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Article

RESPONSE OF WHEAT PLANTS TO COMBINATIONS OF HERBICIDES WITH INSECTICIDES AND FUNGICIDES

Resposta de Plantas de Trigo a Associação de Herbicidas com Inseticidas e Fungicidas

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ABSTRACT - The combination of herbicides and other pesticides can influence weed and crop management. This study aimed to evaluate the response of the combination of different herbicides with insecticides or fungicides of different chemical groups on the development and yield of wheat grains of the cultivar ORS Vintecinco. The experimental design was a randomized block design with three replications. The experiment consisted of 33 treatments, as follows: control without application and application of the herbicides 2,4-D (1,005 g a.e. ha^{-1}), bentazon (756 g a.i. ha^{-1}), metsulfuron-methyl (3 g a.i. ha^{-1}), and pyroxsulam (16 g a.i. ha^{-1}), isolated or combined with the insecticides chlorfluazuron (15 g a.i. ha^{-1}), chlorpyrifos (720 g a.i. ha^{-1}), deltamethrin (5 g a.i. ha^{-1}), and methomyl (5 g a.i. ha^{-1}) or with the fungicides azoxystrobin (100 g a.i. ha^{-1}), carbendazim (250 g a.i. ha^{-1}), and propiconazole (190 g a.i. ha^{-1}). Relative tolerance (RT), shoot dry matter (SDM), yield components, and grain yield were evaluated. In general, pyroxsulam had the highest number of synergistic interactions with insecticides and fungicides, reducing RT and SDM, especially the combination pyroxsulam + chlorpyrifos due to a lower RT level (45%) and higher SDM reduction (52%). All combinations of herbicides with the insecticide chlorpyrifos and most of the combinations with the fungicide propiconazole led to a reduction of grain yield when compared to their isolated application or to control without application.

Keywords: *Triticum aestivum*, synergism, antagonism, tolerance, grain yield.

RESUMO - A associação entre herbicidas e demais pesticidas pode causar reflexos tanto no manejo de plantas daninhas quanto de cultivadas. O objetivo deste trabalho foi avaliar a resposta da associação de diferentes herbicidas com inseticidas ou fungicidas pertencentes a diferentes grupos químicos sobre o desenvolvimento e rendimento de grãos de trigo, cultivar ORS Vintecinco. O delineamento experimental foi o de blocos ao acaso com três repetições. O experimento foi constituído por 33 tratamentos: testemunha sem aplicação, aplicação dos herbicidas 2,4-D (1.005 g e.a. ha^{-1}), bentazon (756 g i.a. ha^{-1}), metsulfuron-methyl (3 g i.a. ha^{-1}) e pyroxsulam (16 g i.a. ha^{-1}), de forma isolada ou associados com os inseticidas chlorfluazuron (15 g i.a. ha^{-1}), chlorpyrifos (720 g i.a. ha^{-1}), deltamethrin (5 g i.a. ha^{-1}) e methomyl (5 g i.a. ha^{-1}), ou com os fungicidas azoxystrobin (100 g i.a. ha^{-1}), carbendazim (250 g i.a. ha^{-1}) e propiconazole (190 g i.a. ha^{-1}). Avaliou-se a tolerância relativa (TR), a massa da parte aérea seca (MPAS) das plantas e os componentes de rendimento e rendimento de grãos. De modo geral, pyroxsulam apresentou maior número de interações sinérgicas com os inseticidas e fungicidas utilizados, reduzindo dessa forma a TR e MPAS das plantas, destacando-se a associação pyroxsulam + chlorpyrifos pelo

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menor nível de TR (45%) e maior redução de MPAS (52%). Todas as associações de herbicidas com o inseticida chlorpyrifos e a maioria das associações com o fungicida propiconazole resultaram em redução do rendimento de grãos em relação à aplicação isolada dos mesmos herbicidas ou à testemunha sem aplicação.

Palavras-chave: *Triticum aestivum*, sinergismo, antagonismo, tolerância, rendimento de grãos.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important grain crops in the Brazilian agricultural scene, being the main species grown in the winter (Conab, 2016). In addition to the economic point of view, this crop is an integrating factor of cropping systems as a whole, especially in crop rotation systems.

The need for weed control in wheat is routine. Species such as ryegrass (*Lolium multiflorum*), turnip (*Raphanus* spp.), and oat (*Avena* spp.) stand out like weeds in wheat fields in southern Brazil (Agostinetto et al., 2008; Rigoli et al., 2008; Paula et al., 2011). Some herbicides used in weed management of this crop are 2,4-D, metsulfuron, bentazon, and pyroxsulam (Vargas and Roman, 2005; Galon et al., 2015).

Regarding the wheat pests, caterpillars and aphids are agents that usually demand chemical management as they have high geographical distribution, high prolificacy, and frequently reach levels that require control (Pereira et al., 2015). Insecticides such as chlorfluazuron, chlorpyrifos, deltamethrin, and methomyl are indicated to control these pests (AGROFIT, 2018). On the other hand, leaf blotch, rust, powdery mildew, and scab are recurrent diseases in wheat (Cromey et al., 2001; Correa et al., 2013), being controlled with the fungicides azoxystrobin, carbendazim, and propiconazole (AGROFIT, 2018).

Weeds, pests, and diseases cause quantitative/qualitative reductions in crop yields, often coinciding. The reduction of grain yield in the wheat crop due to the concomitant incidence of weeds, pests, and diseases is estimated at 50% (Oerke, 2006). When these biotic agents coexist in the production field, a combination of pesticides is necessary to improve control efficiency with fewer applications, thus reducing operating costs and crop losses. A survey indicates that approximately 97% of Brazilian farmers mix pesticides, mainly for the mentioned reasons, but about 72% of them are not fully aware of the effects of these combinations (Gazziero, 2015).

The interaction resulting from the combination of herbicides with pesticides may influence both weed and crops. According to Colby (1967), the combination of products can be characterized as synergistic, antagonistic, or additive. Synergism is described as a superior response of a combination when compared to an isolated application of products; antagonism, on the other hand, is the reverse, i.e., when the mixture results in an inferior response when compared to products in isolation. When the combined use does not result in increased or decreased response, an additive interaction is characterized.

A synergistic combination is considered advantageous for weed control. However, a synergistic combination increases toxicity to cultivated plants, which may compromise grain yield. On the contrary, an antagonistic combination presents as advantage lower toxicity to the crop but leading to a reduction of herbicide efficiency on weeds. The ideal situation is that the interaction reduces the injury on the crop, but without modifying or even increasing the herbicide action on weeds.

