doi: 10.1590/S0100-83582016340300012

Change in Physiological Features in Ryegrass Biotypes in Competition with Soybean Due Resistance to Glyphosate[1]

Alterações de Caracteristicas Fisiológicas em Biótipos de Azevém em Competição com Soja em Função da Resistência ao Glyphosate

AGOSTINETTO, D.[2], OLIVEIRA, C.[2], LANGARO, A.C.[2], NOHATTO, M.A.[2], and MANICA-BERTO, R.[2]

ABSTRACT - Herbicide resistance can cause biochemical, physiological, morphological and phenological changes in weeds, altering their competitive ability against crops. The aim of the study was to assess physiological changes and cellular damage in susceptible and resistant biotypes of ryegrass and soybeans under competition. Three experiments were conducted in a greenhouse using a completely randomized design with four replications performed in a replacement series. The ratios of soybean and to susceptible (experiment 1) and resistant ryegrass (experiment 2) and of susceptible to resistant (experiment 3) ryegrass were 100:0, 75:25, 50:50, 25:75 and 0:100. Forty days after the emergence of the soybean crop and 60 days after the emergence of the ryegrass plants, the leaves of the competing plants were collected. The following variables were assessed: the levels of carotenoids, chlorophylls and total phenolic compounds; amount of hydrogen peroxide; degrees of lipid peroxidation and electrolyte leakage; and activity of enzymes catalase, superoxide dismutase and ascorbate peroxidase. The production of phenols and increased oxidative damage due to interspecies competition with ryegrass was, in general, observed in the soybeans; the ryegrass biotypes, susceptible and resistant to glyphosate, coexisting with soybean have generally higher oxidative damage due to intraspecific competition; since when these biotypes coexist not present modifications of these parameters.

Keywords: oxidative stress, antioxidant enzymes, adaptability of herbicide resistant plants.

RESUMO - A resistência aos herbicidas pode causar mudanças bioquímicas,fisiológicas, morfológicas ou fenológicas nas plantas daninhas alterando a capacidade competitiva com as culturas.O objetivo do estudo foi verificar alterações na fisiologia e os danos celulares em biótipos de azevém resistente ou suscetível e na soja,sob competição. Conduziram-se em casa de vegetação três experimentos em delineamento completamente casualizado, com quatro repetições, sendo esses realizados em série de substituição. As proporções entre plantas de soja e azevém suscetível (experimento 1) e resistente (experimento 2); além de azevém suscetível e resistente (experimento 3) foram de 100:0, 75:25, 50:50, 25:75 e 0:100, respectivamente. Aos 40 dias após a emergência (DAE) da cultura da soja e aos 60 DAE das plantas de azevém, realizou-se a coleta de folhas da cultura e das plantas daninhas. As variáveis avaliadas foram teores de clorofilas e carotenoides, compostos fenólicos totais, teor de peróxido de hidrogênio, peroxidação lipídica, extravasamento de eletrólitos, além da atividade das enzimas catalase, ascorbatoperoxidase e superóxido dismutase. Observou-se que em plantas de soja a produção de fenóis e os danos oxidativos aumentaram em decorrência da competição interespecífica com azevém; os biótipos de azevém, suscetível e resistente ao herbicida glyphosate, convivendo com a cultura da soja, apresentam em geral, maior dano oxidativo em decorrência da competição intraespecífica; já quando os biótipos competem entre si não apresentam modificações destes parâmetros.

Palavras-chave: estresse oxidativo, enzimas antioxidantes, adaptabilidade de plantas.

[1] Recebido para publicação em 2.12.2015 e aprovado em 15.3.2016.

[2] Universidade Federal de Pelotas, Pelotas-RS, Brasil, <agostinetto.d@gmail.com>.

INTRODUCTION

Understanding the effects that resistance will have on the competitive ability of plants is important for predicting the dynamic evolution of resistance to herbicides and for devising strategies against the selection of resistant plants (Walsh and Powles, 2007). The competition between culture and weeds is very common in agricultural systems, resulting in reduced productivity and quality of the final product (Barroso et al., 2010); however, the effects vary and depend on the intensity of the competition.

