

Role of metabolism and conjugation of herbicides in tolerance of white oats to 4-Hydroxyphenylpyruvate Dioxygenase (HPPD) enzyme inhibitors

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Abstract

The success of chemical control is dependent on crop tolerance to herbicides and their efficiency in controlling weeds. Thus, knowledge of the tolerance level of cultivated plants and the tolerance mechanism involved is crucial for safe herbicide use. The objective of this study was to evaluate the effect of metabolism (malathion) and conjugation (NDB-Cl) inhibitors on the tolerance of white oats to the herbicides mesotrione and tembotrione. The experiment was carried out under field conditions in the 2021 crop, in an RBD, with nine treatments, namely: T0- control (no application); T1- malathion; T2- NBD-Cl; T3- mesotrione; T4- mesotrione+malathion; T5- mesotrione+NBD-Cl; T6- tembotrione; T7- tembotrione+malathion and T8- tembotrione+NBD-Cl. The plant characteristics were evaluated for plant intoxication, SPAD index, levels of chlorophyll *a*, *b* and total carotenoids, yield components, yield and industrial grain quality. Symptoms of intoxication in oat plants started at 7 DAA and increased up to 14 DAA in treatments containing mesotrione or tembotrione. Plant recovery started at 21 DAA, and it was intensified at 28 DAA. Intoxication levels were not affected by the addition of the metabolism inhibitors malathion (43.1%) and NDB-Cl (36.2%). There was no change in other variables tested under the control treatment. Based on these findings, it can be concluded that there was no change in the level of tolerance of white oats to the herbicides mesotrione or tembotrione after application of malathion and NDB-Cl. This outcome suggests that metabolism or conjugation is not the main mechanisms that make white oat tolerant to HPPD enzyme inhibitors.

Keywords: *Avena sativa*; differential metabolism; GSTI; herbicide detoxification; malathion; NDB-Cl; P450.

Abbreviations: AGROFIT_Informações sobre agrotóxicos fitossanitários registrados no Ministério da Agricultura, Pecuária e Abastecimento; ALS_acetolactate synthase enzyme; AVENACOR_industrial grain yield; *Ca*_chlorophyll *a*; *Cb*_chlorophyll *b*; CFB_Clima temperado com verão ameno e falta de estação seca; CONAB_Companhia Nacional de Abastecimento; CQFS_Comissão de Química e Fertilidade do Solo; CT_total carotenoids; DAA_days after application; DHI_dehulling index; G>2MM_grains>2mm; GSTI_glutathione S-transferase; HI_harvest index; HPPD_hydroxyphenylpyruvate dioxygenase enzyme; HW_hectoliter weight; IBGE_Instituto Brasileiro de geografia e estatísticas; MDA_malonaldehyde; NDB-Cl_4-chloro-7-nitrobenzofurazan; NGS_number of grains per spikelet; NPM_number of panicles per m²; NSP_number of spikelets per panicle; RDB_randomized block design; SPAD_Soil Plant Analysis Development; TGW_thousand-grain weight; UPFA_Universidade de Passo Fundo; U%_grain harvest moisture.

Introduction

White oat (*Avena sativa*) is widely used in crop rotation, mulching, pasture, silage, hay and grain production systems for animal (Pacheco et al., 2021) and human feed (Kim et al., 2021). In Brazil, white oat is grown during the winter, mostly in the states of Rio Grande do Sul, Paraná and Mato Grosso do Sul, with an expected production of 1.15 million tons for the 2022 harvest (Conab, 2022).

Historically, there was a significant increase in oat-growing areas in Brazil, from 40.3 thousand hectares

year⁻¹ in the 1970s to 503.4 thousand hectares year⁻¹ in the 2022 harvest, i.e., the harvested area increased by 12.5 times (IBGE, 2022). Yield in the same period increased from 1,000 kg ha⁻¹ to 2,281 kg ha⁻¹ (Conab, 2022). Despite the increased production of oat grains, there are factors that pose limitations to crop production, for example, weed interference, which reduces the yield and quality of crops of agricultural interest (Kadam et al., 2021).

Integrated weed management is used in oat cultivation to reduce the negative effects of interference, but it needs to be complemented with chemical control, an important method in modern agriculture (Fu et al., 2019). However, there is a lack of selective herbicides available for post-emergence use in oat crops; therefore, control is limited to two mechanisms of action: auxin-mimicking and acetolactate synthase (ALS)-inhibiting herbicides (Agrofit, 2022), which have specific control for Magnoliopsida (Dicots) weeds.

