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Article

Interference of Volunteer Corn from Different Origins and Emergence Time on Soybean Yield and Stress Metabolism

Interferência de Milho Voluntário de Diferentes Origens e Épocas de Emergência no Rendimento de Grãos e no Metabolismo do Estresse da Soja

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ABSTRACT - Volunteer corn occurrence with soybean is favored by the glyphosate-resistant (GR) corn cultivation preceding soybean and no-tillage systems. Volunteer corn interference causes significant losses in soybean grain yield. The levels of crop losses change with the corn density, origin, and time of emergence. High levels of weed interference in crops can result in the production of reactive oxygen species and lead to the occurrence of oxidative stress. The objectives of this study were to evaluate the effects of interference of (1) different origins (individual plants and clumps) and times of emergence of volunteer corn on soybean growth, yield components, and grain yield loss; and (2) if the volunteer corn interference causes oxidative stress in soybean. Field experiment and laboratory analyses were performed. The evaluated variables were soybean yield components, grain yield, hydrogen peroxide - H_2O_2 content, and antioxidant enzyme superoxide dismutase - SOD, catalase - CAT, and ascorbate peroxidase - APX activities. Volunteer corn interference reduced the yield components and soybean yield. The highest yield losses were observed with volunteer corn clumps regarding individual plants. The interference of volunteer corn emerged 10 days before or on the same day as soybean caused the greater yield losses than those emerged 10 days after, independently of its origin. The content of H_2O_2 and enzyme SOD, CAT and APX activities changed in soybean leaves in response to the interference of volunteer corn plants and clumps. However, the results indicate that the volunteer corn interferences does not cause oxidative stress in soybean.

Keywords: individual plants, clumps, reactive oxygen species (ROS), soybean yield loss.

RESUMO - A ocorrência de milho voluntário em soja é favorecida pelo cultivo de milho tolerante ao glyphosate antecedendo a soja e pelo sistema de semeadura direta. A interferência de milho voluntário causa significativas perdas no rendimento de grãos de soja. Os níveis de perdas no rendimento da cultura variam com a densidade do milho voluntário, origem e época de emergência. Altos níveis de interferência de plantas daninhas nas culturas podem resultar na produção de espécies reativas de oxigênio e levar à ocorrência de estresse oxidativo. Os objetivos deste estudo foram avaliar os efeitos da interferência de (1) diferentes origens (plantas individuais e touceiras) e épocas de emergência de milho voluntário no crescimento, nos componentes do rendimento e no rendimento de grãos de soja; e (2) se a interferência de milho voluntário causa estresse oxidativo na soja. Foram realizados experimento em campo e análises em laboratório. As variáveis avaliadas foram os componentes do rendimento e rendimento de grãos de soja, conteúdo de

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peróxido de hidrogênio – H_2O_2, bem como as atividades das enzimas antioxidantes superóxido dismutase – SOD, catalase – CAT e ascorbato peroxidase – APX. As interferências causadas pelo milho voluntário resultaram na redução dos componentes do rendimento e do rendimento de grãos de soja. As mais altas perdas no rendimento de grãos de soja foram observadas para a interferência de touceiras em relação a plantas individuais. Independentemente da origem do milho, as perdas foram maiores quando a emergência ocorreu aos 10 dias antes e no mesmo dia que a soja, em relação aos emergidos 10 dias após. O conteúdo de H_2O_2 e a atividade das enzimas SOD, CAT e APX variaram em resposta às interferências causadas por plantas e touceiras de milho voluntário. No entanto, os resultados indicam que a interferência do milho voluntário não resultou em estresse oxidativo na soja.

Palavras-chave: plantas individuais, touceiras, espécies reativas de oxigênio (EROS), perdas no rendimento de grãos de soja.

INTRODUCTION

Volunteer corn (VC) originate from unharvested seeds (abandoned areas) or those lost during poor harvest process. These seeds lost occur individually, which produce individual plants, or as rachis segments or whole ears, producing clumps (i.e., several plants at a single point) (Beckett and Stoller, 1988). VC occurrence in soybean crops is favored mainly by factors like production systems with corn cultivated preceding soybean (Deen et al., 2006), and no-tillage systems (seeds are not buried) (Steckel et al., 2009). In soybean crops, VC had been reported before the transgenic crops conferring herbicide-tolerance (Andersen, 1976). However, the introduction of glyphosate-resistant (GR) corn in production systems has been associated with a higher occurrence of VC (Davis et al., 2008).

