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Article

# QUICK TEST FOR DETECTING GLYPHOSATE-RESISTANT RYEGRASS SEEDS

*Teste Rápido de Detecção de Resistência à Glifosato em Sementes de Azevém*

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ABSTRACT - The commercialization of ryegrass seeds with the presence of resistant biotypes is a serious issue that increses the dispersion of resistance to new areas without this problem. Therefore, this study aimed to develop a quick test to detect susceptible and resistant seeds to the glyphosate herbicide in order to identify contaminated seed lots with glyphosate resistance. Three experiments were carried out, one in greenhouse and the other two in the seed laboratory. First, the resistance factor (RF) was determined by a dose-response curve experiment in biotypes suspected to be resistant and susceptible to glyphosate. Then, the germination test was conducted with the selected biotypes under increasing glyphosate rates (0, 3.5, 6.9, 13.9, 27.8, 55.5, 111, 222, 445, 890, and 1,780 mg a.e. $L^{-1}$), as the second experiment. The third experiment was made to verify the methodological efficiency of the germination test with glyphosate to identify different contamination ratios (0, 4, 12, 36, and 100%) of the resistant biotype in the seed lot. The different levels of susceptibility of the biotypes to glyphosate were confirmed by the RF of 154.7 based on $C_{50}$. Germination of the susceptible biotype was inhibited more than 99% by the rate of 127 mg a.e. $L^{-1}$ while the resistant biotype was a little affected. The germination test with 127 mg a.e. $L^{-1}$ of glyphosate showed contaminations of 2, 5, 19, 39, and 86% in lots with 0, 4, 12, 36, and 100% of contaminated seeds, respectively. This methodology can detect glyphosate susceptible and resistant seeds and identify contaminated seed lots with resistant glyphosate biotypes.

**Keywords:** *Lolium multiflorum*, germination, herbicide, methodology.

*RESUMO - A comercialização de sementes de azevém com a presença de biótipos resistentes promove a dispersão da resistência para áreas isentas do problema. Diante disso, o objetivo deste trabalho foi desenvolver teste rápido para diferenciação de sementes suscetíveis e resistentes ao herbicida glifosato, visando identificar lotes contaminados com sementes de biótipos resistentes. Foram realizados três experimentos, sendo um em casa de vegetação e dois em laboratório. No primeiro, determinou-se o fator de resistência (FR) em experimento de curva dose-resposta em biótipos com suspeita de serem resistente e suscetível. No segundo, realizou-se teste de germinação dos biótipos, sob doses crescentes de glifosato (0; 3,5; 6,9; 13,9; 27,8; 55,5; 111; 222; 445; 890; e 1.780 mg e.a. $L^{-1}$. No terceiro, avaliou-se a eficiência da metodologia do teste de germinação com glifosato para identificação de diferentes proporções de contaminação (0, 4, 12, 36 e 100%) do biótipo resistente no lote de sementes. A suspeita dos biótipos foi comprovada em razão dos diferentes níveis de suscetibilidade ao herbicida glifosato, sendo o FR de 154,7 com base na $C_{50}$. A dose de 127 mg e.a. $L^{-1}$ inibiu em mais de 99% a germinação do biótipo suscetível, enquanto o biótipo resistente foi pouco afetado. A partir do teste de germinação, identificou-se contaminação de 2, 5, 19, 39 e 86%*

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*em lotes com a presença de 0, 4, 12, 36 e 100% de sementes contaminadas, respectivamente, quando expostas à dose de 127 mg e.a. $L^{-1}$. A metodologia testada possibilitou diferenciar sementes suscetíveis e resistentes ao herbicida glifosato e identificar lotes de sementes contaminadas com biótipos resistentes.*

**Palavras-chave:** *Lolium multiflorum*, germinação, herbicida, metodologia.

## INTRODUCTION

The South of Brazil shows low temperatures from May to September, which limits the forage production of native grasses because it is mainly formed by summer species. This forage deficit leads the farmers to search for more alternatives to overcome this problem and the use of winter grass crops rise as a viable option to keep high zootechnical indices (Hellbrugg et al., 2008).

