Occurrence of glyphosate resistant annual ryegrass (*Lolium rigidum*) on fence lines of South Australian cropping fields

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Summary Glyphosate resistance in annual ryegrass has been reported at 63 fence line sites in Australia. As growers continue to rely heavily on this herbicide to control annual ryegrass on their fence lines, the number of resistant populations is expected to increase. A survey was undertaken to assess annual ryegrass from fence lines in South Australia that had been treated with glyphosate for five or more years. Plants were collected from fence lines and tested for resistance to glyphosate using the Syngenta Quick Test. Of the fifteen fence lines surveyed, ten had annual ryegrass plants with resistance to glyphosate. A dose response experiment conducted on one putative resistant population collected from a fence line in Clare, South Australia, showed 11-fold greater resistance to glyphosate than the susceptible control.

Keywords Annual ryegrass, glyphosate resistance, fence lines.

INTRODUCTION

Annual ryegrass is one of the most economically important weeds in the southern grain regions of Australia (Gill 1996). It is a major weed of cereal and horticultural crops and also occurs on fence lines of many crop fields in Australia. Fence lines are important areas of cropping fields because they act as firebreaks around crops and also reduce the invasion of pests into crops by serving as alternative habitat. Before the introduction of herbicides, annual ryegrass was controlled by tillage operations, grazing and burning (Reeves and Smith 1975). With the introduction of herbicides, the control of this weed species with non-chemical practices has reduced greatly.

Glyphosate is used by many growers in controlling annual ryegrass on their fence lines because it is safe, easy to use and cheaper than most other herbicides. However, with its intensive use as the only weed control strategy, glyphosate resistant annual ryegrass has evolved on the fence lines of many Australian cropping fields. Currently 63 fence lines in Australia have been confirmed with glyphosate resistant annual ryegrass (Preston 2012) and this number is increasing rapidly with more sites being identified every year.

This paper reports the results of a recent survey of annual ryegrass from fence lines of fields in South Australia. Plants were sampled from fence lines with a history of intensive glyphosate use over a period of five years or longer and tested for resistance using the Syngenta Quick test (Boutsalis 2001). A dose response study was also conducted on one population collected from a fence line at Clare, South Australia which had survived glyphosate application.

MATERIALS AND METHODS

Survey and resistance testing Fence lines at fifteen field sites were surveyed in 2011. At these sites farmers had been using glyphosate to control annual ryegrass for at least five years. At most of these sites, there had been reports of inadequate annual ryegrass control with glyphosate application. Samples of annual ryegrass were collected from the fence lines and tested for resistance to glyphosate (Roundup® 540 g a.e ha^{-1}) as described by Boutsalis *et al*. (2006). The plants were grown in 17 cm diameter pots filled with potting mix with one population per pot and maintained outdoors. The number of plants per pot varied from 5 to 9 according to location. Glyphosate (810 g a.e ha^{-1}) was applied using a laboratory moving boom pesticide sprayer one week after planting in the pots when the plants had regenerated fresh leaf tissue. The output of the sprayer was 109 L ha^{-1} at a pressure of 250 kPa and a speed of 1 m s^{-1} using a Tee-Jet 001 nozzle. The plants were returned outdoors after spraying and survival was assessed 28 days after the herbicide treatment. Annual ryegrass populations were counted as resistant if 20% or more of the plants survived the glyphosate treatment.

Dose response experiment An annual ryegrass population collected from a fence line at Clare, South Australia ('Clare' 2009) was used in this study. There had been a report of failure to control this population with glyphosate. One susceptible ('SLR4') and one known glyphosate resistant population ('SLR76') were used as controls. Seeds were germinated on agar as described by Lorraine-Colwill (2001) and transferred to 10 cm diameter pots (12 seedlings of a population per pot and four replications) containing standard potting mix and maintained outdoors between August and October 2011.

Herbicide treatment Glyphosate (PowerMax®, 540 g a.e L^{-1}) was applied to plants at 2 to 3-leaf stage and at rates ranging from 0 to 3600 g a.e ha^{-1} as described above. The plants were maintained outdoors and the response to the herbicide treatment assessed after 28 days. Plants showing active growth with new tillers were considered survivors (Powles *et al*. 1998, Wakelin *et al*. 2004). This experiment was repeated.

Data analysis The experiment was laid out in a Randomised Complete Block Design with four replicates. Mortality data were analysed using PriProbit and the dose required to control 50% of the plants (LD_{50}) calculated.