The interference of VC in density lower than one plant or clump m^{-2} has been reported to causes significant decreases in soybean grain yield (Andersen et al., 1982; Chahal and Jhala 2016; Piasecki et al., 2018a). Piasecki et al. (2018a) reported that when densities of individual plants and clumps (emerged together with the crop) increased from 0.5 to 12 m^{-2} reduce the soybean (0.5 m row spaced) yield from 22.2 to 91.9%, and 46.4 to 100%, respectively, in South of Brazil.

Clumps of VC predominate on soybean plantations (Deen et al., 2006), cause the highest grain yield losses (Marquardt et al., 2012; Chahal and Jhala, 2016; Piasecki et al., 2018a), and are more difficult to control because seed germination is nonuniform and resulting in different emergence flushes (López-Ovejero et al., 2016). Control of VC is challenging due to the limited effectiveness of pre-emergence herbicides (Chahal et al., 2014; Piasecki and Rizzardi, 2016), being necessary post-emergence treatment using of acetyl-coenzyme A carboxylase (ACCase) inhibiting-herbicides, that is the only option for controlling GR volunteer corn in post-emergence of soybean (Marquardt and Johnson, 2013; Chahal et al., 2014). On the other hand, VC can also host crop disease pathogens and insect pests, becoming a source of infestation for the next cropping season (Summers et al., 2004; Krupke et al., 2009).

Many studies have reported higher reactive oxygen species (ROS) production with a variety of stresses, including cold, salinity, and herbicide exposure, especially herbicides such as carotenoid synthesis and photosystems inhibitors (Knorzer et al., 1999; Porcel et al., 2003; Langaro et al., 2017). However, the literature is not available regards to the production of ROS in function of VC interference in soybean. ROS are highly reactive toxic molecules, causing damage to cellular structures and even cell death. The main ROS is hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide radical (O_2^-), and hydroxyl radical (OH^-) (Apel and Hirt, 2004; Gill and Tuteja, 2010; Caverzan et al., 2016a). On the other hand, to avoid potential ROS-induced damage, plants evolved antioxidant defense systems. Antioxidant mechanisms include the enzymes superoxide dismutase (SOD), catalases (CAT), and ascorbate peroxidase (APX) (Caverzan et al., 2016a).

To our knowledge there are no others reseacrhes that reported the effects of differents origins of VC in soybean according time of emergence, as well as their effects on soybean estress metabolism. The present work hypothesized that the interference of VC originated from individual plants or clumps, and emerged on different times relative to the crop have different effects on the soybean yield, and cause oxidative stress on soybean. Thus, the objectives of the present study were to evaluate the effects of interference of (1) different origins (individual plants and clumps) and times of emergence of volunteer corn on soybean growth, yield components, and grain yield loss; and (2) if the volunteer corn interference causes oxidative stress in soybean.

