PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Influence of Glyphosate on Susceptible and Resistant Ryegrass Populations to Herbicide

Influência do Glyphosate em Populações de Azevém Suscetível e Resistente ao Herbicida

PICOLI JR, G.J.[1]*
CARBONARI, C.A.[1]
MATOS, A.K.A.[1]
RODRIGUES, L.F.O.S.[1]
VELINI, E.D.[1]

ABSTRACT - In Brazil, ryegrass (*Lolium multiflorum*) has been identified as resistant to glyphosate, becoming a major problem, especially in crops cultivated in the winter season. This herbicide can indirectly affect photosynthesis by inhibiting biosynthesis of many compounds. Thus, the aim of this study was to investigate the influence of glyphosate on the physiological profile of susceptible and resistant ryegrass populations to the herbicide. The experimental design was completely randomized with two treatments (720 and 1,080 g e.a. ha^{-1}) and four replications plus control with no treatment. Two ryegrass populations were sown, one susceptible and another one resistant to glyphosate. After the treatments, evaluations were carried out at 1, 3, 7 and 28 days after application (DAA). Variables analyzed were: CO_2 net assimilation rate, stomatal conductance, CO_2 internal concentration, transpiration, water use efficiency and instantaneous carboxylation efficiency. The glyphosate herbicide caused irreversible damage in a susceptible population which at 28 DAA in all variables analyzed this population was already dead and it was impossible to analyze it, but it was shown that the effects of this herbicide were intensified from the third day after application. In the case of the resistant population, at 3 DAA in all variables, it suffered significant effects comparing to the control, showing that even with a high level of resistance the herbicide can affect its metabolism.

Keywords: herbicide resistance, photosynthesis, *Lolium multiflorum*.

*RESUMO - No Brasil, o azevém (**Lolium multiflorum**) foi identificado como resistente ao glyphosate, tornando-se problema, principalmente, em lavouras cultivadas na estação de inverno. Esse herbicida pode afetar indiretamente a fotossíntese, através da inibição da biossíntese de diversos compostos. Com isso, o objetivo deste trabalho foi investigar a influência do glyphosate no perfil fisiológico de populações de azevém suscetível e resistente ao herbicida. O delineamento experimental utilizado foi inteiramente casualizado (DIC) com dois tratamentos (720 e 1.080 g e.a. ha^{-1}) e quatro repetições, mais uma testemunha. Foram semeadas duas populações de azevém, sendo uma suscetível e outra resistente ao glyphosate. Após a aplicação dos tratamentos, foram feitas avaliações aos 1, 3, 7 e 28 dias após a aplicação (DAA). As variáveis analisadas foram: taxa de assimilação líquida de CO_2, condutância estomática, concentração interna de CO_2 e transpiração, eficiência do uso da água e eficiência instantânea de carboxilação. O herbicida glyphosate causou danos irreversíveis à população suscetível, a qual, aos 28 DAA, em todas as variáveis analisadas, já se encontrava morta, impossibilitando assim sua análise; contudo foi mostrado que os efeitos desse herbicida foram intensificados a partir do terceiro dia após a aplicação. No caso da população resistente, aos 3 DAA, nas variáveis analisadas, verificaram-se efeitos significativos em relação à testemunha, mostrando que, mesmo apresentando elevado grau de resistência, o herbicida é capaz de afetar seu metabolismo.*

Palavras-chave: resistência a herbicidas, fotossíntese, *Lolium multiflorum*.

*** Corresponding author:**
<gilmarpicoli@yahoo.com.br>.

Received: May 3, 2016
Approved: July 28, 2016

Planta Daninha 2017; v35:e017163391

[1] Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Botucatu-SP, Brasil.

Doi: 10.1590/S0100-83582017350100055

INTRODUCTION

Photosynthesis, the main biochemical process that occurs in autotrophic organisms, is known to be affected by several biotic and abiotic factors. Among abiotic factors, herbicides can be mentioned. Some of these directly affect photosynthesis, disrupting the transport of electrons, such as paraquat, an inhibitor of photosystem I. Other herbicides, such as glyphosate, indirectly affect by inhibiting the biosynthesis of carotenoids, chlorophyll, fatty acids, etc. (Olesen and Cedergreen, 2010; Gomes et al., 2014).

As a competitive inhibitor, glyphosate blocks the shikimic acid pathway, inhibiting the biosynthesis of secondary metabolites in plants, including compounds related to photosynthesis, such as quinones (Dewick, 1998). However, it is unclear how glyphosate drives plants to death. Hypotheses such as the depletion of protein stocks and carbon drainage from other pathways can be questioned (Duke and Powles, 2008). Some studies have shown a reduction in the photosynthetic rate of weed species after the application of the herbicide glyphosate (Mateos-Naranjo et al., 2009; Yanniccari et al., 2012a).

Glyphosate may also affect photosynthesis, modifying carbon metabolism in plants. Thus, after its application, CO_2 assimilation capacity is reduced, which leads to an increase in intracellular CO_2 concentration, also reducing stomatal conductance (Mateos-Naranjo et al., 2009; Ding et al., 2011). In addition, ribulose 1,5-biphosphate carboxylase oxygenase (RuBisCO) enzyme activity, in addition to ribulose 1,5-biphosphate (RuBP) and 3-phosphoglycerate acid (PGA) levels can be reduced after exposure to glyphosate (Servaites et al., 1987). According to De María et al. (2006), there was a reduction of approximately 26% of the RuBisCO activity in leaves of *Lupinus albus* seven days after the application of 10 mM of glyphosate.

Glyphosate is the most widely used herbicide in the world. Because it is applied frequently, it has facilitated the occurrence of many cases of weed resistance, generating negative impacts on agriculture. Among the species with resistance there is ryegrass (*Lolium multiflorum*), weed which constitutes a major problem for wheat crops (Roman et al., 2004) and orchards, especially in places with a temperate climate, such as Brazilian states of Rio Grande do Sul and Santa Catarina (Vargas et al., 2005). The increased presence of this species in commercial crops, coupled with the low prospect of launching new herbicidal molecules specifically for the control of ryegrass, represents great economic and technical impacts on Brazilian agriculture (Dionisio et al., 2013).

Several studies have been carried out to understand the plants physiology after the application of herbicides (Zobiole et al., 2010a; Orcaray et al., 2012; Yanniccari et al., 2012a, b; Vargas et al., 2014). Physiological knowledge about weeds – in this case, ryegrass is important for an efficient management, as it may help in understanding glyphosate resistance mechanisms, as well as the intensity of effects on susceptible and resistant biotypes. Explanations for the effects after inhibition of EPSPs (5-enolpyruvylshikimate-3-phosphate) are not fully understood and plant death is not only related to the blockade of the aromatic amino acid biosynthesis pathway but also to the failure to produce a large number of secondary compounds, the disruption in the carbon flow in the plant and the reduction of protein synthesis (Velini et al., 2009). Therefore, the objective of this study was to investigate the influence of glyphosate on the physiological profile of ryegrass populations susceptible and resistant to the herbicide.

