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Mixture of glufosinate and atrazine for ryegrass (*Lolium multiflorum* Lam.) control and its effect on seeds' quality

Mezcla de glufosinato y atrazina para el control de raigrás (*Lolium multiflorum* Lam.) y su efecto en la calidad de semillas

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ABSTRACT

Ryegrass management has been difficult by the occurrence of resistant biotypes to several herbicides with different action mechanisms. Since herbicides mixes and rotations are an important alternative for resistant weed management, the objective of this work was to evaluate the interaction of the dose of the herbicides glufosinate and atrazine on ryegrass control and its seeds' quality exposed to their association. For this study, three experiments were carried out using factorial design in field, laboratory, and greenhouse conditions. Two factors (A and B) were evaluated in each experiment, where factor A and B represented the doses of glufosinate and atrazine, respectively. Ryegrass control was evaluated in field experiment, while germination percentage and Emergence Speed Index (ESI), were obtained in laboratory and greenhouse analyses, respectively. The data were submitted to variance analysis ($P \leq 0.05$) and the significant results were analyzed through response surface graphs. For ryegrass control data, the effect of the interaction was analyzed by the Colby method; glufosinate provides efficient ryegrass control, but its association with atrazine reduces the efficiency, being characterized as an antagonism between molecules. Glufosinate herbicide application, independent of atrazine presence, reduced the ryegrass seeds quality at the post-flowering stage.

Keywords:
Antagonism
Glutamine synthetase inhibitor
Lolium multiflorum
Photosystem II inhibitor

RESUMEN

El manejo de raigrás es difícil debido a la ocurrencia de biotipos resistentes a diversos herbicidas con diferentes mecanismos de acción. Dado que la mezcla de herbicidas y sus rotaciones son una alternativa importante para el manejo de malezas resistentes, el objetivo de este trabajo fue evaluar la interacción de las dosis de los herbicidas glufosinato y atrazina para el control de raigrás y la calidad de sus semillas expuestas a su asociación. Se realizaron tres experimentos usando diseño factorial en condiciones de campo, laboratorio e invernadero. Dos factores (A y B) fueron evaluados en cada experimento, donde el factor A y B representaron las dosis de glufosinato y de atrazina, respectivamente. El control de raigrás fue evaluado en el experimento de campo, mientas que el porcentaje de germinación y el Índice de Velocidad de Emergencia (IVE) se determinaron en laboratorio e invernadero, respectivamente. Los datos fueron sometidos a análisis de la varianza ($P \leq 0,05$) y los resultados significativos fueron analizados a través de gráficos de superficie de respuesta. Para los datos de control, el efecto de las interacciones fue analizado por medio del método Colby; el glufosinato proporcionó un eficiente control de raigrás, pero su asociación con atrazina reduce su eficacia, siendo caracterizando como antagonismo entre moléculas. La aplicación del herbicida glufosinato, independiente de la presencia de atrazina, redujo la calidad de las semillas de raigrás en la fase de post-floración.

Palabras clave:
Antagonismo
Inhibidores de glutamina sintetasa
Lolium multiflorum
Inhibidores del fotosistema II

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Ryegrass (*Lolium multiflorum* L.) is a winter grass, with annual cycle, which has high biomass, production of seeds and tolerance to grazing and soil humidity. Ryegrass is widely used as animal fodder, as well as an autumn-winter cover crop providing a high volume of residue/straw to the no-tillage system (Christoffoleti and López, 2003). This species is easily dispersed by biotic and abiotic factors and is characterized as an important weed in crops like wheat, soybean, maize and orchards in different countries (Brunharo and Hanson, 2018; Fernández *et al*., 2017; Peterson *et al*., 2018; Ruchel *et al*., 2015; Yanniccari *et al*., 2015).