MATERIALS AND METHODS

Field experiment and data collection

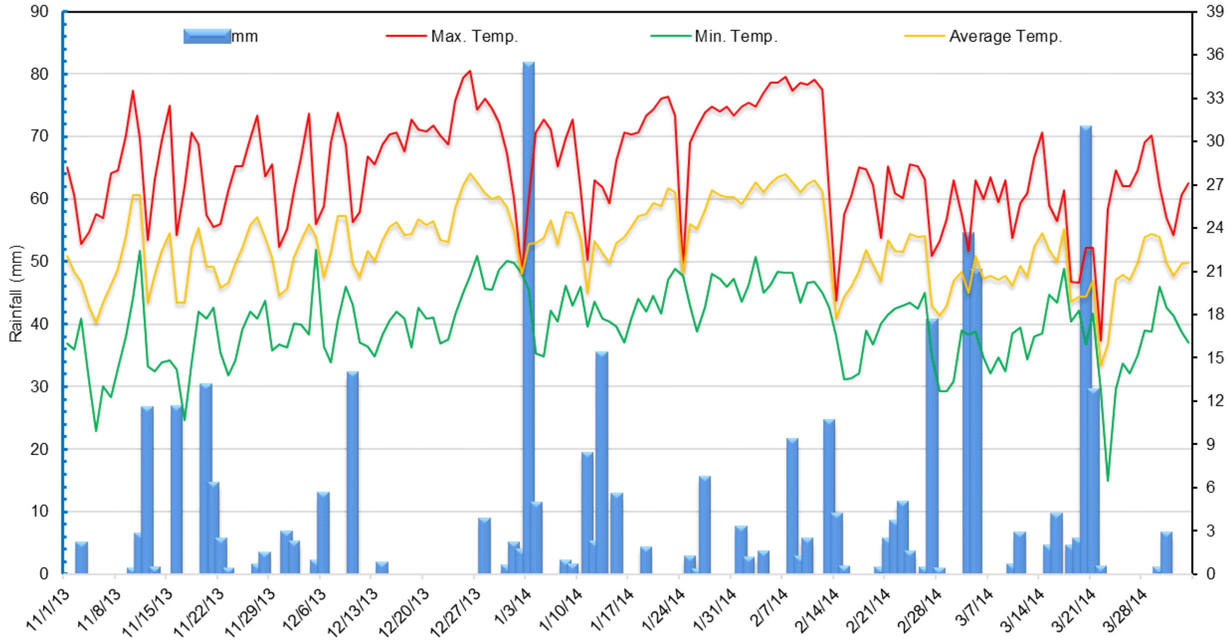
To achieve the objectives a field experiment and laboratory analysis were performed. The experiment was established at the Centro de Extensão e Pesquisa Agropecuária (CEPAGRO) of the Universidade de Passo Fundo (UPF), Passo Fundo city, state of Rio Grande do Sul (28º13’26.7" S; 52º23’03.6" W), Brazil (Figure 1). The experiment was conducted in a no-tillage system, in an area with black oat (*Avena strigosa*) and ryegrass (*Lolium multiflorum*) remains, controlled in a reproductive stage with the tank mix of herbicides clethodim 76.2 g active ingredient (a.i.) ha^{-1} (Select, Arysta Lifesciences Corporation Tokyo, 104-601 - Japan) and glyphosate 720 g acid equivalent (a.e.) ha^{-1} (Monsanto Company Iowa - 52.761 - EUA). The fertilization used on soybean was 5.6 kg N ha^{-1}, 78 kg P_2O_5 ha^{-1}, and 50 kg K_2O ha^{-1}. Soybean seeds were treated with insecticides and fungicides and inoculated with *Bradyrhizobium japonicum* (300 mL 100 kg^{-1} - Nitro-Liq Rizobacter S.A.). Seeds of GR VC used were originated from the corn hybrid AG 8088 $PRO_2^{®}$ F_2 generation, previously harvested. Glyphosate (720 g a.e. ha^{-1}) was sprayed once at 30 days after soybean emergence to control other weeds.

Figure 1 - Geographical localization from the region where experiments were performed.

The experiment was conducted using a randomized block experimental design, arranged in a factorial scheme 2 x 3 + 1, with four replicates. The first factor comprised two origins of VC GR F_2: individual plants or clumps; the second factor comprised three VC emergence times relative to soybean emergence: 10 days before, on the same day, and 10 days after; and the additional treatment was a control kept free of VC and other weeds interference.

In experimental units of 17.5 m^2 (3.5 x 5 m) kernels of VC plants or clumps were manually randomly distributed, according to each treatment and buried at a depth of approximately 3.5 cm. Initially was distributed the equivalent to ten plants or clumps m^{-2}. Immediately after corn emergence in each treatment, a standard VC density was manually adjusted to nine individual plants or clumps m^{-2}. Each clump was fixed to contain seven plants of corn. According to each treatment, VC density and number of plant per clump were manually adjusted twice trough rip out plants. The first fit was performed at three days after corn emergence and readjusted seven days after emergence. The date of VC sowing and emergence were respectively: a) 10 days before – Nov. 16 and Nov. 22; b) at the same day – Nov. 26 and Dec. 02; c) 10 days after soybean emergence – Dec. 06 and Dec. 12. The soybean cultivar BMX Turbo GR was sowed at a density of 30 plants m^{-2} in narrow rows 50 cm apart. The climate conditions during the experiment conduction are presented in Figure 2.