MATERIAL AND METHODS

Ryegrass populations

Two populations of ryegrass were selected (*Lolium multiflorum*), one being susceptible (S) and the other resistant (R) to herbicide glyphosate, in 1-L pots, in Carolina (sphagnum peat, expanded vermiculite, organic residue of roasted rice husk, dolomitic limestone, agricultural gypsum and NPK fertilizer) substrate with pH 5.5. The susceptible population was acquired from company Agro Cosmo, in the Brazilian city of Engenheiro Coelho, SP, and the resistant population was obtained from Embrapa [Empresa Brasileira de Pesquisa Agropecuária (Brazilian Government Agricultural Research Corporation)] Trigo (Wheat), in the Brazilian city of Passo Fundo, RS.

Dose-response curve

A dose-response curve experiment was performed to verify the level of resistance of the two ryegrass populations. For this, the populations mentioned above were sown in 0.5 L pots using the same substrate (Carolina). Herbicide was applied when the plants had approximately six tillers.

The experimental design was a completely randomized design (CRD) with eight treatments and four replications. Treatments consisted of increasing doses of glyphosate: 0, 135, 270, 540, 1,080, 2,160, 4,320 and 8.640 g a.e. ha^{-1}, which correspond to the doses in percentages of 0, 12.5, 25, 50, 100, 200, 400 and 800%, respectively. The value of 100% represents the dose recommended of the product (1,080 g a.e. ha^{-1}). The commercial product used was Roundup Original (360 g a.e. L^{-1}).

In spraying the treatments, a stationary sprayer was used consisting of a 1.5 m wide metallic structure that supports the spray boom, which travels lengthwise by a 6.0 m^2 floor area. The boom is driven by an electric motor and frequency modulator set, making it possible to control its working speed. The boom was equipped with four XR 11002 VS spray tips spaced 0.5 m apart, arranged at a 0.5 m height in relation to the plants. The working pressure used by the equipment was 2.0 kgf cm^{-2}, with a speed of 3.6 km h^{-1} and spray mix consumption of 200 L ha^{-1}.

Visual control evaluations of the plants were carried out at 21 days after application (DAA) according to a percentage scale of grades, where 0 corresponds to no control and 100 means the death of plants, according to Sociedade Brasileira da Ciência das Plantas Daninhas (Brazilian Society of Weed Science) (SBCPD, 1995).

Physiological evaluations

The experimental design was a completely randomized design (CRD) with three treatments (0, 720 and 1,080 g a.e. ha^{-1}) and four replications. For each population, a control without application was used. The commercial product used was Roundup Original (360 g a.e. L^{-1}) and the doses were selected according to the registration of this commercial product for the control of ryegrass. Seeding was carried out on September 9, 2014, and herbicide application was on October 15, 2014, with a temperature of 26.8 ^oC and 56% relative humidity. Immediately after emergence of the plants (two weeks after sowing), the plants were thinned, leaving only four plants per pot. In spraying the treatments, the same sprayer described above was used. After the application on random leaves, gas exchanges were carried out at 1, 3, 7 and 28 DAA.

For the physiological determinations, an LI 6400 xt (LI-COR) model IRGA portable meter with environment CO_2 was used. Flux density of photosynthetically active photons was adjusted and fixed by the radiation affecting on the different days of evaluation of the gas exchanges. The variables analyzed were: net assimilation rate of CO_2 (photosynthesis), stomatal conductance, internal concentration of CO_2 and transpiration. With these variables it was possible to calculate the water-use efficiency (WUE) (net assimilation rate of CO_2/transpiration) and the instantaneous carboxylation efficiency (RuBisCO) calculated by the net assimilation rate of CO_2/internal concentration of CO_2.

Data analysis

The data from the dose-response curve experiment were submitted to analysis of variance and application of the F test at p>0.05. As the effects were significant, the data were adjusted to the log-logistic type nonlinear regression model proposed by Streibig et al. (1988):

$$y = \frac{a}{\left[1 + \left(\frac{x}{b}\right)^c\right]}$$

where: *y* = percentage of control or mass; *x* = dose of the herbicide (g a.e. ha^{-1}); *a*, *b* and *c* = they are parameters of the equation, where *a* = asymptote between the maximum and minimum point of the variable, *b* = dose that provides 50% of the asymptote (corresponds to C_{50} or GR_{50}) and *c* = slope of the curve. Based on the logistic equation, the curves were developed using statistical software Sigmaplot 12.0.

As for the physiological evaluations data, the confidence interval was established by the t test at p>0.1. To determine the confidence interval, the following equation was used:

IC = (t x standarddeviation)/ root nr

where: *CI* = confidence interval; *t* = matched t value, at the level of p>0.1; *standarddeviation* = standard deviation; and *root no.* = square root of the number of repetitions.

RESULTS AND DISCUSSION

Dose-response curve

The control percent data can be viewed in Figure 1. According to the coefficient of determination (r^2), the results showed adjustments close to 1 by the model proposed by Streibig et al. (1988). It was observed that 25% of the dose recommended (270 g a.e. ha^{-1}) was sufficient to obtain control above 80% in the susceptible (S) population, thus demonstrating a great susceptibility of this one in relation to herbicide glyphosate. However, the same was not observed for the resistant (R) population, where twice the dose was not sufficient to gain control over 80%. Therefore, the R population can be considered resistant to herbicide glyphosate, as it presented a high level of resistance (Table 1).

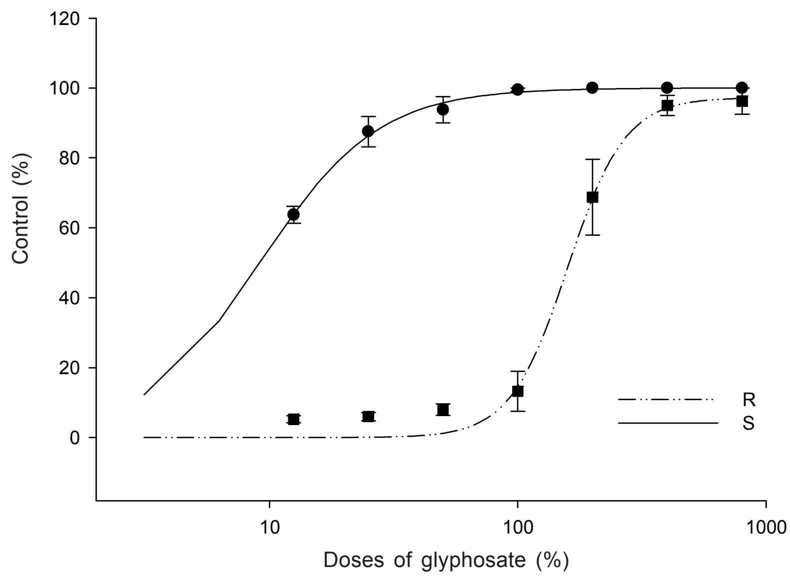


Figure 1 - Control of ryegrass susceptible (S) and resistant (R) after the application of doses of herbicide glyphosate at 21 DAA. Botucatu, SP

Table 1 - Estimates of parameters a, b, c and the coefficient of determination (r^2) of the log-logistic model for the populations of *Lolium multiflorum* in relation to the percentage of control at 21 DAA. Botucatu, SP

Variable	Population	a	b (C_{50})	c	r^2	RF*
Control (%)	R	97.25	158.76	-3.74	0.99	17.39
	S	100.01	9.13	-1.83	0.99	

Equation of the model: $y = a/(1+((x/b)^c))$.