Various pesticide classes (herbicides, fungicides, and insecticides) may present different mechanisms and modes of action in plants. That is, these products can lead to different morphological, physiological or biochemical responses in plants. Thus, their simultaneous use makes the analysis of these interactions more complex and valuable since they directly influence the development of crops and weed control.

The combination of herbicides with specific insecticides may result in the increased herbicidal activity. The effect is reported mainly when herbicides inhibiting acetolactate synthase (ALS), some of photosystem II (PSII; bentazon and propanil), and acetyl CoA carboxylase (ACCase) are combined with insecticides from the organophosphorus and carbamate groups (McRae et al., 1964; Matsunaka, 1968; Campbell and Penner, 1982; Biediger et al., 1992; Baerg et al., 1996; Ahrens and Panara, 1997). Studies have indicated that the combination of the herbicide ethoxysulfuron with organophosphorus insecticides (malathion + chlorpyrifos) increased injuries caused by herbicide on the bean crop when compared to the isolated herbicide application (Pagnoncelli Jr et al., 2016). Similar results have been observed in corn with the combination of nicosulfuron and chlorpyrifos (Silva et al., 2005), as well as in wheat with the application of imazamox and malathion (Rojano-Delgado et al., 2015). Few studies have evaluated the effect of combining herbicides with insecticides, such as deltamethrin (pyrethroid) and chlorfluazuron (benzoylurea), or fungicides, such as propiconazole (triazole) and carbendazim (benzimidazole).

The effects of the combination of herbicides with fungicides have been little explored when compared to herbicides and insecticides. However, studies have indicated that some combinations may reduce the phytotoxicity of certain herbicides. The control efficiency of the weed *Senna obtusifolia* with 2,4-D was reduced by approximately 15% when combined with the fungicide azoxystrobin, while its combination with tebuconazole did not lead to a difference in weed control in relation to its isolated application (Lancaster et al., 2005). The combination of fenoxaprop-p-ethyl and picoxystrobin reduced the injury on wheat, leading to a higher plant development when compared to the isolated herbicide application (Nader et al., 2015).

This study hypothesized that the insecticides chlorpyrifos (organophosphorus) and methomyl (carbamate) might increase the injury caused by the herbicides pyroxsulam, metsulfuron (ALS inhibitors), and bentazon (FS2 inhibitor) on wheat (synergistic effect), as already observed with other cultivated species. However, it is believed that the use of the fungicide azoxystrobin (strobilurin) may provide a protective effect, reducing injuries caused by the herbicides pyroxsulam, metsulfuron, bentazon, and 2,4-D on the wheat crop (antagonistic effect), and even stimulate plant development. This study aimed to evaluate the combination of the herbicides 2,4-D, bentazon, metsulfuron, and pyroxsulam with the insecticides chlorfluazuron, chlorpyrifos, deltamethrin, and methomyl and the fungicides azoxystrobin, carbendazim, and propiconazole, belonging to different chemical groups, on the development and grain yield of the wheat crop.

MATERIAL AND METHODS

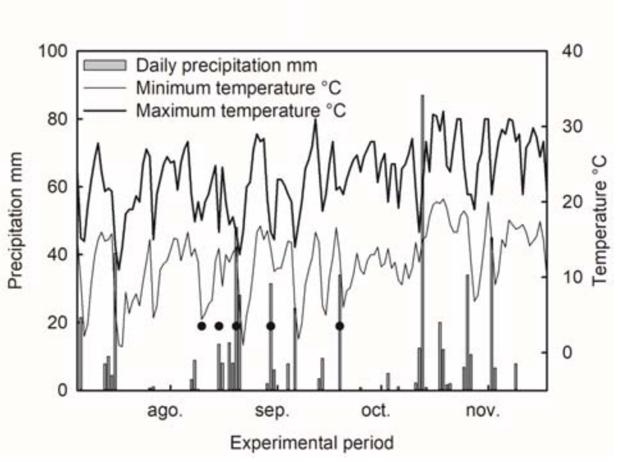
The experiment was conducted under field conditions in the municipality of Pato Branco (26°10’28.2" S and 52°41’12.9" W) between June and November 2016. The soil of the area is classified as a dystroferric Red Latosol (Oxisol), whose textural composition and chemical characteristics are shown in Table 1. Climate conditions in the experimental period are shown in Figure 1.

The experimental design was a randomized block design with three replications. The experiment consisted of 33 treatments composed of control without application and

Table 1 - Textural composition and chemical characteristics of dystroferric Red Latosol (Oxisol)

Textural composition		Chemical characteristics	
Fraction	%	Properties	Values
Clay	55.7	OM $^{(1)}$	49.50
Sand	3.0	$P_2O_5$$^{(2)}$	14.32
Silt	41.3	K_2O $^{(3)}$	0.70
		CEC $^{(4)}$	17.63
		pH$^{(5)}$	5.6
		H+Al $^{(6)}$	5.35

(1) Organic matter (g dm^{-3}); (2) Phosphorus (mg dm^{-3}); (3) Potassium (cmol$_c$ dm^{-3}); (4) Cation exchange capacity (cmol$_c$ dm^{-3}); (5) pH of soil ($CaCl_2$ 0,01 mol L^{-1}); (6) Exchangeable acidity (cmol$_c$ dm^{-3}).



Source: IAPAR – Instituto Agronômico do Paraná.

Figure 1 - Precipitation and minimum and maximum temperatures in the period of conduction of the experiment. The points represent the time of application and the evaluation periods.

application of the herbicides 2,4-D (1,005 g a.e. ha^{-1}), bentazon (756 g a.i. ha^{-1}), metsulfuron (3 g a.i. ha^{-1}), and pyroxsulam (16 g a.i. ha^{-1}), isolated or combined with the insecticides chlorfluazuron (15 g a.i. ha^{-1}), chlorpyrifos (720 g a.i. ha^{-1}), deltamethrin (5 g a.i. ha^{-1}), and methomyl (5 g a.i. ha^{-1}) or with the fungicides azoxystrobin (100 g a.i. ha^{-1}), carbendazim (250 g a.i. ha^{-1}), and propiconazole (190 g a.i. ha^{-1}).

The wheat cultivar used was the ORS Vintecinco, which has an early cycle and a semi-erect growth habit. Each experimental unit consisted of 8 m^2 (4 × 2 m). Weeds were manually removed from each experimental unit during the entire experimental period.