Plant metabolism plays an important role in the interaction of biotic, like competition (Ormeño et al., 2007), abiotic, as drought (Ramakrishna and Ravishankar, 2011) and xenobiotic, as herbicide application (Song et al., 2007) factors in the competition between plants (Ormeño et al., 2007). Among responses that plants have against these stresses include increased membrane peroxidation, which affects the structure and function of the photosynthetic apparatus, inactivating the reaction centers of the photosystems (Tripathy et al., 2007).

Competition can cause irreversible damage to cell membranes, similar to herbicide application (Xiao et al., 2008), via the production of reactive oxygen species (ROS), such as O_2^- (superoxide), H_2O_2 (hydrogen peroxide) and $^{\bullet}OH$ (hydroxyl radicals). These ROS can interact with and damage the structure and organization of cell membranes, altering the function of enzymes and membrane-bound receptors (Suzuki et al., 2012). Such damage can be detected by quantifying the amount of hydrogen peroxide and lipid peroxidation and evaluating the relative permeability of the membranes, determined by measuring electrolyte leakage.

To avoid such damage, plants have enzymatic and non-enzymatic antioxidant defense systems that allow the elimination of ROS and protect the cells from oxidative damage. The enzymatic defense system of plants includes several antioxidant enzymes in different cellular compartments. Among the main defense mechanisms are superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11) and catalase (CAT; EC 1.11.1.6). The degree of oxidative cell stress is determined by the amounts of O_2^-, H_2O_2 and $^{\bullet}OH$. Therefore, the balance of the activities of SOD, APX and CAT is crucial to suppress toxic levels of ROS in the cell (Karuppanapandian et al., 2011).

The aim of the study was to investigate the changes in the secondary metabolic compounds and the cellular damage in ryegrass biotypes (resistant and susceptible) and soybeans under competition.

MATERIALS AND METHODS

The experiments were conducted in the greenhouse and laboratory at the Faculdade de Agronomia Eliseu Maciel (FAEM) of the Universidade Federal de (UFPel), Municipality of Capão do Leão – RS/BR in 2011 and 2012. We used seeds of soybean cultivar CD226RR. Susceptible ryegrass seeds came from plants in an area where glyphosate had never been used (30°58'54"S and 54°40'39"W); the biotype of the ryegrass resistant to glyphosate was obtained from the City of Tuparendi-RS (27°45'26"S and 54°34'27"W).

To determine the metabolic variations between the coexisting biotypes of the susceptible and resistant ryegrass, as well as those of soybean, three experiments were conducted in a completely randomized design with four replications. The species were sown in pots with a capacity of 8 liters and diameter of 23 cm, which were filled with soil.

The first two experiments were conducted in a replacement series soybean biotypes and susceptible or resistant ryegrass were installed with a population of 24 plants per pot (578 plants m^{-2}), which was determined in a previous experiment in monoculture (data not shown). The soybean planting was carried out 20 days after the sprouting of the ryegrass, to simulate the escapes or regrowth of plants that occurs in crops sown after pre-drying of the previous crop. The third experiment was conducted in a replacement series, with biotypes of ryegrass and a population of 36 plants per pot (866 plants m^{-2}).

The ratios of soybean to susceptible (experiment 1) and resistant ryegrass (experiment 2) and of susceptible to resistant

ryegrass (experiment 3) were 100:0, 75:25, 50:50, 25:75 and 0:100. Forty days after the emergence (DAE) of soybeans and 60 DAE of ryegrass, leaves of the plants sowed separately or in competition were collected, and a composite sample was produced from all plants in the plot; the sample was stored at -80 ºC until the time of measurement of the variables (De Vos et al., 2007).

The chlorophyll and carotenoid contents were determined in samples with 0.1 g and calculated by the formula of Lichtenthaler (1987), expressing the results in mg g^{-1} fresh weight (FW). The determination of the total phenolic compounds was performed according to the method described by Singleton and Rossi (1965), using 1 g of plant material.

Cellular damage to tissues was determined by the content of hydrogen peroxide (H_2O_2), as described by Sergiev et al. (1997), and of thiobarbituric acid reactive substances (TBARS) via accumulation of malondialdehyde (MDA), as described by Heath and Packer (1968). The cell damage was also assessed by the relative membrane permeability, determined by means of electrolyte leakage, as described by Tarhanen et al. (1999).

To determine the activity of the antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), first step is quantification of the protein samples was made following the method of Bradford (1976). The APX and CAT activities were determined according to (Azevedo et al., 1998), and the methodology for determining SOD was adapted from Giannopolitis and Ries (1977), for these analyzes was used in the sample extraction of 0.2 g of plant material.