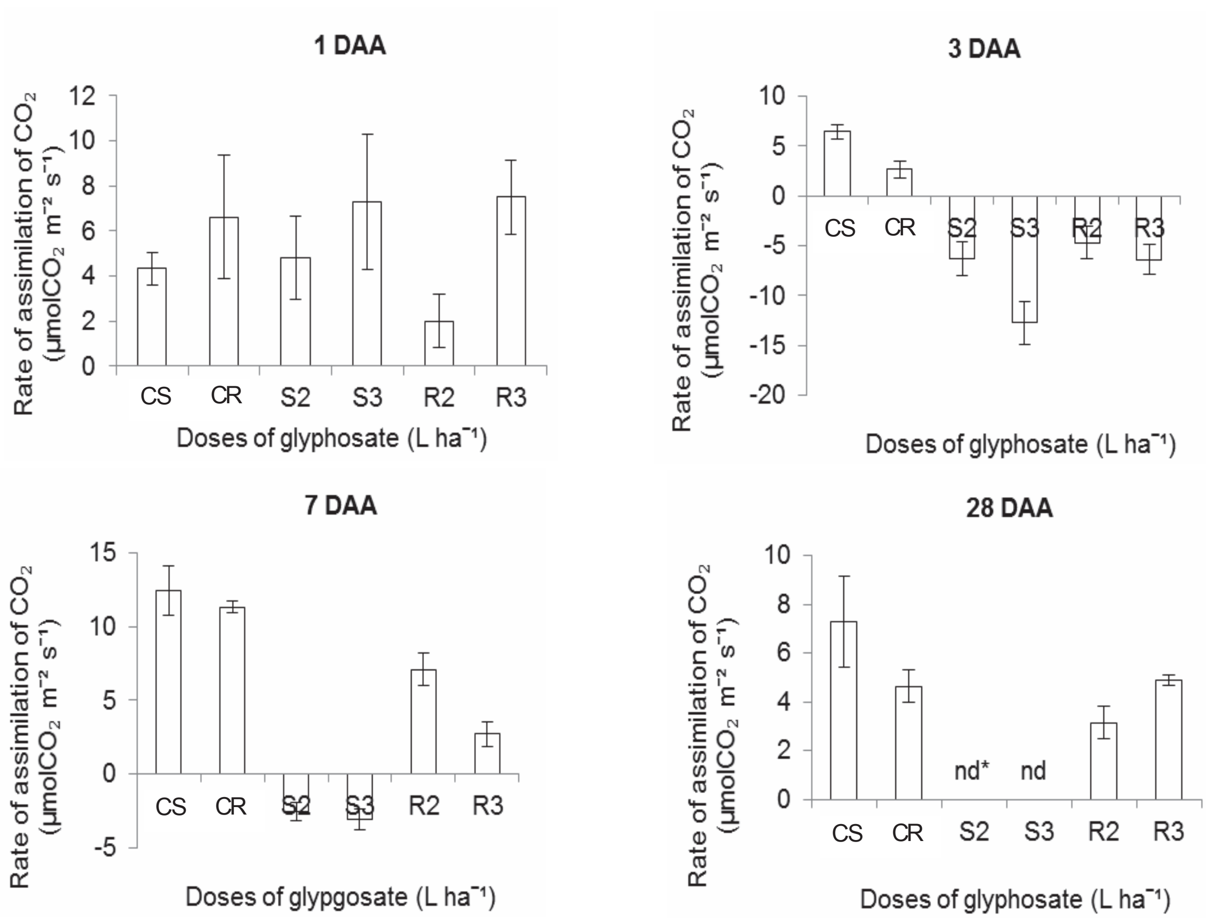
* Resistance Factor = C50R/C50S.

The high level of resistance shown in the R population shows that the resistance mechanism involved may be the result of the action of two or more mechanisms. Some studies have reported that the change in Proline in position 106 (Pro106), together with differentiated translocation or another unknown mechanism, provides a higher degree of resistance than one mechanism alone (Bostamam et al., 2012; Nandula et al., 2013). In other cases, gene duplication or site-of-action changes were detected but did not seem to fully explain the higher levels of resistance (Collavo and Sattin, 2012). The occurrence of multiple mechanisms of action is common, especially in cross-pollinated species such as ryegrass, producing a higher level of resistance in response to the selection pressure imposed by the constant application of the herbicide (Sammons and Gaines, 2014).

Management of this weed should be based on herbicides with different mechanisms of action, since increasing the dose of glyphosate is not economically feasible based on the dose-response curve and the application of twice the recommended dose was not enough to efficiently control this population.

Physiological evaluations

On the first day after glyphosate herbicide application, the rate of assimilation of CO_2 (A) (Figure 2) was positive for all treatments. This means that one day was not enough for glyphosate to affect photosynthesis, regardless of whether the plant is susceptible or resistant to it. This



The vertical bars indicate the confidence interval. Botucatu, SP. * nd: non-detectable

Control susceptible (CS), control resistant (CR), susceptible population submitted to 720 g a.e. ha^{-1} (S2), susceptible population submitted to 1,080 g a.e. ha^{-1} (S3), resistant population submitted to 720 g a.e. ha^{-1} (R2), resistant population submitted to 1,080 g a.e. ha^{-1} (R3).

Figure 2 - Rate of assimilation of CO_2 ($\mu molCO_2\ m^{-2}\ s^{-1}$) in ryegrass plants susceptible and resistant after the application of herbicide glyphosate at 1, 3, 7 and 28 DAA.

result is acceptable, since this herbicide has slow action in the plant, presenting symptoms days after the application.

