Revista Brasileira de Herbicidas, v.14, n.1, p.47-53, jan./mar. 2015 (ISSN 2236-1065)
DOI: http://dx.doi.org/10.7824/rbh.v14i1.287 www.rbherbicidas.com.br

Ryegrass biotypes sensitivity to glyphosate, iodosulfuron-methyl and clethodim[1]

Sensibilidade de biótipos de azevém a glyphosate, iodossulfurom-metílico e clethodim

Jônatas Galvan[2]; Leandro Oliveira da Costa[3]; Mauro Antônio Rizzardi[4]; Sabrina Tolotti Peruzzo[5]

Abstract - Ryegrass (*Lolium multiflorum)* is an annual grass, traditionally used as forage that has become weed in crops in the south of Brazil. It has been observed that there are ryegrass biotypes resistant to glyphosate that survive the application of herbicides that inhibit ALS and ACCase. Therefore, the objective was to evaluate the sensitivity of different ryegrass biotypes to glyphosate, iodosulfuron-methyl and clethodim herbicides. The experiment was carried out in a greenhouse, in randomized blocks, in a triple factorial arrangement with three repetitions. Four ryegrass biotypes were used, where: biotype 1 (B1), susceptible to glyphosate; biotype 2 (B2), resistant to glyphosate; biotype 3 (B3) and biotype 4 (B4), biotypes suspected to be resistant to iodosulfuron-methyl. The doses were standardized in 0; 0.25; 0.5; 1; 2; 4 and 8 times the commercial doses of glyphosate (1080 g ha^{-1} a.i.) and clethodim (96 g ha^{-1} a.i.). The variables evaluated were control, in a percentage scale, and dry shoot biomass (DSB) of the plants at 28 days after the application of treatments (DAT). With that data, we calculated the parameters of the equation, from which the necessary dose was determined to reduce the DSB in 50% comparing to the non-treated plants (GR_{50}) and the resistance factor (F). Biotypes B2, B3 and B4 obtained Resistance factors (F) above 10 comparing to glyphosate and iodosulfuron-methyl herbicides, B3 being the highlighted biotype because, besides being resistant to those two herbicides, also showed an F value close to the clethodim threshold, which can characterize a low resistance level to this herbicide. All biotypes, except the control one, were resistant to the iodosulfuron-methyl and glyphosate herbicides, which characterizes multiple resistance to those herbicides. The clethodim herbicide can be an alternative for the control of biotypes resistant to glyphosate and iodosulfuron-methyl; however, attention should be paid to the chemical handling of these populations, because we identified biotypes with low resistance level to this herbicide.
Keywords: multiple resistance; resistance fator; *Lolium multiflorum*

Resumo - O azevém (*Lolium multiflorum*) é uma gramínea anual, tradicionalmente utilizada como forrageira que se tornou planta daninha em lavouras no sul do Brasil. Tem-se observado biótipos de azevém, resistentes ao glyphosate, sobrevivendo à aplicação de herbicidas inibidores

[1] Received for publication on 12/11/2014 and approved on 28/08/2015.
[2] Eng. Agr., Dr., Universidade de Passo Fundo, PPGAgro-UPF. Passo Fundo-RS. Email: galvan.j@gmail.com.
[3] Eng. Agr., Me., Doctor student in Agronomy at the Universidade de Passo Fundo, PPGAgro-UPF. Passo Fundo-RS. Email: leandro.jari@gmail.com.
[4] Eng. Agr., Dr., Professor at the Universidade de Passo Fundo, PPGAgro-UPF, CNPq researcher, Post Office Box 611, 99052-900, Passo Fundo-RS. E-mail: rizzardi@upf.br.
[5] Eng[a]. Agr[a]., Master student in Agronomy at the Universidade de Passo Fundo, PPGAgro-UPF. Passo Fundo-RS. Email: peruzzo.sabrinat@gmail.com.