The evaluated variables at a field experiment were VC and soybean plant height; soybean leaf area index (LAI), shoot dry weight (SDW), number of pods and grains per plant, and grain yield. At 20 and 62 days after emergence (DAE), during the V4 and R2 soybean stages, respectively, the soybean and VC heights were measured in ten randomly selected plants or clumps per plot. The height of plants was determined from the ground to the tip of the last fully expanded trifoliate leaves in soybean and corn. The average height was considered for the VC clumps.



Weather data were obtained from SOMAR meteorology (http://www.somarmeteorologia.com.br).

Figure 2 - Daily total rainfall (mm) and daily average air temperature (°C) in 2013 and 2014 growing season during the conduction of the field experiment at Centro de Extensão e Pesquisa Agropecuária (CEPAGRO) of the Universidade de Passo Fundo (UPF), Passo Fundo, State of Rio Grande do Sul, Brazil.

The LAI was evaluated at full flowering (R2 stage) in 10 consecutive soybean plants collected without their petioles of the same central sowing row per plot. The leaf area was determined with an electronic planimeter and used to calculate the soybean LAI. The soybean SDW was measured employing the same total plants used for the determination of the LAI. The plants were dried in an oven at 58 °C until constant weight. The SDW m^{-2} (g) was calculated using the weight of the ten plants by the rule of three, considering that the sample collected corresponded to 0.33 m^2.

The number of pods and grains per plant were quantified manually in 10 randomly plants per plot, gathered on the day of the harvest. A sample area of 7.5 m^2 in each experimental unit was harvested and used to calculate the soybean yield. Following the harvest, the grain moisture content was measured and corrected to 13%, and the grain yield per hectare (kg ha^{-1}) was estimated.

H_2O_2 content and enzyme assays

For the determination of H_2O_2 concentrations and SOD, CAT, and APX activities, leaves were randomly collected from soybean plants 46 DAE during the R_2 stage (full flowering). The last fully expanded trifoliate leaf was obtained from five plants (composite sample) aleatory selected from of useful area in each experimental unit. The material was stored at -20 °C.

Cellular tissue damage was determined according to levels of H_2O_2, as described by Sergiev et al. (1997). The solution of 10 mL of 0.1% trichloroacetic acid (TCA) was added to 1.0 g of processed leaf tissue and vortexed. Samples were centrifuged at 5600 rpm for 25 min at 4 °C. For H_2O_2 quantification, the supernatant was collected and five technical samples of 0.2 mL added to 0.8 mL of phosphate buffer 10 mM (pH 7.0), and 1.0 mL of potassium iodide 1 M. The solution was allowed to stand for 10 min at room temperature. Absorbance was recorded at 390 nm. The concentration of H_2O_2 was determined using a standard curve with known concentrations of H_2O_2 and expressed in millimoles per gram of fresh weight (mM g^{-1} FW).

For enzyme assays extraction was performed to determine the activity of antioxidant enzymes SOD, CAT, and APX, and from this extract, the enzymes activities were calculated and expressed in active units (AU) per milligram of fresh weight (AU mg^{-1} FW). To 1.0 g of processed leaf tissue with 0.1 g of polyvinylpyrrolidone (PVP), 4.5 mL of 200 mM phosphate buffer (pH 7.8), 90 µL of 10 mM EDTA, 900 µL of 200 mM ascorbic acid, and 3.51 mL of ultrapure water was added and vortexed. The homogenates were centrifuged at 5600 rpm at 4 °C for 25 min. The supernatant was collected and used as the crude enzyme extract for all enzyme assays.