The data were analyzed for normality by the Shapiro-Wilk test and for homoscedasticity by the Hartley test; then, they were subjected to analysis of variance ($p \leq 0.05$). The effects of proportion, compared to monoculture (control) were assessed by using Dunnett test ($p \leq 0,05$) and the test between the proportions in the mixture by the Duncan test ($p \leq 0,05$); the tests were conducted separately for each competitor. The presence of correlations between the dependent variables of the study was analyzed using the Pearson correlation coefficient ($p \leq 0,05$).

RESULTS AND DISCUSSION

The levels of variable chlorophyll (Chl *a*), chlorophyll (Chl *b*) and chlorophyll total (Chl *a*+*b*) in soybeans in competition with the susceptible biotype were reduced compared to the control (Table 1). For susceptible ryegrass intercropped with soybean, none of the proportions of the tested plants differed from the control. There were no differences between the proportions of soybeans, while susceptible ryegrass at a ratio of 75:25 showed more photosynthetic pigments, demonstrating that in this case, competition among species favored susceptible ryegrass.

Soybeans living with the ryegrass biotype susceptible to variable carotenoids (CAR) showed no statistical significance for the ratios of plants in relation to the control or among the proportions (Table 1). For the susceptible ryegrass biotype, no significant differences in the proportions relative to their monoculture were verified. When analyzing the differences between proportions, plants grown at a ratio of 75:25 showed higher concentrations of CAR than the others, as was observed for the other pigments analyzed.

When coexisting with the largest proportions of the resistant biotype, soybean showed reduced contents of Chl *a,* Chl *b*, and Chl *a* + *b* and CAR in relation to the monoculture (Table 1). Comparing the effect of the proportions of plants, those grown at a ratio of 75:25 showed higher levels of photosynthetic pigments than the others; that is, competition with resistant ryegrass harms soybean. For ryegrass, the proportions of plants did not show statistical significance for the levels of Chl *a* and Chl *b* compared with the control. However, the content Chl *a*+*b* and that of CAR were higher in the 50:50 and 75:25 proportions in comparison with the monoculture. Furthermore, it was found that for the 25:75 ratio, the contents of all pigments decreased, demonstrating that competition among species favored resistant ryegrass, similar to the finding for the susceptible biotypes. Overall, there were reductions in soybean chlorophyll and carotenoids when in competition with susceptible and resistant ryegrass, as well as in the susceptible or

Table 1 - Contents of chlorophyll *a* (Chl *a*), *b* (Chl *b*), total (Chl *a* + *b*) and carotenoids (CAR) (mg^{-1} g) extracted from the leaves of soybean and ryegrass susceptible or resistant to glyphosate for a four proportions replacement series