de ALS e ACCase. Assim, objetivou-se avaliar a sensibilidade de diferentes biótipos de azevém aos herbicidas glyphosate, iodosulfurom-metílico e clethodim. O experimento foi conduzido em casa de vegetação, em blocos casualizados, arranjo fatorial triplo com três repetições. Foram utilizados quatro biótipos de azevém, sendo: B1, suscetível ao glyphosate; B2, resistente ao glyphosate; B3 e B4, biótipos suspeitos de resistência ao iodosulfurom-metílico. As doses foram padronizadas em 0; 0,25; 0,5; 1; 2; 4 e 8 vezes as doses comerciais dos herbicidas glyphosate (1080 g ha^{-1}e.a.), iodosulfurom-metílico (5 g ha^{-1} i.a.) e clethodim (96 g ha^{-1} i.a.). As variáveis avaliadas foram controle, em escala percentual, e massa seca da parte aérea (MSPA) das plantas aos 28 dias após a aplicação dos tratamentos (DAT). Com esses dados, foram calculados os parâmetros da equação, a partir dos quais, determinou-se a dose necessária para reduzir a MSPA em 50% em relação às plantas não tratadas (GR_{50}) e o fator de resistência (F). Os biótipos B2, B3 e B4 obtiveram fatores de Resistência (F) acima de 10 em relação aos herbicidas glyphosate e iodosulfurom-metilico, destacando-se o biótipo B3, que além de possuir resistência a esses dois herbicidas, também demonstrou valor de F próximo do limiar para o clethodim, o que pode caracterizar um baixo nível de resistência a esse herbicida. Todos os biótipos, exceto o controle, foram resistentes aos herbicidas iodosulfurom-metílico e glyphosate, o que caracteriza a resistência múltipla a esses herbicidas. O herbicida clethodim pode ser uma alternativa para o controle de biótipos resistentes ao glyphosate e iodosulfurom-metílico, porém, deve-se ter atenção no manejo químico dessas populações, pois se identificou biótipos com baixo nível de resistência a esse herbicida.

Palavras-chaves: resistência múltipla; fator de resistência; *Lolium multiflorum*

Introduction

Ryegrass (*Lolium multiflorum* Lam.) is a cold season annual grass, widely disseminated in the south region of Brazil, maturing mainly during autumn and spring (Roman et al., 2004). Besides being used as forage, it frequently becomes weedy in crops mainly wheat and corn It is controlled mostly with the application of inhibitor herbicides of the enzyme acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS) and 5 enolpyruvylshikimate-3-phosphate synthase, glyphosate (EPSPs), the latter used for its low cost and advancement of genetically modified crops resistant to glyphosate.

The extended use of herbicides throughout time has resulted in the selection of weeds that are resistant to the main action mechanisms (Owen, 2008). This happens due to the use of herbicides as the only control method with little or no diversity of other agricultural practices (Beckie et al., 2004). It has been shown that for ryegrass in Brazil, herbicide use has created resistance to three herbicides action mechanisms, isolated resistance to the inhibitor herbicides of the ACCase and EPSPs. In Chile, this weed has multiple resistance to the mechanisms with herbicide action ALS, ACCase and EPSPs (Heap, 2014).

It is highlighted the importance of knowing the frequency and the resistance patterns of weeds to herbicides in certain regions in order to develop efficient control strategies. The quantification of the extension and distribution of the resistance also provides a starting point for the monitoring of evolution of the resistance that can be essential for the future (Rauch et al., 2010).

Multiple resistance can occur by means of selection imposed by the frequent application of herbicides with different action mechanisms through the accumulation of resistance genes to herbicides (Mallory-Smith and Sanchez-Olguin, 2011; Beckie and Tardif, 2012). The rotation of herbicides with different action mechanisms and mixtures have a greater effect in delaying the selection of resistant plants, mainly when the mechanism that gives resistance is an altered action or translocation site (Beckie 2006). Ryegrass is characterized for being a weed with cross-pollination, which enables a greater gene flow of resistance among the populations via pollen, facilitating the evolution of the resistance to herbicide (Liu et al., 2014).

