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Full Length Research Paper

Glufosinate-Resistant Italian Ryegrass Populations Emerge from Glyphosate-Resistant Populations in Japan

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To evaluate glufosinate resistance, we conducted a dose-response bioassay using two glyphosate-resistant Italian ryegrass (Lolium multiflorum Lam.) populations (S63 and S64) in Shizuoka, Japan. Two additional cultivars, Waseyutaka and Common, were used as the susceptible control in the experiment. At the five to six leaf stage, glufosinate was applied at a rate of 0, 0.25, 0.5, 1.0, or 2.0 kg active ingredient (a.i.) ha^{-1} for each of the susceptible cultivars, at a rate of 0, 0.5, 1.0, 2.0, or 4.0 kg a.i. ha^{-1} for S63, and at a rate of 0, 1.0, 2.0, or 4.0 kg a.i. ha^{-1} for S64. Almost all individuals of the two susceptible cultivars died after the application of 1.0 kg a.i. ha^{-1}, the recommended rate for pest control, but 33 and 23% of individuals survived from the S63 and S64 populations, respectively. The ratios of the LD50 values for resistant vs. susceptible populations (R/S ratios) for S63 and S64 were 1.3 and 1.8, respectively. However, because Italian ryegrass is an obligate out crossing, wind-pollinated species, the S63 and S64 populations included both susceptible and resistant individuals and thus the R/S ratios may have been underestimated.

Keywords: Glufosinate, Glyphosate, Herbicide resistance, Italian ryegrass, Lolium multiflorum, Multi-resistance.

INTRODUCTION

Herbicides have made important contributions to world food production. Among commercially available herbicides, glyphosate and glufosinate are the most widely used, nonselective, post-emergence herbicides around the world (Green, 2014).

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and reduces the production of aromatic amino acids (Duke and Powles, 2008). For more than 20 years after its introduction, few weed species developed resistance to glyphosate. However, since glyphosate-resistant rigid ryegrass (Lolium rigidum Gaud.) was first reported in 1998 (Powles et al., 1998), 35 glyphosate-resistant weed species have been documented (Heap, 2017). These occurrences may indicate that the exclusive and repeated use of glyphosate has created a strong selective pressure and has accelerated the evolution of glyphosate resistance in weeds around the world (Green, 2014).

Glufosinate inhibits glutamine synthetase (GS), which catalyzes the assimilation of ammonium (Manderscheid, 1993; Manderscheid et al., 2005). The inhibition of GS leads to the accumulation of ammonium and causes the structural and functional destruction of plant cells.

Glyphosate-resistance in weeds in Japan was first reported in Italian ryegrass (L. multiflorum Lam.) populations on the levees of rice paddies in 2013 (Niinomi et al., 2013). To control these glyphosate-resistant populations, glufosinate was used as a glyphosate alternative, but it was noted that some plants survived glufosinate treatment.

Multiple resistance to glyphosate and glufosinate has also been reported in goosegrass (Eleusine indica (L.) Gaertn.) in Malaysia (Seng et al., 2010; Jalaludin et al., 2015), perennial ryegrass (L. perenne L.) in the US

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(Avila-Garcia and Mallory-Smith, 2011), and Italian ryegrass in New Zealand (Ghanizadeh et al., 2015). The mechanism of this resistance remains poorly understood. The emergence of multi-resistant biotypes to glyphosate and glufosinate has very serious implications for crop production.

Understanding the degree of herbicide resistance is important, as it provides insight into the mechanism of resistance and may subsequently assist in the development of an herbicide-resistant weed management system. In this study, we subjected two glyphosate-resistant Italian ryegrass populations to glufosinate and paraquat dose-response assays.

This is the first report of the emergence of glyphosate and glufosinate multiple resistant weeds in Japan.

MATERIALS AND METHODS

Plant Materials

Seeds from two glyphosate-resistant populations of Italian ryegrass (S63 and S64) (Ichihara et al., 2016) were collected in Iwata, Shizuoka, Japan and used in the experiment. Seeds of the Italian ryegrass cultivars Waseyutaka (TAKII) and Common (Snow Brand Seed) were used as the susceptible control in the experiments.