Sowing was carried out with a seed drill, with an interrow spacing of 17 cm, resulting in a density of 350,000 plants per ha. Base fertilization consisted of 300 kg ha^{-1} of the formulation 2-20-20 (N-P_2O_5-K_2O). Soon after the emission of the first tiller, 300 kg ha^{-1} of the formulation 36-00-12 (N-P_2O_5-K_2O) was applied. Treatment application was carried out when wheat plants started emitting the third tiller, which coincides with the initial stage of determination of the number of spikelets per spike and spikes per plant (Zadoks et al., 1974). For this, a CO_2-pressurized backpack sprayer was used at a constant pressure of 300 kPa, applied volume of 200 L ha^{-1}, and application speed of 3.6 km h^{-1}. The application boom was composed of three fan tips XR 110.02, spaced 0.5 m from each other. The mean environmental conditions during application were relative air humidity of 60% and an air temperature of 15 °C.

The relative tolerance (ability of wheat plants to withstand the application of different treatments when compared to the control) was evaluated at 5, 10, 20, and 40 days after treatment application (DAA) using the scale of Frans et al. (1986) with modifications, where 100 corresponds to the absence of symptoms (complete tolerance) and 0 corresponds to plant death (complete sensitivity).

At 20 and 40 DAA, the shoot dry matter (SDM) of wheat plants was determined by collecting plants from an area of 0.25 m^2 within the useful area of each plot. The plants were cut close to the soil and taken to a drying chamber at 60 °C until constant weight. SDM data were transformed into a percentage in relation to the control without treatment application.

Yield components and grain yield were determined at physiological maturation. Plants from four rows of 1 m in length (0.7 m^2) of each plot were threshed to obtain the one thousand-grain weight and grain yield, which was extrapolated to kg ha^{-1}. The total number of spikes were counted in 1 m^2 at a row of each plot at harvest time.

The data were submitted to analysis of variance by the F test ($p \leq 0.05$) using the software R (RStudio 2016) and the package ExpDes.pt (Ferreira et al., 2011). The means of treatments were grouped by the Scott-Knott test ($p \leq 0.05$).

The GGE Biplot technique (Yan et al., 2000) was used to evaluate the effect of combinations of herbicides with fungicides and insecticides on grain yield. Combinations were interpreted based on the cosine of the angle between vectors, being positive if the angle between vectors is <90°, negative if >90°, and zero if the angle is ≅ 90°. The Scaled notation = 0 indicates that the data were not transformed. The Centered notation informs the used model, where Centered = 2 represents GGE (genotype + genotype × environment interaction). SVP (singular value partitioning) is a matrix decomposition technique; if SVP = JK, it has a focus on genotypes (herbicides). Combination analysis was carried out with the software R using the package GGEBiplotGUI (Frutos et al., 2014).

RESULTS AND DISCUSSION

The analysis of variance resulted in the significance of treatments for all the variables, indicating their differentiated effects on wheat plants. Among the main symptoms caused by the application of isolated or associated herbicides were epinasty (2,4-D), toppling (2,4-D and bentazon), chlorosis (metsulfuron and pyroxsulam), necrosis (bentazon and pyroxsulam), and reduction in height (pyroxsulam). These symptoms were more intense at first evaluation times (5, 10, and 20 DAA). In most treatments, the injury was higher in the combinations of herbicides with insecticides or fungicides when compared to the isolated herbicide application.

The lowest relative tolerance (RT) level was 45% (Table 2), which was observed in plants sprayed with the combination of pyroxsulam with chlorpyrifos at 20 DAA. This combination provided the highest injury level on plants, regardless of the evaluation time. Wheat plants sprayed with 2,4-D and bentazon in isolation and with the combinations 2,4-D + chlorfluazuron, bentazon + chlorfluazuron, 2,4-D + deltamethrin, bentazon + deltamethrin, 2,4-D + methomyl, bentazon + methomyl, bentazon + carbendazim, and bentazon + propiconazole presented RT similar to the control at all the evaluation times.

In isolated applications, metsulfuron and pyroxsulam reduced RT of wheat plants to 87 and 89%, respectively, at 5 DAA, while pyroxsulam reduced it to 86% at 10 DAA (Table 2). However, no significant effect of these herbicides was observed on RT of plants in subsequent evaluations.

Table 2 - Relative tolerance (%) (RT) at 5, 10, 20 e 40 days after application of the treatments (DAA) in wheat plants of ORS Vintecinco cultivar

Treatment			Relative tolerance (%)			
	Herbicide	Insecticide Fungicide*	5 DAA	10 DAA	20 DAA	40 DAA
1	Control treatment		100.00 a	100.00 a	100.00 a	100.00 a
2	2,4-D	-	95.17 a	93.21 a	86.86 a	97.76 a
3	Bentazon	-	95.67 a	97.00 a	100.00 a	98.33 a
4	Metsulfuron	-	86.67 b	96.33 a	96.67 a	98.33 a
5	Pyroxsulam	-	89.00 b	85.67 b	91.67 a	95.00 a
6	2,4-D	Chlorfluazuron	93.33 a	97.33 a	91.67 a	100.00 a
7	Bentazon	Chlorfluazuron	91.64 a	95.74 a	94.44 a	97.76 a
8	Metsulfuron	Chlorfluazuron	83.33 b	95.00 a	95.00 a	96.67 a
9	Pyroxsulam	Chlorfluazuron	75.00 c	65.00 c	85.00 b	95.00 a
10	2,4-D	Chlorpyrifos	85.00 b	90.67 a	93.33 a	95.00 a
11	Bentazon	Chlorpyrifos	75.47 c	82.1 b	96.96 a	95.23 a
12	Metsulfuron	Chlorpyrifos	66.67 d	60.00 c	68.33 c	85.00 a
13	Pyroxsulam	Chlorpyrifos	60.00 d	48.33 d	45.00 d	50.00 b
14	2,4-D	Deltamethrin	93.33 a	98.33 a	93.33 a	100.00 a
15	Bentazon	Deltamethrin	95.00 a	94.67 a	100.00 a	100.00 a
16	Metsulfuron	Deltamethrin	83.33 b	94.00 a	100.00 a	100.00 a
17	Pyroxsulam	Deltamethrin	73.33 c	63.33 c	81.67 b	93.33 a
18	2,4-D	Methomyl	97.33 a	99.00 a	98.33 a	97.33 a
19	Bentazon	Methomyl	95.67 a	95.67 a	100.00 a	98.33 a
20	Metsulfuron	Methomyl	82.33 b	95.00 a	96.67 a	100.00 a
21	Pyroxsulam	Methomyl	68.33 d	73.33 c	83.33 b	95.00 a
22	2,4-D	Azoxystrobin	71.67 c	80.00 b	81.67 b	95.00 a
23	Bentazon	Azoxystrobin	78.33 c	80.00 b	93.33 a	98.33 a
24	Metsulfuron	Azoxystrobin	84.33 b	86.67 b	98.33 a	100.00 a
25	Pyroxsulam	Azoxystrobin	70.00 d	61.67 c	76.67 b	88.33 a
26	2,4-D	Carbendazim	85.67 b	98.00 a	95.67 a	95.67 a
27	Bentazon	Carbendazim	96.67 a	97.00 a	99.00 a	100.00 a
28	Metsulfuron	Carbendazim	86.67 b	95.67 a	96.67 a	100.00 a
29	Pyroxsulam	Carbendazim	73.33 c	61.67 c	80.00 b	90.00 a
30	2,4-D	Propiconazole	80.00 c	88.33 b	95.00 a	95.67 a
31	Bentazon	Propiconazole	92.33 a	95.67 a	100 a	100.00 a
32	Metsulfuron	Propiconazole	80.67 c	93.33 a	100 a	100.00 a
33	Pyroxsulam	Propiconazole	74.00 c	68.33 c	80.00 b	96.67 a
CV(%)**			7.83	7.84	6.04	5.28