However, recent studies have recommended herbicides that inhibit the biosynthesis of carotenoids and plastoquinones, particularly the inhibitors of 4-hydroxyphenyl-pyruvatedioxygenase (HPPD), because they are selective for winter crops and efficient in controlling weeds, and they offer low toxicity to the environment (Ahrens et al., 2013; Lindell et al. 2015; Ndikuryayo et al., 2017; Schmitz et al., 2015). Also, they have been shown to be highly selective for oat crops (Buba et al., 2021; Soltani et al., 2011; Pedroso et al., 2021).

HPPD-inhibiting herbicides include the herbicides mesotrione and tembotrione, which are widely used in maize as they have a broad spectrum of weed control, including Poaceae (Williams et al., 2010). These herbicides inhibit the HPPD enzyme, which converts 4-hydroxyphenyl pyruvate into homogentisate - a precursor in the biosynthesis of plastoquinone and tocopherol in plants (Lee et al., 1998). Plastoquinone is required for the biosynthesis of carotenoids, which protect chlorophyll by absorbing excited electrons released during photosynthesis. Depletion of carotenoids leads to photo-oxidation of chlorophyll, which results in bleaching, followed by necrosis and plant death (Dankov et al., 2009).

The selectivity of HPPD-inhibiting herbicides is mostly due to differential metabolism in tolerant plants, such as corn (Matte et al., 2018; Lu et al., 2020), and resistant biotypes of wild radish (*Raphanus raphanistrum*) (Lu et al., 2020) and grain sorghum (*Bicolor sorghum*) (Pandian et al., 2020). This could be the mechanism responsible for the selectivity of these herbicides to white oat.

Differential metabolism in herbicide-tolerant plants, also called detoxification or herbicide metabolism is the main mechanism responsible for the selectivity of herbicides used at post-emergence in crops of agricultural interest (Nandula, 2017). Differential metabolism is characterized by the ability of plants to rapidly metabolize the phytotoxic chemical (herbicide) into a less toxic or non-toxic product (Edwards et al., 2000; Hatzios et al., 2004).

According to Jablonkai (2015), herbicide detoxification can be divided into three phases in plants, namely: Phase I: also called metabolization, in which primary metabolism converts biologically active molecules into less active compounds through hydroxylation performed by Cytochrome P450 monooxygenases; Phase II: the products resulting from phase I through

oxidation, reduction and hydrolysis, are converted into less toxic molecules; the best known process is conjugation to glutathione, carried out by the enzyme glutathione S-transferase - GSTI or glucosyl transferase - GT (Cobb et al., 2011); Phase III: Phase II conjugates are transformed into non-toxic secondary conjugates or linked to insoluble residues. Phase IV: Phase III products can be compartmentalized in the cell (compartmentalization).

Differential metabolism in herbicide-tolerant or resistant plants can be investigated by using malathion and/or 4-chloro-7-nitrobenzofurazan (NDB-Cl). These products are used for inhibiting the cytochrome P450 enzymes and the activity of the glutathione-S-transferase enzyme, respectively; they inhibit phases I and II of the detoxification process, reversing maize tolerance to HPPD inhibitors (Ma et al., 2012; Oliveira et al., 2018), wild radish (Lu et al., 2020) and grain sorghum (Pandian et al., 2020).

Thus, the objective of this study was to evaluate the effect of metabolization (malathion) and conjugation (NDB-Cl) inhibitors on the tolerance of white oat to HPPD-inhibiting herbicides.

Results

Oat intoxication

There was a significant treatment effect for the variable intoxication of white oat plants at 7, 14, 21 and 28 DAA (Table 1). The first symptoms of plant intoxication were found at 7 DAA in treatments containing the herbicides mesotrione or tembotrione. The control, malathion and NBD-Cl treatments showed no injuries to the oat plants (Table 1). Treatments containing mesotrione (mesotrione, mesotrione+malathion and mesotrione+NBD-Cl) showed the highest levels of intoxication with 33.1, 38.8 and 36.2% respectively, compared to treatments with tembotrione (tembotrione, tembotrione+malathion and tembotrione+NBD-Cl), which showed only 5.8, 6.0 and 6.4% of intoxication (Table 1).