Glufosinate Dose-Response Bioassay

Seeds from each resistant population and the susceptible cultivars were sown in cell trays (1.7 × 1.7 × 5.0 cm) in April 2015. At the three- to four-leaf stage, seedlings were transplanted into cell trays, at one plant per cell; 4.0 × 4.0 × 5.0-cm cells were used. In each tray, 20 plants for Waseyutaka, Common, and S63; and 10 plants for S64 were transplanted and grown in an experimental field at Kyoto University, Kyoto. At the five- to six-leaf stage, glufosinate [Basta, 200 g active ingredient (a.i.) L^{-1}, Bayer CropScience] was applied. Application rates varied according to population: 0, 0.25, 0.5, 1.0 (recommended rate), or 2.0 kg a.i. ha^{-1} was applied for Waseyutaka and Common; 0, 0.5, 1.0, 2.0, or 4.0 kg a.i. ha^{-1} was applied for S63; and 0, 1.0, 2.0, or 4.0 kg a.i. ha^{-1} was applied for S64. In the 0 g a.i. ha^{-1} treatments, water alone was applied. Trays were arranged in a completely randomized design with three replicates.

Fourteen days after treatment, the numbers of surviving (completely or partially surviving) vs. completely dead plants were counted. The survival data were then used to estimate the $LD_{50,}$ or the application rate at which 50% mortality was achieved. The R/S ratio, which refers to the ratio of the LD_{50} of each resistant population against that of susceptible populations, was defined as the resistance index (RI).

Paraquat Dose-Response Bioassay

Seeds from each resistant population and susceptible cultivar were sown as described above. At the three- to four-leaf stage, 20 seedlings of S63 and the susceptible cultivars, and 8 seedlings of S64 were transplanted into 5.5 × 5.5 × 5.0-cm cell trays.

At the five- to six-leaf stage, paraquat (Preeglox-L, Syngenta Crop Protection) was applied as follows: 0, 313, 625 (recommended rate), 1250, or 2500 g a.i. ha^{-1} for S63, Waseyutaka, and Common; and 625, 1250, or 2500 g a.i. ha^{-1} for S64. In the 0 g a.i. ha^{-1} treatments, water alone was applied. Trays of S63 and the susceptible cultivars were arranged in a completely randomized design with three replicates. S64 were not replicated due to insufficient seedlings for the bioassay. Fourteen days after treatment, the number of surviving plants was determined and the resistance level was estimated.

Statistical Analysis

All statistical analyses were conducted using the R (ver.3.2.0) software package (Knezevic et al., 2007). The survival rate of each biotype at the recommended application rate was compared by fitting a generalized linear model with a logit link function and a binomial error distribution and then performing a Tukey-Kramer multiple comparison tests.

Dose-response curves on the survival rate were also obtained using a four-parameter logistic model. According to the model (Streibig et al., 1993), the LD_{50} was estimated as follows:

$$Y = \mathrm{c} + \left\{\frac{d-c}{1+exp\,[b\,(x-e)]}\right\},$$

Where, Y represents the survival rate at herbicide application rate x, and e is the LD_{50} value. The upper limit is d, the lower limit is c, and b indicates the slope of the line at the LD_{50}.

RESULTS AND DISCUSSION

In the glufosinate bioassay, almost all plants of the two susceptible cultivars survived at the lower application rate (0.25 kg a.i. ha^{-1}), but more than half of the plants died at 0.5 kg a.i. ha^{-1}. 97 to 98% plants of the two susceptible cultivars died at application rates of more than 1.0 kg a.i. ha^{-1}. In contrast, 68% plants of the S63 plants survived at 0.5 kg a.i. ha^{-1}, and 33 and 23% of plants in the S63 and S64 populations, respectively,