In order to solve this forage trouble, ryegrass (*Lolium multiflorum*) cultivation is an alternative due to the resistance to low temperatures, high bromatological quality and good dry matter production (Freitas et al., 2003; Noro et al., 2003). However, it is considered the main weed in winter cereals and makes harder the establishment of summer crops, because of the resistance to several herbicides sites of action. In addition, the seed bank persistence and the irregular seed production affect the adoption of efficient managment practices to reduce the weed interference in crop development and productivity (Tironi et al., 2014).

There are ryegrass biotypes with simple and multiple resistances to herbicides inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) enzyme, acetyl coenzyme-A carboxylase (ACCase), and acetolactate synthase (ALS) enzymes (Heap, 2017). Also, crosses between resistant and susceptible populations are getting this situation worst, as a result of the dissemination via pollen of the resistance genes to the offspring (Vargas et al., 2007; Mariani et al., 2015). Thus, integrated weed management is needed to be taken to avoid the overspread of the resistant biotype in areas already infested and/or the dispersion to new regions.

Among these integrated actions, preventive management should be encourage as an additional tool to reduce the dissemination of resistant seeds since it aims to avoid the introduction of seeds with resistant biotypes in areas not contaminated yet. However, the current seed rules for marketing do not guarantee a seed lot free of resistant biotypes, because there is not appropriated and available procedures to quickly verify a contaminated seed lot.

The present methods to detect resistance in weeds are performed by the dose-response curve experiments at early development stages of the weed. However, this methodology is not functional in seed laboratories for routine testing, because it spends a lot of time and requires additional resources. Therefore, the aim of this study was to develop a quick test for detecting glyphosate-resistant ryegrass biotypes in order to identify contaminated seed lots with resistant biotypes.

## MATERIAL AND METHODS

This study was conducted at the Seed Teaching Laboratory of FAEM/UFPel and in a greenhouse of the CEHERB/FAEM/UFPEL in 2016 and 2017. First, suspected ryegrass seeds of resistant and susceptible were previously collected in the field in Passo Fundo, RS (28’13"49° S and 52’24"23° W), sown in trays, and sprayed them with glyphosate at 2,160 g a.e. $ha^{-1}$ (Glifosato Atanor 48®) rate in the 3 to 4 leaf stage. The surviving plants were considered resistant and the biotypes in which all plants died were considered susceptible. From these results, crosses between susceptible biotypes (Sc × Sc) and between resistant biotypes (Rr × Rr) were carried out in individualized and sealed greenhouses in order to avoid contamination with foreign pollen. Thus, high homozygosity of susceptible and resistant seeds were obtained, being stored at 10 °C and 40% relative humidity for five months for subsequent use in the experiments, as described below.

### Experiment 1 – Dose-response curve of susceptible and resistant biotypes

Seeds of the biotypes were sown on white blotter paper to germinate at the seed laboratory. After germination, a single seedling of susceptible and resistant biotypes was transplanted into

750 mL capacity pots filled with a 3:1 mixture of soil and commercial substrate. Plants were grown until the beginning of the tillering stage and then the glyphosate was applied. The experimental design was a completely randomized design in a factorial scheme (2 × 11) with four replications. Factor A was composed of susceptible and resistant biotypes, and factor B increasing of glyphosate rates (0, 33.7, 67.5, 135, 270, 540, 1,080, 2,160, 4,320, 8,640, and 17,280 g a.e. $ha^{-1}$), using the 2,160 g a.e. $ha^{-1}$ rate as the commercial standard rate.

The herbicide was applied with a calibrated $CO_2$ pressurized backpack sprayer to reach a spray solution volume of 120 L $ha^{-1}$. Control at 28 days after application (DAA) and shoot dry weight at 28 DAA were the variables assessed. Scores from zero to 100 in a percentage scale were assigned in the control assessment, which zero was considered the absence of control and 100 the plant death (SBCPD, 1995). Dry weight assessment was performed by cutting the plants close to the soil surface, packing them in properly labeled paper bags, and drying them in a forced air circulation oven at 60 ± 5 °C until constant weight.