In the south of Brazil, it has been observed the occurrence of ryegrass biotypes that have survived the application of the main herbicides used for its control such as the EPSPs, ALS and ACCase inhibitors. Therefore, the objective of this paper was to evaluate the sensitivity of the ryegrass biotypes, collected in different places, to the glyphosate, iodosulfuron-methyl and clethodim herbicides.

Material and Methods

The experiment was carried out in a greenhouse in Passo Fundo, RS, at coordinates 28° 13' 01" S and 52° 23' 37" W.

The experimental outline used was of randomized blocks, in a triple factorial design with three repetitions. The treatments were composed of four ryegrass biotypes: biotype 1 (B1), susceptible to glyphosate, (certified seed); biotype 2 (B2), biotype with known resistance to glyphosate; biotype 3 (B3) and biotype 4 (B4), biotypes suspected of being resistant to iodosulfuron-methyl and glyphosate, collected in wheat crop rows. All biotypes, except B1, were collected in agricultural areas of the municipality of Passo Fundo-RS.

The plants were grown in plastic vases with a volumetric capacity of 0.5 L., with two plants per vase, filled out with commercial substrate Turfa Fértil®. The plants were watered intermittently when needed, except 24 hours after the application of the herbicides. In the development stage of four to five true leaves, there was the application of herbicides with a knapsack precision sprayer pressurized at CO_2, using flat spray tips XR 110.02, at a height of 50 cm above the target, with working pressure of 2 kgf cm² and application volume of 200 L ha^{-1} The herbicides used and their respective recommended commercial doses were; glyphosate (Roundup Original®, dose 1080 g ha^{-1} a.e.); iodosulfuron-methyl (Hussar®, dose 5 g ha^{-1} a.i.) + Hoefix®, dose 0,5% of water volume; clethodim (Select®, dose 96 g ha^{-1} a.i.) + Assist®, dose 0,5% of water volume. The doses were standardized in 0; 0.25; 0.5; 1; 2; 4 and 8 times the commercial doses recommended by the manufacturer

At 28 days after the application (DAA) control was evaluated visually using a percentage scale where 0% corresponds to no effect of the herbicide and 100% to total death of the plants. Dry shoot biomass (DSB), where the plants were cut close to the soil and dried in oven at 65°C for 72 hours, until reaching constant weight, was calculated. The DSB values were converted into percentages starting from the principle that plants without application of the herbicide had 100% DSB and the treated had percentages of this biomass, (Beckie et al., 2012).

The data was submitted to variance analysis with the application of the F test. When significant, the averages were compared through the Tukey test (P<0.05). The data was adjusted to the non-linear log-logistic regression models of three parameters (Equation 1) (Streibig, 1988).

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

Where:

y = control percentage, reduction of dry mass of the aerial part; *x* = herbicide dose; and *a*, *b* and *c* = parameters of the curve, in which *a* is the difference between the maximum and minimum point of the curve, *b* is the dose that provides 50% of variable response (GR_{50}), and *c* is the declivity of the curve.

Through the equation parameters, the necessary dose to reduce the DSB of the aerial part in 50% compared to the non treated plants (GR_{50}) and the resistance factor (F) were calculated. The F factor corresponds to the ratio between the GR_{50} of the resistant biotype and the GR_{50} of the susceptible biotype ($F= GR_{50\ Biotype\ R} / GR_{50\ Biotype\ S}$). The F factor expresses the number of times the dose necessary to control 50% of the resistant biotype is greater to the dose that controls 50% of the susceptible biotype (Hall et al., 1998).

Results and Discussion

The statistical analysis has shown significant effects both in biotypes and the doses of herbicides used, as well as, in some cases, interactions between both factors. In the present paper, each herbicide was evaluated separately, having as factors, therefore, the biotypes and the doses used.