The efficacy of a given weed management program in a cultivated area is the key for weed population dynamics because plants that survive to herbicide treatment can complete their life cycle, disperse seeds, and restock the soil seed bank (Rizzardi *et al*., 2015). In this way, burndown herbicide applications, at an advanced stage of plant growth, have great importance in the weed seed inviability, with impact on the soil seed bank (Bae *et al*., 2017), as well as to guarantee a better establishment of a given crop.

For burndown, it is recommended to use broad-spectrum herbicides that promote high control, for this purpose glyphosate is the most used. However, the repetitive use of this herbicide has generated resistant biotypes of weeds, and it is necessary to adopt herbicides with a different action mechanism. Regarding ryegrass, it is reported that it has developed resistance to eight action mechanism in several countries such as Argentina, Brazil, Chile, Denmark, France, Italy, Japan, New Zealand, Spain, Switzerland, United Kingdom, and United States. It was found a ryegrass biotype in Brazil, which presented resistance to the 5-enolpyruvylhikimate-3-phosphate synthase (EPSPs), acetolactate synthase (ALS) and acetyl coenzyme-A carboxasease (ACCase) inhibitors, showing multiple resistant forms among them (Barroso *et al.,* 2018; Henckes, 2018; Mariani *et al*., 2016; Schneider *et al.,* 2016; Heap, 2018). This situation makes it challenging to manage ryegrass, reducing the effective options for its control.

To reduce the initial interference of weeds, many farmers use herbicide mixtures with non-selective herbicides and residual activity on the soil at pre-sowing applications. For ryegrass management, atrazine is one of the best suitable alternatives. It belongs to the Photosystem II Inhibitors (PSII) action mechanism and is mainly absorbed by plant roots (Silva *et al*., 2013). This herbicide can be mixed with non-selective herbicides such as glufosinate, to be applied before corn sowing, without risk of residual activity on the crop establishment. Glufosinate is a herbicide that inhibits the activity of Glutamine Synthetase (GS), acts on the ammonia incorporation in cells by contact with plant tissue due to its low translocation (Latorre *et al*., 2013; Shaner, 2014).

Although herbicide mixtures constitute an important alternative in weed management, it is necessary to know the type of interaction resulting from the mixture. A herbicide mixture is considered synergistic when its added effect is higher than the response predicted by the effect of the isolated applications of each herbicide; additive effect, when the added effect of the isolated applications is similar to the expected effect; and antagonistic when the effect of the blend is less than the effect of individual applications (Staker and Oliver, 1998). Thus, the knowledge of the interaction effects of herbicides mixture is essential for making recommendations of weed management strategies, especially in the current weed resistance scenario.

On fields where EPSPs, ACCase and ALS inhibitors resistant ryegrass were found, it is recommended to use contact herbicides for burndown applications which association usually has an antagonistic effect by destroying the foliar tissues limiting the absorption and translocation of other herbicides in mixes (Hydrick and Shaw, 1994; Vidal *et al*., 2016). The research hypothesis is that the mixture of glufosinate and atrazine may be an alternative for ryegrass management in pre-sowing, especially in areas where multiple herbicide resistant ryegrass biotypes are present. The contact action of glufosinate and the residual atrazine effect in the soil can provide a more extended control period, with influence on seed production of weeds. The objectives of this work were to evaluate the interaction of glufosinate and atrazine herbicides in the mixture on 1) the control of ryegrass biotypes, and 2) the seed quality of ryegrass biotypes exposed to different doses of the herbicide mixture.

MATERIALS AND METHODS

Three experiments were carried out between October 2013 and April 2014. The first experiment (Experiment I) was in field conditions using a randomized complete block

design with four replications. The second (Experiment II) and the third (Experiment III) experiments were carried out in laboratory and greenhouse, respectively, at Universidade Federal de Pelotas in Capão do Leão/RS (31°48'04.13"S, 52°30'09.22"W). A completely randomized design with three and five replications for experiments II and III were used. Different glufosinate dosage (Factor A: 0, 200, 400 and 600 g a.i. ha^{-1}) and atrazine (Factor B: 0, 1000, 2000 and 3000 g a.i. ha^{-1}.), measured in grams of active ingredient, were arranged in a factorial design. The control was evaluated by the zero doses of atrazine and glufosinate in the factorial design.

