GLYPHOSATE SUSCEPTIBILITY OSTARTF ANNUAL RYEGRASS (LOLIUM MULTIFLORUM L.) POPULATIONS IN NO-TILLAGE SYSTEMS IN URUGUAY

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ABSTRACT

The response of annual ryegrass (Lolium multiflorum L.) populations to different alvphosate rates was assessed in highly infested sites of the Western agricultural area of Uruguay. Six sites with previous information regarding crop rotation history, years under no-tillage and glyphosate usage were selected for this study. Seven glyphosate rates were tested: 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 kg a.i./ha and a control treatment without herbicide treatment was also included. Visual estimations at 10, 20, 30 and 40 days after the applications were conducted to determine control efficiency of glyphosate rates. Results showed significant differences in the speed of control among different rates. No satisfactory controls were achieved at the lowest herbicide rates at any of the evaluation times, Furthermore, the increase of glyphosate rates reduced the differences on control efficiency between sites. Ten days after herbicide treatments the control efficiency was extremely variable across sites. However, after 30 days from the herbicide treatments, rates of 4.0, 6.0, 8.0 kg a.i./ha achieved more than 90% of control efficiency at all sites. No glyphosate-resistant ryegrass populations were found in this study. The susceptibility of annual ryegrass populations was highly dependent on phenological stage when the herbicide treatments were applied, as well as dry matter per hectare present in each of the sites. It was observed that some ryegrass seedlings survived high glyphosate rates showing a tolerance response and suggesting the need to frequently monitor these sites in future.

Key words: Chemical control, glyphosate, resistance, annual ryegrass

INTRODUCTION

In Uruguay, crop production has traditionally been undertaken by rotating beef cattle production and crops in the same area across years. This system has been demonstrated to be environmentally sustainable as soil erosion is reduced and nutrients are recycled. However, in last ten years the Western agricultural area of Uruguay has experienced an intensification partially associated with high adoption of no-tillage systems together with transgenic glyphosateresistant soybean crops. The usage of transgenic glyphosate-resistant soybean cultivars in Uruguay was accepted in 1999 and since then, the

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sowing area of this crop has increased dramatically (Department of Agril. Statistics – DIEA, 2010).

The strong dependence on using herbicides in no-tillage systems and introduction of glyphosate herbicide-resistant cultivars has increased frequency of the herbicides' treatments, imparting a high selection pressure to those weed species more tolerant to this chemical. In the short term, these systems face the risk of weed shifts to glyphosate-tolerant species and in the long term, a higher risk of occurrence of glyphosate-resistant weed biotypes (Powles, 2008). Previous results have suggested shifts of weed flora in the region, showing an increased abundance of weeds belonging to Asteraceae family and which seeds are wind scattered (Tuesca and Puricelli, 2001; Rios et al., 2005; Rios et al., 2008). Within the Poaceae family, annual species such as summer grass (Digitaria sanguinalis L. Scop) also increased their abundance. Perennial species such as Johnsongrass (Sorghum halepense L.) Persoon) and purple nutsedge (Cyperus rotundus L.) have also shown an increase in their presence as they are naturally tolerant to glyphosate (Tuesca and Puricelli, 2001; Rios et al., 2005; Vila-Aiub et al., 2007; Rios et al., 2008).

The present study aimed to evaluate the susceptibility of several annual ryegrass populations to increasing glyphosate rates during winter fallows. This species has already developed glyphosate resistance in other parts of the world (Powles *et al.*, 1998; Heap, 2011) and its population dynamic under Uruguayan conditions poses it as the main threat for the occurrence of glyphosate-resistant biotypes. Furthermore, annual ryegrass has been identified as the weed species with highest current presence in country's no-tillage systems during winter fallows (Rios *et al.*, 2008). Thus, we believe it is crucial to regularly monitor the current response of the species to the most common herbicide used in the region.

MATERIALS AND METHODS Study Sites

The experiments were installed in West agricultural area of Uruguay (Lat. 33° 15′ 8″S Long. 58° 1′ 41″W), in departments of Paysandú, Río Negro and Soriano. This region has historically concentrated its major agricultural area and has generally adopted a no-tillage system from the 90's. Paddocks under no-tillage for a number of years and with available information regarding glyphosate applications and crop rotation were selected. From this selection, six sites at different farmers' properties were included for the study as they showed high infestation levels of annual ryegrass.

The climate is characterized by temperate conditions; from a 30 year average the annual precipitation was 1236.5 mm, occurring

evenly throughout the year. However, the annual rainfall was well below this level for surveyed year (i.e. 862.4 mm in 2010). The mean night temperatures are lower than 5.5°C in winter and above 30°C in day in summer (GRAS, National Agriculture Institute –INIA- 2011).

Assessment of the Species Susceptibility to Glyphosate

In order to characterize the annual ryegrass populations prior to the application of the treatments, the species biomass was sampled by using five quadrats (30×30 cm) per site. Upon cutting, plants were partitioned into above-ground and below-ground plant material and these two classes were put into separate brown paper bags. When returning to the National Agriculture Institute (INIA) in Colonia, Uruguay, samples were carefully washed using a grid underneath to prevent losses and dried in an oven at 60 ± 5 °C for 72 hours.

Within each of the six selected sites, experiments were set up using a completely randomized block design with three repetitions (i.e. rectangular plots of 2 x 5 m size) and seven glyphosate rates: 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 kg a.i./ha plus a control treatment without herbicide. The herbicide applications were performed between 28 August 2010 and 17 September 2010 depending on weather conditions in each site. The glyphosate formulation contained potassium Nphosphonomethyl glycine salt at a concentration of 500 g a.i./L. Herbicide treatments were performed using CO_2 spraying equipment with Teejet AI 110 02 flat spray tips and 110 L/ha of water. Weather conditions, such as air humidity, wind speed and temperature were recorded at the time each of treatment applications.