* Insecticides = chlorfluazuron, chlorpyrifos, deltamethrin e methomyl. Fungicides = azoxystrobin, carbendazim e propiconazole. (1) The averages followed by the same letter in the column do not differ by the Scott-Knott test (p≤0,05). ** CV = coeficiente of variation.

In general, the combination of pyroxsulam with insecticides and fungicides reduced RT of wheat plants, especially for the evaluation performed at 5 DAA (Table 2). The herbicides bentazon and 2,4-D in combination or not with insecticides and fungicides, in general, presented less negative impact on RT of wheat plants. Except for plants sprayed with the combination of pyroxsulam + chlorpyrifos, all the others showed transitory injuries, which were little perceptible or imperceptible in the evaluation of RT performed at 40 DAA.

Pyroxsulam and metsulfuron, even when applied alone, reduced shoot dry matter (SDM) of wheat plants by 25% at 40 DAA when compared to the control (Table 3). The herbicide pyroxsulam presented the highest number of synergistic interactions between combinations with the insecticides chlorfluazuron, chlorpyrifos, and methomyl and the fungicides azoxystrobin and carbendazim, reducing SDM in a higher proportion when compared to other treatments. This effect occurred at 20 DAA (51 to 83%) and reached a higher magnitude at 40 DAA (47 to 68%). Among all treatments, the highest reduction of SDM was observed in plants treated with the combination of the herbicide pyroxsulam with the insecticide chlorpyrifos, reaching 52% at 40 DAA (Table 3).

Table 3 **-** Shoot dry matter relative to the control treatment (SDM) (%) at 20 e 40 days after application of the treatments (DAA) in wheat plants of ORS Vintecinco cultivar

Treatment			SDM (%)	
	Herbicide	Insecticide or Fungicide*	20 DAA	40 DAA
1	Control treatment		100.00 a	100.00 b
2	2,4-D	-	109.79 a	95.59 b
3	Bentazon	-	86.94 a	87.79 b
4	Metsulfuron	-	97.17 a	75.41 c
5	Pyroxsulam	-	132.33 a	74.87 c
6	2,4-D	Chlorfluazuron	94.98 a	90.34 b
7	Bentazon	Chlorfluazuron	103.90 a	79.22 c
8	Metsulfuron	Chlorfluazuron	96.44 a	80.68 d
9	Pyroxsulam	Chlorfluazuron	83.12 b	94.32 b
10	2,4-D	Chlorpyrifos	90.30 a	81.54 c
11	Bentazon	Chlorpyrifos	106.03 a	80.08 c
12	Metsulfuron	Chlorpyrifos	70.22 b	70.74 d
13	Pyroxsulam	Chlorpyrifos	51.43 b	47.73 d
14	2,4-D	Deltamethrin	90.58 a	83.53 c
15	Bentazon	Deltamethrin	94.28 a	80.40 c
16	Metsulfuron	Deltamethrin	90.84 a	93.18 b
17	Pyroxsulam	Deltamethrin	104.9 a	85.80 c
18	2,4-D	Methomyl	90.66 a	108.13 a
19	Bentazon	Methomyl	108.99 a	127.45 a
20	Metsulfuron	Methomyl	95.97 a	73.30 c
21	Pyroxsulam	Methomyl	80.86 b	68.18 d
22	2,4-D	Azoxystrobin	80.31 b	83.24 c
23	Bentazon	Azoxystrobin	64.39 b	82.96 c
24	Metsulfuron	Azoxystrobin	98.30 a	84.66 c
25	Pyroxsulam	Azoxystrobin	73.09 b	68.47 d
26	2,4-D	Carbendazim	97.44 a	78.98 c
27	Bentazon	Carbendazim	93.09 a	94.04 b
28	Metsulfuron	Carbendazim	108.28 a	93.47 b
29	Pyroxsulam	Carbendazim	70.99 b	60.51 d
30	2,4-D	Propiconazole	87.77 a	90.91 b
31	Bentazon	Propiconazole	98.86 a	99.90 b
32	Metsulfuron	Propiconazole	85.68 a	87.50 b
33	Pyroxsulam	Propiconazole	96.97 a	94.89 b
CV(%)**			14.75	13.81

* Insecticides = chlorfluazuron, chlorpyrifos, deltamethrin e methomyl. Fungicides = azoxystrobin, carbendazim e propiconazole. (1) The averages followed by the same letter in the column do not differ by the Scott-Knott test ($p \leq 0,05$). ** CV = coeficiente of variation.

The combinations of the herbicide metsulfuron and the insecticide chlorpyrifos at 20 and 40 DAA and the herbicides 2,4-D and bentazon with the fungicide azoxystrobin at 20 DAA also stood out due to reductions in SDM (Table 3). The combination of the herbicides 2,4-D and bentazon with the insecticide methomyl led to increases of 8 and 27% in SDM at 40 DAA, respectively, when compared to the control.