Data were analyzed for normality (Shapiro-Wilk test), with a subsequent analysis of variance (ANOVA) ($p \leq 0.05$). Regression analysis was performed for significant values between factors biotype and rate, also the values of the rate required to 50% ($C_{50}$) control and the rate required to reduce dry weight production ($GR_{50}$) by 50% of the biotypes were compared, by the means of arithmetic calculation of the value needed to promote 50% of response, according to the parameters generated in the equations. The regression analysis was performed by fitting the data to the sigmoidal logistic regression equation $y = a/[1 + (x/x0)^b]$, which y is the percentage of control or reduction of dry weight, x is the herbicide rate, and a, x0 and b are parameters of the equation, which a is the difference between the maximum and minimum points of the curve, x0 is the rate that provides 50% of response of the variable, and b is the slope of the curve. From the values of $C_{50}$ and $GR_{50}$, the resistance factor (RF), with a confidence interval of ≥0.95, for the studied biotypes was performed.

**Experiment 2 – Dose-response curve in germination test to identify susceptible and resistant biotypes to the glyphosate herbicide**

Following the criterions for normal and abnormal seedling, and dead seeds described in the Rules for Seed Testing (RST) (Brasil, 2009), the rates of glyphosate herbicide were used to detect susceptible and resistant biotypes. For this, treatments were arranged in a factorial scheme (2 × 11) in a completely randomized design with four replications of 50 seeds. Susceptible and resistant biotypes were factor A, whereas increasing and exponential glyphosate rates (0, 3.5, 6.9, 13.9, 27.8, 55.5, 111, 222, 445, 890, and 1,780 mg a.e. $L^{-1}$) were factor B. Two white blotter paper were used as a substrate for germination, which was weighed and placed in a gerbox box. The paper of each box was soaked by adding the corresponding solution to each herbicide rate, with a volume of 2.5 times the mass of the paper used. The biotypes were sown on the white blotter paper, placed them for germination in a BOD (Biochemical Oxygen Demand), at daytime temperature of 30 °C (8 hours) and nighttime temperature of 20 °C (16 hours). The assessments were performed at 5 and 14 days after sowing (DAS). Only normal seedlings were randomly chosen from the first count of germination (5 DAS), root (RL) and shoot length (SL) and the total dry weight (DW) were determined per seedling. Then, germination (G), abnormal seedlings (AS), and dead seeds (DS) were measured at 14 DAS. The means of RL and SL were expressed as centimeters, DW as milligrams, and G, AS, and DS as a percentage. The data were assessed for normality and submitted to ANOVA ($p \geq 0.05$) and, if significant, regression analysis was made ($p \geq 0.05$).

**Experiment 3 – Verification of the germination test efficiency to identify susceptible and resistant seeds to glyphosate**

In order to evaluate the germination test efficiency to detect resistant seeds, known mixtures between resistant and susceptible ryegrass were made. Ratios of 0, 4, 12, 36, and 100% of resistant ryegrass seeds as were established as treatments, in a completely randomized design with four replications. The ratios composed a mean sample of 60 grams, which was reduced to 6 grams in a soil divisor to constitute the work sample, according to RST (Brasil, 2009). Subsequently, the germination test was carried out using white blotter paper as substrate soaked 2.5 times its

mass with 127 mg a.e. $L^{-1}$ of glyphosate. The rate of 127 mg a.e. $L^{-1}$ was used because provided an inhibition of germination higher than 99% in the susceptible biotype to glyphosate, according to the calculated values from experiment 2. The conditions were the same as described in the previous experiment and the variables analyzed in the germination test were G, AS, and DS 14 DAS, which were expressed as a percentage. The expected values were compared with those observed by means of the coefficient of variation in order to demonstrate the dispersion in relative terms of their mean value observed between the replications.

## RESULTS AND DISCUSSION

The results and discussion will be presented following the sequence of activities detailed in the section Material and Methods.

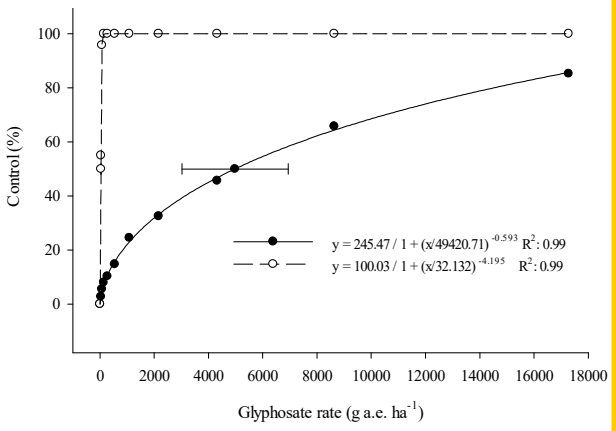
### Experiment 1 – Dose-response curve of susceptible and resistant biotypes