Proportion	Chl *a*	Chl *b*	Chl *a* + *b*	CAR
	(mg g^{-1} FM[1/])			
	Soybean- experiment I (soybean: susceptible ryegrass)			
100: 0	1.45	0.65	2.12	0.39 NS
75: 25	0.80 * NS	0.39 * NS	1.18 * NS	0.30 NS
50: 50	0.85 *	0.33 *	1.18 *	0.25
25: 75	0.65 *	0.26 *	0.94 *	0.24
CV (%) Dunnet/Duncan	10.1/6.2	29.0/12.5	9.2/7.7	15.3/7.6
	Susceptible ryegrass-Experiment I (soybean: susceptible ryegrass)			
0: 100	0.37 NS	0.26 NS	0.68 NS	0.07 NS
25: 75	0.26 b	0.25 b	0.50 b	0.04 b
50: 50	0.34 b	0.22 b	0.56 b	0.06 b
75: 25	0.50 a	0.34 a	0.83 a	0.12 a
CV (%) Dunnet/Duncan	18.5/8.1	18.1/41.2	6.9/10.6	20.8/15.9
	Soybean- experiment II (soybean: resistant ryegrass)			
100: 0	0.80	0.39	1.18	0.30
75: 25	0.76 ns a	0.25 ns a	1.01 * a	0.31 ns a
50: 50	0.39 * b	0.06 * b	0.41 * b	0.16 * b
25: 75	0.38 * b	0.17 * b	0.55 * b	0.15 * b
CV (%) Dunnet/Duncan	11.1/11.0	12.9/12.9	10.5/11.2	15.9/16.7
	Resistant ryegrass- experiment II (soybean: resistant ryegrass)			
0: 100	0.73 NS	0.40 NS	1.13	0.14
25: 75	0.59 b	0.36 b	0.95 ns b	0.15 ns b
50: 50	0.86 a	0.56 a	1.42 * a	0.22 * a
75: 25	0.96 a	0.63 a	1.26 * a	0.19 * a
CV (%) Dunnet/Duncan	26.1/12.1	32.24/25.82	18.14/17.21	14.2/14.77
	Susceptible ryegrass - experiment III (susceptible ryegrass: resistant ryegrass)			
100: 0	0.86	0.35	1.21	0.25
75: 25	1.10 ns a	0.50 ns a	1.60 ns a	0.31 ns a
50: 50	0.96 ns a	0.42 ns a	1.38 ns a	0.29 ns a
25: 75	0.42 * b	0.18 * b	0.60 * b	0.12 * b
CV (%) Dunnet/Duncan	22.4/22.2	16.9/16.93	20.2/19.8	259/26.6
	Resistant ryegrass - experiment III (susceptible ryegrass: resistant ryegrass)			
0: 100	0.44	0.18	0.62	0.14
25: 75	0.43 ns b	0.19 ns b	0.62 ns b	0.16 ns b
50: 50	0.79 * a	0.34 * a	1.13 * a	0.23 * a
75: 25	1.00 * a	0.40 * a	1.40 * a	0.32 * a
CV (%) Dunnet/Duncan	19.5/19.5	21.5/21.0	19.2/19.1	17.2/17.3

[1/] FM = fresh matter; * or ns means (n=4) differs or not from the control (ratio 0: 100 or 100: 0) in the column by the Dunnett test ($p \leq 0.05$). Means followed by the same letter in the column in the presence of competitors do not differ for the Duncan method ($p \leq 0.05$). NS means that the F test results are not significantly different ($p \leq 0.05$).

resistant biotypes when there was an increase in the ryegrass ratio (Table 1).

For the coexistence of ryegrass biotypes, decreases in chlorophylls and carotenoids occurred in the susceptible plants when they were grown at a ratio of 25:75 compared with the control. The plants of the resistant biotype grown at ratios of 50:50 and 75:25 showed greater amounts of photosynthetic pigments than the control (Table 1). When analyzing the effect of proportion, it was observed that both the susceptible and resistant biotypes grown at a ratio of 25:75 contained less photosynthetic pigments than those grown at the other proportions. These results show that both the susceptible and resistant biotypes coexist better with susceptible ryegrass plants. The differences in pigment most likely occurred because even biotypes belonging to the same species (*L. multiflorum*) may present morphological differences in plant height and the width of leaves. These differences most likely arose because of the different environmental conditions in the different collection locations for the biotypes.

The chlorophyll and carotenoid pigments are connected to photosynthetic efficiency and, therefore, to plant growth and adaptation to different environments (Foyer and Noctor, 2009). Thus, the decline of these compounds may compromise photosynthetic activity, hindering the development of the plants.

The content of the total phenolics in soybean, when competing with the susceptible ryegrass biotype, was not statistically significant for the different proportions of the plants compared to the control or between the proportions of competitors (Table 2). For soybeans competing with the resistant biotype, there was an increase in total phenols when ryegrass was in equal or greater proportion in the mixture. Therefore, the increase of the population of resistant ryegrass resulted in an increase of phenols in the culture, or the interspecific competition with resistant ryegrass altered the metabolism of soybean.

For the ryegrass biotypes in competition with the soybean, the susceptible biotype showed lower concentrations of phenols when grown at a ratio of 75:25, relative to the control. This ratio also resulted in decreased levels of phenols compared to the other ratios when analyzing the effect of interspecific competition (Table 2). The resistant biotype, in turn, had a reduction of total phenols in all proportions compared to the control, while in the comparison between proportions, the 25:75 ratio led to greater amounts of phenols in relation to the other cases. In general, a decrease of the total phenols was observed in both biotypes of ryegrass when the number of soybean plants in the proportions was increased.

When ryegrass biotypes coexisted, there was no significance of the proportions compared to the monoculture or between the proportions of the plants of the competitors (Table 2), demonstrating that there are no changes in the concentration of total phenolics in ryegrass biotypes when these compete with soybean.