The control of biotype B1, with 0.25 times the recommended dose of glyphosate was close to 100%. The other biotypes, on the other hand, even with double that dose, were not controlled. For the B3 biotype it was necessary four times the recommended dose to get close to 100% control (Table 1). Similar results were found by Vargas et al. (2004) when they identified the resistance of ryegrass to the glyphosate herbicide, they observed that the susceptible biotype was controlled in 100%, using 0.25 times the record dose, but for the resistant biotype, when applied 8 times the recommended dose, it got to a maximum of 45% control.

Table 1. Visual control of ryegrass biotypes at 28 days after the application of glyphosate, iodosulfuron-methyl, and clethodim herbicides. Passo Fundo-RS, 2014.

Dose [1/]	Glyphosate				Iodosulfuron-methyl				Clethodim			
($g\ ha^{-1}$)	B1 [2/]	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
0	A 0 b*	A 0 d	A 0 c	A 0 b	A 0 b	A 0 b	A 0 c	A 0 b	0[ns]	0	0	0
0.25	A 98 a	C 12 cd	B 57 b	C 22 b	A 87 a	B 22 b	B 27 bc	B 10 b	97	98	95	97
0.5	A 100 a	B 28 bc	B 48 b	A 82 a	A 92 a	B 28 b	B 30 bc	B 20 b	99	98	97	98
1	A 100 a	B 50 b	A 88 a	A 97 a	A 97 a	C 32 b	B 48 ab	AB 75 a	99	98	98	98
2	A 100 a	A 100 a	A 92 a	A 99 a	A 99 a	B 68 a	B 52 ab	AB 77 a	100	99	99	100
4	A 100 a	A 100 a	A 99 a	A 100 a	A 100 a	AB 72 a	B 53 ab	AB 82 a	100	100	100	100
8	A 100 a	A 100 a	A 100 a	A 100 a	A 100 a	A 91 a	A 80 a	A 99 a	100	100	100	100
CV (%)	14.0				26.0				1.4			

*Averages followed by the same lower case letter, on the column, or preceded by the same upper case letter on the line do not differ among themselves by the Tukey test ($p<0.05$). [ns] Non-significant differences between the herbicide doses by the Tukey test ($p\leq 0,05$); [1/] Values multiplied by the commercial dose (Recommended) of each herbicide, in $g\ ha^{-1}$ a.e. or $g\ ha^{-1}$ a.i.; [2/] Ryegrass biotypes.

For the iodosulfuron-methyl herbicide, with 0.25 times the recommended dose, the control of B1 biotype was greater than 86%, not being statistically different from the more elevated doses. The B2 and B3 biotypes did not reach 50% of the control on the recommended dose, and the B3 biotype control in the commercial dose did not differ statistically from the control when a higher dose of the herbicide was used (Table 1). In the same table, we can observe that for B4, the commercial dose also did not differ from the higher dose. When clethodim herbicide, was used 0.25 times the recommended dose, control was equal to or greater than 95% in all four biotypes, and there was no significant difference among doses. This suggests that the biotypes evaluated did not develop resistance to this herbicide.

Rauch et al. (2010) in surveying 75 populations of ryegrass in wheat areas observed that, among the herbicides of the cyclohexanediones group, clethodim was the one which presented a lower percentage of populations resistant to this herbicide, only 5% of the populations. This is mainly due to its non-selection to wheat and therefore is not used in the wheat rotation. When clethodim becomes used in wheat the selection pressure will increase, and the number of resistant populations will also gradually increase

Of the ryegrass plants were treated with glyphosate and B2 and B3 biotypes were reduced by 50% only with doses greater than 1500 and 1700 g ha^{-1} a.e., respectively. It is also observed that B4 biotype needed a dose of 370 g ha^{-1} a.e. to reach 50% of the control compared to biotype B1 Factor F of biotypes B2 and B3 were close to 100, showing an elevated resistance to the glyphosate herbicide (Figure 1 and Table 2). Biotype B4 was also resistant to glyphosate, but with a lower F factor.