ANOVA indicated an interaction between the factors tested for all variables analyzed in experiments 1 and 2. A satisfactory fit of the data was performed to the sigmoidal regression model, with a 0.99 coefficient of determination ($R^2$) (Figure 1). A lower control of the resistant ryegrass biotype was observed when compared to the susceptible ryegrass biotype, with a control over 90% only 28 DAA at the highest glyphosate rate (Figure 1). On the other hand, for the susceptible biotype, the lowest rate resulted in 55% control and above 95% of control at 67.5 g a.e. $ha^{-1}$, which was equivalent to 3% of the recommended herbicide rate. Vargas et al. (2004) and Galvan et al. (2015) reported similar results, which the susceptible biotype was controlled 100% using 0.25 times the registration rate, but for the resistant biotype, a maximum of 45% control was reached, when the recommended rate were applied eight times.

The required rate to provide 50% of control ($C_{50}$) of the susceptible biotype was 32.2 g a.e. $ha^{-1}$, whereas for the resistant biotype was 4975.0 g a.e. $ha^{-1}$ (Table 1). Therefore, the rate required to control 50% of the resistant biotype is 154.7 times higher when is compared to the rate required to obtain the same control for the susceptible biotype. Based on the value of the calculated resistance factor (RF) (154.7), is considered high and significant due to the absence of overlapping confidence intervals in relation to the susceptible biotype, thus the high level of resistance of this biotype is confirmed (Figure 1).

The data of DW corroborate the control results for both biotypes, which showed a lower DW reduction for the resistant biotype up to the rate of 8,640 g a.e. $ha^{-1}$ (Figure 2). In general, from the rate of 135 g a.e. $ha^{-1}$, a reduction of DW above 80% was observed for the susceptible biotype, indicating an efficient control of the biotypes at low rate. Thus, to reduce 50% of the dry weight ($GR_{50}$) of the resistant biotype was needed an herbicide rate of 2,000.0 g a.e. $ha^{-1}$, approximately 99% higher than the susceptible biotype (0.3 g a.e. $ha^{-1}$) (Table 2).

Based on the values of $GR_{50}$ for RF, a difference was observed between biotypes due

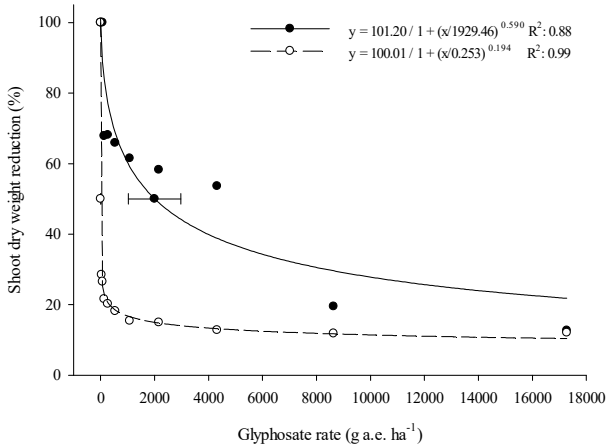



Dots represent the mean values of replications and the horizontal bars represent the confidence intervals for the rate that causes 80% control, with 95% significance.

***Figure 1*** - Control (%) of resistant (●) and susceptible (O) ryegrass biotypes in response to the application of different rates of the glyphosate herbicide assessed at 28 DAT.

***Table 1*** - Herbicide rate that promotes a 50% control of the population ($C_{50}$) and resistance factor (RF) of ryegrass biotypes in response to the application of different rates of the glyphosate herbicide assessed at 28 DAT

| Biotype | $C_{50}$ | | RF |
|---|---|---|---|
| | g. a.e. $ha^{-1}$ | 95% CI | |
| Susceptible | 32.2 | 31.4–33.0 | – |
| Resistant | 4975.0 | 3015.1–6935.0 | 154.7 |




Dots represent the mean values of replications and the horizontal bars represent the confidence intervals for the rate that causes 80% reduction in dry weight, with 95% significance.