The number of fertile tillers (NFT) treated with 2,4-D, bentazon, and metsulfuron in isolation was lower than that observed in plants of the control without application (Table 4). Reductions in NFT of treatments with combinations of the herbicide bentazon with the insecticides chlorpyrifos and methomyl and the fungicides carbendazim and propiconazole also stood out. The combinations of the herbicide metsulfuron with the insecticide deltamethrin and the herbicide pyroxsulam with the fungicide propiconazole also reduced NFT. For these treatments, NFT ranged from 216 to 425; for the others, it ranged from 456 to 633.

Table 4 **-** Number of fertile tillers (m^{-2}) (NFT), number of grains per spike (NGS), thousand-grain weight (g) (TGW) e grain yield (kg ha^{-1}) (GY) depending the application of the treatments in wheat plants of ORS Vintecinco cultivar

Treatment			Yield components			
Herbicide		Insecticide or Fungicide*	NFT (no. m^{-2})	NGS (no. m^{-2})	TGW (g)	GY (kg ha^{-1})
1	Control treatment		479.87 a	19.16 c	31.88 a	2976.92 a
2	2,4-D	-	335.75 b	44.68 a	33.36 a	4372.80 a
3	Bentazon	-	424.80 b	32.61 b	29.77 b	3527.84 a
4	Metsulfuron	-	216.33 b	32.35 b	30.66 a	2136.03 b
5	Pyroxsulam	-	503.47 a	21.61 c	33.46 a	3557.32 a
6	2,4-D	Chlorfluazuron	481.83 a	22.33 c	28.39 b	2956.72 a
7	Bentazon	Chlorfluazuron	580.17 a	17.40 c	31.02 a	3083.69 a
8	Metsulfuron	Chlorfluazuron	470.03 a	20.96 c	28.33 b	2795.58 b
9	Pyroxsulam	Chlorfluazuron	499.53 a	16.13 c	26.07 b	2126.37 b
10	2,4-D	Chlorpyrifos	623.43 a	15.65 c	28.46 b	2793.28 b
11	Bentazon	Chlorpyrifos	311.90 b	27.75 b	33.11 a	2585.62 b
12	Metsulfuron	Chlorpyrifos	491.67 a	17.28 c	32.86 a	2802.94 b
13	Pyroxsulam	Chlorpyrifos	597.87 a	16.15 c	27.28 b	2555.08 b
14	2,4-D	Deltamethrin	456.27 a	20.32 c	33.22 a	2947.92 a
15	Bentazon	Deltamethrin	475.93 a	17.83 c	29.02 b	2444.07 b
16	Metsulfuron	Deltamethrin	391.37 b	31.52 b	25.94 b	2928.59 a
17	Pyroxsulam	Deltamethrin	536.90 a	14.66 c	28.49 b	2271.35 b
18	2,4-D	Methomyl	475.93 a	22.71 c	28.75 b	3237.88 a
19	Bentazon	Methomyl	298.93 b	18.87 c	26.91 b	1536.78 b
20	Metsulfuron	Methomyl	521.17 a	20.89 c	31.48 a	3469.85 a
21	Pyroxsulam	Methomyl	501.50 a	17.36 c	28.52 b	2425.99 b
22	2,4-D	Azoxystrobin	544.77 a	21.69 c	32.11 a	3788.80 a
23	Bentazon	Azoxystrobin	493.63 a	20.80 c	31.97 a	3208.88 a
24	Metsulfuron	Azoxystrobin	548.70 a	15.10 c	27.60 b	2087.71 b
25	Pyroxsulam	Azoxystrobin	548.70 a	13.84 c	31.65 a	2377.67 b
26	2,4-D	Carbendazim	483.80 a	23.62 c	29.38 b	3358.70 a
27	Bentazon	Carbendazim	393.33 b	25.19 c	30.49 a	2960.30 a
28	Metsulfuron	Carbendazim	489.70 a	19.01 c	30.04 b	2677.29 b
29	Pyroxsulam	Carbendazim	570.33 a	18.71 c	30.97 a	3291.68 a
30	2,4-D	Propiconazole	472.00 a	25.48 c	33.55 a	3730.81 a
31	Bentazon	Propiconazole	418.90 b	16.85 c	27.77 b	2019.61 b
32	Metsulfuron	Propiconazole	633.27 a	10.91 c	28.69 b	1981.39 b
33	Pyroxsulam	Propiconazole	424.80 b	21.52 c	29.61 b	2677.29 b
CV(%)**			20.48	33.83	8.23	21.6

* Insecticides = chlorfluazuron, chlorpyrifos, deltamethrin e methomyl. Fungicides = azoxystrobin, carbendazim e propiconazole. (1) The averages followed by the same letter in the column do not differ by the Scott-Knott test ($p \leq 0,05$). ** CV = coeficiente of variation.

The highest number of grains per spike (NGS) was observed in plants treated with 2,4-D in isolation (approximately 45 grains/spike) (Table 4), followed by treatments with the isolated application of metsulfuron and bentazon and the combinations of bentazon + chlorpyrifos and metsulfuron + deltamethrin (approximately from 28 to 33 grains/spike). NGS of the other treatments, including control, was lower, with values ranging from 11 to 25.

The amplitude of values of thousand-grain weight (TGW) was lower than that observed in other yield components (Table 4). Plants sprayed with 2,4-D, metsulfuron, and pyroxsulam in isolation or with combinations of 2,4-D + deltamethrin, bentazon + chlorpyrifos, metsulfuron + chlorpyrifos, bentazon + chlorfluazuron, metsulfuron + methomyl, 2,4-D + azoxystrobin, bentazon + azoxystrobin, pyroxsulam + azoxystrobin, bentazon + carbendazim, pyroxsulam + carbendazim, and 2,4-D + propiconazole showed TGW similar to that found in control plants, with values ranging from 30 and 33 g (Table 4). For the other treatments, TGW ranged from 26 to 30 g.

The Scott-Knott test differentiated two groups regarding grain yield (Table 4). The isolated treatments of 2,4-D, bentazon, and pyroxsulam and the combinations of 2,4-D + deltamethrin, metsulfuron + deltamethrin, 2,4-D + methomyl, metsulfuron + methomyl, 2,4-D + chlorfluazuron, bentazon + chlorfluazuron, bentazon + azoxystrobin, 2,4-D + azoxystrobin, 2,4-D + propiconazole, 2,4-D + carbendazim, bentazon + carbendazim, and pyroxsulam + carbendazim stood out positively for the highest values of grain yield, being similar to that of the control, with values between 2,948 and 4,373 kg ha^{-1}. Grain yield in the other treatments ranged from 1,536 to 2,802 kg ha^{-1}. Also, all combinations of herbicides with the insecticide chlorpyrifos and most combinations with the fungicide propiconazole led to a reduction in grain yield when compared to the control.