Phenols are products of the secondary metabolism of plants, which acts to combat free radicals (Goufo et al., 2014); they are a mechanism of chemical defense against insects, weeds and plant pathogens. The results of studies show that when soybeans are combined with high proportions of the resistant biotype or are subject to interspecific competition, they activate these defense mechanisms. For ryegrass biotypes (susceptible and resistant), the higher proportions of plants caused an increase in total phenols under the effect of intraspecific competition. Thus, both soybean and ryegrass show increased defenses when coexisting with neighboring ryegrass plants.

Soybean coexisting with the susceptible ryegrass showed increased levels of H_2O_2 and TBARS for the plant ratio of 25:75 compared to the control and compared to other ratios (Table 2). The susceptible ryegrass showed no significant differences for the different proportions of plants compared to the control, with the exception of the ratio of 25:75, for which an increase in the variables was observed.

When the coexistence of soybean and resistant ryegrass was assessed, it was found that the lower proportions of soybeans (50:50 to 25:75) had a higher concentration of H_2O_2 and TBARS than the monoculture (Table 2),

Table 2 - Levels of total phenols (mg GAE g^{-1} FW), hydrogen peroxide (H_2O_2) (mM g^{-1}) and thiobarbituric acid reactive substances (TBARS) (nM MDA g^{-1} FW) extracted from leaves of soybean cultivar CD226RR and ryegrass susceptible or resistant to glyphosate according to four sets of replacement series

Proportion	Total phenols (mg GAE[1/] g^{-1} FM[2/])	H_2O_2 (mM g^{-1})	TBARS (nMMDA[3/] g^{-1}MF)
	Experiment I (soybean: susceptible ryegrass)		
	Soybean		
100: 0	32.47 NS	1.1	23.5
75: 25	33.51 NS	1.0 ns b	24.4 ns b
50: 50	32.64	1.1 ns b	24.6 ns b
25: 75	34.47	1.5 * a	26.4 * a
CV (%) Dunnet/Duncan	9.3/10.4	8.7/7.1	6.6/7.3
	Susceptible ryegrass		
0: 100	24.93	1.0 NS	18.5 NS
25: 75	23.22 ns a	0.9 a	17.0 a
50: 50	21.37 ns a	0.7 b	14.0 b
75: 25	17.09 * b	0.7 b	15.0 b
CV (%) Dunnet/Duncan	10.3/7.2	17.48/18.8	23.5/14.6
	Experiment II (soybean: resistant ryegrass)		
	Soybean		
100: 0	32.47	1.0	30.8
75: 25	32.46 ns b	1.0 ns b	31.2 ns b
50: 50	40.37 * a	1.2 * a	35.8 * a
25: 75	38.53 * a	1.5 * a	38.4 * a
CV (%) Dunnet/Duncan	7.0/7.7	10.1/11.4	5.7/6.8
	Experiment II (soybean: resistant ryegrass)		
0: 100	20.00	0.9	24.0
25: 75	14.86 * a	0.6 * a	18.9 ns a
50: 50	11.93 * b	0.6 * a	17.0 ns a
75: 25	9.92 * b	0.2 * b	9.3 * b
CV (%) Dunnet/ Duncan	14.1/8.7	7.6/13.8	11.8/7.8

[1/] GAE = gallic acid. [2/] FM = fresh matter. [3/] MDA= malondialdehyde.* or ns * or ns means (n=4) differs or not from the control (ratio 0: 100 or 100: 0) using the Dunnett test ($p \leq 0.05$). Mean followed by the same letter in the column in the presence of competitors do not differ by the Duncan test ($p \leq 0.05$). NS means no significant difference in the results of the F test.

and both variables were reduced at a ratio of 75:25. The resistant ryegrass showed reductions in the levels of H_2O_2 when planted together with soybean plants. For the TBARS variable, only the 75:25 case differed from the control. When comparing the proportions, the 75:25 condition had lower levels of H_2O_2 and TBARS than the others.