***Figure 2*** - Shoot dry weight reduction (%) of resistant (●) and susceptible (O) ryegrass biotypes in response to the application of different rates of the glyphosate herbicide assessed at 28 DAT.

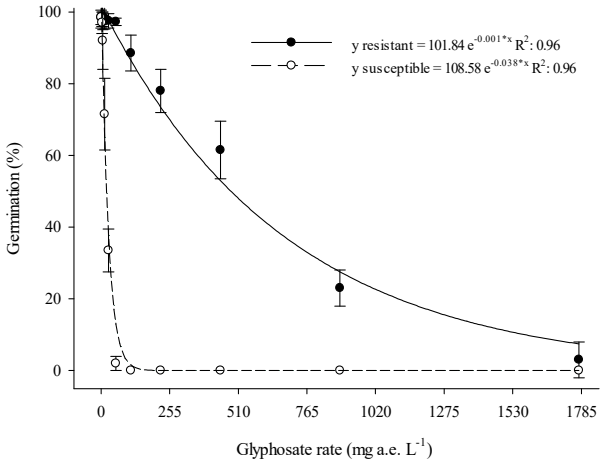
***Table 2*** - Herbicide rate needed to reduce dry weight production by 50% ($GR_{50}$) and resistance factor (RF) of ryegrass biotypes in response to the application of different rates of the glyphosate herbicide assessed at 28 DAT

| Biotype | $GR_{50}$ | | FR |
|---|---|---|---|
| | g. a.e. $ha^{-1}$ | 95% IC | |
| Susceptible | 0.3 | 0.14–0.36 | – |
| Resistant | 2000 | 1024.7–2975.3 | 6666.7 |

to the absence of overlap of confidence intervals, which RF value was 6,666.7 (Figure 2; Table 2). Therefore, the biotype is resistant to glyphosate since according to Heap (2017), one of the criteria to document the resistance is when the RF is equal or higher than 10, based on $C_{50}$ or $GR_{50}$. This high RF corroborates the results found in the literature, especially when the mechanism of resistance is related to overexpression or mutation in the EPSPS gene (Ghanizadeh et al., 2014, 2016; Salas et al., 2015). However, due to the high resistance factor, it is supposed that more than one mechanism of resistance is occurring: one related to the action site and another not related to the site of the herbicide action (Sammons and Gaines, 2014).

## Experiment 2 – Dose-response curve in germination test to identify susceptible and resistant biotypes to the glyphosate herbicide

Results from germination were fitted to the two-parameter exponential decreasing model y = a*e("b*x), and 0.96 of $R^2$ value (Figure 3). Substituting the values of y, a 10% reduction was observed for germination of the resistant biotype when the substrate was soaked in 127 mg a.e. $L^{-1}$ of glyphosate, while the susceptible biotype reduced germination by more than 99% (Figure 3).

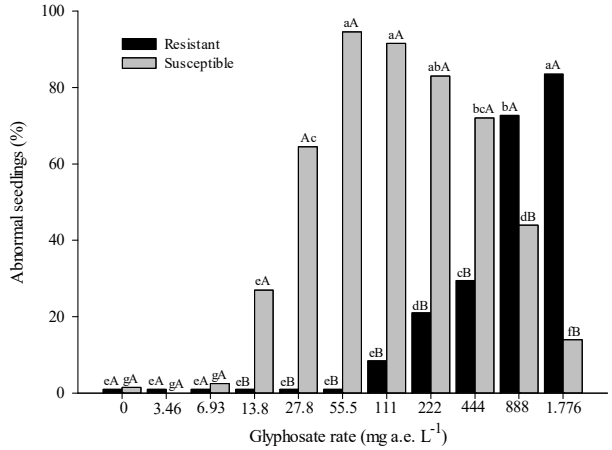



Dots represent the mean values of replications of each biotype at each rate and bars represent their respective confidence intervals ($p<0.05$).