At 3 DAA, only the controls (without application) remained with positive values, that is, besides the susceptible plants, the resistant ones also presented negative values. During this period, glyphosate had already begun to have an effect on the plants. This case indicates that, specifically at that time, the two populations were breathing more than performing photosynthesis, thus reducing the ability to accumulate mass. Glyphosate can affect photosynthesis by reducing RuBisCO activity and synthesis of 3-phosphoglyceric acid, as well as chlorophyll synthesis, besides increasing the rate of cellular respiration (Flexas et al., 2005; Ahsan et al., 2008). Breathing is an important process for plants because it is from this reaction that energy is produced in the form of ATP (Adenosine triphosphate) using the carbohydrates produced in photosynthesis, where this one can be used for plant maintenance and development (Taiz and Zeiger, 2013). The balance between photosynthesis and respiration determines the amount of photoassimilates required for normal plant growth. Therefore, if some abiotic or biotic factors impair this balance, there can be negative consequences, which can lead to its death.

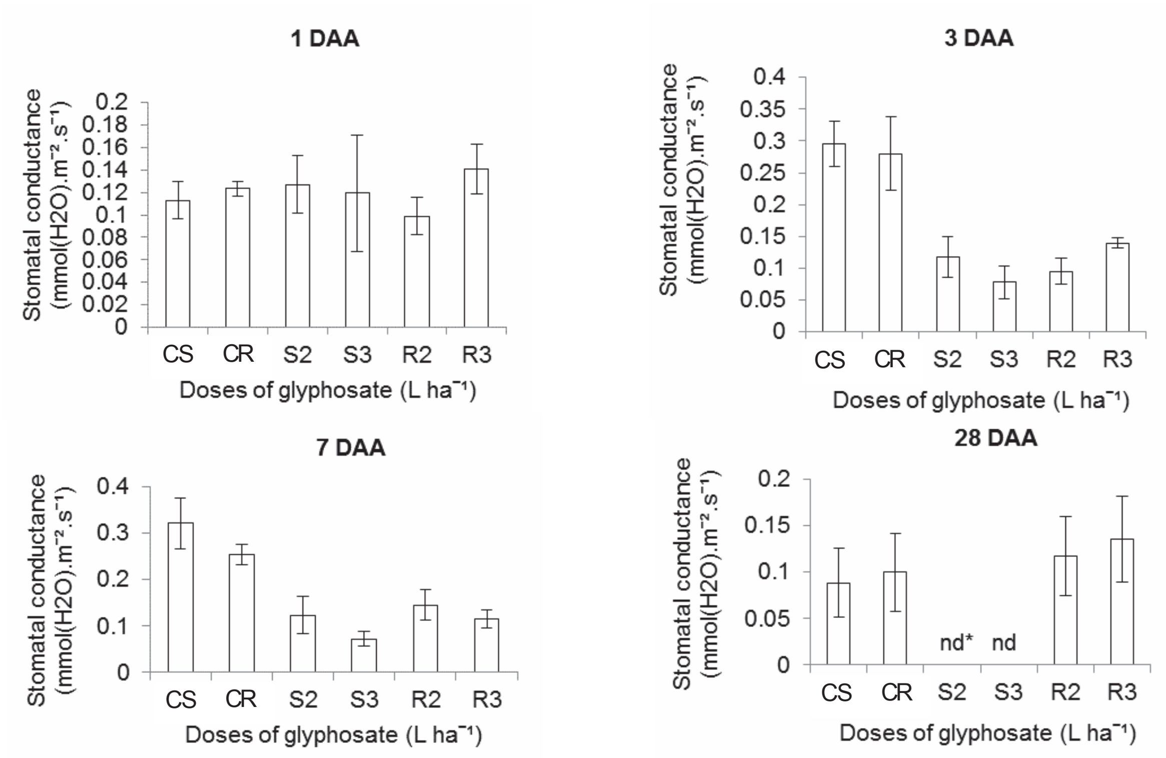
Although at 3 DAA the resistant population was breathing more than performing photosynthesis, at 7 DAA it recovered, presenting positive values. This fact was not observed in the population considered susceptible, since it continued to show negative values. In susceptible plants, glyphosate may inhibit the synthesis of chlorophyll and carotenoids, considered as the basic photosynthetic unit (Kaspary et al., 2014), and reduce assimilation of CO_2 (Ding et al., 2011), among others. The consequence of these effects can be seen at 28 DAA, when the susceptible population was completely controlled by the herbicide regardless of the dose applied and it was not possible to make measurements. The resistant population, however, remained with positive values similar to those of the resistant control.

Similar results were found by Santos et al. (2014) when evaluating the effects of glyphosate on the photosynthesis in horseweed resistant and susceptible to this herbicide. These authors observed that, at 3 DAA, both biotypes showed a reduction in the assimilation of CO_2 in relation to the control and this result was extended up to 10 DAA. But at 14 DAA, only the resistant one recovered. In addition, both susceptible and resistant biotypes did not present negative values, regardless of the evaluation period, which differs from the results verified here.

The reduction of CO_2 assimilation in the R population, even in a short time, can be considered advantageous in practical terms, since, in addition to decreasing the production of carbohydrates, when competing with a crop, the weed can have its growth reduced. In this way, the crop may have greater development, thus shading the weed, which hinders its growth. Silva et al. (2014) have observed a reduction in photosynthesis, stomatal conductance and transpiration in glyphosate resistant soybeans due to glyphosate resistant horseweed interference. Thus, any management that may hinder the development of the weed may be beneficial to the crop.

As with CO_2 assimilation, stomatal conductance has not undergone significant changes on 1 DAA (Figure 3). However, at 3 DAA there was a significant reduction both for the susceptible population as for the resistant one, extending up to 7 DAA. Yanniccari et al. (2012a) have observed greater reduction in the stomatal conductance in biotypes of *L. rigidum* susceptible to glyphosate in relation to the resistant ones at 7 DAA. When the plant is under stress, it tends to close the stomata as a defense mechanism against water loss, thus increasing resistance, which reduces stomatal conductance (Taiz and Zeiger, 2013), a phenomenon that can be observed after the application of herbicides. Some studies have shown this effect after the application of glyphosate (Zobiole et al., 2010a; Silva et al., 2014). At 28 DAA, the resistant population showed conductance similar to that of the controls, which demonstrates its recovery power.