In general, it appears that inter- and intraspecific competition results in increases in H_2O_2 and TBARS in both the culture and the weed. This result may indicate that in these situations, a state of stress is induced that is associated with damaged membranes, leading to reductions in the levels of photosynthetic pigments (Table 1). H_2O_2 and other ROS are strong oxidants that can initiate oxidative damage, which leads to disturbances in metabolic functions and to the loss of cellular integrity, which are cumulative (Karuppanapandian et al. 2011; Petrov and

Breusegem, 2012). In cells or tissues where the H_2O_2 concentration is relatively low, an antioxidant response (Foyer and Shigeoka, 2011) characterized by the production of phenols or the activity of antioxidant enzymes is observed.

Competition between susceptible and resistant ryegrass biotypes did not result in significant concentrations of H_2O_2 and TBARS for the mixed plants compared with the control or in the combinations of plants of both biotypes, which demonstrates that competition may not have been the cause of oxidative damage in the ryegrass biotypes under coexistence.

The variable leakage of electrolytes for soybeans and susceptible and resistant ryegrass biotypes showed no significance in terms of competition. Thus, it appears that either the oxidative stress caused by competition caused no degradation of cell membranes due to the efficiency of the antioxidant system of the plants (both non-enzymatic and enzymatic), which acted effectively to prevent cell damage, or that the method used in the study to quantify the variable was not sufficiently sensitive to detect cell extravasation.

The levels of ROS are under enzymatic and non-enzymatic control, and the increase in certain intermediates, such as H_2O_2, may result in increases in enzyme activity (such as CAT, SOD and APX), or metabolites, such as ascorbate. It was found that, in soybean in competition with the sensitive ryegrass biotype, there was a reduction in CAT activity for ratios of 50:50 and 25:75 relative to the monoculture, while the activity of APX was lower in these associations relative to the monoculture (Table 3). Comparing proportions with the presence of competitors, CAT activity was higher for the 75:25 ratio than the others, while for the APX enzyme, there was no significant difference between the proportions.

The decreased CAT activity in situations of stress due to interspecies competition was most likely due to an increase of H_2O_2 because CAT can be inactivated by binding with H_2O_2 (Barbosa et al., 2014). High stress can also inhibit the synthesis of the enzyme or lead to changes in the assembly of its subunits, which may explain the decline in the activity of the enzymes tested in both species when subjected to competitive stress. It is believed that the protection of plants against the accumulation of H_2O_2 results from the joint action of the CATs and the APXs. The affinity of different APXs and CATs for H_2O_2 suggests that APX is responsible for the fine modulation signal of the reactive species, while catalase removes excess H_2O_2 during stress (Gill and Tuteja, 2010; Sharma et al., 2012.

Increases of SOD activity were observed in various proportions among the soybean plants coexisting with ryegrass compared to the control plants (Table 3). When the effect of the ratio was analyzed, it was found that for the soybean coexisting with the susceptible biotype, the ratio of 75:25 resulted in lower enzyme activity than the other SOD; however, when coexisting with the resistant ryegrass, there was no significant difference.

For the susceptible ryegrass competing with soybeans, there was no significant difference in the activity of the SOD between the proportions tested and the control or between the proportions (Table 3). For resistant ryegrass, SOD activity decreased in all proportions compared with the control. However, in interspecific competition, the ratio of 75:25 resulted in a lower activity of this enzyme. Thus it is observed that the resistant biotype appears to activate more efficiently against the stress caused by intraspecific competition when compared to susceptible biotype, however it should be noted that this response cannot be linked to the fact that resistance to glyphosate but the environment that these biotypes have evolved.

For the soybean plants coexisting with resistant ryegrass, the SOD behavior was reversed compared to that found for i.e. SOD; CAT and APX behavior increased when soybean and the resistant biotype were in proportions that contained more ryegrass (Table 3). The highest activity of this enzyme in these circumstances is credited to the great stress that these plants were exposed to in the corresponding treatments. As a result of the stress, the species activated their enzymatic defense systems, increasing the

Table 3 **-** Activity of the enzymes catalase (CAT) (UA mg^{-1} prot min^{-1}), ascorbate peroxidase (APX) and superoxide dismutases (SOD) extracted from leaves of soybean cultivar CD226RR and ryegrass susceptible or resistant to glyphosate ratios according to four sets of replacement series