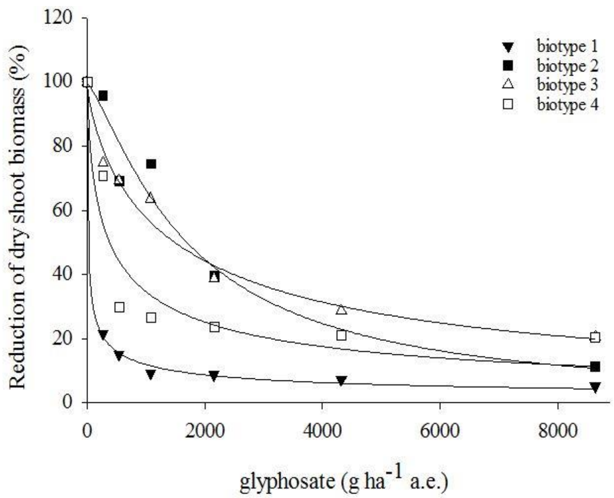

Figure 1. Reduction of dry shoot biomass of four ryegrass biotypes as a function of increased dose of glyphosate herbicide. Passo Fundo-RS, 2014.

The reduction of DSB of the plants treated with the herbicide iodosulfuron-methyl shows a different response between the biotypes. The DSB of Biotype B1 was reduced to 50% with only 0.04 g ha^{-1} a.i., below the recommended does for control of this weed. This shows that it is extremely sensitive to this herbicide. On the other hand, the other biotypes need doses greater than 100 times the ones necessary to reduce in 50% the DSB of the sensitive biotype (Figure 2 and Table 2).

According to Heap (2014) the criteria to document resistance is when the resistance factor (F) is equal to or greater than 10, based on GR_{50} or DL_{50}. Therefore, biotypes B2, B3 and B4 have a resistance to the iodosulfuron-methyl herbicide, B3 reaching the F factor greater than 700, characterizing the elevated resistance to this herbicide. Through this study, it was observed that biotypes B2, B3 and B4 showed multiple resistance to herbicides that inhibit ALS and EPSPs.

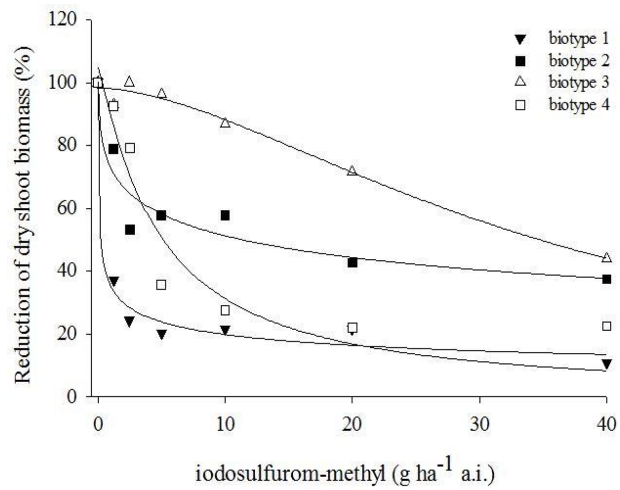

Figure 2. Reduction of dry shoot biomass of four ryegrass biotypes as a function of increasing dose of iodosulfuron-methyl herbicide. Passo Fundo-RS, 2014.

In Brazil, the use of herbicides that inhibit ALS has been increasing both in winter and summer crops. The selection pressure imposed by herbicides of the same action mechanism makes it easier to select new populations resistant to these herbicides (Monquero and Christoffoleti, 2001; Costa and Rizzardi, 2014). According to Saari et al. (1994) modeling suggests that the mutation frequency of initial resistance for herbicides that inhibit ALS may be 10^6 or less, and the first biotype resistant may become apparent only 5 years after its initial use.