At 14 DAA, intoxication levels were higher, except for the control, malathion and NBD-Cl treatments, which did not show symptoms of intoxication (Table 1). The highest levels of intoxication were found in treatments containing the herbicide mesotrione (mesotrione at 63.1%, mesotrione+malathion at 68.1% and mesotrione+NBD-Cl at 65%) compared to those containing the herbicide tembotrione (tembotrione at 6.8%, tembotrione+malathion at 7.3% and tembotrione+NBD-Cl at 6.6%) (Table 1).

At 21 DAA, there was a reduction in the levels of intoxication of the treatments compared to the previous period, indicating the recovery of injuries by the oat plants. The highest levels of intoxication were found in plants treated with mesotrione+malathion (43.1%) and mesotrione+NBD-Cl (36.2%), followed by mesotrione at 28.1%, and later with treatments containing the herbicide tembotrione (tembotrione+malathion at 2.6%, tembotrione at 2.4% and tembotri-

Table 1. Intoxication of white oat plants (%) at 7, 14, 21 and 28 days after application (DAA) of herbicides applied alone or previously treated with a metabolism or conjugation inhibitor. 2021/2021 season, Lages – SC.

Treatment	Intoxication (%)							
	7 DAA		14 DAA		21 DAA		28 DAA	
Control	0.0	c	0.0	c	0.0	d	0.0	b
Malathion	0.0	c	0.0	c	0.0	d	0.0	b
NBD-Cl	0.0	c	0.0	c	0.0	d	0.0	b
Mesotrione	33.1	a	63.1	a	28.1	b	6.5	a
Mesotrione + Malathion	38.8	a	68.7	a	43.1	a	10.4	a
Mesotrione+NDB-Cl	36.2	a	65.0	a	36.2	a	8.2	a
Tembotrione	5.8	b	6.8	b	2.4	c	0.4	b
Tembotrione+Malathion	6.0	b	7.3	b	2.6	c	0.5	b
Tembotrione+NDB-Cl	6.4	b	6.6	b	1.5	c	0.3	b
<i>p-value</i>	<0.001*		<0.001*		<0.001*		<0.001*	
C.V. (%)	11.7		7.8		12.3		27.5	

*Significant at $p < 0.05$ by the F-test; ^{ns} non-significant by the F-test; identical letters in the column do not differ from each other at $p < 0.05$ by Tukey's test; CV: coefficient of variation.

one+NBD-Cl at 1.5%), which did not differ from each other (Table 1).

At 28 DAA, there was a reduction in the levels of plant intoxication in comparison to the other periods of assessment, indicating more intense plant recovery. The highest levels of intoxication were found in the treatments mesotrione+malathion at 10.4%, mesotrione+NDB-Cl at 8.2% and mesotrione at 6.5%, compared to the other treatments that did not differ from each other (Table 1) and resulted in nearly zero intoxication.

Photosynthetic pigments

There was a significant effect ($p < 0.05$) of treatment for the SPAD index of white oat plants only at 7 and 14 DAA (Table 2). At 7 DAA, the highest SPAD index was measured in the following treatments: control (39.41), malathion (39.42) and NBD-Cl (39.65), while the lowest value was measured in the Mesotrione treatment (31.31) (Table 2). However, the other treatments showed no significant difference from the untreated control (Table 2).

At 14 DAA, treatments containing mesotrione (mesotrione with 12.78, mesotrione+malathion with 10.46 and mesotrione+NDB-Cl with 14.31) had the lowest SPAD index compared to the other treatments, which did not differ from each other (Table 2). At 21 and 28 DAA, there was no difference ($p > 0.05$) between treatments (Table 2); they showed a mean SPAD index of 38.9 and 45.5, respectively.

For concentrations of chlorophyll *a* (Ca), chlorophyll *b* (Cb), total carotenoids (CT) and malonaldehyde (MDA) in white oat plants at 21 DAA, there was no significant difference ($p < 0.05$) between treatments (Supplementary 1). The average treatment values corresponded to 106.4, 30.6 and 26.7 $\mu\text{g g FM}^{-1}$ for Ca, Cb and CT respectively, and 22.9 nmol ml^{-1} of MDA.