The herbicide application in Experiment I was made using a backpack precision sprayer pressurized with CO_2, equipped with a nozzle-type fan 110.015 and calibrated to provide 150 L ha^{-1} of spraying volume. In all treatments, 2% mineral oil adjuvant was added to the herbicide treatment. The experimental units consisted of plots of 6 m^2 (3.0x2.0 m), in an area with a natural occurrence of ryegrass weed which did not have cultivation before. At application time, ryegrass plants were in the post-flowering stage.

The percent of weed control at 8, 16 and 24 Days After Treatment (DAT) on a scale of 0 to 100% was recorded. Zero meant the absence of injuries and 100% the death of all plants. To correct the weed phenological stage, it was considered that the plants of the control plots presented approximately 40, 60 and 75% of senescence in the respective evaluation periods.

At 24 DAT, time that coincided with seed maturation, 20-plant seeds of each plot were sampled for experiments II and III. Samples of the replicates of each treatment were grouped to constitute a seed sample per treatment. These seed samples were used to determine the percent of germination (experiment II) and the Emergence Speed Index (ESI) (experiment III) after 131 days of harvest.

For experiment II, the experimental unit was carried out using transparent plastic boxes (i.e. 'gearbox' type), containing two sheets of blotting paper moistened with distilled water in the proportion of 2.5 times the weight of the dry paper. Fifty seeds per treatment were seeded in each 'gearbox' and located in the BOD-type germination chamber at 20 °C during a 12-hour photoperiod. Germination was evaluated by counting germinated seeds according to the Rules for Seed Analysis (RAS) (Ministério da Agricultura, Pecuária e Abastecimento, 2009). Germination results were showed as a percentage of germinated seed with respect to the initial sowed seeds.

Experiment III was carried out in 6 L (40.0x25.0x7.0 cm) volume plastic trays filled with a 50:50 soil and commercial substrate mixture, which constituted the experimental units. Each band, five rows were sown, spaced at 10 cm, where twenty ryegrass seeds were seeded according to the treatment.

To calculate the ESI, daily emergence of emerged seedlings (aerial emergence greater than 1 cm) was achieved until the stabilization of the emergence occurred 25 Days After Sowing (DAS). The ESI was calculated according to the equation proposed by Maguire (1962) as follows:

$$SG=(G1/N1)+(G2/N2)+(G3/N3)+...+(Gn/Nn)$$

Where:
SG: Emergence Speed Index
G1, G2, G3, ..., Gn: Number of seedlings computed in the first, second, third and last count.
N1, N2, N3, ..., Nn: Number of sowing days in the first, second, third and last count.

Data were analyzed using the two-way ANOVA ($P \leq 0.05$). Significant results were analyzed through the construction of response surface graphs, where the atrazine doses were arranged on the x-axis, glufosinate doses on the y-axis and the response variables on the z-axis. To compare the treatments, the values of the minimum significant differences ($P \leq 0.05$) were calculated.

The effect of interactions between the herbicide doses was analyzed by the Colby method (Colby, 1967). In this method, the effect of the mixtures is evaluated by the equation:

$$E=100-\{[(100-X)\times(100-Y)]/100\}$$

Where:
E: Expected value for the herbicide mixture in each dose combination.
X and Y: Control with each herbicide alone.
When the observed effect (O) resulting from the application

of the X+Y mixture is higher, lower or equal to the expected value (E) the synergism, antagonism, or additivity occur respectively. The expected and observed values were compared by *t*-test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Interactions were observed between the factors tested for all variables analyzed. The control percentage of ryegrass plants at 8 DAT increased as glufosinate doses increased; however, they decreased as the atrazine doses increased (Figure 1A). At 16 and 24 DAT, a similar result was observed on glufosinate doses; however, it was observed an increasing percent of control as atrazine doses increased (Figures 1B and 1C). This response may be due to the slower action of atrazine since this herbicide needs to be absorbed by the roots and translocated via xylem to the active site (Silva *et al*., 2013). On the other hand, with glufosinate (a contact action herbicide) shows phytotoxicity in few days after the application.