When 0.25 the recommended dose is used for clethodim herbicide, DSB reduced the values less than 50% in all biotypes (Figure 3). This is because the biotypes are extremely sensitive to this herbicide, and only biotype B3 reached an F factor value close to 10 (Table 2). Thus, biotypes B1, B2, and B4 are sensitive to the clethodim herbicide, as results of this herbicide becoming the tool to control ryegrass resistant to glyphosate and iodosulfuron-methyl. Regarding, it is important to highlight how close the F factor is to 10 in biotype B3. This ryegrass population has a low resistance level to this herbicide, and would classify as having multiple resistance to the three action mechanisms (EPSPs, ALS, and ACCase) of the herbicides being studied. Further studies are need as well as monitoring by producers in areas where this ryegrass population is predominant.

Table 2. Necessary dose to reduce the dry shoot biomass in 50% comparing to the non-treated plants (GR50) and resistant factor (F) of the ryegrass biotypes at 28 days after the application of herbicides glyphosate, iodosulfuron-methyl, and clethodim. Passo Fundo-RS, 2014.

Ryegrass biotypes	Glyphosate [1/]		Iodosulfuron-methyl [2/]		Clethodim [2/]	
	GR50 [3/]	F	GR50	F	GR50	F
B1	17.47	---	0.045	---	2.38	---
B2	1712.16	98.01	9.69	215.9	0.86	0.36
B3	1528.98	87.53	35.43	789	21.45	8.99
B4	369.71	21.16	4.75	105.8	0.0013	0.01
	Equation parameters					
	a	c	a	c	a	c
B1	100.7930	0.4964	129.9868	0.3170	111.5966	0.2769
B2	99.4979	1.3031	103.1293	0.3883	115.7317	0.2737
B3	98.9556	0.7961	98.2758	1.7079	101.8952	0.3874
B4	101.2164	0.6558	104.6337	1.1478	196.0611	0.1362

[1/] Dose of a.e. g ha^{-1}; [2/] Dose of a.i. g ha^{-1}; [3/] GR_{50}= obtained from parameter *b* of the equation.

Ryegrass biotypes resistant to glyphosate survived doses of up to 1440 g ha^{-1} a.e. and did not affecting the production of dry mass. Clethodim resistant biotypes required a dose of 79.2 g ha^{-1} a.i. for to exert 90% control (Roman et al., 2004).

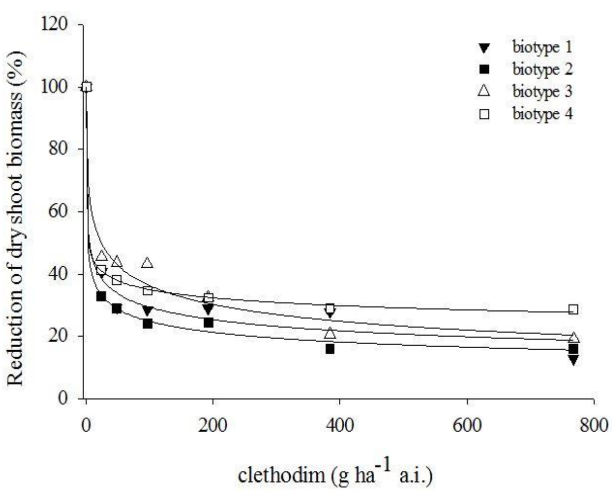

Figure 3. Reduction of dry shoot biomass of four ryegrass biotypes as a function of the increased dose of clethodim herbicide. Passo Fundo-RS, 2014.

According to Powles and Yu (2010), the greatest challenge regarding the resistance of weeds to herbicides is the accumulation of more than one resistance mechanism in the same individual. This is clear in *Lolium rigidum* in Australia, which makes it almost impossible to control this weed with herbicides.

Herbicides inhibitors of ACCase are used during the desiccation of ryegrass resistant to glyphosate, which exerts strong selection pressure. Therefore, these results reinforce the need for producers to control ryegrass using a rotation of herbicides of varying chemical groups.

Conclusions

The B2, B3, and B4 ryegrass biotypes have an elevated resistance to iodosulfuron-methyl, and glyphosate, characterizing them with multiple resistance to the action mechanisms ALS and EPSPs.