***Figure 3*** - Germination (%) of resistant (●) and susceptible (O) biotypes exposed to increasing glyphosate rates.

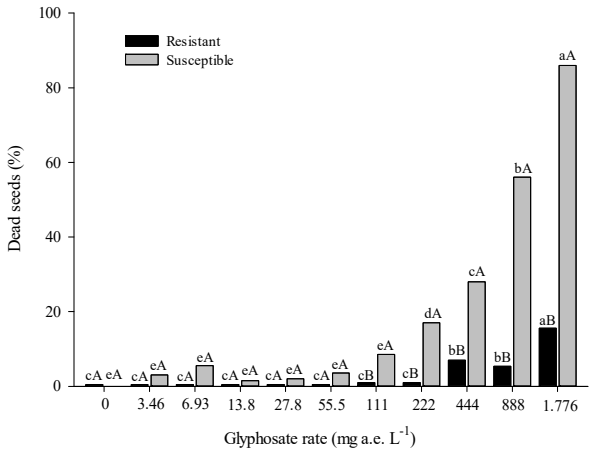
At the rate of 127 mg a.e. $L^{-1}$, the 10% of the resistant biotype that did not grow normal seedlings, died or developed abnormal seedlings due to the glyphosate herbicide in the substrate of the soaking solution. Although the plants are resistant, part of the population can remain in segregation, requiring several generations to reduce the value close to zero (Mariani et al., 2015). In the preliminary study, 10% of the plants of the resistant biotype did not survive at 2.160 g a.e. $L^{-1}$ of glyphosate applied at the 3-4 leaf stage (data not shown), which corroborates the results found for the germination test and confirms the 9/1 segregation between resistant and susceptible plants, respectively.

Data from the variables abnormal seedlings, dead seeds, and seedling dry weight, did not fit to the exponential model, so multiple comparisons were used to analyze (Figures 4, 5, and 6). In relation to the abnormal seedlings, both biotypes showed differences from the rate




Uppercase letters comparing biotypes at the same glyphosate rate and lowercase letters comparing glyphosate rate within each biotype do not differ from each other by the Tukey's test ($p<0.05$).

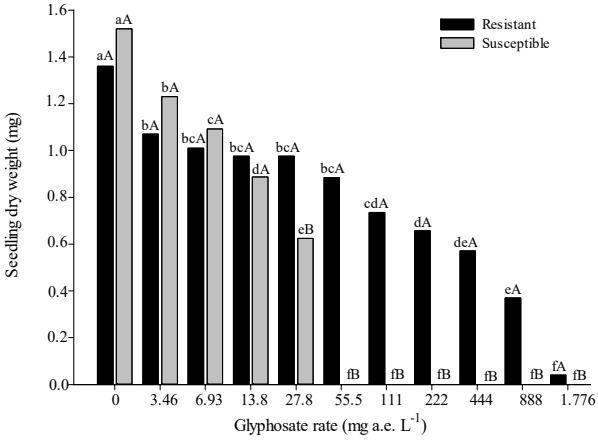
***Figure 4*** - Abnormal seedlings (%) of resistant (■) and susceptible (□) biotypes exposed to increasing glyphosate rates.




Uppercase letters comparing biotypes at the same glyphosate rate and lowercase letters comparing glyphosate rates within each biotype do not differ from each other by the Tukey's test ($p<0.05$).

***Figure 5*** - Dead seeds (%) of resistant (■) and susceptible (□) biotypes exposed to increasing glyphosate rates.

of 6.93 mg a.e. $L^{-1}$ of glyphosate. The susceptible biotype exhibited the highest number of abnormal seedlings, up to the rate of 55.5 mg a.e. $L^{-1}$, while the resistant biotype remained statistically equal to the control up to the rate of 111 mg a.e. $L^{-1}$ (Figure 4). A decrease for the number of abnormal seedlings of the susceptible biotype from the rate of 111 mg a.e. $L^{-1}$ due to an increase in the number of dead seeds was also observed (Figures 4 and 5). On the other hand, the number of abnormal seedlings for the resistant biotype increased from the rate of 222 mg a.e. $L^{-1}$, reaching 80% as the maximum value when the substrate was soaked with 1,776 mg a.e. $L^{-1}$ (Figure 4). Thus, the highest glyphosate rates (444, 888, and 1,776 mg a.e. $L^{-1}$) for the resistant biotype caused an increase in the number of dead seeds of 7, 5, and 16%, respectively (Figure 5).




Uppercase letters comparing biotypes at the same glyphosate rate and lowercase letter comparing glyphosate rates within each biotype do not differ from each other by the Tukey's test ($p<0.05$).