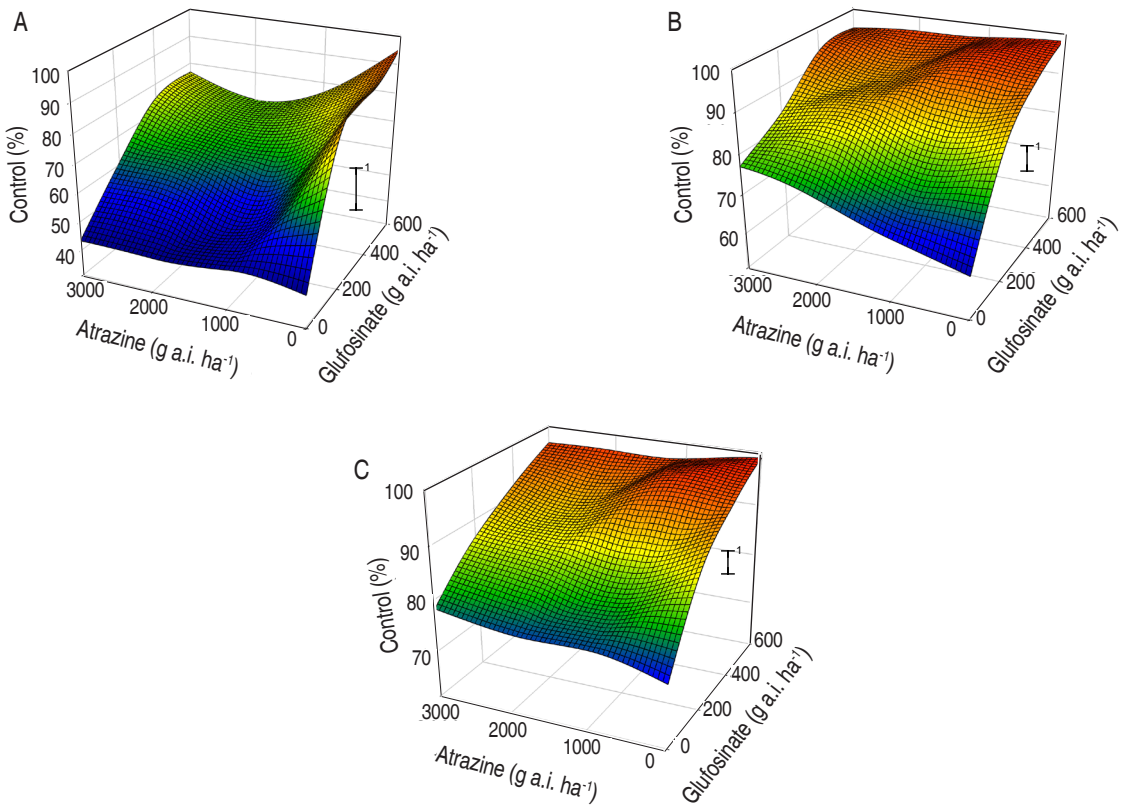

Figure 1. Control percent of ryegrass (*Lolium multiflorum* L.) at A. 8; B.16; C. 24 days after application (DAT) of mixture of glufosinate and atrazine herbicides. Different colors represent a significant difference ($P \leq 0.05$).