Growth, yield and yield components

There was no significant effect ($p > 0.05$) of treatment for the following variables: plant height, harvest index (HI), number of panicles per m^2 (NPM), number of spikelets per panicle (NSP) and number of grains per spikelet (NGS) (Supplementary 2). The average value of the treatments corresponded to 107.8 cm for plant height, 0.47 for HI, 522 NPM, 30.3 NSP and 1.1 NGS. There was also no significant effect ($p > 0.05$) for grain harvest moisture (U%), thousand-grain weight (TGW) and grain yield, and the mean values were 11.7% of U%, 37 g for TGW and 5907 kg ha^{-1} for grain yield (Supplementary 3).

Grain quality

There was no significant effect ($p > 0.05$) for the variables related to quality and industrial yield of white oat grains owing to the treatments tested (Supplementary 4). The average values of the treatments corresponded to: grains higher than 2 mm by sieve ($G > 2$) = 90.5%; hectoliter weight (HW) = 50.5 kg hL^{-1} ; dehulling index (DHI) = 75.4%; industrial grain yield (Avenacor) = 4034 kg ha^{-1} of caryopsis.

In summary, the findings of this study indicate a significant effect ($p < 0.05$) for plant intoxication (Table 1) and SPAD index (Table 2) as a result of the application of the herbicides mesotrione and tembotrione, either alone or with previously treated with malathion or NBD-Cl. These results clearly show the appearance of symptoms in the plants and their subsequent recovery, allowing the white oat plants to complete their cycle without affecting grain yield and yield components, and without affecting grain quality. The herbicides mesotrione and tembotrione were selective to white oat crop.

Table 2. SPAD index of white oats at 7, 14, 21 and 28 days after application (DAA) of herbicides applied alone or previously treated with a metabolization or conjugation inhibitor. 2021/2021 season, Lages – SC.

Treatment	7 DAA		14 DAA		21 DAA		28 DAA	
	SPAD Chlorophyll Index							
Control	39.41	a	37.29	a	37.25	^{ns}	44.07	^{ns}
Malathion	39.42	a	38.21	a	40.79		46.34	
NBD-Cl	39.65	a	38.55	a	39.72		44.21	
Mesotrione	31.31	b	12.78	b	36.85		46.29	
Mesotrione + Malathion	36.16	ab	10.46	b	38.63		46.20	
Mesotrione+NDB-Cl	33.80	ab	14.31	b	38.97		47.95	
Tembotrione	37.38	ab	37.22	a	39.40		45.44	
Tembotrione+Malathion	34.41	ab	36.92	a	41.02		44.63	
Tembotrione+NDB-Cl	35.50	ab	35.12	a	37.32		44.53	
<i>p-value</i>	0.003*		<0.001*		0.506 ^{ns}		0.279 ^{ns}	
C.V. (%)	7.8		10.4		8.1		4.9	

*Significant at $p < 0.05$ by the F-test; ^{ns} non-significant by the F-test ($p > 0.05$); Different letters in the column differ from each other at $p < 0.05$ by Tukey's test; CV: coefficient of variation.

Discussions

Symptoms of intoxication started at 7 DAA, in treatments containing the herbicides mesotrione or tembotrione, alone or with previous treatment using malathion or NBD-Cl (Table 1). Visual symptoms were characterized by bleaching and/or purplish stains on tissues produced after application of the treatments. The symptoms are similar to those described by De David et al. (2017) for *Illex paraguariensis*, Pandian et al. (2020) for sorghum, Teixeira et al. (2017) for sweet sorghum and Wang et al. (2020) for wheat, sprayed with carotenoid biosynthesis inhibitor herbicides.

Tissue bleaching is due to the photo-oxidation of chlorophyll which results from the absence of carotenoids that protect chlorophyll from the excited electrons released during photosynthesis (Dankov et al., 2009; Wang et al., 2020). Carotenoid depletion is the result of applying the HPPD-inhibiting herbicides mesotrione and tembotrione. HPPD is responsible for converting 4-hydroxyphenyl pyruvate into homogentisate, i.e., the precursor of plastoquinone, which is required for carotenoid biosynthesis (Lee et al., 1998; Godar et al., 2015).

To assess the intoxication of oat plants at 7 DAA, the treatments were separated into three groups (Table 1) in terms of increasing levels of intoxication, namely: without herbicides (0%), treatments containing tembotrione (5.8 to 6.4%) and treatments containing mesotrione (33.1 to 38.8%). It was found that intoxication levels were not affected by the metabolization inhibitors tested, only by the herbicides. A similar behavior was found at 14 DAA, except for the increase in intoxication levels in treatments containing tembotrione (6.6 to 7.8%) and

mesotrione (63.1 to 68.7%) in comparison to the previous assessment period (Table 1).