***Figure 6*** - Seedling dry weight (mg) of resistant (■) and susceptible (□) biotypes exposed to increasing glyphosate rates.

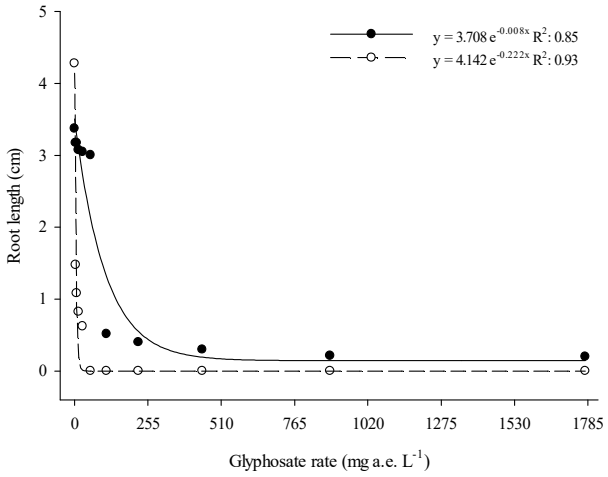
Glyphosate inhibits the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) by interrupting the shikimic acid pathway, responsible for the 20% of the carbon flux fixed by the photosynthetically active plants (Orcaray et al., 2010). From germination, seedlings develop numerous metabolic alterations, which make them autotrophic and independent from the reserves contained in the seeds and among these alterations the shikimic acid route is included (van Zanten et al., 2013). Thus, regardless of the biotype, more than 70% of the seeds emitted the radicle up to the rate of 444 mg a.e. $L^{-1}$ of glyphosate (Figures 3 and 4). However, the susceptible biotype had higher percentage of abnormal seedlings due to the negative effects caused by the inhibition of the EPSPs enzyme. These effects include a reduction of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) activity (Ahsan et al., 2008) and disorganization of the photosynthetic apparatus (María et al., 2005), resulting in induction of oxidative stress and lipid peroxidation (Ahsan et al., 2008).

Seedlings dry weight of susceptible and resistant biotypes was reduced by soaking the substrate and increasing the rate of glyphosate (Figure 6). For the susceptible biotype, the reduction was

major than the resistant biotype, which 55.5 mg a.e. $L^{-1}$ rate was enough to completely inhibit the weight accumulation by the seedlings. For the resistant biotype, in spite of the reduction of the dry weight with an increase of the glyphosate rate, the dry weight ranged from 1 to 0.4 mg per seedling between the rates of 55.5 and 888 mg a.e. $L^{-1}$.

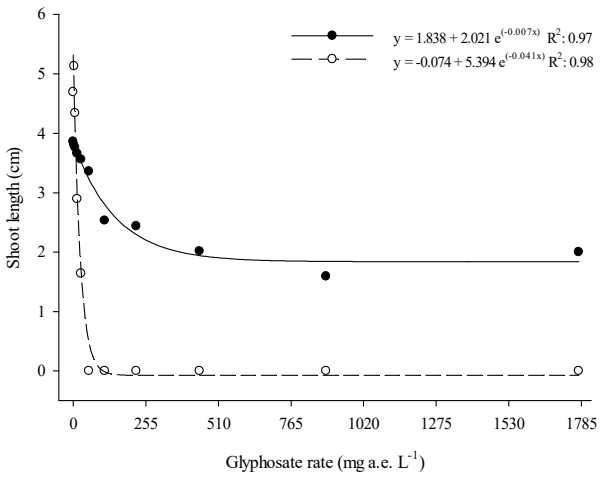
The resistance mechanism of ryegrass to glyphosate has not yet been fully understood (Vargas et al., 2016). Some studies have suggested a reduction in the absorption and translocation (Ghanizadeh et al., 2016; Fernández-Moreno et al., 2017) and others the overexpression of the EPSPs enzyme (Salas et al., 2015) and the sequestration of the herbicide in the vacuole (Ge et al., 2012). Thus, it can be suggested that the reduction of the absorption and translocation is not the mechanism involved in the resistance of the tested biotype since both coleoptile and radicle were subject to glyphosate absorption soon after the development of the embryo, as well as fresh tissues have limited barriers to the absorption and translocation of herbicides.