Jones *et al*. (2001) showed an increase of 14% in the control of *Amaranthus palmeri* S. Watson with a glufosinate+atrazine mixture in comparison to individual glufosinate. Similarly, Stephenson *et al*. (2015) demonstrated an increase of around 10% of the control of *Abutilon theophrasti* Medik, *Amaranthus palmeri*, and *Ipomoea hederacea* var. *integriuscula* Gray with a mixture of atrazine+glufosinate. However, these researchers also reported no differences between the application of atrazine alone and in mixture with glufosinate in the tank (Stephenson *et al*., 2015). On the other hand, results on species belonging to Poaceae family are different, where glufosinate+atrazine mixture did not adequately control *Sorghum halepense* (L.) Pers and *Urochloa ramosa* (L.) Nguyen, promoting control up to 89%, and these results did not differ from the control by using glufosinate alone (Stephenson *et al*., 2015), evidencing

the low atrazine effect for the control of these species. Therefore, the effect of the interaction between atrazine and glufosinate may be synergistic or antagonistic, depending on the application conditions and weed species.

Concerning the interaction between the two molecules, a decrease in glufosinate efficiency was observed due to the increase atrazine dose, most of the comparisons evidenced antagonism independent of the doses tested (Table 1). Alike results were found for the association of glufosinate and metribuzin where antagonism was observed at low doses of the former (Hydrick and Shaw, 1994), this can be because atrazine and metribuzin have the same action mode.

Table 1. Observed (Obs) and Expected (Exp) control (%) of *Lolium multiflorum* at diferents glufosinate and atrazine doses and the interaction (Int) between herbicides at 8, 16 and 24 Days After Treatment (DAT).

	Glufosinate (g a.i·ha^{-1})											
Atrazine	**0**		**200**			**400**			**600**		**Average**	**Average**
(g a.i.·ha^{-1})	**Obs[1]**	**Obs**	**Exp[1]**	**Int[1]**	**Obs**	**Exp**	**Int**	**Obs**	**Exp**	**Int**	**Obs**	**Exp**
8 DAT												
0	43	86	-		89	-		95	-		78	-
1000	45	53	92*	Ant[2]	69	93*	Ant	77	97*	Ant	61	94
2000	43	59	92*	Ant	67	93*	Ant	70	97*	Ant	60	94
3000	44	61	92*	Ant	75	93*	Ant	77	97*	Ant	64	94
Average	43	65	92		75	93		80	97		66	94
16 DAT												
0	62	85	-		94	-		98	-		85	-
1000	66	84	95*	Ant	95	98*	Ant	97	99*	Ant	85	97
2000	70	87	95*	Ant	90	98*	Ant	96	99^{ns}	Adit	85	98
3000	77	81	97*	Ant	94	99*	Ant	95	100*	Ant	86	98
Average	68	84	96		93	98		96	99		85	98
24 DAT												
0	73	89	-		95	-		99	-		89	-
1000	76	83	97*	Ant	96	99*	Ant	97	100*	Ant	88	99
2000	76	85	97*	Ant	90	99*	Ant	97	100*	Ant	87	99
3000	78	84	98*	Ant	92	99*	Ant	97	100*	Ant	88	99
Average	76	85	97		93	99		97	100		88	99

[1] Obs, exp and nt mean the values observed in the experiment, the values expected by the Colby method and the result of the interaction based on the expected values, respectively. [2]Ant. represents the result of the interaction between the antagonistic herbicides. Adit. indicates the additive interaction. * and ^{ns} indicate significant difference or not between the values observed and expected by the t test ($P \leq 0.05$).

When a mixture of glufosinate and glyphosate was used, a reduction in the control efficacy of *A. theophrasti*, *Chenopodium album* L., *Eleusine indica* Gaert. and *Setaria faberi* Herrm was observed compared to the separate application of glyphosate (Bethke *et al*., 2013; Chuah *et al*., 2008). The predominant antagonistic interactions in the association of these herbicides may occur because molecules with contact action rapidly destroy the leaf tissue, impairing the absorption and translocation of systemic herbicides (Vidal *et al*., 2016).