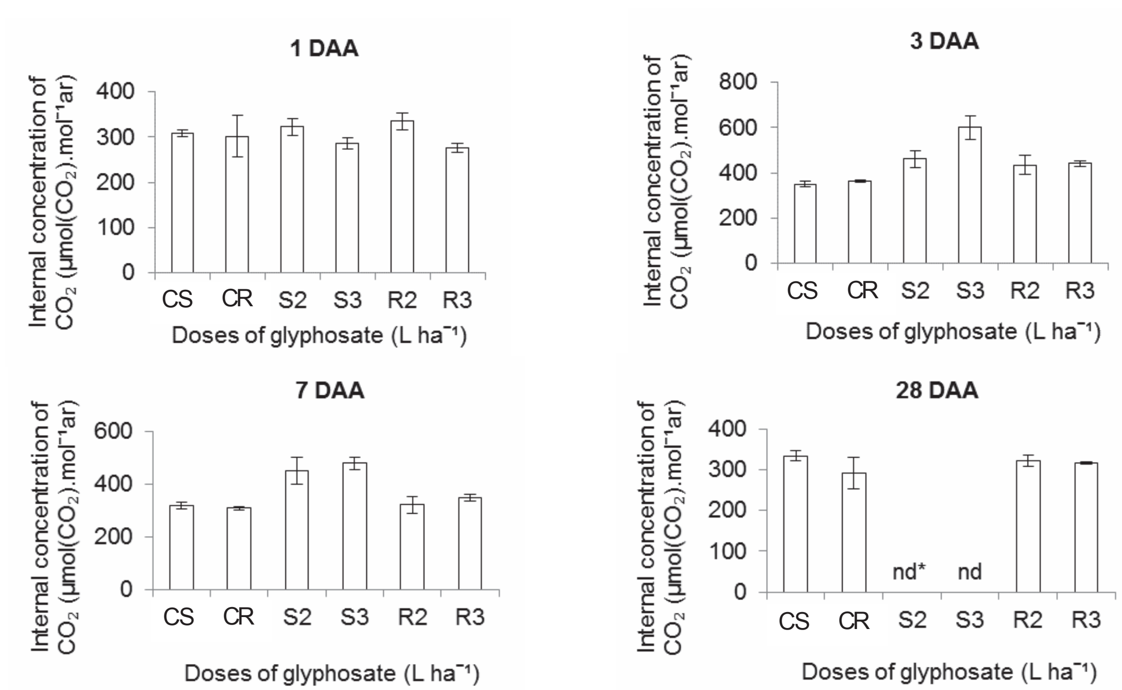
It was observed at 3 days after application of glyphosate that there was an increase in the internal concentration of CO_2 (Ic) in both populations (Figure 4). The population susceptible at 1,080 g a.e. ha^{-1} (S3) showed the higher value of Ic and this result was similar to the one found at 7 DAA. In *Abutilon theophrasti* medikus, Fuchs et al. (2002) have shown that the reduction of stomatal conductance was accompanied by the reduction of Ic after application of glyphosate, which at 5 DAA reached zero – results contrary to those observed here. Concenço et al. (2008) have observed that the biotype of resistant ryegrass, under competitive conditions, presented higher concentrations of CO_2 in the leaf mesophyll compared to the susceptible biotype, in an



The vertical bars indicate the confidence interval. Botucatu, SP. * nd: non-detectable.

Control susceptible (CS), control resistant (CR), susceptible population submitted to 720 g a.e. ha^{-1} (S2), susceptible population submitted to 1,080 g a.e. ha^{-1} (S3), resistant population submitted to 720 g a.e. ha^{-1} (R2), resistant population submitted to 1,080 g a.e. ha^{-1} (R3).

Figure 3 - Stomatal conductance (mmolH_2O m^{-2} s^{-1}) in susceptible and resistant ryegrass plants after the application of herbicide glyphosate at 1, 3, 7 and 28 DAA.



The vertical bars indicate the confidence interval. Botucatu, SP. * nd: non-detectable

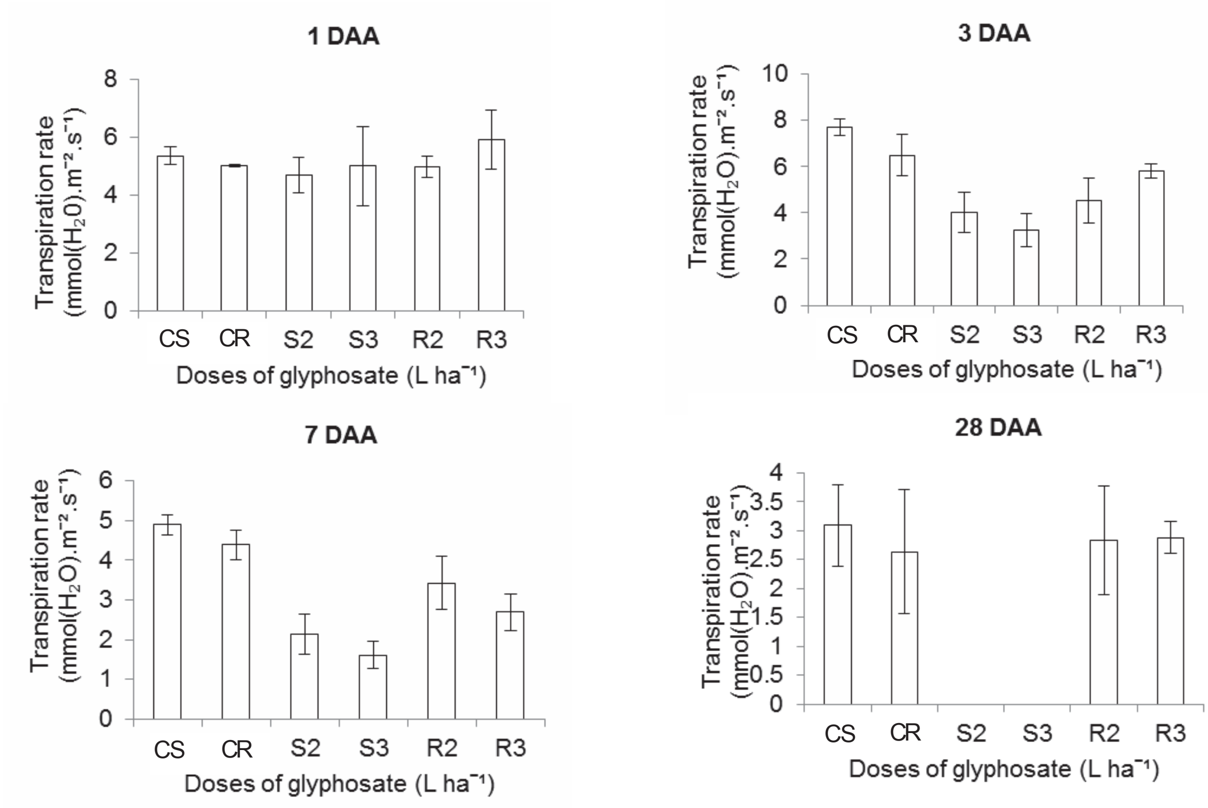
Control susceptible (CS), control resistant (CR), susceptible population submitted to 720 g a.e. ha^{-1} (S2), susceptible population submitted to 1,080 g a.e. ha^{1} (S3), resistant population submitted to 720 g a.e. ha^{-1} (R2), resistant population submitted to 1,080 g a.e. ha^{-1} (R3).

Figure 4 - Internal concentration of CO_2 in the sub-stomatal chamber (µmol(CO_2) mol^{-1} ar) in susceptible and resistant ryegrass plants after the application of herbicide glyphosate at 1, 3, 7 and 28 DAA.

attempt to reduce the stress generated by the competition with the plants of both the same biotype and the opposite one. Ic is considered a physiological variable influenced by environmental factors, such as water availability and light. The internal concentration of CO_2 in the leaf reflects the availability of substrate for photosynthesis, where during the gas exchanges the stomata regulate this concentration, keeping it constant (Farquhar and Sharkey, 1982). After suffering stress, the plant can modify its constant pattern, reducing or increasing Ic. Again at 28 DAA, the R2 population presented values similar to those of the control.