Root length of both biotypes was reduced with the increasing of the glyphosate rate (Figure 7). The deficient development of the root system was the main reason why seedlings of the resistant biotype were considered abnormal from a rate of 222 mg a.e. $L^{-1}$, thus fulfilling the requirements proposed by RST (Brasil, 2009). The reduction of the root system may be a strategy that resistant plants have to reduce the exposure to the herbicide since the shoot length of this biotype remained practically constant, with a value around 2.5 cm, due to an increase of the rate from 222 to 1,776 mg a.e. $L^{-1}$, while in the same situation, a complete inhibition of the shoot development occurred for susceptible biotype (Figure 8).




Dots represent the mean values of replications of each biotype at each rate and bars represent their respective confidence intervals ($p<0.05$).

***Figure 7*** - Root length (cm) of resistant (●) and susceptible (○) biotypes exposed to increasing glyphosate rates.




Dots represent the mean values of replications of each biotype at each rate and bars represent the respective confidence intervals presented by each treatment ($p<0.05$).

***Figure 8*** - Shoot length (cm) of resistant (●) and susceptible (○) biotypes exposed to increasing glyphosate rates.

**Experiment 3 – Verification of the germination test efficiency to identify susceptible and resistant seeds to glyphosate**

In order to verify the possibility to detect the pre-established percentages of seed contamination in ryegrass lots with resistant seeds, the rate that would identify the biotypes by the germination test through the soaking of the white blotter paper with glyphosate was established. From the equation parameters of the dose-response curve for the variable germination, the rate of 127 mg a.e. $L^{-1}$ promoted more than 99% inhibition of germination to the susceptible biotype and to the segregated seeds of the resistant biotype (10%) (experiment 1), thus this rate was used in this experiment.

The germination test could identify the resistant biotypes in the sample. When the contamination was 4, 12, and 36% for the susceptible biotype with resistant seeds, the germination test detected 5, 19, and 39% contamination, respectively (Table 3).

***Table 3*** - Verification of germination test efficiency in substrate soaked with 127 mg a.e. $L^{-1}$ of glyphosate for identifying susceptible and resistant biotypes in lots with different contamination ratios

| Ratio (resistant:susceptible) | Germination (%) | CV[(1)] (%) | Abnormal (%) | CV (%) | Dead (%) | CV (%) |
|---|---|---|---|---|---|---|
| 0:100 | 2 | 54.7 | 93 | 2.7 | 5 | 51.6 |
| 4:96 | 5 | 21.6 | 88 | 5.0 | 7 | 36.8 |
| 12:88 | 19 | 33.2 | 70 | 20.2 | 11 | 83.3 |
| 36:64 | 39 | 11.9 | 47 | 23.1 | 14 | 50.4 |
| 100:0 | 86 | 3.5 | 3 | 43.3 | 11 | 27.3 |

[(1)] Coefficient of variation ($p<0.05$).

For the ratio of 0% contamination, the expected value was the total inhibition of germination, but 2% of germination was observed, because the rate of 127 mg a.e. $L^{-1}$ inhibited 99% of the germination to the susceptible biotype and not all. Germination of 86% in the ratio of 100% was observed. These results were expected since 10% of these seeds were segregated for the susceptibility of the herbicide, while the remaining 4% could be composed of non-viable seeds present in the sample.

Results from the second and third experiments, showed the best strategy to identify resistant biotypes through the germination test and soaked substrate with glyphosate is only adequate to assess shoot development because the increase of the glyphosate rate for the resistant biotype caused reduction for the root system and according to RST those seedlings are classified as abnormal. Assessing only the shoot, it would be possible to use higher glyphosate rates (between 300 and 500 mg a.e. $L^{-1}$), thus avoiding errors that could occur in seed lots that present different levels of susceptibility to the herbicide.

In synthesis, resistant plants in the field show different RF and the seeds can have others responses when are exposed to glyphosate concentrations in the soaked substrate. Thus, further studies of biotypes with different RF could assist to determinate the required rate to identify susceptible and resistant biotypes, thus ensuring a higher safety in seed laboratories of to routine analyses. Therefore, susceptible and resistant seeds to glyphosate could be identified through the method of soaking the white blotter paper. This method is efficient to identify contaminated seed lots, enabling them to be disposed of before being marketed.

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