The externalization of symptoms after 7 DAA, together with increased levels of symptoms up to 14 DAA, is due to the mechanism of action of the herbicides mesotrione and tembotrione; they inhibit the synthesis of new carotenoids after application, but they do not affect the carotenoids formed prior to application (Dan Hess, 2000; Grossmann et al., 2007; Hawkes, 2007; De Paula et al., 2020). Thus, plants continue to grow, but new tissues are produced without the presence of carotenoids, resulting in increased levels of intoxication. A similar behavior was reported by De David et al. (2017) for *Illex paraguariensis* and, by Teixeira et al. (2017) for sweet sorghum under increasing rates of tembotrione.

At 21 DAA, there were reduced intoxication levels for treatments containing tembotrione (1.5 to 2.6%) and mesotrione (28.1 to 43.1%) in comparison to the previous evaluation period (Table 1). This finding suggests the onset of plant recovery, which is intensified at 28 DAA (Table 1). Plant recovery occurs as a result of the production of new tissues with the absence of visual symptoms of intoxication, leading to a reduction in intoxication levels. A similar behavior was found in wheat cultivars "Quartzo" and "Pioneiro" sprayed with 0.8 L ha⁻¹ of clomazone; however, recovery started at 14 DAA, and it was intensified at 21 DAA (Galon et al., 2015).

Plant recovery resulting from the production of new tissues (green) without symptoms is a strong indication that the herbicides being used are not inhibiting the site of action; the route is reestablished and starts producing carotenoids again. The non-inhibition of the herbicidal site of action on the plant may be due to reduced translocation, and/or increased differential metabolism and/or

compartmentalization of the herbicide by the plant in vacuoles or cell walls (Délye et al., 2013). The mechanisms of detoxification by the action of cytochrome P450 are responsible for the selectivity of HPPD-inhibiting herbicides in crops of agricultural interest (Ahrens et al., 2013), e.g., corn (Lu et al., 2020). They also thought to be responsible for the resistance of wild radish (*Raphanus raphanistrum*) (Lu et al., 2020), grain sorghum (*Sorghum bicolor*) (Padian et al., 2020) and Palmer amaranth (*Amaranthus palmeri*) (Küpper et al., 2018) biotypes to HPPD-inhibiting herbicides; However, there is no information available on white oat crops.

To test differential metabolism in plants, experiments were conducted with the C¹⁴-labeled herbicide molecule, which was applied to tolerant and susceptible plants. It was subsequently assessed and quantified for absorption, translocation and metabolism (Nandula et al., 2017; Shergill et al., 2018; Lu et al., 2020). As an alternative, differential metabolism in plants can be investigated by means of metabolization inhibitors, a method applied in this work. The application of malathion and/or 4-chloro-7-nitrobenzofurazan (NDB-Cl) inhibits the cytochrome P450 enzymes and the activity of the glutathione-S-transferase enzyme, respectively, inhibiting phases I and II of the metabolism process, thus reversing tolerance by plants (Shergill et al., 2018).

The application of malathion (43.1%) or NDB-Cl (36.2%) reduced the recovery speed of plants sprayed with mesotrione, compared to this herbicide alone (28.1%) at 21 DAA (Table 1), but the intoxications for the other treatments and evaluation periods did not change (Table 1). This result indicates a possible effect of detoxification on the tolerance of white oat to mesotrione. However, the same outcome did not occur for tembotrione. Unlike the findings in this work, the application of malathion or NDB-Cl reverses the tolerance of the crop to HPPD inhibitors in maize (Ma et al., 2012; Oliveira et al., 2018), wild radish (Lu et al., 2020), grain sorghum (Padian et al., 2020) and Palmer amaranth (Shergill et al., 2018; Küpper et al., 2018).

The onset of intoxication symptoms at 7 DAA and their intensification at 14 DAA in treatments containing the herbicides mesotrione and tembotrione, followed by the recovery process at 21 and 28 DAA, can also be evidenced by the SPAD Index (Table 2). It is measured by the difference in light transmitted through a leaf using two detectors at wavelengths from 650 to 940 nm, generating a leaf greenness index (Blackmer et al., 1995). In other words, the SPAD index is positively correlated with leaf greenness and chlorophyll, which is degraded by the absence of carotenoids in the tissues sprayed with carotenoid biosynthesis inhibitor herbicides. This variable was also not affected by the application of malathion or NDB-Cl metabolizing inhibitors (Table 2).