Glyphosate drastically reduced the transpiration rate in the susceptible population at 3 DAA in relation to the control (Figure 5). However, the same was not observed for the resistant population. This behavior was maintained until the 7 DAA and at 28 DAA the R population presented values similar to those of the control. Transpiration is the process of loss of water by the vegetable in the form of vapor through the stomatal pores, which at the same time allow the entrance of CO_2 (Vavasseur and Raghavendra, 2005). Depending on the type of stress, the plant closes the stomata in order to avoid water loss. As observed in Figure 3, after application of glyphosate there was a reduction of stomatal conductance. With this, the stomata closed, hindering the exit of water, thus reducing perspiration.

The water-use efficiency (WUE) showed a similar pattern to the assimilation of CO_2 (A) after the application of glyphosate for both populations and in all times assessed (Figure 6). WUE is the amount of carbon fixed in the photosynthetic process by the amount of water lost in the transpiration process, i.e., dry matter mass produced per gram of water transpired. This fact explains why this variable has negative values. Under normal conditions, the plant opens its stomata, allowing the exit of water in the form of vapor and at the same time the entry of CO_2, which shall be used for the formation of carbohydrates. However, it has previously been shown



The vertical bars indicate the confidence interval. Botucatu, SP. * nd: non-detectable

Control susceptible (CS), control resistant (CR), susceptible population submitted to 720 g a.e. ha^{-1} (S2), susceptible population submitted to 1,080 g a.e. ha^{-1} (S3), resistant population submitted to 720 g a.e. ha^{-1} (R2), resistant population submitted to 1,080 g a.e. ha^{-1} (R3).

Figure 5 - Transpiration rate (µmol(H_2O) m^{-2} s^{-1}) in susceptible and resistant ryegrass plants after the application of herbicide glyphosate at 1, 3, 7 and 28 DAA.

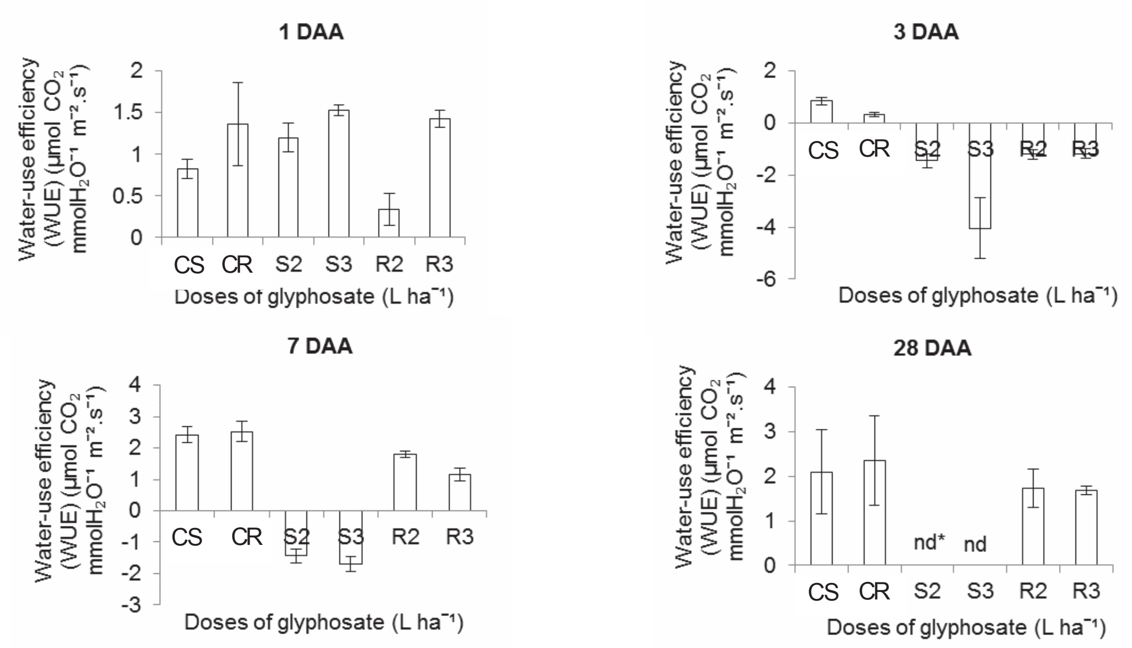
that after the application of glyphosate there was a reduction in CO_2 assimilation and transpiration in both populations but the resistant one demonstrated its recovery power. As expected, plants considered susceptible were killed and at 3 DAA they would already present negative values, especially at 1,080 g a.e. ha^{-1}. On the other hand, the resistant population, although showing negative values at 3 DAA, at 7 DAA recovered.

Vargas et al. (2014) have not found differences in water-use efficiency (WUE) at 3 DAA of glyphosate between the biotypes of horseweed susceptible and resistant to it. However, at 7 DAA there was a large difference. Biotype R showed higher WUE, keeping values significantly equal to the ones of the control up to 14 DAA. Zobiole et al. (2010a) have concluded that soybeans resistant to glyphosate reduced WUE after the application of this herbicide. Despite showing reduction of WUE, at 28 DAA the resistant ryegrass showed values similar to the ones of the control. Water-use efficiency (WUE) is directly related to stomatal opening time because while the plant absorbs CO_2, water is lost by transpiration with variable intensity, following a current of water potentials between the leaf and the atmosphere (Pereira-Neto, 2002). Throughout the evaluations, the resistant population assimilated CO_2 and transpired more than the susceptible population, thus presenting greater WUE. The data presented in this experiment were negative for this variable although the values found by other authors were positive. This is probably due to the degree of susceptibility and the type of species involved.

Like in WUE, carboxylation efficiency presented a pattern similar to that of the assimilation of CO_2 (Figure 7). This variable indirectly represents the activity of RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase). This enzyme is responsible for incorporating the CO_2 absorbed by stomata into ribulose-1,5-biphosphate (RuBP) in the Calvin cycle (Taiz and Zeiger, 2013). Servaites et al. (1987) have proposed that glyphosate induces depletion of carbon or phosphate or both from the carbon reduction cycle, reducing the regeneration of RuBP and, consequently, photosynthesis. At 3 DAA, the results showed that RuBP regeneration limited glyphosate-induced photosynthesis in the two populations. Under conditions limiting RuBP regeneration, the RuBisCO enzyme uses RuBP faster than it is synthesized. Thus, CO_2 assimilation does not respond to the increase in Ic (Caemmerer and Farquhar, 1981). This fact can be observed in this experiment because after the application of glyphosate there was an increase of internal CO_2 concentration (Figure 4) but the plant did not take advantage of this event since the rate of CO_2 assimilation was reduced after the application of this herbicide (Figure 2).