Proportion	CAT	APX	SOD
	(UA[1/] mg^{-1} prot. min^{-1})		
	Experiment I (soybean: susceptible ryegrass)		
Soybean			
100: 0	0.48	2.90	7.71
75: 25	0.40 ns a	2.21 * NS	11.59 * b
50: 50	0.26 * b	2.15 *	13.77 * a
25: 75	0.23 * b	1.18 *	16.45 * a
CV (%) Dunnet/Duncan	18.7/24.5	12.4/12.8	26.2/25.4
Susceptible ryegrass			
0: 100	0.56 NS	5.96	19.48 NS
25: 75	0.72 b	7.44 * b	20.73 NS
50: 50	0.62 b	7.32 * b	19.20
75: 25	0.96 a	15.50 * a	19.8
CV (%) Dunnet/Duncan	14.6/22.9	28.9/29.9	24.7/22.9
Experiment II (soybean: resistant ryegrass)			
Soybean			
100: 0	0.48	2.90	7.17
75: 25	0.38 ns NS	2.86 ns a	10.46 * NS
50: 50	0.36 *	2.66 ns a	10.23 *
25: 75	0.33 *	1.84 * b	11.28 *
CV (%) Dunnet/Duncan	14.5/26.9	13.9/15.6	18.6/18.8
Resistant ryegrass			
0: 100	0.30 NS	6.62	39.80
25: 75	0.25 b	6.80 ns b	30.73 * a
50: 50	0.27 b	8.16 ns b	29.00 * a
75: 25	0.40 a	15.84 * a	19.51 * b
CV (%) Dunnet/Duncan	25.1/8.5	27.8/29.6	22.6/14.3

[1/] UA = active unit. * or ns means (n=4) differs from control (ratio 100: 0 and 100: 0) in the column by Dunnett test ($p \leq 0.05$). Means followed by the same letter in the column in the presence of competitors do not differ by the Duncan test ($p \leq 0.05$). NS averages do not differ in the column by the F test.

activity of SOD, which is the first enzyme in the detoxification process; however, other enzymes analyzed were not affected.

Studies performed using leaf discs of maize have shown correlations between the tolerance of plants to oxidative stress and the activity of SOD, as conducted with rice (Damanik et al., 2012); cucumber (Naliwajski and Sklodowska, 2014) and wheat (Rajabi et al., 2012).

In neither of two biotypes did the competition between the ryegrass glyphosate susceptible and resistant biotypes modify the activity of the antioxidant enzymes studied. These results partly corroborate those found for the contents of phenols, H_2O_2 and TBARS, showing no disturbance in oxidative damage when competing with the ryegrass plant.

Based on the Pearson correlation in this study for soybeans competing with susceptible

ryegrass, there were positive correlations between the activities of APX and CAT enzymes and total chlorophyll content (r =0.98; r = 0.84, respectively) and carotenoids (r = 0.96; r = 0.95, respectively). There was a negative correlation with the SOD enzyme activity with the variables total chlorophyll (r = -0.92) and carotenoids (r = -0.96). For soybeans coexisting with resistant ryegrass, negative correlations between total phenolics and total chlorophyll variables (r = -0.98) and carotenoids (r = -0.97) were observed. Additionally, negative correlations between TBARS and chlorophyll a (r = -0.96) and carotenoids (r = -0.95) were found. These results emphasize that the different secondary metabolites produced in situations of stress are directly linked to each other and have close relationships in reducing the levels of photosynthetic pigments in the system.

Based on the results of this research, it can be inferred that competition stimulates the secondary metabolism of soybean plants through the synthesis of antioxidant molecules and that stress due to competition is comparable to the stresses induced by temperature, pathogens, insects, ultraviolet radiation, drought and nutrient deficiency (Kumar et al., 2012; Suzuki et al., 2012; Mandal et al., 2013).

In conclusion the interspecific competition with ryegrass, soybeans produce phenols and increased oxidative damage due to interspecies competition with ryegrass was, in general, observed in the soybeans; the ryegrass biotypes, susceptible and resistant to glyphosate, coexisting with soybean have generally higher oxidative damage due to intraspecific competition; since when these biotypes coexist not present modifications of these parameters.

ACKNOWLEDGEMENTS

FAPERGS and CNPq for financial support through the edictal REPENSA (22/2010) and FAPERGS and CAPES for grant scholarship DOCFIX.

REFERENCES

Azevedo R.A. et al. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. **Physiol Plant.** 1998;104:280-92.

Barbosa M.R. et al. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Cienc Rural**. 2014;44:453-60.