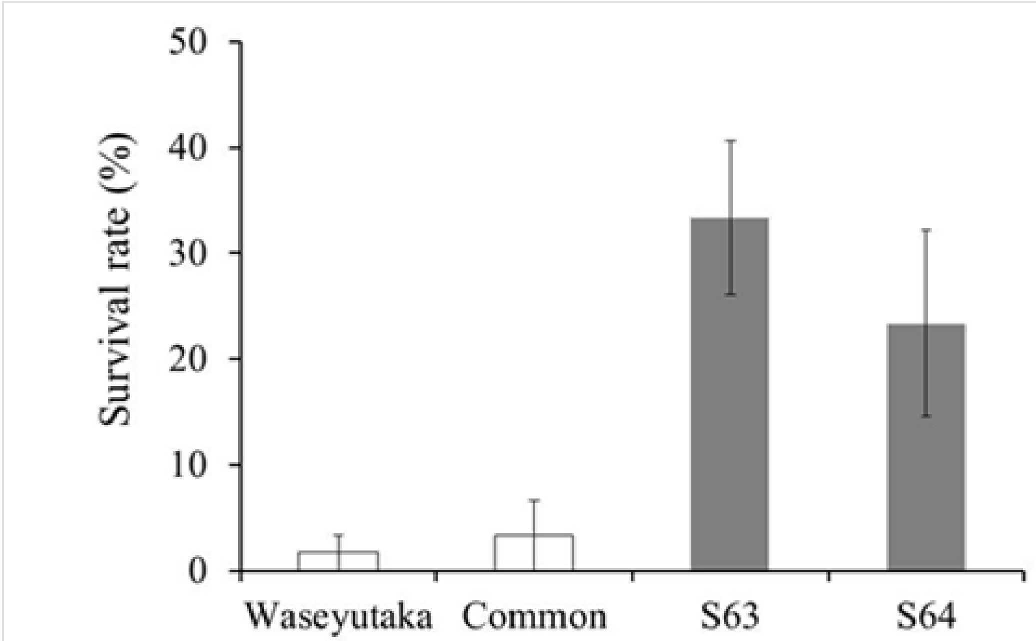

Figure 1. Survival rate of two cultivars (Waseyutaka® and Common®) and two resistant populations (S63 and S64) of Italian ryegrass at 1.0 kg a.i. ha^{-1} of glufosinate, the recommended rate for weed control.

Table 1. Parameters for the analysis of glufosinate dose-response by fitting the survival rate data to a four-parameter logistic model for two cultivars and two resistant populations of Italian ryegrass

Population		Parameter	Estimate	Standard error	t-value	p-value	R/S ratio
Cultivar							
	Waseyutaka®	*b'*	16.75	16.55	1.01	0.3167	
		c'	0.92	3.45	0.27	0.7911	
		d'	100.04	5.06	19.79	0.0000	
		$e'=LD_{50}$	0.49	0.02	26.93	0.0000	
	Common®	*b'*	14.83	4.28	3.47	0.0011	
		c'	1.64	3.45	0.47	0.6378	
		d'	100.20	5.16	19.43	0.0000	
		$e'=LD_{50}$	0.42	0.03	14.76	0.0000	
		LD_{50}	0.46				1.00
Resistant Population							
	S63	*b'*	2.50	0.77	3.26	0.0020	
		c'	-0.81	4.12	-0.20	0.8444	
		d'	121.81	22.39	5.44	0.0000	
		$e'=LD_{50}$	0.61	0.18	3.39	0.0014	1.33
	S64	*b'*	6.02	33.70	0.18	0.8590	
		c'	-0.03	3.68	-0.01	0.9928	
		d'	100.80	27.84	3.62	0.0007	
		$e'=LD_{50}$	0.80	1.16	0.69	0.4949	1.75

†S* refers to the average of LD50 of two cultivars.

‡R/S* ratio = $\frac{\text{LD50 of each resistant population}}{\text{LD50 of S*}}$

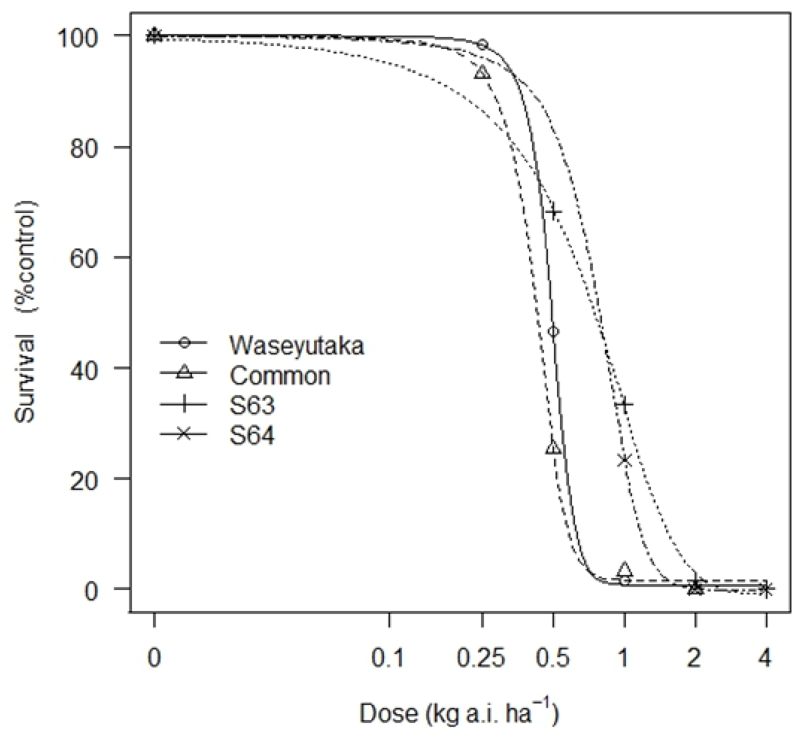

Figure 2. Glufosinate dose-response for survival rate of two cultivars (Waseyutaka® and Common®) and two resistant populations (S63 and S64) of Italian ryegrass at 14 days after treatment.