Total SOD activity was measured according to Peixoto (1999) in a 2 mL reaction mixture containing 1.0 mL phosphate buffer 100 mM (pH 7.8), 400 µL of methionine 70 mM, 20 µL of ethylenediaminetetraacetic (EDTA) 10 µM, 390 µL of ultrapure water, 150 µL ρ-nitro blue tetrazolium chloride (NBT) 1 mM, 20 µL of enzyme extract. The reaction was initiated by adding 20 µL of riboflavin 0.2 mM last. The samples were placed under fluorescent lamps at 4000 lx, 15 Watts for 10 min and the absorbance at 560 nm was recorded. One activity unit (AU) of SOD was equal to the amount of enzyme necessary to cause 50% inhibition of NBT reduction at 560 nm. The activity was expressed in AU mg^{-1} of fresh weight min^{-1} (AU mg^{-1} FW min^{-1}).

Catalase and ascorbate peroxidase activities were determined according to Azevedo et al. (1998). Catalase activity was measured following the decline in absorbance for 1.5 min at 240 nm resulting from H_2O_2 consumption (extinction coefficient: 39.4 mM cm^{-1}). The 2 mL reaction mixture contained 1.0 mL of potassium phosphate buffer 200 mM (pH 7.0), 850 µL of ultrapure water, 100 µL of H_2O_2 250 mM, and 50 µL of enzyme extract last to initiate the reaction.

Ascorbate peroxidase activity was measured following the decrease in absorbance at 290 nm for 1.5 min. The 2.0 mL reaction mixture contained 1.0 mL of potassium phosphate buffer 200 mM (pH 7.0), 750 µL of ultrapure water, 100 µL of ascorbic-acid (ASC) 10 mM, 50 µL of enzyme extract, and 100 µL of H_2O_2 2.0 mM last to initiate the reaction. The activity of APX was calculated using an extinction coefficient of 2.9 mM^{-1} cm^{-1}.

For both CAT and APX activities, for calculation purposes, it was considered that the decrease of one absorbance unit is equivalent to one active unit (AU). The activities of total extract were determined by calculating the amount of extract that reduced the absorbance reading by one AU and expressed in AU mg^{-1} of fresh weight min^{-1} (AU mg^{-1} FW min^{-1}).

Statistical Analysis

The statistical program CoStat (Costa and Castoldi, 2009) was used for statistical analysis. Data were tested for normality using the Shapiro-Wilk's test as well as homoscedasticity using Hartley's test. The factorial results were subjected to ANOVA followed by Tukey's test at $p<0.05$. For evaluate the treatments in contrast to treatment control, the Dunnett's test was used at $p<0.05$.

RESULTS AND DISCUSSION

Soybean and volunteer corn height

In a field experiment, a significant interaction between factors was observed for soybean height at 20 and 62 DAE, whereas significant differences in corn height were only observed between different emergence times (Table 1). At 20 DAE the soybean height varied with the VC origin and emergence time. In this evaluate and was more strongly affected by the presence of clumps compared with individual plants and by VC emerged 10 days before or on the same day as soybean compared with 10 days after soybean. The corn height varied with the emergence time and was higher for VC emerged before rather than after or at the same day as soybean. Significant differences in soybean height about the control were only observed for soybean in interference with clumps emerged 10 days before, at 20 and 62 DAE.

The greater height of VC em relation to soybean conferred to VC preferential access to light and shaded the crop. Independently of the VC origin and time of emergence, the shading of soybean by corn resulted in a series of adverse effects, such as a decreased LAI (lower light interception) and lower SDW accumulation, resulting in reduced soybean yield components and grain yield (Tables 1 to 4) (Silva et al., 2009).

Table 1 - Average height (cm) of soybean and corn plants at 20 and 62 days after soybean emergence as a function of interference by volunteer corn GR F_2 that emerged 10 days before, at the same day, or 10 days after soybean emergence

Volunteer corn	Times of emergence of volunteer corn regard to soybean			Mean
	Soybean height at 20 DAE (cm)			
	10 days before(1)	Same day	10 days after	
Individual plants	ns20.8 bA	ns20.1 bA	ns19.5 aA	
Clumps	*24.5 aA	ns22.4 aA	ns19.0 aB	
Control	19.9			
CV%(2)	7.3			
	Volunteer corn height at 20 DAE (cm)			
Individual plants	68	46	20	45ns
Clumps	64	38	22	42
Mean	66 A	42 B	21 C	
Control	-			
CV%(2)	12.9			
	Soybean height at 62 DAE (cm)			
Individual plants	ns110 aAB	ns118 aA	ns108 aB	
Clumps	*96 bB	ns119 aA	ns111 aA	
Control	112			
CV%(2)	4.6			
	Volunteer corn height at 62 DAE (cm)			
Individual plants	192	192	153	179ns
Clumps	178	175	154	169
Mean	185 A	184 A	154 B	
Control	-			
CV%(2)	7.8			

