Differential response of two sourgrass populations to glyphosate

Paulo Roberto Fidelis Giancotti*
Bruna Pires da Silva
Núbia Maria Correia
São Paulo State University, Jaboticabal, SP, Brazil

Leonardo Bianco de Carvalho
Santa Catarina State University, Lages, SP, Brazil

Pedro Luis da Costa Aguiar Alves
São Paulo State University, Jaboticabal, SP, Brazil

ABSTRACT

The repetitive use of glyphosate may cause increase on the resistance of sourgrass (*Digitaria insularis*) through mechanisms of natural selection. The aim of this study was to verify the response of two populations of sourgrass (one collected from nonagricultural area and the other one from area suspected of glyphosate resistance) to increasing doses of glyphosate. The experimental design was completely randomized with four repetitions. For both populations, glyphosate was sprayed at 10 doses (0D, D/16, D/8, D/4, D/2, D, 2D, 4D, 8D, and 16D; so that D is the dose of 1.08 kg e.a. ha⁻¹). The treatments were sprayed when the plants had shown 3-5 tillers. The population collected in the nonagricultural area was slightly more sensible to the herbicide glyphosate than the population originated from an area where the herbicide application is common, not indicating glyphosate resistance.

Keywords: *Digitaria insularis*, N-(phosphonomethyl)glycine, dose-response.

* Corresponding author: paulogiancotti@gmail.com

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INTRODUCTION

Sourgrass (*Digitaria insularis*) is herbaceous, perennial, erect, and rhizomatous species with striated culms and height of 0.5 to 1 m (Kissmann and Groth 1997). It is an important weed infesting several crops (Azevedo 2003); and the use of herbicides, like glyphosate, to control this weed in field crops is an activity widespread around the world (Fontes et al. 2001).

Glyphosate is a non-selective and systemic herbicide used to control annual and perennial weeds and to burndown cover crops (Timossi et al. 2006, Rodrigues and Almeida 2011). This herbicide inhibits the enzyme EPSPs (5-enolpyruvylshikimate-3-phosphate synthase) which participates on the

pathway of aromatic amino acids synthesis, such as phenylalanine, tyrosine e tryptophan.

There are evidences that the repeated use of glyphosate have been resulting in higher resistance of sourgrass through mechanisms of natural selection, that can improve resistant biotypes present in the population (Christoffoleti et al. 2008). Consequently, the population of resistant plants can increase until affects negatively the weed control in fields.

This research aimed to verify the response of two sourgrass populations to increasing glyphosate doses.

MATERIAL AND METHODS

The assay was carried out between August and November of 2010, in Jaboticabal, SP, Brazil, testing

two populations of sourgrass. Seeds of one population (P1) was collected at urban area of Jaboticabal (21° 14' 30.64" S and 48° 18' 21.26" WGr), never previously treated with glyphosate. The other one (P2) was collected in an orange orchard area in Matão, SP, Brazil (21° 37' 43.94" S and 48° 28' 46.12" WGr), being suspected to be resistant to glyphosate. After collecting, seeds were placed in paper bags and labeled for been transported to the lab. The storage was done at dried site with ambient temperature until the sourgrass sowing.

Seeds of the two populations were placed to germinate in styrofoam trays filled with commercial substrate. When emerging seedlings showed two true leaves, they were transplanted to plastic 5 L-pots at a density of one plant per pot. Pots were filled with a soil collected at tillable layers of field areas of Jaboticabal and classified as an Oxisol, being daily watered.

The experimental design was completely randomized with four replications. A commercial formulation of glyphosate herbicide (Roundup WG®, 720 g e.a. ha⁻¹) was used. For both sourgrass populations, we used nine doses of glyphosate (D/16, D/8, D/4, D/2, D, 2D, 4D, 8D e 16D; in which D is a dose of 1.08 kg e.a. ha⁻¹), keeping a no-herbicide check. When the plants showed 3-5 tillers, we performed the application of the treatments.

Herbicide spraying was performed in a closed spraying room, using a CO₂ backpack sprayer with a nozzle XR 110.02, delivering 200 L ha⁻¹. After the herbicide application, pots were transported outside and maintained in natural conditions.

Evaluation of control efficacy was done at 7, 14, 21, and 26 days after application (DAA). To control efficacy, control level notes were given at 0% in the case of absence of symptoms caused by glyphosate and 100% when cause plant death. F test was performed and mean notes compared by Tukey test at 5% of probability.

In addition, at 30 DAA, fresh mass of aboveground was determined. Averages were submitted to regression analyzes, using the software OriginPro 8. The ED₅₀ (herbicide dose that providing 50% of reduction of fresh mass accumulation) was calculated. After that, we calculated the resistance factor (RF) as being the ratio between ED₅₀ of the resistant population and the ED₅₀ of the susceptible population.

RESULTS AND DISCUSSION

For doses below the field dose (1.08 kg a.e. ha⁻¹), no symptoms of injury were observed in plants of both populations (P1 and P2) when submitting to glyphosate spraying at 0.07 and 0.14 kg a.e. ha⁻¹ in the four periods of evaluation (Table 1). In addition, from 14 DAA, P1 and P2 plants submitted to 0.27 kg a.e. ha⁻¹ recovered of the initial symptoms of injury as well as plants submitted to 0.54 kg a.e. ha⁻¹, from 21 DAA, showing no more symptoms of injury.

