

Characterisation and Management of Glyphosate Resistant Fence Line Populations of Annual Ryegrass (*Lolium rigidum* Gaud.)

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ABSTRACT

Glyphosate resistant weeds have evolved because of over-reliance on this herbicide without integration with other effective weed control methods. Since the first incidence of glyphosate resistance in 1996, 24 weed species have been confirmed with glyphosate resistance in 18 countries. Australia is one of the countries that is most affected by glyphosate resistance with resistance reported in 6 weed species. Glyphosate resistance in *L. rigidum* remains the greatest problem with 347 documented populations. These resistant populations have mostly been reported from cropped situations with heavy glyphosate use, such as chemical fallows, orchards, and vineyards. Recently populations of glyphosate resistant *L. rigidum* have started appearing on fence lines where glyphosate is applied once annually late in the cropping season. The aim of this project was to investigate the evolution and management of glyphosate resistance in *L. rigidum* on fence lines.

Dose response of *L. rigidum* to glyphosate was investigated in two fence line populations from Clare, South Australia (Clare 2010 and Clare 2009), two known resistant populations (SLR 76 and SLR 77) and one susceptible population (SLR 4). In these experiments, the susceptible population (SLR4) was completely controlled at the recommended glyphosate rate of 450 g a.e ha⁻¹. However, the resistant populations required much higher rates of glyphosate to achieve control. The fence line populations required 9 to 15-fold higher glyphosate dose to achieve 50% weed kill (LD₅₀) than SLR4. The standard resistant population SLR 77 had 5 to 7-fold greater resistances than SLR4, while SLR 76 was 20 to 30-fold more resistant than the susceptible control.

The pattern of glyphosate translocation in *L. rigidum* plants was investigated by examining the movement of ¹⁴C-labeled glyphosate. There were no significant differences between *L. rigidum* populations in the amount of herbicide absorbed 48 hours following application. Plants of populations SLR 76, Clare 2009 and Clare 2010 retained significantly more herbicide in their treated leaves than did the susceptible plants 48 h after the treatment. The amount of herbicide translocated to the stem and untreated leaves of the susceptible plants was significantly greater than that translocated into these tissues in the resistant plants. These differences in translocation pattern of glyphosate in these fence line populations of *L. rigidum* suggest that resistance is associated with an altered translocation mechanism. The pattern of glyphosate translocation in SLR 77 was similar to the susceptible population SLR4 indicating presence of a different resistance mechanism. To determine if these fence line populations have target-site mutation, the populations were assayed through DNA sequencing to

determine any alterations in the EPSPS gene. Results from this screening indicated, the fence line populations did not have a mutation at Pro 106 of the ESPS gene observed in glyphosate resistant weeds with target site resistance.

A farmer survey was conducted on selected fence lines sites in South Australia where glyphosate had been sprayed for five years or more. *L. rigidum* plants were sampled from these fence lines and tested for resistance to glyphosate (810 g a.e ha⁻¹) using the Syngenta Quick Test. Plants sampled from ten out of the fifteen surveyed sites were confirmed with resistance to glyphosate. Survival in these populations ranged between 23 to 98%. Fence lines with plant survival less than 20% were considered not to have resistance. The herbicide programs used on these sites showed that sites which had been treated with herbicide mixtures or non-glyphosate herbicides in the last three years did not have resistance. This confirmed that intensive and repeated use of glyphosate on fence lines is the major determinant of evolution of resistance.

Control of glyphosate resistant *L. rigidum* on fence lines is important to prevent spread of resistance into adjacent crops. To identify alternative herbicide strategies to control glyphosate resistant *L. rigidum* along fence lines, a range of herbicides were examined at two different fence line sites. It was observed that the control of these resistant weeds requires mixtures of broad spectrum contact herbicides with residual herbicides or sequential application of contact herbicides 14 days apart. The results of this study also showed that glyphosate-resistance in *L. rigidum* had moved at least 50 m from the fence lines into adjacent crops.

DECLARATION

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