Control efficiency at each site was evaluated at 10, 20 and 30 days after herbicide treatments using a visual estimate of senescence rate of annual ryegrass plants. The visual scale was: very poor control (59% or less of the plants yellow); poor control (between 60 to 79% of the plants yellow); poor to satisfactory control (between 80 to 94% of the plants yellow) and excellent control (more than 95% of the plants yellow).

Statistical Analysis

All data sets were analysed by an analysis of variance using a General Linear Model in S.A.S (SAS Institute Inc, Cary, NC, USA). The general linear model was set up with sites and glyphosate rates as factors for analysis. According to normality tests a root-arcsine transformation of data was performed into x/100.

Data were analysed using an Adjusted Sum of Squares approach with 95.0% confidence intervals. When significant treatment effects were detected, pair wise comparisons of the means were undertaken using Tukey HSD method with 95.0% confidence intervals.

RESULTS

Significant differences were found in the speed of control across rates at all evaluation times (i.e. 10, 20 and 30 days after herbicide treatments). Within each site, at three evaluation times, the amplitude of control efficiency range decreased as the herbicide rates increased. No satisfactory control was achieved at lowest herbicide rate at any time after herbicide treatments. In the evaluation performed 10 days after the herbicide treatments the percentages of control that were achieved with 0.5 kg a.i./ha glyphosate were lower than 42% (Fig. 1, Table-1). Furthermore, the increase of glyphosate rates reduced the differences on control efficiency between sites (Table-1). Glyphosate rates of 1.0 and 1.5 kg a.i./ha showed equal amplitude of control efficiency ranges across sites (ca. 30%), as well as rates of 6.0 and 8.0. kg a.i./ha (40%). After 30 days from the herbicide treatments, rates of 4.0, 6.0, 8.0 kg a.i./ha achieved more than 90% of control efficiency at all sites (Table-1). Treatment rates of 0.5 kg a.i./ha showed poor control for all sites, except sites 5 and 6, where efficiencies of 78% and 85% were recorded, respectively. Excellent control was achieved with treatments of 1.0 kg a.i./ha for sites five and six, with efficiencies of 96% and 97% respectively. With the exception of site 1, the remaining tested sites averaged 70% control efficiency. Rates of 1.5 kg a.i./ha also achieved excellent controls for sites 1, 6 and 5 while remaining sites ranged between 53% to 77% of control efficiency. For all rates higher than 4.0 kg a.i./ha control surpassed 90% of efficiency.

times: 10, 20 and 50 days after herbicides treatments.										
	Evaluation time (days after herbicide treatment)									
Herbicide dose (Kg a.i./ha)	10		20		30					
	Control efficiency range across sites (%)									
	Min	Мах	Min	Мах	Min	Max				
0.5	6.6	42	22	53	37	85				
1.0	20	50	33	88	47	97				
1.5	28	60	45	93	53	97				
2.0	40	77	62	96	78	99				
4.0	50	87	78	99	90	100				
6.0	50	90	92	100	96	100				
8.0	53	93	94	100	99	100				

Table-1.	Contro	l efficie	ncy rang	ges acros	s the six	sites for the
	seven	herbicic	le rates	and for	the thre	e evaluation
	times:	10, 20 a	nd 30 d	avs after l	herbicide	s treatments.

When relating the results on control efficiencies with dry biomass of ryegrass populations from each site (data not presented) at the time of herbicide treatments, those sites with higher available biomass (sites 2 and 4, with 694 y 578 g DM. m^{-2} respectively) where the ones with less control efficiencies overall. Conversely, sites one, five and six, which recorded the lower biomass figures (461, 450 and 433 g DM. m^{-2} , respectively) achieved best control efficiencies, even at lower glyphosate rates and as soon as 20 days after herbicide treatments. However, site three, which presented the lowest available biomass (228 g DM. m^{-2}) due to previous grazing, achieved the worse control efficiencies.

No clear trends were detected between the above and/or below-ground biomass of annual ryegrass plants and the obtained control efficiencies.



Figure 1. Control efficiency (%) for the six evaluated sites and 0.5 kg a.i./ha of glyphosate (lowest dose) at 10 (), 20 () or 30 () days after herbicide treatments. Different letters above bars represent significant differences across sites and between evaluation times calculated through Tukey HSD method with 95.0% confidence intervals.

DISCUSSION

Results revealed that glyphosate rates of 1.0 kg a.i./ha and lower would provide an unsatisfactory control as various tolerant ryegrass plants survived the treatments. These circumstances would not only promote the presence of weed but also increase the frequency of glyphosate-tolerant biotypes.

No glyphosate-resistant ryegrass populations were observed in this study. The susceptibility of annual ryegrass populations was highly dependent on phenological stage when the herbicide treatments were performed, as well as the dry matter per hectare present in each of the sites. Even though better controls were achieved with less available biomass it is essential that sufficient above-ground plant material is exposed to the herbicide treatment. Thus grazing of fallows should be restricted previous to herbicide treatments. If this is possible to accomplish, rates of 1.5 and 2.0 kg a.i./ha combined with 20 days of fallow period might be enough to reach satisfactory control efficiencies.

It was observed that some ryegrass seedlings survived high glyphosate rates showing a tolerance response and suggesting the need to frequently monitor these sites in future.

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