a	106.38 ± 15.14 A a	56.38 ± 7.43 B b
CD 104	64.74 ± 2.82 A c	129.38 ± 7.78 A a	98.42 ± 13.98 A b	41.03 ± 3.94 C d
CEP 24	44.64 ± 4.58 C c	84.34 ± 8.13 C a	45.65 ± 2.85 C c	65.98 ± 7.98 A b
IAPAR 78	48.48 ± 4.71 BC b	72.93 ± 11.92 D a	54.46 ± 2.59 B b	39.88 ± 1.47 C c
Rubi	50.20 ± 2.92 B b	68.69 ± 5.63 D a	43.15 ± 3.63 C c	52.98 ± 7.06 B b

[1/] GST activity is defined as mmol min^{-1} per mg protein; [2/] Data represent the mean ± S.E. of six replicates; [3/] Means followed by the same letter (uppercase in column and lowercase in row) did not differ significantly according to Duncan's test. Standard error = 0.4307; α = 0.05; CV = 5.36%; Degrees of freedom = 100.

relationship between tolerance and high GST activity was found for the varieties studied in this present work (Table 2).

It has been demonstrated in wheat that the metabolism of diclofop, which has the same mechanism of action as fenoxaprop-*p*-ethyl, may be mediated by different enzymes, possibly P450-dependent monooxygenases, and the role of GST in diclofop metabolism in wheat has not been demonstrated (Romano et al., 1993). However, the selectivity of the aryloxyphenoxy propionate herbicide class can be increased with the addition of specific safeners. Studies have shown that safener-induced catabolism of fenoxaprop-*p*-ethyl is associated with an increase in GST activity (Cummins et al., 1997).

Our results indicated that the mefenpyr-diethyl safener applied alone and in premix with fenoxaprop-*p*-ethyl was not responsible for inducing GST activity. According to Matola & Jablonkai (2007), the protective efficacy of dichloroketal safeners in maize does not seem to be associated with enhanced herbicide detoxification by glutathione conjugation. On the other hand, several studies have reported that safeners increase herbicide tolerance given that they selectively induce GST activity. Therefore, safeners lead to a high detoxification rate through conjugation with glutathione (Hatzios & Wu, 1996; Kreuz et al., 1996; Marrs, 1996; Hess & Weller, 2000). It should also be taken into account that GSTs include constitutive isozymes as well as safener-inducible isozymes (Potter et al., 1995).

Mefenpyr-diethyl is a molecule that has been recently replaced by fenchlorazole-ethyl as a safener for fenoxaprop-*p*-ethyl in wheat crops. Yaacoby et al. (1991) used the commercial formulation of fenoxaprop-*p*-ethyl with the safener fenchlorazole (fenoxaprop-*p*-ethyl 60 g a.i. L^{-1} plus fenchlorazole 15 g L^{-1}) at the same concentration used in this present study (fenoxaprop-*p*-ethyl 60 g a.i. L^{-1} plus mefenpyr-diethyl 18.75 g a.i. L^{-1}). According to these authors, the safener might protect wheat plants from the phytotoxic action of fenoxaprop-*p*-ethyl by increasing both the deesterification rate of fenoxaprop-*p*-ethyl to fenoxaprop and the metabolism of fenoxaprop to other metabolites.

Lipid content differed among the wheat varieties (Table 3). Relative to control plants, the lipid content was lower in all wheat varieties (except for IAPAR 78) treated only with fenoxaprop-*p*-ethyl, and major reductions in lipid content were found in CD 104 (72%) and BRS 49 (63%). Fenoxaprop-*p*-ethyl plus mefenpyr-diethyl increased lipid content in all varieties compared to plants treated with fenoxaprop-*p*-ethyl alone; whereas mefenpyr-diethyl used alone led to the greatest increase in lipid content. With regard to the protective action of the safener mefenpyr-diethyl, the premix with fenoxaprop-*p*-ethyl increased the lipid content compared to the fenoxaprop-*p*-ethyl treatment alone.

Based on the results of lipid content (Table 3) correlated with the visual injury (Table 1), BRS 49 and CD 104 are probably the wheat varieties that are most susceptible

Table 3 - Lipid content as a percentage of wheat cultivars shoots at 14 DAT

Variety	Treatments			
	Untreated/ Control[1/]	Fenoxaprop-*p*-ethyl (69 g ha^{-1})	Fenoxaprop-*p*-ethyl + mefenpyr-diethyl (69 g + 18.75 g ha^{-1})	Mefenpyr-diethyl (18.75 g ha^{-1})
BRS 49	8.27 ± 0.16 BC[2/]c	3.03 ± 0.10 C d	10.30 ± 0.50 A b	11.45 ± 0.28 C a
CD 104	8.56 ± 0.44 BC b	2.47 ± 0.14 D c	8.56 ± 0.18 C b	9.33 ± 0.08 E a
CEP 24	11.06 ± 0.34 A b	9.31 ± 0.12 A c	11.06 ± 0.50 A b	15.33 ± 0.15 A a
IAPAR 78	8.65 ± 0.40 B c	8.11 ± 0.69 A c	10.77 ± 0.61 A b	13.50 ± 0.12 B a
Rubi	8.16 ± 0.34 C c	6.99 ± 0.08 B d	9.61 ± 0.06 B b	10.70 ± 0.21 D a

[1/] Data represent the mean ± S.E. of six replicates; [2/] Means followed by the same letter (uppercase in column and lowercase in row) did not differ significantly according to Duncan's test. Standard error = 0.0543; $\alpha = 0.05$; CV = 1.81%; Degrees of freedom = 100.

to the action of fenoxaprop-*p*-ethyl. Thus, lipid level quantification can be used as a biochemical marker for herbicide susceptibility. Fenoxaprop-*p*-ethyl acts as a lipid biosynthesis inhibitor (Vidal & Merotto Jr., 2001). In the present study, lipid levels increased in plants treated with mefenpyr-diethyl alone and also in those subjected to herbicide plus safener (Figure 2 and Table 3), suggesting that mefenpyr-diethyl safener protects plants by enhancing lipid biosynthesis. These results suggest that the mechanism of action of mefenpyr-diethyl is related to lipid content increase. Mefenpyr-diethyl probably plays a protective role by altering cuticle and plasma membrane composition. Hess & Weller (2000) reported that only 1-6% fenoxaprop-*p*-ethyl can translocate through the plant vascular system and that any decrease in this translocation rate may lead to higher tolerance to the herbicide in wheat.

In conclusion, our results demonstrate that lipid quantification can be used to determine the differential susceptibilities of wheat varieties to fenoxaprop-*p*-ethyl. This quantification will be useful in future studies to detect the lowest lipid levels in response to herbicide treatment, quickly identifying the highest tolerance of wheat varieties to the herbicide. Thus, this criterion can be used to screen new varieties developed through genetic methods. Furthermore, this biochemical evaluation can be used to select appropriate safeners for the aryloxyphenoxy propionic acid chemical family and to identify resistant weeds tolerant to this herbicide class. Although the development of herbicide safeners and the understanding of their mechanisms of action have advanced, further studies are needed to clarify how these chemicals act at the molecular level to protect plants against herbicide injury.

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