survived at 1.0 kg a.i. ha^{-1} (recommended application rate) (Figure 1). At 2.0 kg a.i. ha^{-1}, survival dropped to 1.7% plants in the S63 populations. All plants of the two resistant populations died at an application rate of 4.0 kg a.i. ha^{-1}.

R/S ratios are often calculated based on the dose required to cause a 50% decrease in shoot weight (GR_{50}). However, because GR_{50} can be affected by both environmental conditions and growth stage (Jalaludin et al., 2015), we instead estimated the R/S ratio based on LD_{50} data. This was necessary because the early growth of two susceptible cultivars was much faster than that of the S63 or S64 populations.

Based on the R/S ratios, the S63 and S64 populations showed 1.3- and 1.8-fold greater resistance to glufosinate, respectively, compared to susceptible populations (Table 1 and Figure 2). However, these ratios may be underestimated due to a number of factors, including the characteristics of the species. Italian ryegrass is an obligate outcrossing species and each plant is heterozygous such that seedlings from resistant populations are usually segregated for resistance to glufosinate. The S63 and S64 populations included both susceptible and resistant individuals; furthermore, resistant individuals may show differing levels of resistance. More accurate estimation of resistance using logistic regression modeling is possible if larger numbers of individuals are analyzed and if treatments at the lower end of the range of concentrations (0–1.0 kg a.i. ha^{-1}) are performed.

In the paraquat bioassay, all individuals of the susceptible cultivars and the S63 and S64 populations died at an application rate of 625 g a.i. ha^{-1} (the recommended rate), and no significant difference in survival rate was observed among the susceptible and resistant populations (data not shown). These data demonstrate that the S63 and S64 have not evolved resistance to paraquat.

Glufosinate resistance as assessed in our study was roughly similar to that described in previous studies examining Italian ryegrass, which reported two- to four-fold greater resistance compared to susceptible populations (Avila-Garcia and Mallory-Smith, 2011; Avila-Garcia et al., 2012; Ghanizadeh et al., 2015). However, our results were relatively low compared to those observed for goosegrass in Malaysia, which showed 5- to 14-fold greater resistance compared to susceptible populations (Jalaludin et al., 2015). Thus, the S63 and S64 populations examined here should be considered moderately resistant populations.

Glufosinate resistance is a relatively new herbicide-resistant biotype. Since 2009, the glufosinate-resistant biotype has been reported in only goosegrass and Italian ryegrass. These reports demonstrated resistance using whole-plant bioassays and the mechanisms of resistance are somewhat unknown. Work on a glufosinate-resistant population of Italian ryegrass in the US has suggested that its resistance is the result of an amino acid substitution in glutamine synthetase (GS2) (Avila-Garcia et al., 2012). In another population, resistance is thought to be conferred by limiting translocation (Avila-Garcia and Mallory-Smith, 2011). Additional research on the mechanisms responsible for conferring glufosinate resistance is necessary.

Resistance to both glyphosate and glufosinate resulting from the repeated use of these herbicides has been reported in goosegrass in Malaysia (Seng et al., 2010; Jalaludin et al., 2015), Italian ryegrass in the US (Avila-Garcia and Mallory-Smith, 2011), and Italian ryegrass and perennial ryegrass in New Zealand (Ghanizadeh et al., 2015). Glyphosate and glufosinate are the most widely used herbicides in the world. Accordingly, clarifying the mechanism of multi-resistance is important to establishing management programs for these weeds.

This is the first report of glufosinate resistance as well as multi-resistance across glyphosate and glufosinate in Japan. Italian ryegrass is a wind-pollinated, outcrossing species, and the pollen may travel great distances. For example, the pollen of rigid ryegrass, which is the same genus as Italian ryegrass, can be distributed up to 3 km from its source (Busi et al., 2008). Accordingly, monitoring the spread of resistant individuals and/or gene(s) is necessary.

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