The concentrations of chlorophyll *a* (Ca), chlorophyll *b* (Cb) and total carotenoids (TC) in white oat plants at 21 DAA, were not altered by the application of the herbicides mesotrione and tembotrione, alone or with previous treatment using malathion or NDB-Cl (Supplementary 1). This outcome indicates recovered tissue and absence of symptoms at the apex of the plant. The same occurred for malonaldehyde (MDA) concentration at 21 DAA (Supplementary 1). MDA is commonly used as a measure of oxidative stress in physiological systems (Ghani, 2017) as it is generated from lipid peroxidation in plant cells under different types of stress (Carvalho et al., 2011). The findings show the absence of lipid peroxidation in the evaluated tissue of white oat plants as a result of the treatments when compared to control (Supplementary 1). This outcome allows the plant to complete its cycle without interfering with yield. This behavior is opposite to what is expected in plants treated with carotenoid biosynthesis inhibitor herbicides, which reduce tocopherol and plastoquinone levels, leading to carotenoid depletion and consequently leaf bleaching (reduction in chlorophyll and carotenoid levels) and plant death arising from oxidative stress (lipid peroxidation) (Beaudegnies et al., 2009; Dankov et al., 2009; Wang et al., 2020).

The assessment of herbicide selectivity must be based on plant intoxication symptoms and oxidative stress parameters (Agostinetto et al., 2016). In addition, crop yield and product quality need to be quantified. There are products that affect crop yield without causing visual symptoms, while others cause injuries that are recoverable, thus not affecting yield (Negrisoli et al., 2004). Also, they may stimulate plant development when applied at specific rates (Duke et al., 2006). The parameters relative to yield components (Supplementary 2), yield (Supplementary 3), and grain quality (Supplementary 4) did not differ from those of the control when applying the herbicides mesotrione and tembotrione, either alone or in previous treatments with malathion or NDB-Cl. These findings indicate crop tolerance to the application of the herbicides mesotrione and tembotrione alone and absence of tolerance reversal after application of the metabolization inhibitors malathion and NDB-Cl.

Differential metabolism is considered to be the main mechanism responsible for tolerance and resistance of species to HPPD inhibitors, but this fact was not found to occur in this work. However, Kramer et al. (2014) reported that the HPPD isoenzyme, encoded by the gene *avhppd-03* in *Avena sativa*, has a reduced binding affinity for mesotrione; thus, it is more easily tolerated by the species. In addition, Matringe et al. (2005), Siehl et al. (2014), Dreesen et al. (2018) and Hanwkes et al. (2019) reported the use of the HPPD-resistant gene of *Avena sativa* in association with a modified gene of *Pseudomonas fluorescens* for the development of transgenic soybean (*Glycine max*)

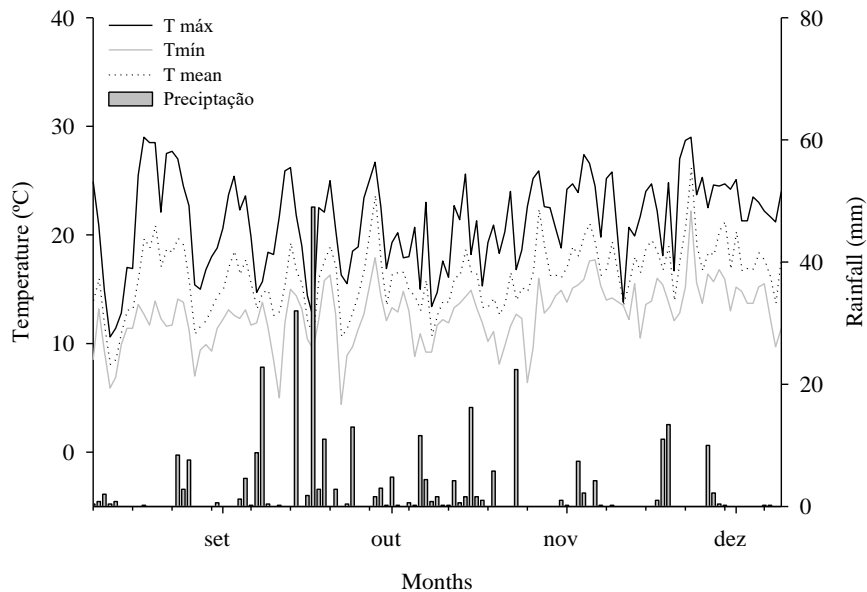


Fig 1. Rainfall, maximum, minimum and mean air temperatures from sowing to harvesting the experimental white oat growing during the 2021/2022 season, in Lages - SC. T max; Maximum temperature; T min: minimum temperature; T mean: mean temperature.