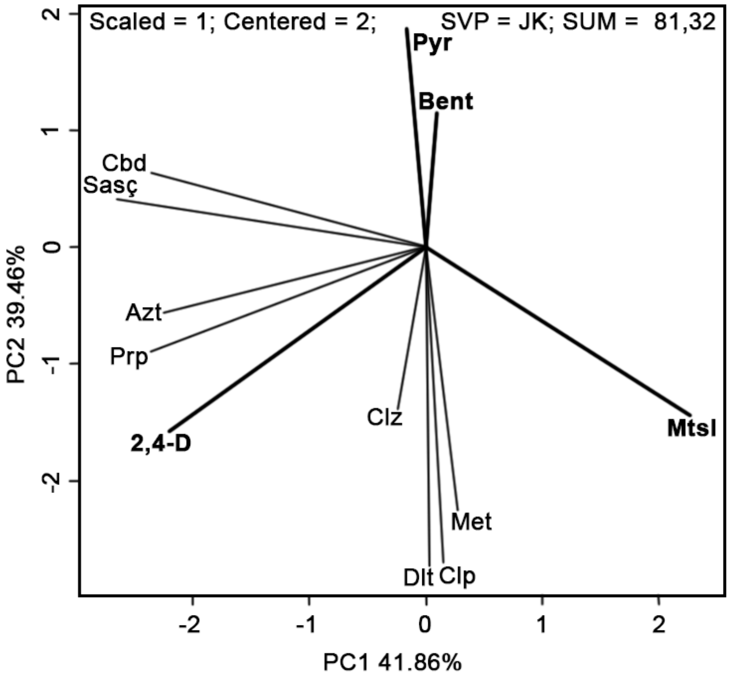
In general, the combinations previously mentioned with a predominance of low grain yields presented at least two yield components with lower values in comparison to treatments with higher grain yield (Table 4). Moreover, plants treated with metsulfuron in isolation showed a lower grain yield when compared to the control without application. This treatment provided the lowest NFT among all the treatments, which was probably determinant for the low grain yield.

The GGE Biplot analysis was complementary to the Scott-Knott test (Table 5), assisting in identifying the positive (antagonistic or additive) or negative (synergistic) combinations of pesticides on wheat grain yield. The GGE Biplot model showed that the herbicide 2,4-D presented a positive association (strong or weak) with all insecticides/ fungicides (angle<90º) (Figure 2). It is an indication that, among all the combinations, those involving the herbicide 2,4-D affected wheat grain yield in a lower intensity. These results are similar to the data shown in Table 4. The herbicides pyroxsulam and bentazon showed a weak association with the fungicide carbendazim (angle ≅ 90º), but negative with the other fungicides and insecticides (angle>90º). The herbicide metsulfuron showed a weak positive association with the insecticides methomyl, chlorpyrifos, deltamethrin, and chlorfluazuron, but negative with the fungicides propiconazole, azoxystrobin, and carbendazim, as well as for the isolated application. It suggests that the combination of metsulfuron with specific insecticides can increase grain yield, as shown in Table 3.

Table 5 **-** Summary of positive (+) and negative (-) effects among the association of four herbicides, with four insecticides and fungicides, obtained trhough Scott-Knott clustering means and GGE Biplot methods, for grain yield in the wheat plants of ORS Vintecinco cultivar

Insecticide/fungicide	Pyroxsulam		Metsulfuron		Bentazon		2,4-D	
	S-K(1)	GGE(2)	S-K	GGE	S-K	GGE	S-K	GGE
Without association	+(3)	+(4)	-	-	+	+	+	+
Chlorfluazuron	-	-	-	+	+	-	+	+
Chlorpyrifos	-	-	-	+	-	-	-	+
Deltamethrin	-	-	+	+	-	-	+	+
Methomyl	-	-	+	+	-	-	+	+
Azoxystrobin	-	-	-	-	+	-	+	+
Carbendazim	+	+	-	-	+	+	+	+
Propiconazole	-	-	-	-	-	-	+	+

(1) S-K = Scott-Knott; (2) GGE = GGE Biplot; (3) Considering the clustering generated by Scott Knott test “a = +” e “b = -”; (4) Considering positive associations with angle <90º, regardless of whether it is strong or weak.



Sasç: without association, Bent: bentazon, Mtsl: metsulfuron, Pyr: pyroxsulam, Clz: chlorfluazuron, Clp: chlorpyrifos, Dlt: deltamethrin, Met: methomyl, Azt: azoxystrobin, Prp: propiconazole e Cbd: carbendazim.

Figure 2 - Plot of the main component scores (PC) for the grain yield variable of the ORS25 wheat cultivar, according to the GGE Biplot model, regarding the association between four different herbicides and eight insecticides/fungicides.

The GGE Biplot analysis indicated a positive association between metsulfuron and chlorpyrifos for grain yield. Based on the mean yield of all combinations, it occurred because the combination metsulfuron + chlorpyrifos provided a slightly higher grain yield (Table 4). It was due to the compensation of NFT, which was higher when there was a combination with chlorpyrifos when compared to the isolated application of metsulfuron.

According to other studies, injuries at early stages of wheat development are not always related to a reduction in grain yield, since they were transitory in most treatments, i.e., plants presented high recovery due to the phenotypic plasticity of the crop (Provenzi et al., 2012; Fioreze and Rodrigues, 2014), being able to compensate the moderate injury observed at early development stages. Similar results were obtained using combinations of the herbicides fenoxaprop-p-ethyl, prosulfuron + bromoxynil, and fluroxypyr + MCPA with the fungicides pyraclostrobin + metconazole, trifloxystrobin + propiconazole, azoxystrobin + propiconazole or picoxystrobin (Nader et al., 2015) in wheat. However, dichlorprop/2,4-D + azoxystrobin/propiconazole reduced grain yield of wheat plants by 10% when compared to their isolated application (Robinson et al., 2013).

The high injury observed with the combination pyroxsulam + chlorpyrifos is in accordance with other studies carried out under field conditions. Reddy et al. (2010) also observed synergism in the combinations of these pesticides, reducing RT of wheat plants, but without compromising grain yield. Several factors may influence plant response to an herbicide or product combination, such as application stage, climate conditions, and even the differential tolerance presented by cultivars (Everitt and Keeling, 2009; Galon et al., 2010; Robinson et al., 2015).