(1) 10 days before: corn emergence 10 days before that of soybean; same day: corn and soybean emergence at the same day; 10 days after: corn emergence 10 days after soybean. (2) CV: coefficient of variation (%). Means followed by different lower case letters in the same column and upper case letters in the same row differ significantly according to the Tukey test ($p<0.05$); ns to the right of a number: not significantly different according to the Tukey test ($p<0.05$); ns to the left of a number: not significantly different from the control according to the Dunnett test ($p<0.05$); and * to the left of a number: significantly different from the control according to the Dunnett test ($p<0.05$). DAE - days after soybean emergence; GR - glyphosate resistant.

Table 2 - Leaf area index (LAI) and shoot dry weight (SDW) of soybean at stage R_2 as a function of interference by volunteer corn originating from individual plants or clumps and emerged 10 days before, at the same day as, or 10 days after soybean

Volunteer corn	Times of emergence of volunteer corn regard to soybean			Mean
	LAI (m^2 m^{-2}) at the R2 soybean stage			
	10 days before(1)	Same day	10 days after	
Individual plants	*3.5 aB	*3.4 aB	ns4.9 aA	
Clumps	*1.0 bC	*2.9 aB	ns4.2 aA	
Control	5.2			
CV%(2)	21			
	SDW (g m^{-2}) on R_2 stage			
Individual plants	*152	*180	ns306	213 a
Clumps	*33	*153	*217	134 b
Mean	92 C	166 B	262 A	
Control	366			
CV%(2)	24.8			

(1) 10 days before: corn emergence 10 days before that of soybean; same day: corn and soybean emergence at the same day; 10 days after: corn emergence 10 days after that of soybean. (2) CV: coefficient of variation (%). Means followed by different lower case letters in the same column and upper case letters in the same row differ significantly according to the Tukey test ($p<0.05$); ns to the right of a number: not significantly different according to the Tukey test ($p<0.05$); ns to the left of a number: not significantly different from the control according to the Dunnett test ($p<0.05$); and * to the left of a number: significantly different from the control according to the Dunnett test ($p<0.05$).

Leaf Area Index (LAI) and Shoot Dry Weight (SDW)

A significant interaction between factors was observed for the soybean LAI, whereas only significant simple effect occur for the SDW (Table 2). The most pronounced decrease in the soybean LAI occurred under interference with the VC clumps that emerged 10 days before the soybean. The soybean LAI was less affected when the corn emerged 10 days after the soybean compared with the remaining VC emergence times and origins that were tested and did not differ significantly from the control without VC interference. The LAI determines the ability of the canopy to intercept photosynthetically active radiation and therefore determines the dry weight accumulation by plants (Silva et al., 2009).

The soybean SDW was lowest in the soybean competing with clumps and with corn emerged 10 days before the soybean emergence (Table 2). When compared with control, soybean SDW did not differ significantly only for individual plants emerged 10 days after soybean (Table 2). The soybean SDW was 63% lower when competing with clumps in a direct comparison with individual plants. On average soybean, SDW were 172 and 273% lower than control when competing with individual plants and clumps, respectively. Beside this, independently of the corn origin, the higher soybean SDW losses correlated with earlier corn emergence relative to that of soybean.