Exactly for the field dose, different response was observed between P1 and P2 populations, so that P1 was better controlled than P2 (Table 1). Similar result occurred for the double field dose (2.16 kg a.e. ha⁻¹). However, increasing the dose after the double field dose, P1 and P2 responded similarly to glyphosate spraying, showing that there were no differences in plant injury if high doses of glyphosate were used to control those sourgrass populations. In spite of that, differences observed in the field dose and in the double field dose (most common to be used in field conditions) indicates different behavior of both P1 and P2 populations.

Analyzing the dose response at 30 DAA in relation to fresh mass accumulation, we can observed slightly differences between P1 and P2 populations, corroborating the previous results on plant injury (weed control) (Figure 1), so that P2 tolerated higher doses of glyphosate than P1. The ED₅₀ of P1 was,

Table 1. Percentage of control of two sourgrass (*Digitaria insularis*) populations (P1 and P2) submitted to spraying of increasing doses of glyphosate, within four periods of evaluation (days after application – DAA).

Doses (kg a.e. ha ⁻¹)	7 DAA		14 DAA		21 DAA		26 DAA	
	P1	P2	P1	P2	P1	P2	P1	P2
0.00	0.00 ¹	0.00	0.00	0.00	0.00	0.00	0.0	0.00
0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.27	1.25 C	0.50 D	0.00	0.00	0.00	0.00	0.00	0.00
0.54	2.50 C	1.25 D	2.50 E	5.00 D	0.00	0.00	0.00	0.00
1.08	12.50 C	10.00 CD	21.25 D	12.50 D	30.00 B	5.00 C	30.00 B	18.75 C
2.16	38.75 B	15.00 C	55.00 C	30.00 C	73.75 B	55.00 B	77.50 A	62.50 B
4.32	35.00 B	33.75 B	65.00 BC	65.00 B	88.00 A	86.25 A	88.75 A	86.25 A
8.64	58.75 A	48.75 A	81.25 B	75.00 B	95.75 A	95.50 A	97.50 A	95.50 A
17.28	73.75 A	51.25 A	96.25 A	90.00 A	100.00 A	98.75 A	100.00 A	98.75 A
CV (%)	32.04	28.5	23.63	21.28	29.85	17.7	28.84	23.86
F	59.72**	82.65**	101.65**	145.75**	60.58**	216.22**	64.74**	104.33**

**Significant at $\alpha = 0.01$.¹Within a column, means followed by the same letter are not significantly different according to Tukey F-protected test at $P = 0.05$.

1,211.0 g a.e. ha⁻¹, whereas it was 2,192.5 g a.e. ha⁻¹ for P2 (Table 2). Therefore, the P2 has a resistance factor of 1.8, meaning that P2 population tolerated glyphosate doses closed to two times higher than P1. This low resistant factor did not indicated P2 as a resistant population, but the control of these plants cannot be good using the field dose, providing a plant selection in time.

Figure 1. Dose-response curve of the fresh mass of two populations of sourgrass (percentage of fresh mass accumulation in relation to nontreated plants) at 30 days after glyphosate application.

Table 2. Parameters of the log-logistic^a equation used to calculate the dose of glyphosate required to reduce aboveground fresh mass of two populations of sourgrass by 50% (ED₅₀).

Population ^b	a	B	ED ₅₀	c	R ^{2/c}	RF ^d
P1	1.5	101.9	1,211.0	0.0	0.99**	-
P2	1.6	94.7	2,191.5	8.5	0.99**	1.8

^a Equation $Y = a + (a - b) / (10^{(ED_{50} - x)^c})$ in which Y indicates the sourgrass fresh mass, a and b are coefficients that express the minimum and maximum values, ED₅₀ is the inflexion point that represents the glyphosate dose reducing fresh mass by 50%, and c is the steepness of the curve.

^b P1 is a population collected from nonagricultural area and P2 is one from field with frequently glyphosate application.

^c R² is the coefficient of determination and ** indicates that fitting adjustment is significant at 1% of probability.

^d RF is the resistance factor expressed by the ratio ED₅₀(P2)/ED₅₀(P1).

We cannot forget that an inadequate control of sourgrass in fields can be due to an application of glyphosate out of a better time for killing plants. Timossi et al. (2009) showed that there is no effective control of sourgrass when glyphosate is sprayed at late post-emergence or after rhizomes development. In our assay, the population supposed to be the susceptible

one showed any tolerance at late post-emergence application, since it was not controlled by the recommended dose of glyphosate. So, it indicates that the time of application (3-5 tillers) is too late to control sourgrass using glyphosate, so that the lack of control is not related to herbicide resistance.

Resistant populations of sourgrass were previously identified in Brazil (Carvalho et al. 2011) and the mechanisms of resistance were identified as associated to limitation of glyphosate absorption and translocation, enhanced glyphosate metabolism, and amino acid changes (Carvalho et al. 2012).

CONCLUSION

Sourgrass populations from different places can show distinct response to glyphosate; however the different responses of sourgrass populations cannot be associated to herbicide resistance.

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