The application of glufosinate, regardless of atrazine doses in the mixture, when the ryegrass plants were in the post-flowering stage, caused a reduction in the quality of the ryegrass seed, evidenced by germination and ESI decreasing (Figure 2). A similar result was observed with the application

of 600 g a.i. ha^{-1} of glufosinate, which provided an absence of germination in ryegrass seeds (de Campos *et al*., 2012), characterizing as a promising alternative for the management of the species and the reduction of seeds in the soil. For the management of *Eriochloa villosa* Thunb, it was verified a reduction in the viability of seeds when glyphosate was applied, which efficiency only occurred when the application occurred shortly before the inflorescence emission (Nurse *et al*., 2015), demonstrating the importance of the stage at the time of application.

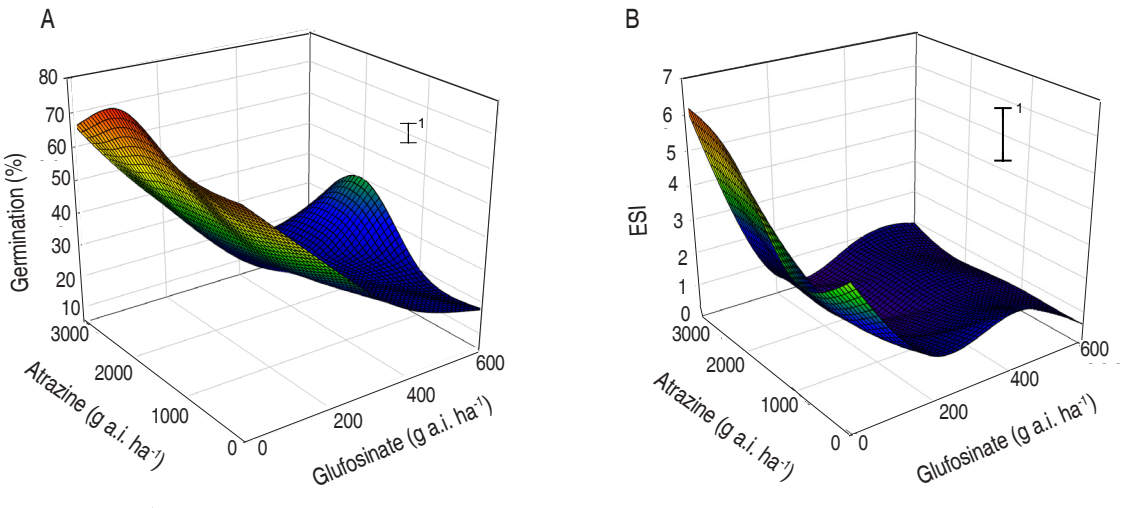

Figure 2. A. Percentage of germination; B. Emergence Speed Index (ESI) of seeds from ryegrass (*Lolium multiflorum* L.) harvested from plants exposed to doses of glufosinate and atrazine herbicides. Different colors represent a significant difference ($P \leq 0.05$).

For *Ambrosia artemisiifolia* L. the stage of development was determinant for the application of For *Ambrosia artemisiifolia* L. the stage of development was determinant for the application of glyphosate and glufosinate to reduce the production of viable seeds and pollen (Gauvrit and Chauvel, 2010). Thus, the burndown with the use of glufosinate at the appropriate time can make the weed seeds unviable, reducing the deposit of viable propagules in the soil seed bank. Although, without interference in the viability of ryegrass seeds, the association with atrazine may be detrimental to the management of the species by reducing control efficiency.

CONCLUSIONS

Glufosinate provides an efficient control of ryegrass at doses of 400 and 600 g a.i.·ha^{-1}; however, the tank mix with atrazine is generally antagonistic and decrease the control efficacy. Ryegrass plants exposed to glufosinate, loss seed viability, reducing germination and Emergence Speed Index (ESI) independently of atrazine.

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