In this study, treatment application was carried out at the initial stage of tillering, which coincided with the beginning of determination of the number of spikelets per spike and spikes per plant (Zadoks et al., 1974). The high injury (reduction of RT and SDM), mainly due to the combination pyroxsulam + chlorpyrifos at the beginning of crop development, compromised especially NGS, reducing productive crop potential. Also, minimum temperatures lower than 10 ^{o}C were registered in the first days after treatment application (Figure 1). Low temperatures can reduce plant metabolism and consequently its growth rate, directly increasing sensitivity to herbicides (Robinson et al., 2015).

Most of the treatments in which a reduction was observed in grain yield showed no high levels of visual injury. However, the injury can be manifested through biochemical and physiological changes, without influencing the morphological characteristics of the plant. In a sugarcane genotype sprayed with the herbicides ametryn (PSII inhibitor) and trifloxysulfuron-sodium (ALS inhibitor), no reduction was observed in shoot dry matter of plants, but there was a reduction of the photosynthetic rate and increased internal CO_2 concentration (Galon et al., 2010).

Negative effects provided by combinations between the herbicides pyroxsulam, metsulfuron, and bentazon, the insecticides chlorpyrifos and methomyl, and the fungicide propiconazole may be related to the main mechanism of tolerance of plants to these herbicides: herbicide detoxification by the action of cytochrome P450 enzymes (Yuan et al., 2007; Rojano-Delgado et al., 2015). It is the main enzymatic cluster responsible for detoxification of xenobiotics by plants. Insecticides belonging to the organophosphorus and carbamate groups and fungicides of the triazole group inhibit P450 enzymes (Werck-Reichhart et al., 2000; Sun et al., 2007; Chai et al., 2009; Rojano-Delgado et al., 2015). However, studies involving the use of triazole have been conducted only in vitro (Sun, 2007; Chai et al., 2009), without field studies, indicating the effect of the combination with herbicides. The inhibition process results in increased plant sensitivity to these herbicides, as observed in the present study. The differential response provided by chlorpyrifos, methomyl, and propiconazole may be related to the multiple forms of P450, as presented by Yun et al. (2001). Each of these pesticides may inhibit specific forms of P450, which do not necessarily act on the herbicides or act with a different inhibition level.

Physiological and biochemical mechanisms responsible for increasing injuries due to herbicides combined with the insecticides deltamethrin and chlorfluazuron and the fungicides azoxystrobin and carbendazim are unknown. However, physical and chemical interactions at the time of mixing them may have an influence, but it was not investigated in the present study.

Among the tested hypotheses, we confirmed that organophosphorus (chlorpyrifos) and carbamate (methomyl) insecticides might have increased the injury of the herbicides inhibiting ALS (pyroxsulam and metsulfuron) and PSII (bentazon) on wheat. However, although elevated, the injury provided by the combination metsulfuron + methomyl was not able to compromise crop grain yield. The hypothesis that strobilurin (azoxystrobin) would reduce herbicide injury was rejected in the present study, as RT and SDM of wheat plants were reduced in all combinations of herbicides with azoxystrobin. However, grain yield was only reduced when azoxystrobin was combined with the herbicides pyroxsulam and metsulfuron.

For weed control, the synergism of pesticide combinations may have advantages, but it is not feasible when it results in compromised crop yield. Therefore, combinations of the herbicides bentazon, metsulfuron, and pyroxsulam with most of the insecticides/fungicides evaluated in this study can cause losses, being safer their use in isolation.

Metsulfuron adversely affected grain yield of the cultivar ORS Vintecinco, even when applied alone. The combinations of this herbicide with methomyl and deltamethrin presented a greater safety since they did not compromise grain yield. Among the tested treatments, those using 2,4-D resulted in lower/no impact on the wheat crop, indicating that its combination with the evaluated insecticides/fungicides can be positive, mainly from an operational point of view and reduction of crushing losses.

The negative implications that combinations of the organophosphorus insecticide chlorpyrifos can cause on the wheat crop should be highlighted since it increased injuries and compromised grain yield, regardless of the combined herbicide. Propiconazole was the fungicides that most affected grain yield when associated with bentazon, metsulfuron, and pyroxsulam. In general, among the evaluated insecticides/fungicides, carbendazim led to a lower interference in grain yield, suggesting that its combination with 2,4-D, bentazon, metsulfuron, and pyroxsulam would imply lower risks in crop management.

REFERENCES

Agostinetto D, Rigoli RP, Schaedler CE, Tironi SP, Santos LS. Período crítico de competição de plantas daninhas com a cultura do trigo. Planta Daninha. 2008;26(2):271-8.

Ahrens WH, Panaram WR. Basis for thifensulfuron-insecticide synergism in soybeans (*Glycine max*) and corn (*Zea mays*). Weed Sci. 1997;6(4):648-53.

Baerg RJ, Barret M, Polge D. Insecticide and insecticide metabolite interactions with cytochrome P450 mediated activities in maize. Pest Biochem Physiol.1996;55(1):10-20.

Biediger DL, Baumann PA. Weaver DN. Chandle JM. Interactions between primisulfuron and selected soil applied insecticides in corn (*Zea mays*), Weed Technol. 1992;6(4):807-12.

Companhia Nacional de Abastecimento – Conab. Quarto levantamento, janeiro de 2016. [acesso em: 24 abr. 2017]. Disponível em: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_01_12_09_00_46_boletim_graos_janeiro_2016.pdf.

Sistema de Agrotóxicos Fitossanitários. – AGROFIT. [acesso em: 11 mar. 2018]. Disponível em: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons.

Campbell JR, Penner D. Enhanced phytotoxicity of bentazon with organophosphate and carbamate insecticides. Weed Sci. 1982;30(3):324-6.

Chai X, Zhang J, Hu H, Yu S, Sun Q, Dan Z, et al. Design, synthesis, and biological evaluation of novel triazole derivatives as inhibitors of cytochrome P450 14a-demethylase. Eur J Med Chem. 2009;44(5):1913-20.

Colby SR. Calculating synergistic and antagonistic responses of herbicide combinations. Weed Sci Soc Am. 1967;15(1):20-2.

Correa D, Nakai EH, Marco Junior J, Costa Junior AC. Eficiência de fungicidas no controle de doenças foliares do trigo no Paraná. Acta Iguazu. 2013;2(1):20-8.