RESULTS AND DISCUSSION

Resistance screening with Quick test at 810 g ha^{-1} glyphosate (Table 1), confirmed resistance in ten out of the fifteen populations of annual ryegrass with survival ranging from 23% to 96%. Fence lines with less than 20% survival were considered to be susceptible. The intensive use of glyphosate without any other weed control strategies appears to be the main factor contributing to glyphosate resistance in weeds (Powles and Preston 2006). The site with the highest plant survival (96%) had a history of intensive glyphosate use for more than five years. The repeated use of this herbicide would have imposed intense selection pressure and increased the frequency of resistant individuals until they dominated the population (LeBaron and McFarland 1990). The sites with less than 20% survival also had a history of intensive glyphosate use; however in the recent years these populations had been treated with glyphosate mixed with other herbicides. This may be why glyphosate resistance had not yet become a problem at these sites.

In the dose response study, the susceptible annual ryegrass population 'SLR4' was completely controlled by glyphosate at the recommended rate of 450 g a.e ha^{-1} (Figure 1). The known resistant population ('SLR76') required much higher rates of glyphosate to achieve any control with no control achieved at doses below 1800 g a.e ha^{-1}. The putative resistant population ('Clare' 2009) survived glyphosate doses that completely controlled the susceptible population, but was not as resistant as 'SLR76'. The LD_{50} values calculated for each population in the experiment showed that Clare 2009 required eleven times more herbicide than the susceptible population to control 50% of the population (Table 2) and 'SLR76' required 29 times more herbicide. Consistency in results was observed in the repeat of this experiment.

Fence lines or firebreaks appear to be major contributors to areas where glyphosate resistance occurs in weeds in Australia. At present there are 63 confirmed

Table 1. Response of fence lines' populations of annual ryegrass to glyphosate. Data is presented as percentage survival of plants treated with 810 g a.e ha^{-1} glyphosate in outdoor pot trials in the spring of 2011.

Nearest town	Paddock fence line	Survival (%)	Rating
Tanunda	A115	70	RR
Brinkworth	North Alms	96	RRR
	Abbot Pad 8A	36	R
Greenock	Brown	42	R
	Elson	10	S
	BAG	11	S
	Cabaret	43	RR
	Moppa	33	R
	Jolly	23	R
Meningie	Coorang	92	RR
Eudunda	Gwynganna	0	S
Ungarra	U 2011	73	RR
Murraytown	A1380	11	S
	A 1379	0	S
Roseworthy	A1334	27	R
Standard resistant	SLR 76(R)	98	RRR
Standard susceptible	SLR 4 (S)	0	S

RRR – actively growing plants with no signs of wilting, RR – signs of stunted growth with new tillers, R – some wilting, stunted growth and regeneration of new shoots, S – mortality.

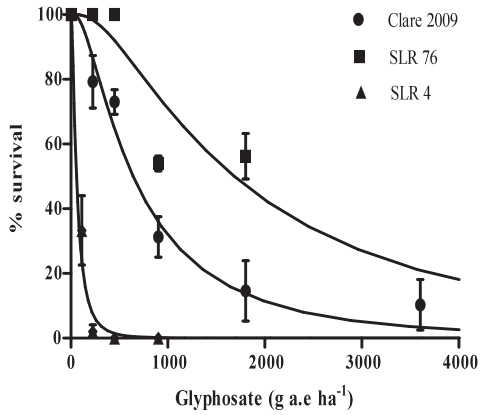

Figure 1. The response of resistant and susceptible populations of annual ryegrass to glyphosate. Error bars are SEM.

Table 2. The rate of glyphosate required for 50% mortality of resistant and susceptible annual ryegrass population. R/S is the ratio of LD_{50} of resistant population compared with the susceptible population.

Population	LD_{50} (g a.e ha^{-1})	R/S
Clare 2009	641.9	11
SLR 4	57.8	1
SLR 76	1683.4	29

cases of glyphosate resistance in annual ryegrass on fence lines in Australia (Preston 2012). The results of the survey reported here indicate that this number will continue to increase so long as growers rely solely on glyphosate for weed management. Populations where resistance was not detected came from farms where glyphosate has been used in a mixture with other herbicides over the last three years. There are reports in literature that use of appropriate herbicide mixtures can delay the onset of resistance in weeds (Christoffoleti *et al*. 2005). It is therefore important that growers rotate herbicides, apply herbicides in mixtures or practice other weed management strategies to either avoid or slow down the evolution of resistant weeds on their fence lines.

In conclusion, whilst most growers prefer glyphosate to other herbicides for controlling annual ryegrass on their fence lines, evolution of resistance is making this herbicide less effective. The challenge for the whole industry is to sustain glyphosate efficacy through rotation with other herbicides, use of herbicide mixtures and adoption of integrated weed management strategies.

ACKNOWLEDGMENTS

This research was partially supported by the Grains Research and Development Corporation and AusAID. The authors wish to thank the farm owners for providing plant samples for the study and also providing information about herbicide use.

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