Number of grains and pods per plant and grain yield

No significant interactions between factors origins and relative time of emergence were observed for the average number of grains per plant or the soybean grain yield, whereas a significant interaction between factors occurred for the number of pods (Table 3). The number of grains and the soybean grain yield was on average 50% and 54% lower, respectively, for soybean under interference with clumps compared with individual plants. No differences were observed in the number of grains or soybean grain yield between origins when VC emerged 10 days before

Table 3 - Average number of grains and pods per soybean plant and soybean grain yield (kg ha^{-1}) as a function of interference by volunteer corn originating from individual plants or clumps and emerged 10 days before, at the same day as, or 10 days after soybean

Volunteer corn	Times of emergence of volunteer corn regard to soybean			Mean
	Number of grains per soybean plant			
	10 days before[(1)]	Same day	10 days after	
Individual plants	*33	*31	*43	36 a
Clumps	*10	*10	*32	18 b
Mean	22 B	21 B	38 A	
Control	64			
CV%[(2)]	28.8			
Number of pods per soybean plant				
Individual plants	*15 aA	*14 aA	*19 aA	
Clumps	*5 bB	*5 bB	*18 aA	
Control	27.7			
CV%[(2)]	22.6			
Soybean grain yield (kg ha^{-1})				
Individual plants	*780	*972	*2359	1370 a
Clumps	*98	*271	*1871	747 b
Mean	439 B	621 B	2115 A	
Control	3243			
CV%[(2)]	19.1			

[(1)] 10 days before: corn emergence 10 days before that of soybean; same day: corn and soybean emergence at the same day; 10 days after: corn emergence 10 days after that of soybean. [(2)] CV: coefficient of variation (%). Means followed by different lower case letters in the same column and upper case letters in the same row differ significantly according to the Tukey test ($p<0.05$); ^{ns} to the right of a number: not significantly different according to the Tukey test ($p<0.05$); ^{ns} to the left of a number: not significantly different from the control according to the Dunnett test ($p<0.05$); and * to the left of a number: significantly different from the control according to the Dunnett test ($p<0.05$).

or at the same day as soybean. When the VC emerged 10 days before or at the same day as soybean, the number of pods was lower for the treatments with clumps compared with individual plants, whereas when the corn emerged 10 days after soybean, no differences in the number of pods were observed between VC origins (Table 3). By the present study, lower losses in soybean grain yield have been previously reported when VC emerged after compared with before or on the same day as soybean emergence (Marquardt et al., 2012).

Significant differences in the number of grains, number of pods, and soybean grain yield were observed between the control without VC interference and all the remaining treatments (Table 3). On average, when soybean competing respectively with individual plants or clumps, the number of grains reduced 1.8 – 3.6 times, while grain yield was 2.4 – 4.3 times lower than control. In soybean, the number of pods is the parameter most affected by interference between species, whereas the number of grains per pod and the grain weight is more directly associated with genetic factors, varying little with the environment (Board et al., 1995).

Overall, the most significant decreases in yield components and soybean grain yield were observed in interference with VC clumps, for all emergence times tested. This result occurred because more plants were located at a single point with the clumped compared with the individual VC plant treatment (Piasecki et al., 2018a).

Enzyme activity

For the variables related to oxidative stress in soybean, significant differences in the H_2O_2 concentration and CAT and SOD activities were observed among different corn emergence times. However, no significant differences were observed between different corn origins and a significant interaction between factors for the APX activity (Table 4). The highest H_2O_2 concentrations occurred for soybean under interference with VC emerged 10 days after soybean, and these were not significantly different from those of the control without corn interference. In turn, the H_2O_2 concentrations were lower with corn emerged 10 days before or at the same day as soybean and differed significantly from those of the control. The higher H_2O_2 levels observed in the soybeans grown with corn emerged 10 days after soybean than with corn emerged at the remaining times may have been due to the exposure of these soybeans to a higher light incidence and temperature, considering that soybean shading was more intense with an earlier corn emergence time.

Plant natural processes like photosynthesis are a potential source of ROS production which is subsequently eliminated by the antioxidant systems (Gill and Tuteja, 2010). It is known that high light intensity and temperature (heat or cold) are factors of abiotic stresses that lead to an increase in ROS production (Caverzan et al., 2016a). That way, Somporn et al. (2012) observed differences among shading conditions for antioxidant activity in coffee beans. The lower incident radiation on soybean in interference with corn emerged 10 days before or on the same day as soybean may result in the establishment of milder microclimates at the plant canopy. Ultraviolet-B (UV-B) radiation is an exogenous source of ROS, especially H_2O_2 (Alexieva et al., 2001) and changes in H_2O_2 level was showed in soybean and maize plants submitted to the UV-B application (Shen et al., 2015).