At 7 DAA, population R returned to positive values, but much lower than those of the control, especially at 1,080 g a.e. ha^{-1}. Even though the population was resistant, glyphosate reduced carboxylation efficiency. Results found by De Maria et al. (2006) in leaves of *Lupinus albus* have shown a 26% reduction in RuBisCO activity five days after application of glyphosate. Zobiole et al. (2010b) have also found a reduction of RuBisCO activity in soybean resistant to glyphosate after its application. With this, there was a reduction of the biomass since the diffusion of CO_2 to the chloroplast is essential for the photosynthesis. At 28 DAA, again the resistant population showed recovery, presenting values close to those of the control.

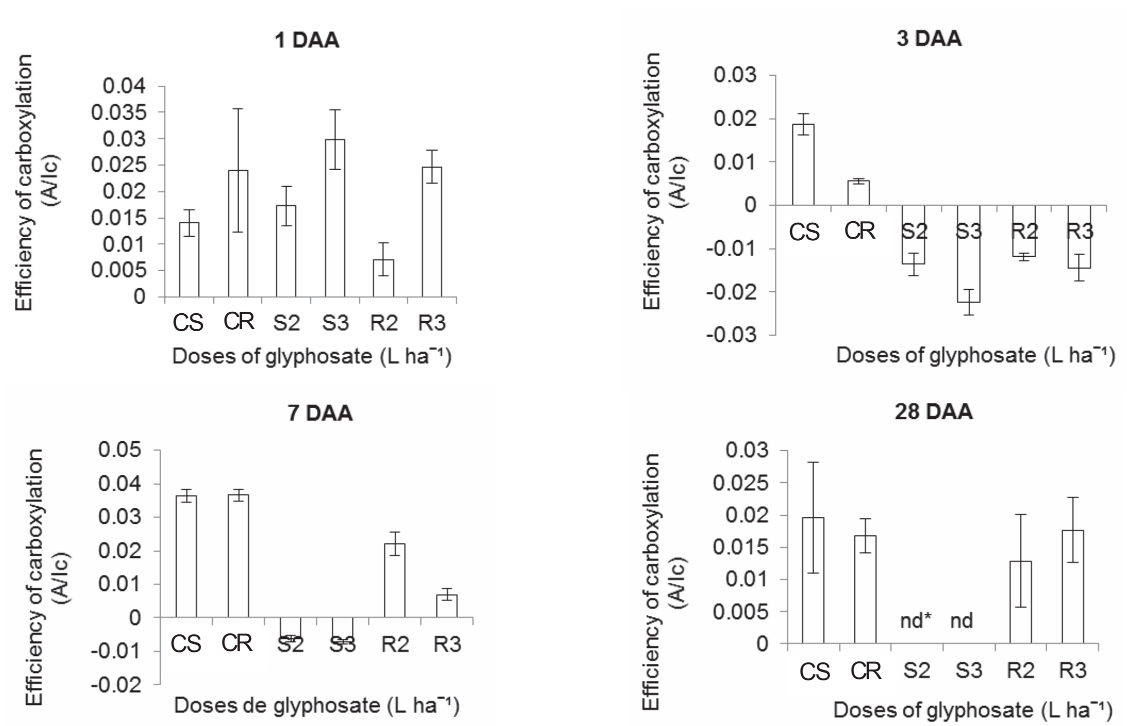
One of the hypotheses that has advanced to explain the effect of glyphosate on carbon metabolism is related to the carbon flow to the shikimic acid pathway due to the disruption of this one. The effect caused by glyphosate on this pathway leads to an accumulation of shikimic acid, becoming a carbon drainage (Duke and Powles, 2008). Arogenate is a byproduct of chorismate and an inhibitor of DAHP (3-Deoxy-D-arabinoheptulosonate 7-phosphate) synthase, the first enzyme of the shikimate pathway (Siehl, 1997). According to this author, the inhibition of the synthesis of DAHP synthase by the arogenate is the key in the regulation of the shikimate pathway. With the inhibition of the EPSPs enzyme caused by glyphosate, there is an interference in carbon entry into the shikimate pathway due to increased DAHP synthase activity (Devine et al., 1983). Increase of this enzyme is due to the low levels of arogenate, because the production of its precursor has been reduced. Thus, the enzyme continues to act, causing high levels of shikimic acid. The amount of shikimic acid accumulated by the disruption of the pathway represents a strong carbon drainage due to the deviation of erythrose-4-phosphate, which would be used in the regeneration of ribulose-1,5-bisphosphate, drastically reducing photosynthesis (Geiger et al., 1986; Servaites et al., 1987; Siehl, 1997).



The vertical bars indicate the confidence interval. Botucatu, SP. * nd: non-detectable

Control susceptible (CS), control resistant (CR), susceptible population submitted to 720 g a.e. ha^{-1} (S2), susceptible population submitted to 1,080 g a.e. ha^{-1} (S3), resistant population submitted to 720 g a.e. ha^{-1} (R2), resistant population submitted to 1,080 g a.e. ha^{-1} (R3).

Figure 6 - Water-use efficiency (WUE) (µmol CO_2 mmol H_2O^{-1}) in susceptible and resistant ryegrass plants after the application of herbicide glyphosate at 1, 3, 7 and 28 DAA.



The vertical bars indicate the confidence interval. Botucatu, SP. * nd: non-detectable.

Control susceptible (CS), control resistant (CR), susceptible population submitted to 720 g a.e. ha^{-1} (S2), susceptible population submitted to 1,080 g a.e. ha^{-1} (S3), resistant population submitted to 720 g a.e. ha^{-1} (R2), resistant population submitted to 1,080 g a.e. ha^{-1} (R3).

Figure 7 - Efficiency of carboxylation (A/Ic) in susceptible and resistant ryegrass plants after the application of herbicide glyphosate at 1, 3, 7 and 28 DAA.

As expected, in the susceptible population the effects of glyphosate were intensified from 3 DAA in all variables analyzed. This is due to the slower translocation of the herbicide in the plant, which at 28 DAA caused irreversible damage to it, which was already dead, thus making its analysis impossible. In the case of the resistant population, at 3 DAA, in the variables analyzed, there were significant effects in relation to the control, showing that, even though it presented a high degree of resistance, the herbicide was able to affect its metabolism. However, from the 7 DAA it showed its recovery power and at 28 DAA it presented values similar to those of the control.

In view of the above, differences were observed in the physiological profile of ryegrass populations susceptible and resistant to glyphosate. The resistant biotype showed a high resistance factor, with a high capacity of recovery of the photosynthetic activity after the application of the herbicide, able to return to a physiological state similar to that for the plants that did not receive the product.