Source: Inmet, 2021;

resistant to HPPD inhibitors or, for the same purpose, the use of only the HPPD “*Avena sativa* 339” with deletion of a single amino acid in the N-terminal domain (Hawkes et al., 2016). Thus, the low enzymatic affinity of the HPPD isoenzyme of *A. sativa* may be the main mechanism responsible for the tolerance of the crop to the herbicides mesotrione and tembotrione. This may explain the occurrence of some symptoms of intoxication and subsequent recovery.

Based on these findings, further research should be conducted on the use of (C^{14})-labeled herbicide molecules to evaluate their differential absorption and translocation and to prove the occurrence of differential metabolism in oat plants. There should be studies on the enzymatic affinity of HPPD in oats, and on the sequencing of the HPPD gene in oats and susceptible plants, to elucidate the complete mechanism of crop tolerance to this group of herbicides.

Materials and Methods

Characterization of the field

The experiment was carried out at an experimental site at the State University of Santa Catarina CAV/UDESC, in the city of Lages – SC, under the following geographical coordinates: 27° 52' South latitude; 50° 18' West Longitude; and average altitude of 930 m, during the crop year of 2021. The climate of the region, according to the Köppen classification, is Cfb (temperate climate, with mild summers). Figure 1 shows the meteorological data (maximum, average, and minimum temperatures, and rainfall) in Lages

during the time when the experiments were conducted.

The experiment was implemented in an aluminic humic Cambisol (Embrapa, 2017) with the following characteristics: pH in water: 6.3; Ca: 9.1 $cmol_c\ dm^{-3}$; Mg: 4.0 $cmol_c\ dm^{-3}$; Al: 0.0 $cmol_c\ dm^{-3}$; K: 143.3 $mg\ dm^{-3}$; P: 35.2 $mg\ dm^{-3}$; CEC: 16.6 $cmol_c\ dm^{-3}$; OM: 2.8% and clay: 50%.

Experimental design and treatments

The crop was sown under a no-tillage system (soybean stubble) using a randomized block design (DBC), with four replications. The treatments are listed below: T0-control (no application); T1-malathion (Dipil®, 1000 g active ingredient (a.i.) ha^{-1}); T2- 4-chloro-7-nitrobenzofurazan – NBD-Cl (Alfa Aesar®, 270 g a.i. ha^{-1}); T3- mesotrione (Callisto®, 168 g a.i. ha^{-1}); T4- mesotrione+malathion (168 + 1000 g a.i. ha^{-1}); T5- mesotrione+NBD-Cl (168 + 270 a.i. ha^{-1}); T6- tembotrione (Soberan®, 88.2 a.i. ha^{-1}); T7- tembotrione+malathion (88.2 + 1000 a.i. ha^{-1}) and T8- tembotrione+NBD-Cl (88.2 + 270 g a.i. ha^{-1}).

Plant material

This experiment was carried out with the white oat cultivar UPFA Fuerza, launched in 2015. It has a semi-erect growth habit, with high stature and medium cycle, and it is resistant to leaf rust (Danielowski et al., 2021).

Conduction of study

Before sowing, the seeds were treated with fipronil+pyraclostrobin+thiophanate-methyl (Standak Top®, 200 ml 100 kg^{-1} of seeds) and sown mechanically

on August 9, 2021; 350 viable seeds per m² of the white oat cultivar UPFA Fuerza were used. Fertilization was performed with 400 kg ha⁻¹ of the N-P₂O₅-K₂O 5-20-10 formula, and later, at the beginning of tillering and stem elongation, topdressing nitrogen fertilization was performed with 45 kg ha⁻¹ of N, following the recommendations of the Soil Chemistry and Fertility Commission (CQFS, 2016) for a productive potential of 5 t ha⁻¹ of seeds. Each plot consisted of 5 rows that were 5 m long, with 0.2 m inter-row spacing, spaced 0.5 m apart from each other.

The phytosanitary management of the crop was carried out by applying fungicides and insecticides whenever necessary (through monitoring) to ensure the intended yield potential, following the technical indications for white oat (Danielowski et al., 2021).