Overall, the results of the present study showed that the APX, CAT, and SOD activities in the soybean grown with corn were higher when the corn emergence time was earlier (Table 4). These enzyme activity increases most likely occurred to eliminate or neutralize H_2O_2 in soybean competing with corn emerged 10 days before or on the same day as soybean (higher interference) and may explain the lower H_2O_2 concentrations observed. The enhance of the antioxidant enzyme activity has been shown in other plant species and stresses conditions (Ashraf and Ali, 2008; Ferreira-Silva et al., 2011; Caverzan et al., 2016b). Also, the balance among CAT, SOD, and APX activities is therefore essential to scavenging toxic levels of ROS (Apel and Hirt, 2004). Piasecki et al. (2018b) reported alterations on H_2O_2 content and SOD, APX, and CAT activities in soybean in response to interference caused by different densities of VC in South of Brazil.

The higher SOD and APX activities observed in the soybean competing with corn emerged 10 days before or on the same day as soybean may have resulted in the transformation of H_2O_2 into other molecules, such as water and O_2. The CAT, SOD, and APX activities were higher in the soybean under higher corn interference but were not significantly different from those of

Table 4 - Leaf hydrogen peroxide (H_2O_2) content (mmol g^{-1} FW), and ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) activities (U mg^{-1} FW min^{-1}) in R_2 stage soybean

Volunteer corn	Times of emergence of volunteer corn regard to soybean			Mean
	H_2O_2 content (mmol g^{-1} FW)			
	10 days before[1]	Same day	10 days after	
Individual plants	*8.5	*8.1	^{ns}17.2	11.3^{ns}
Clumps	*8.4	*6.7	^{ns}15.7	10.2
Mean	8.4 B	7.4 B	16.5 A	
Control	18.1			
CV%[2]	17.5			
APX activity (U mg^{-1} FW $min.^{-1}$)				
Individual plants	*81.2 bA	*50.9 aB	^{ns}32.3 aB	
Clumps	*110.8 aA	^{ns}33.9 bB	^{ns}21.3 aB	
Control	16.5			
CV%[2]	21.6			
CAT activity (U mg^{-1} FW $min.^{-1}$)				
Individual plants	^{ns}11.2	^{ns}6.4	^{ns}6.7	8.1^{ns}
Clumps	^{ns}9.5	^{ns}7.7	^{ns}7.6	8.3
Mean	10.4 A	7.1 B	7.2 B	
Control	9.8			
CV%[2]	29.2			
SOD activity (U mg^{-1} FW $min.^{-1}$)				
Individual plants	^{ns}106.6	^{ns}90.2	^{ns}86.3	94.3^{ns}
Clumps	^{ns}102.2	^{ns}93.2	^{ns}90.5	95.3
Mean	104.4 A	91.7 AB	88.4 B	
Control	85.6			
CV%[2]	11.8			

[1] 10 days before: corn emergence 10 days before that of soybean; same day: corn and soybean emergence on the same day; 10 days after: corn emergence 10 days after that of soybean. [2] CV: coefficient of variation (%). Means followed by different lower case letters in the same column and upper case letters in the same row differ significantly according to the Tukey test ($p<0.05$); ^{ns} to the right of a number: not significantly different according to the Tukey test ($p<0.05$); ^{ns} to the left of a number: not significantly different from the control according to the Dunnett test ($p<0.05$); and * to the left of a number: significantly different from the control according to the Dunnett test ($p<0.05$).

the control treatment, especially those for CAT and SOD (Table 4). However, the consistently observed increase in the activities of these antioxidant enzymes indicates the existence of metabolic changes in soybean plants under interference from VC. Although changes in soybean metabolism occurred, it probably did not causes the oxidative stress because of the H_2O_2 levels reduced with higher interference caused by VC.

The interferences caused by volunteer corn on soybean reduced the yield components and soybean yield. Clumps are more competitive than individual plants and caused the highest losses on soybean. The soybean yield losses increased when the volunteer corn emerged 10 days before or on the same day as soybean, independently of its origin. Interferences caused by volunteer corn on soybean resulted in alterations on metabolism. However, results indicate that the volunteer corn interferences does not cause oxidative stress in soybean.

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