The B3 biotype has low resistance to clethodim herbicide, which characterizes it with multiple resistance to the three action mechanisms, EPSPs, ALS and ACCase.

References

Beckie, H.J.; Warwick, S.I.; Sauder, C.A. Acetolactate Synthase (ALS) Inhibitor-Resistant Wild Buckwheat (*Polygonum convolvulus*) in Alberta. **Weed Technology**, v.26, n.1, p.156-160, 2012.

Beckie, H.J.; Hall, L.M.; Meers, S.; Laslo, J.J.; Stevenson, F.C. Management practices influencing herbicide resistance in wild oat. **Weed Technology**, v.18, n.3, p.853-859, 2004.

Beckie, H.J. Herbicide-resistant weeds: management tactics and practices. **Weed Technology**, v.20, n.3, p.793-814, 2006.

Beckie, H.J; Tardif F.J. Herbicide cross-resistance in weeds. **Crop Protection**, v.35, n.1, p.15-28, 2012.

Costa, L.O.; Rizzardi, M.A. Resistance of *Raphanus raphanistrum* to the herbicide metsulfuron-methyl. **Planta Daninha**, v.32, n.1, p.181-187, 2014.

Hall, L.M., Strome, K.M., Horsman, G.P. Resistance to acetolactate synthase inhibitors and quinclorac in a biotype of false clover (*Gallium spurium*). **Weed Science**, v.46, n.4, p.390-396, 1998.

Heap, I. **International Survey of Herbicide resistant Weeds**. Disponível em: http:<//www.weedscience.org/In.asp>. Acesso em: 03 de jun. 2014.

Liu, M.; Hulting, A.G.; Mallory-Smith, C.A. Characterization of multiple-herbicide-resistant Italian ryegrass (*Lolium perenne* spp. *multiflorum*). **Pest Management Science**, v.70, n.7, p.1145-1150, 2014.

Mallory-Smith, C.A; Sanchez-Olguin, E. Gene flow from herbicideresistant crops: it is not just for transgenes. **Journal of Agricultural and Food Chemistry**, v.59, n.11, p.5813-5818, 2011.

Monquero, P.A.; Christoffoleti, P.J. Manejo de populações de plantas daninhas resistentes aos herbicidas inibidores da acetolactato sintase. **Planta Daninha**, v.19, n.1, p.377-387, 2001.

Owen, M. Weed species shifts in glyphosate-resistant crops. **Pest Management Science**, v.64, n.4, p.377-387, 2008.

Powles, S.; Yu, Q. Evolution in action: plants resistant to herbicides. **Annual Review of Plant Biology**, v.61, n.1, p.317-347, 2010.

Rauch, T.A.; Thill, D.C; Gersdorf, S.A.; Price, W.J. Widespread occurrence of herbicide-resistant Italian ryegrass (*Lolium multiflorum*) in Northern Idaho and Eastern Washington. **Weed Technology**, v.24, n.3, p.281-288, 2010.

Roman, E.S.; Vargas, L.; Rizzardi, M.A.; Mattei, R.W. Resistência de azevém (*Lolium multiflorum*) ao herbicida glyphosate. **Planta Daninha**, v.22, n.2, p.301-306, 2004.

Saari, L.L.; Cotterman, J.C.; Thill, D.C. Resistance to acetolactate synthase inhibiting herbicides. In: Powles, S. B.; Holtum, A. M. **Ed. Herbicide Resistance in Plants: Biology and Biochemistry**. Boca Raton, Lewis Publishers, 1994. p. 83-139.

Streibig, J.C. Herbicide bioassay. **Weed Research**, v.28, n.6, p.479-484, 1988.

Vargas, L.; Roman, E.S.; Rizzardi, M.A.; Silva, V.C. Identificação de biótipos de azevém (*Lolium multiflorum*) resistentes ao herbicida glyphosate em pomares de maçã. **Planta Daninha**, v.22, n.4, p.617-622, 2004.