REFERENCES

Ahsan N. et al. Glyphosate-induced oxidative stress in rice leaves revealed by proteomic approach. **Plant Physiol Biochem.** 2008;46:1062-70.

Bostamam Y. et al. Rigid ryegrass (*Lolium rigidum*) populations containing a target site mutation in EPSPs and reduced glyphosate translocation are more resistant to glyphosate. **Weed Sci.** 2012;60:474-9.

Caemmerer S.V., Farquhar G.D. Some relationships between the biochemistry of photosynthesis and gas exchange pf leaves. **Planta**. 1981;153:376-87.

Collavo A., Sattin M. Resistance to glyphosate in *Lolium rigidum* selected in Italian perennial crops: bioevaluation, management and molecular bases of target-site resistance. **Weed Res.** 2012;52:16-24.

Concenço G. et al. Fotossintese de biótipos de Azevém sob condição de competição. **Planta Daninha.** 2008;26:595-600.

De María, N. et al. New insights on glyphosate mode of action in nodular metabolism: role of shikimate accumulation. **J Agr Food Chem.** 2006;54:2621-8.

Devine M.D., Bandeen J.D., Mckersie B.D. Temperature effects on glyphosate absorption, translocation, and distribution in quackgrass (*Agropyron repens*). **Weed Sci.** 1983;31:461-4.

Dewick P.M. The biosynthesis of shikimate metabolities. **Nat Prod Rep.** 1998;10:173-203.

Dionísio G. et al. Manejo de plantas daninhas resistentes ao glifosato no Brasil. In: Ríos A. **Viabilidad del glifosato em sistemas productivos sustentables**. Montevideo: INIA, 2013. p.111-8.

Ding W. et al. Physiological responses of glyphosate-resistant and glyphosate-sensitive soybean to aminomethylphosphonic acid, a metabolite of glyphosate. **Chemosphere.** 2011; 83:593-8.

Duke S.O., Powles D.O. Glyphosate: a once-in-a-century herbicide. **Pest Manag Sci.** 2008;64:319-25.

Farquhar G.D., Sharkey T.D. Stomatal conductance and photosynthesis. **Ann Rev Plant Phys.** 1982;33:317-45.

Flexas J. et al. The effects of water stress on plant respiration. In: Lambers H., Ribas-Cardo M. **Plant respiration:** from cell to ecosystem. Dordrecht: Springer, 2005. p.85-93.

Fuchs M.A. et al. Mechanisms of glyphosate toxicity I velvetleaf (*Abutilon theophrasti* medikus). **Pestic Biochem Phys.** 2002;74:27-39.

Geiger D.R., Kapiran S.W., Tucci M.A. Glyphosate inhibits photosynthesis and allocation of carbon to start in sugar beet leaves. **Plant Physiol.** 1986;82:468-72.

Gomes M.P. et al. Alteration of plant physiology and its by-product aminomethylphosphonic acid: an overview. **J Exp Bot.** 2014;65:4691-703.

Kaspary T.E. et al. Pigmentos fotossintéticos em azevém suscetível e resistente ao herbicida glyphosate. **Ci Rural**. 2014;44:1901-7.

Mateos-Naranjo E. et al. Effectiveness of glyphosate and imazamox on the controlo f the invasive cordgrass *Spartina densiflora*. **Ecotox Environ Safe.** 2009;72:1694-700.

Nandula V.K. et al. Glyphosate resistance in tal waterhamp (*Amaranthus tuberculatus*) from Mississipi is due to both target-site and nontarget-site mechanisms. **Weed Sci.** 2013;61:374-83.

Olesen C.F., Cedergreen N. Glyphosate uncouples gas exchange and chlorophyl fluorescence. **Pest Manag Sci.** 2010;66:536-42.

Orcaray L. et al. Impairment of carbono metabolismo induced by the herbicide glyphosate. **J Plant Phys.** 2012;169:27-33.

Pereira-Neto A.B. Crescimento e desenvolvimento. In: Wachowicz C.M., Carvalho R.I.N. **Fisiologia vegetal**: produção e pós-colheita. Curitiba: Champagnat, 2002. p.17-42.

Roman E.S. et al. Resistência de azevém (*Lolium multiflorum*) ao herbicida glyphosate. **Planta Daninha.** 2004;22:301-6.

Sammons R.D., Gaines T.A. Glyphosate resistance: state of knowledge. **Pest Manag Sci.** 2014;70:1367-77.

Santos F.M. et al. Effects of glyphosate on the physiological parameters of horseweed. **Rev Bras Cienc Agr.** 2014;9:519-25.

Servaites J.C., Tucci M.A., Geiger D.R. Glyphosate effects on carbon assimilation, ribulose bisphosphate carboxylase activity, and metabolite levels in sugar beet leaves. **Plant Phys.** 1987;85:370-4.

Siehl D. Inhibitors of EPSPs synthase, glutamine synthetase and histidine synthesis. In: Roe R., Burton J., Kuhr R. **Herbicide activity**: toxicology, biochemistry and molecular biology. Amsterdam: IOS Press, 1997. p.37-67.

Silva D.R.O. et al. Glyphosate-resistant hairy fleabane competition in RR soybean. **Bragantia**. 2014;73:451-7.

Sociedade Brasileira da Ciência das Plantas Daninhas – SBCPD. **Procedimentos para instalação, avaliação e análise de experimentos com herbicidas**. Londrina: 1995. 42 p.

Streibig J.C. Herbicide bioassay. **Weed Res.** 1988;28:479-84.

Taiz L., Zeiger E. **Fisiologia vegetal**. 5. ed. Porto Alegre: Artmed, 2013. 820 p.

Vargas L. et al. Alteração das características biológicas dos biótipos de azevém (*Lolium multiflorum*) ocasionada pela resistência ao herbicida glyphosate. **Planta Daninha.** 2005; 23:153-60.

Vargas L. et al. Glyphosate influence on the physiological parameters of *Conyza bonariensis* biotypes. **Planta Daninha**. 2014;32:151-9.

Vavasseur A., Raghavendra A.S. Guard cell metabolism CO_2 sensing. **New Phytol.** 2005;165:665-82.

Velini E.D. et al. Modo de ação do glyphosate. In: Velini, E.D. et al. **Glyphosate**. Botucatu: FEPAF, 2009. 496 p.

Yanniccari M. et al. Glyphosate effects on gas exchange and chlorophyll fluorescence responses of two *Lolium perenne* L. biotypes with differential herbicide sensitivity. **Plant Phys Biochem.** 2012a;57:210-7.

Yanniccari M. et al. Glyphosate resistance in perennial ryegrass (*Lolium perenne* L.) from Argentina. **Crop Prot.** 2012b;32:12-6.

Zobiole L.H.S. et al. Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate. **Pestic Biochem Phys.** 2010a;97:182-93.

Zobiole L.H.S. et al. Effetcs of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans. **Appl Soil Ecol.** 2010b;44:176-80.