Barbosa M.R. et al. Interferência entre espécies de planta daninha e duas cultivares de feijoeiro em duas épocas de semeadura. **Bragantia.** 2010;69:609-16.

Bradford M.A. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding. **Anal Biochem.** 1976;72:248-54.

De Vos R.C.H. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. **Nat Protoc.** 2007;2:778-91.

Damanik R.I. et al. Response of antioxidant systems in oxygen deprived suspension cultures of rice (*Oryza sativa* L.). **Plant Growth Regul.** 2012;67:83-92.

Ferreira E.A. et al. Potencial competitivo de biótipos de azevém (*Lolium multiflorum*). **Planta Daninha**. 2008;26:261-9.

Foyer C.H., Noctor G. Redox regulation in photosynthetic organisms: signaling, acclimation and practical implications. **Antioxid Redox Signal**. 2009;11:861-905.

Foyer C.H., Shigeoka S. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. **Plant Physiol.** 2011;155:93-100.

Giannopolitis C.N., Ries S.K. Superoxide dismutase. **Plant Physiol**. 1977;59:309-14.

Gill S.S., Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiol Biochem.** 2010;48:909-30.

Goufo P. et al. Rice (*Oryza sativa* L.) phenolic compounds under elevated carbon dioxide (CO_2) concentration. **Environ Exp Bot.** 2014;99:28-37.

Health R.L., Packer L. Photoperoxidation in isolated chloroplasts. **Arch Biochem Biophys.** 1968;125:189-98.

Karuppanapandian T. et al. Reactive oxygen species in plants: Their generation, signal transduction, and scavenging mechanisms. **Austr J Crop Sci.** 2011;5:709-25.

Kumar S. et al. Comparative response of maize and rice genotypes to heat stress: Status of oxidative stress and antioxidants. **Acta Physiol Plant.** 2012;34:75-86.

Lichtenthaler H.K. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes**. Methods in Enzymology.** 1987;148C:350-82.

Mandal C. et al. Antioxidative responses of Salvinia (*Salvinia natans* Linn.) to aluminium stress and it's modulation by polyamine. **Physiol Molec Biol Plants**. 2013;19:91-103.

Naliwajski M.R., Sklodowska M. The oxidative stress and antioxidant systems in cucumber cells during acclimation to salinity. **Biol Plant.** 2014;58:47-54.

Ormeño E. et al. Plant coexistence alters terpene emission and content of Mediterranean species. **Phytochemistry**. 2007;68:840-52.

Pedersen B.P. et al.Ecological fitness of a glyphosate-resistant *Lolium rigidum* population: Growth and seed production along a competition gradient. **Basic Appl Ecol.** 2007;8:258-68.

Petrov V.D., Breusegem F.V. Hydrogen peroxide: a central hub for information flow in plant cells. **AOB Plants**. 2012;2012:1-13.

Rajabi R. et al. Effects of stress induced by post-emergence application of metribuzin herbicide on wheat. **Afr J Biotechnol**. 2012;11:3773-8.

Ramakrishna A., Ravishankar G.A. Influence of abiotic stress signals on secondary metabolites in plants. **Plant Signal Behav.** 2011;6:1720-31.

Sergiev I. et al. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. **C R Acad Bulg Sci.** 1997;51:121-4.

Sharma P. et al. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. **J Bot.** 2012:2012:1-26.

Singleton V.L., Rossi J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **Am J Enol Viticult.** 1965;16:144-58.

Song N.H. et. al. Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. **Chemosphere**. 2007;68:1779-87.

Suzuki N. et al. ROS and redox signalling in the response of plants to abiotic stress. **Plant Cell Environ.** 2012;35:259-70.

Tarhanen S. et al. Membrane permeability response of lichen Bryoria fuscescens to wet deposited heavy metals and acid rain. **Environ Pollut.** 1999;104:121-9.

Tripathy B.C. et al. Impairment of the photosynthetic apparatus by oxidative stress induced by photosensitization reaction of protoporphyrin IX. **Biochim Biophys Actal**. 2007;1767:860-8.

Walsh M.J., Powles S.B. Management strategies for herbicide resistant weed populations in Australian dry land crop production systems. **Weed Technol.** 2007;21:332-8.

Xiao L.Y. et al. Toxic reactivity of wheat (*Triticum aestivum*) plants to herbicide isoproturon. **J Agr Food Chem.** 2008;56:4825-31.