Herbicide application (mesotrione or tembotrione) was carried out when 50% of the plants had 3-4 fully expanded leaves (09/11/2021), and 0.5% v/v vegetable oil (Áureo®) was added to the spray mixture. The 4-chloro-7-nitrobenzofurazan (NDB-Cl; diluted in acetone) a metabolization inhibitor via glutathione-S-transferase (GST) enzymes was applied 48 hours before herbicide application, while the application of malathion, an inhibitor of cytochrome P450 monooxygenase (P450) enzymes, preceded the application of herbicides by two hours, based on the work by Shergill et al. (2018). The treatments were applied with the aid of a CO₂-pressurized backpack sprayer (Herbicat, Brazil), with a pressure of 30 KPa, fitted with six 80.02 flat jet tips VS (TeeJet®, USA), calibrated for a spray volume of 200 L ha⁻¹.

Traits measured

At 7, 14, 21 and 28 DAA (days after application) of the treatments, plant intoxication was evaluated using the percentage scale from 0 to 100% (SBCPD, 1995), where 0% represents the absence of symptoms and 100%, the death of all plants. Concomitantly, the SPAD index was measured on the last fully expanded leaf of the plant, using a SPAD-502 Plus chlorophyll meter.

At 21 DAA, the last fully expanded leaf was collected from five plants per plot, then frozen in liquid nitrogen and stored in an ultrafreezer at -80 °C for quantification of concentrations of chlorophyll *a* (*Ca*), chlorophyll *b* (*Cb*), total carotenoids (TC) and malonaldehyde (MDA). *Ca*, *Cb* and TC were quantified by using the methodology proposed by Lichtenthaler et al. (2001), while MDA was quantified by the method proposed by Heath et al. (1968).

At stage 89 (Zadocks et al., 1974), oat plants with 0.3 m² (0.5 m x 0.6 m) in each plot were cut close to the ground and packed in paper bags. Using these sub-samples, number of panicles, spikelets and G>2 per spikelet were counted by direct counting. Then, the samples were packed and dried in an oven at 65±3 °C to constant weight. Subsequently, total plant biomass and grain weight were weighed separately on a semi-analytical scale, and the harvest index (HI) was

determined through the ratio: total grain weight (g)/total plant biomass (g).

Grain yield was estimated by harvesting the total area of each plot (5 m²); water content was corrected to 13% and converted into hectare. Thousand-grain weight (TGW) was measured on the basis of the count of 1,000 grains and subsequent weighing on a semi-analytical balance with a precision of 0.0001 g.

Grain thickness greater than two mm (G>2) was determined by weighing and subsequently sieving a sample of grains in a rectangular sieve (40 x 30 cm) with 2 mm x 20 mm thick oblong holes. The sample retained on the sieve was weighed again to estimate the percentage (%) of G>2.

Hectoliter weight (HW) was measured by weighing a grain sample previously sieved G>2 and placed in a tube of known volume (0.25 L), using the Dalle Molle hectoliter weight scale (type 40). Such weight (g) was converted (kg into 100 L), based on specific table for oat crops (Brasil, 2009).

Dehulling index (DHI) was determined by manually dehulling a sample of 5 g of G>2 (hull+caryopsis) previously dried in an oven at 60 °C. After dehulling, the grains were weighed again to determine caryopsis weight.

Industrial yield (AVENACOR, kg ha⁻¹) was estimated by calculating grain yield (GY) x G>2 x DHI.

Statistical analysis

Data were tested as assumptions of analysis of variance (normality and homogeneity), and when satisfied, they underwent analysis of variance (ANOVA) by the F-test (p<0.05). Data on levels of intoxication that did not satisfy the assumptions were transformed by arcsin of (x/100)^{0.5}, prior to ANOVA. The data, when significant, were further analyzed using Tukey's test (p<0.05) and the R software.

Conclusion

The application of metabolization (malathion) and conjugation (NDB-Cl) inhibitors delayed the recovery process of plants sprayed with mesotrione. This result suggests that metabolization may increase the tolerance of white oat to mesotrione. The tolerance of white oat to the herbicides mesotrione and tembotrione was not reversed by the application of metabolization (malathion) and conjugation (NDB-Cl) inhibitors, indicating that differential metabolism is not the main mechanism of tolerance of white oats to HPPD-inhibiting herbicides.

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