Cromey MG, Lauren DR, Parkes RA, Sinclair KI, Shorter SC, Wallace AR. Control os Fusarium head blight of wheat with fungicides. Aust Plant Pathol. 2001;30(4):301-8.

Everitt JD, Keeling JW. Cotton growth and yield response to simulated 2, 4-D and dicamba drift. Weed Technol. 2009;23(4):503-6.

Ferreira EB, Cavalcanti PP, Nogueira DA. ExpDes: Experimental designs package R package version 1.1.1, 2011. [acesso em: 19 out. 2016]. Disponível em: https://cran.r-project.org/web/packages/ExpDes.pt/ExpDes.pt.pdf.

Fioreze SL, Rodrigues JD. Componentes produtivos do trigo afetados pela densidade de semeadura e aplicação de regulador vegetal. Semina: Cienc Agr. 2014;35(1):39-54.

Frans R, Talbert R, Marx D, Crowley H. Experimental design and techniques for measuring and analysing plant responses to weed control practices. In: Camper ND. Research methods in weed science. 3rd. ed. Champaign: Southern Weed Science Society; 1986. p.29-46.

Frutos E, Galindo MP, Leiva V. An interactive biplot implementation in r for modeling genotype-by environment interaction. Stoch Environ Res Risk Assess. 2014;28(7):1629-41.

Galon L, Castoldi CT, Forte CT, Kujawiski R, David FA, Perin GF, et al. Efficacy and phytotoxicity of herbicides applied for the handling of weeds that infest wheat. Rev Bras Herbic. 2015;14(2):128-40.

Galon L, Ferreira FA, Silva AA, Concenço G, Ferreira EA, Barbosa MHP, et al. Influência de herbicidas na atividade fotossintética de genótipos de cana-de-açúcar. Planta Daninha. 2010;28(3);591-7.

Gazziero DLP. Misturas de agrotóxicos em tanque nas propriedades agrícolas do Brasil. Planta Daninha. 2015;33(1):83-92.

Lancaster SH, Jordan DL, Spears JF, York AC, Wilcut JW, Monks DW, Sicklepod (*Senna obtusifolia*) Control and seed production after 2,4-DB applied alone and with fungicides or insecticides. Weed Technol. 2005;19(2):451-55.

Matsunaka S. Propanil hydrolysis: Inhibition in rice plants by insecticides. Science. 1968;160(1):1360-1.

McRae DH, Yih RY, Wilson HF. A biochemical mechanism for the selective action of anilides, Weed Sci Soc Am. 1964;1(1):87.

Nader S, Shropshire C, Sikkema PH. Responses of winter wheat to herbicide-fungicide tankmixes. Agric Sci. 2015;6(11):1352-56.

Oerke EC. Crop losses to pests. J Agric Sci. 2006;144(1):31-43.

Pagnoncelli Jr FDB, Vidal RA, Trezzi MM, Machado A, Gallon M, Xavier E, et al. Tolerance of common bean plants to ethoxysulfuron herbicide and the mechanism involved in the process. Planta Daninha. 2016;34(3):535-43.

Paula JM, Agostinetto D, Schaedler CE, Vargas L, Silva DRO. Competição de trigo com azevém em função de épocas de aplicação de nitrogênio. Planta Daninha. 2011;29(3):557-62.

Pereira PRVS, Marsaro Júnior AL, Lau D, Panizzi AR, Salvador JR. Manejo de insetos-praga. In: Borém A, Scheeren PL. Trigo: do plantio à colheita. Viçosa, MG: Empresa Brasileira de Pesquisa Agropecuária; 2015. p.185-202.

Provenzi FD, Bergamo R, Debastiani W, Balbinot Junior AA. Arranjo espacial de plantas em duas cultivares de trigo. Unoesc Cienc – ACET. 2012;3(1):31-6.

Reddy SS, Stahlman PW, Geier PW. Pyroxsulam and Chlorpyrifos applied the same day injures wheat. Crop Manag. 2010;11(1):31-6.

Rigoli RP, Agostinetto D, Schaedler CE, Dal Magro T, Tironi S. Habilidade competitiva relativa do trigo (*Triticum aestivum*) em convivência com azevém (*Lolium multiflorum*) ou nabo (*Raphanus raphanistrum*). Planta Daninha. 2008;26(1):93-100.

Robinson MA, Cowbrough MJ, Sikkema PH, Tardif FJ. Winter wheat (*Triticum aestivum* L.) tolerance to mixtures of herbicides and fungicides applied at different timings. Can J Plant Sci. 2013;93(3):491-01.

Robinson MA, Letarte J, Cowbrough MJ, Sikkema PH, Tardif FJ. Winter wheat (*Triticum aestivum* L.) response to herbicides as affected by application timing and temperature. Can J Plant Sci. 2015;95:325-33.

Rojano-Delgado AM, Priego-Capote F, Castro MDL, Prado R. Mechanism of imazamox resistance of the Clearfield® wheat cultivar for better weed control. Agron Sust Develop. 2015;*35*(2):639-48.

Silva AA, Freitas FM, Ferreira LR, Jakelaitis A. efeitos de mistura de herbicida com inseticida sobre a cultura do milho, as plantas daninhas e a lagarta-do-cartucho. Planta Daninha. 2005;23(3):517-25.

Sun QY, Xu J-M, CaoY-B, Zhang W-N, Wu Q-Y, Zhang D-Z, et al. Synthesis of novel triazole derivatives as inhibitors of cytochrome P450 14a-demethylase (CYP51). Eur J Med Chem. 2007;42(9):1226-33.

Vargas L, Roman ES. Seletividade de herbicidas em cereais de inverno. Rev Bras Herb. 2005;4(3):1-10.

Werck-Reichhart D, Hehn A, Didierjean L. Cytochromes P450 for engineering herbicide tolerance. Trends Plant Sci. 2000;5(3):116-23.

Yan W, Hunt LA, Sheng Q, Szlavnics Z. Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Sci. 2000;40(3):597-605.

Yuan JS, Tranel PJ, Stewart CN. Non-target-site herbicide resistance: a family business. Trends Plant Sci. 2007;12(1):6-13.

Yun MS, Shim LS, Usui K. Involvement of cytochrome P-450 enzyme activity in the selectivity and safening action of pyrazosulfuron-ethyl. Pest Manage Sci. 2001;(57):283-8.

Zadoks JC, Chang TT, Konzak CF. A decimal code for the growth satges of cereals. Weed Res. 1974;14:415-21.